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G.P. ELLIS

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D.K. LUSCOMBE

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Progress in Medicinal Chemistry 29

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Preface

This volume contains eight reviews on topics of current interest in medicinal chemistry. These cover the following subjects: inhibitors of 5-lipoxygenase and their anti-inflammatory activities; class III electrophysiological agents for therapy of antiarrhythmia; the chemistry and biological activities of non-peptide selective kappa opioid ligands; pyridazine derivatives with cardiovascular and other activities; centrally acting dopamine D2 receptor ligands: agonists; medicinal chemistry of the herb feverfew; ondansetron and related 5-HT_3 antagonists; synthetic inhibitors of bacterial and mammalian interstitial collagenases.

We are grateful to our authors for their critical appraisal of the expanding literature of these fields. We thank owners of copyright material for granting permission to reproduce extracts from their journals, and also the staff of our publishers for their continuing help and encouragement.

June 1991 G.P. Ellis

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1 5-Lipoxygenase Inhibitors and their Anti-inflammatory Activities

DOUGLAS G. BATT, Ph.D.

Inflammatory Diseases Research, The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880-0353, U.S.A.

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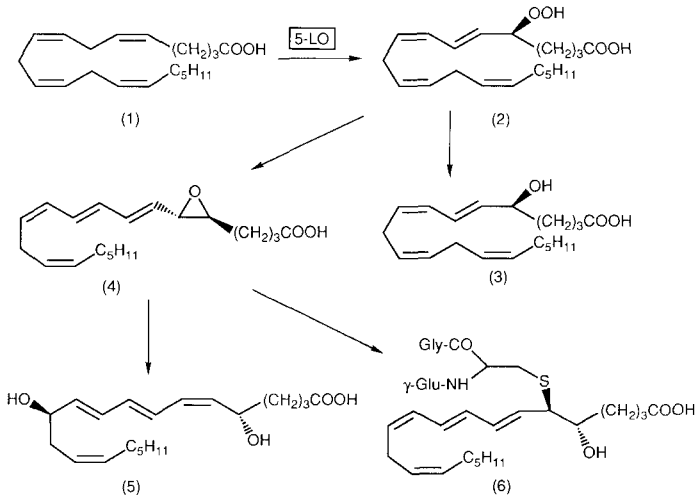
INTRODUCTION

Oxidation of arachidonic acid in mammalian cells gives rise to a number of metabolites, collectively called eicosanoids, which are intimately involved in inflammation and other physiological and pathological processes (for a general overview of eicosanoids, see [1]). Prostaglandins (PGs) were the first members of this family to be identified and characterized, and are involved in inflammatory and pain responses. The clinically useful non-steroidal anti-inflammatory drugs (NSAIDs) of today are considered to act by inhibiting the enzyme cyclo-oxygenase (CO), which converts arachidonic acid to prostaglandins. More recently, a second major pathway of arachidonate metabolism has been characterized, in which arachidonic acid is converted to pro-inflammatory products called leukotrienes (LTs). The enzyme 5-lipoxygenase (5-LO) initiates this cascade. (A number of excellent reviews of the 5-LO pathway and the LTs are available, for instance [2–5].)

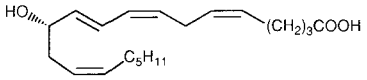
5-LO activity is found in a variety of mammalian cells, most notably leukocytes, with the enzymes from neutrophils and the rat basophilic leukemia (RBL-1) cell line being the most studied; several have been purified and cloned [5]. 5-LO is a cytosolic enzyme which requires calcium, ATP and additional uncharacterized cell components for optimal activity. Characterization and kinetic studies with 5-LO enzymes are often difficult, because of self-inactivation and complex cofactor requirements.

5-LO catalyses the stereospecific conversion of arachidonic acid (1) to a 5-hydroperoxyeicosatetraenoic acid (5-HPETE) (2). The enzyme contains



non-heme iron in the active site, and the mechanism of the transformation probably involves an organoiron intermediate or a dienyl radical which is trapped by molecular oxygen to produce (2) (for discussions and leading references, see [6,7]). Reduction of (2) generates 5-HETE (3), while enzymatic dehydration produces the unstable epoxide leukotriene A_4 (LTA_4) (4). LTB_4 (5) arises from the enzymatic hydration of (4), while addition of glutathione generates LTC_4 (6). The latter compound, along with its proteolytic metabolites LTD_4 and LTE_4, constitutes slow-reacting substance of anaphylaxis (SRS-A).

Additional lipoxygenases are known which oxygenate different positions on the arachidonic acid chain. 12-LO, resulting in the formation of 12-HETE (7), is best known in platelets, while the 15-LO from soybean has been studied in detail for many years [8]. 15-HETE (8) is also produced by mammalian cells; the enzymes from neutrophils and particularly rabbit reticulocytes are the best characterized.



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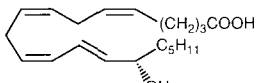

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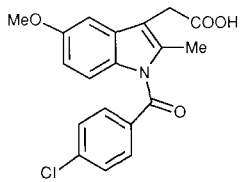
5-LO products (3)–(6) mediate a large number of physiological effects [3,4,9]. Various of the LTs are chemotactic for inflammatory cells, cause smooth muscle contraction leading to such effects as vasoconstriction and bronchoconstriction, and increase capillary permeability, contributing to oedema. More recently, effects on cell proliferation and immune function have been described. Much evidence has accumulated implicating LTs in disease states having inflammatory components [10–12], including arthritis, asthma and allergy [13,14], psoriasis and other inflammatory skin diseases [15–17], inflammatory bowel diseases such as ulcerative colitis and Crohn's disease [18,19], and more recently in circulatory diseases, such as shock and myocardial ischaemia [20,21].

The standard NSAIDs, of which indomethacin (9) is a prototype, do not significantly affect the generation of LTs in most circumstances. In the hope



(9)

of finding anti-inflammatory drugs with reduced side-effects or greater efficacy, a major effort has been mounted by the pharmaceutical industry over the past decade to identify either selective inhibitors of 5-LO, or dual inhibitors of CO and 5-LO.

The literature of leukotrienes and 5-LO inhibition is vast (well over 1000 papers and patents dealing with inhibition of this pathway were found by computer searching of various databases), so a comprehensive analysis of the field is not possible in this short chapter. Several previous reviews have appeared (for instance [22,23]); the broadest overview is that of Fitzsimmons and Rokach [24], with coverage to early 1988. The present survey emphasizes more recent reports, and compounds and series for which anti-inflammatory activity and/or structure–activity relationships (SARs) have been described. Patents have been cited only when they provide structure–activity information which is not available in other published form. The compounds and series discussed are organized roughly along structural and mechanistic lines; this organization is necessarily somewhat arbitrary, due to the large variety of structures for which 5-LO inhibitory activity has been reported. References dealing solely with inhibition of other lipoxygenases (12-LO and 15-LO) are not generally included.

METHODS OF EVALUATION

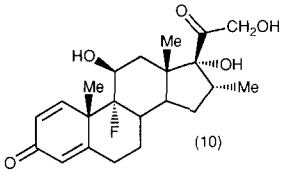
Numerous testing systems and protocols have been used to study 5-LO inhibitors in different laboratories, complicating attempts to compare compounds and series. *In vitro*, a variety of both cell-free and cellular preparations have been employed as primary screens. The most commonly used cell-free system is the crude cytosolic fraction from broken RBL-1 cells [25]; various broken neutrophil preparations are also used, and more recently purified enzymes have occasionally been employed. The formation of 5-LO products is generally determined by radioimmunoassay or (in older work) HPLC or bioassay methods.

Inhibition of the release of 5-LO products from intact stimulated leukocytes is another widely used evaluation method. Most commonly, the calcium ionophore A23187 is used to stimulate the production and release of 5-HETE and LTB_4 by neutrophils (from a variety of species, usually human or rat). Macrophages stimulated with opsonized zymosan (releasing LTC_4 and PGs), and antigen-induced LT release by lung tissue isolated from sensitized guinea-pigs have also been used. The formation of 5-HETE and LTB_4 in ionophore-stimulated whole blood can be useful; when product release in whole blood from drug-treated animals (or humans) is measured, bioavailability can be estimated as well.

While ionophore-stimulated 5-LO product release from neutrophils is often used as an indication of 5-LO inhibition, one must interpret these results cautiously. For example, halothane, an inhalation anaesthetic which may cause membrane perturbation [26], and colchicine, a microtubule disrupter [27], both were active, but presumably not because of 5-LO inhibition. A23187 is assumed to stimulate 5-LO by raising the intracellular calcium level, but this agent causes many other effects which may or may not be related to 5-LO activation, including changes in membrane potential, protein phosphorylation, phospholipid turnover, cyclic nucleotide levels, and DNA and protein synthesis [28]. Also, the effects of some putative 5-LO inhibitors on product release from neutrophils has been shown to vary with the stimulant used [29].

One class of agents which inhibits LT production in stimulated cell systems, but probably not by direct 5-LO inhibition, is the anti-inflammatory corticosteroids, represented by dexamethasone (10) [30–34]. The well-known inhibition of PG production seen with corticosteroids is not due to direct CO inhibition, but has been attributed to the inhibition of arachidonic acid mobilization by phospholipase A_2, caused by enhanced biosynthesis of one or more proteins called lipocortins [35] (although this hypothesis is now being seriously questioned [36]). More recent evidence indicates the possibility of down-regulation of CO enzyme levels [37–39]; similar mechanisms involving altered gene regulation could be involved in the observed effects on LT production as well.

Likewise, retinoic acid (11) and its derivatives [40,41], as well as synthetic aromatic retinoids (arotinoids) [42,43], inhibited A23187-stimulated release



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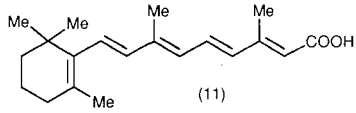


(11)

from both human and rat neutrophils at low concentrations; however, in neutrophil homogenates the potency was 10- to 100-fold less [41]. These effects are probably not due to 5-LO inhibition, since retinoids have a variety of effects on cell proliferation and differentiation, immune function, and inflammation [44], and are thought to mediate these effects by altering gene expression via soluble receptors related to the steroid receptors (see references cited in [45]). In general, 5-LO inhibition by an agent cannot be assumed based on inhibition of the release of products from cells, unless speci-

ficity for LT production or direct 5-LO inhibition in cell-free systems is also demonstrated.

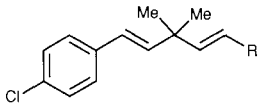
Many different *in vivo* models have been used to evaluate 5-LO inhibitors as potential anti-inflammatories, antipsoriatics, and antiasthmatics. 5-LO products may be involved in the standard anti-inflammatory screening models (such as carrageenan paw oedema and adjuvant arthritis) which are used for NSAIDs [46], but these models appear to be most sensitive to CO inhibitors. Other animal systems which are poorly responsive to NSAIDs have been used to evaluate potential 5-LO inhibitors, including carrageenan pleurisy and sponge models [46], peritoneal passive anaphylaxis in rats (see, for example, [47] and references cited therein), and the pleural reverse passive Arthus reaction in rats (see, for example, [48]). While various types of skin inflammation [49] have been applied to 5-LO inhibitor screening, the premier model is mouse ear oedema induced by topically applied arachidonic acid, with either topical or oral dosing of drugs [50,51]. A commonly used model for asthma is antigen-induced bronchoconstriction in sensitized guinea-pigs, which can be manipulated to indicate inhibition of LT-derived effects (see, for example, [52]). In many of these models, levels of 5-LO products can be measured in exudate fluids or isolated tissues, providing additional evidence for the mechanism of action of the administered agents.

In this review, drug concentrations or doses are assumed to be IC_{50} or ED_{50} values unless indicated otherwise. Abbreviations for routes of administration are p.o. (oral), i.p. (intraperitoneal) and i.v. (intravenous). The following abbreviations are used for common biological testing systems:

AAE: *arachidonic acid-induced ear oedema*
CPE: *carrageenan paw oedema*
cRBL: *crude cytosolic enzyme preparation from RBL-1 cells*
GPB: *antigen-induced guinea-pig bronchoconstriction*
ISN: *ionophore-stimulated neutrophil product release*
RAA: *rat adjuvant-induced arthritis*

SUBSTRATE AND PRODUCT ANALOGUES

Much early effort in 5-LO inhibition centered on substrate and product analogues. One goal, especially of the Corey group at Harvard, was to study the 5-LO reaction by creating mechanism-based irreversible inhibitors, or by removing the substrate protons which are abstracted by the enzyme. These approaches, which included preparation of acetylenic, methylated, cyclized, or thia-analogues of arachidonic acid, and cyclopropyl analogues



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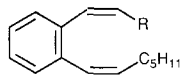

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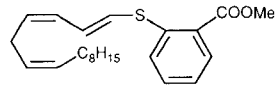
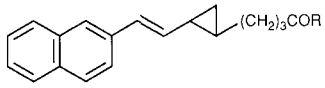


(14)

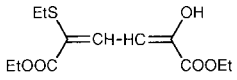
(12), (13): R = $(CH_2)_3COOH$

of LTB_4, have been reviewed previously [24]. Structures (12)–(14) represent newer inhibitors [53–55]. In general, no anti-inflammatory activity has been reported for any of these substrate/product analogues. However, the LTB_4 analogues TEI-8005 (15) and TEI-1338 (16), which inhibited LT production by ionophore-stimulated human blood at micromolar concentrations, also showed oral activity in CPE (1–3 mg/kg) and topical activity in endotoxin-induced endophthalmitis in rabbit eyes [56].

A series of hexadiendioic acid derivatives such as (17) (of uncertain stereochemistry) inhibited cRBL and rat ISN (2 μM in both), and also inhibited AAE (10 μg/ear topically, 20 mg/kg p.o.) [57].The thioether group in (17) was required for activity, but the nature of the alkyl group was unimportant. Benzoyl groups or preferably esters were required at each end of the molecule. *In vivo* activity did not correlate with 5-LO inhibition.



(15) R = OH
(16) R = NHC_6H_4(2-COOMe)



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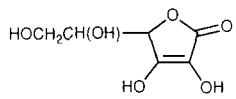
PHENOLIC COMPOUNDS

Beginning as early as 1970 [58], a vast array of phenolic compounds have been explored as inhibitors of lipoxygenases. Since phenols are well-known as reducing agents, activity against an oxidase would not be unexpected. Although the structural details vary widely, all active compounds of this class contain at least one free aromatic hydroxyl group, or a phenolic ester which presumably acts as a prodrug form of the phenol. Consideration of various SARs reveals little in common, although within structural series great specificity may be seen. Lipophilic character is a common feature of most of these inhibitors, as might be expected for activity against an enzyme whose normal substrate is a fatty acid. (Surprisingly, however, incorporation of a carboxyl group into many of these molecules destroys activity.) The requirement for lipophilicity in a reducing agent is demonstrated by the inactivity of ascorbic acid (18), a water-soluble physiological antioxidant, in rat ISN

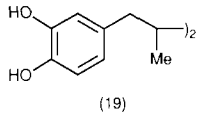
below millimolar concentrations [59,60]. However, a palmitate ester of (18) is a potent inhibitor (1.5 μM) of LTB_4 synthesis in human ISN and in cell homogenates [60].



(18)

CATECHOLS AND *ORTHO*-AMINOPHENOLS

One of the earliest reports of LO inhibition concerned the effects of *ortho*-dihydroxybenzene (catechol) derivatives on soybean 15-LO [58]. Lipophilic catechols, notably nordihydroguaiaretic acid (NDGA) (19), were more potent (10 μM) than pyrocatechol itself. The inactivation was, under some conditions, irreversible, and was accompanied by oxidation of the phenolic compound. The *ortho*-dihydroxyphenyl moiety was required for the best potency, and potency also correlated with overall lipophilicity of the inhibitor [61]. NDGA and other phenolic compounds have been shown by electron paramagnetic resonance spectroscopy to reduce the active-site iron from Fe(III) to Fe(II) [62]; one-electron oxidation of the phenols occurs to yield detectable free radicals [63]. Electron-poor, less easily oxidized catechols form stable complexes with the active-site iron atom [64].



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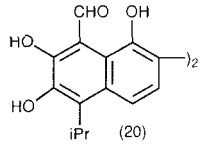

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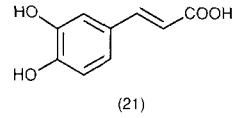


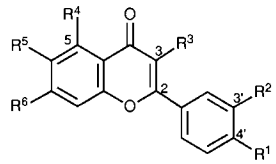
(21)

NDGA also inhibits 5-LO in a variety of systems [65–68]. In vivo, anti-inflammatory activity has been reported in lipopolysaccharide (LPS)-induced knee inflammation in rats [69] and in AAE and croton oil induced ear oedema [70,71]. However, in a 14-day clinical trial in psoriasis, no efficacy was seen following topical application of NDGA [72]. Although this compound has been used as an archetypal 5-LO inhibitor in many studies, the general antioxidant properties of this compound require caution in these interpretations.

A large number of catechol-containing compounds have been reported to inhibit a variety of 5-LOs, most commonly in cRBL and ISN. Many of these are natural products or synthetic analogues, such as gossypol (20) [67,73],

caffeic acid (21) and derivatives [74–79], and a wide variety of other *ortho*-dihydroxyphenyl compounds [76, 80–89]. In general, most inhibit 5-LO (0.1–10 μM) in cells or free enzyme systems, are somewhat less potent against 12-LO, and are often significantly less potent as inhibitors of CO. Potency is often roughly correlated with lipophilicity, and methylation of one of the hydroxyls (giving a guaiacol moiety) reduces but does not abolish activity. No *in vivo* anti-inflammatory effects have been reported for most of these compounds, with a couple exceptions having AAE activity [71,85].

Many reports have appeared over the last decade dealing with 5-LO inhibition by flavonoids. The most studied compound is quercetin (22), which inhibited cRBL (0.2 μM) as well as the generation of 5-LO products by rat peritoneal macrophages (*ca.* 5 μM) [90,91]. Like other catechols, it has been shown to undergo oxidation by soybean 15-LO [92]. Topical anti-inflammatory effects were seen in croton oil ear oedema [71], but surprisingly not in AAE [93]. Activity has been reported in rat carrageenan pleurisy, at doses of 100 mg/kg (p.o.) [91] and 10–20 mg/kg (i.p.) [94]; oedema and cell influx were both inhibited, and concentrations of LTB_4 and (to a lesser extent) $PGF_{2\alpha}$ were reduced.



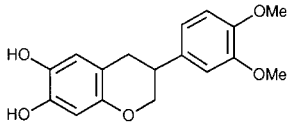
	R^1	R^2	R^3	R^4	R^5	R^6
(22)	OH	OH	OH	OH	H	OH
(23)	OH	OH	H	OH	OMe	OMe
(24)	OPO_3H_2	OH	H	OC_6H_{13}	OMe	OMe
(25)	H	H	H	OH	OH	OH

In one study, cirsiliol (23) was the most potent flavonoid against cRBL (0.1 μM) [95]. Conversion of the 5-hydroxyl to a lipophilic alkyl ether gave more potent analogues (0.01 μM), with 5–10 carbon chains optimal [96]. A phosphate ester derivative (24) was the only one reported to show *in vivo* activity, inhibiting by 80% the ischaemia/reperfusion-induced increase in LTC_4 in gerbil brain when given at 200 mg/kg i.v. [97]. Baicalein (25) is another example of a flavonoid inhibiting the 5-LOs from rat and human neutrophils and lymphocytes [98–100]. This compound showed no effect in CPE or cotton pellet granuloma, but did give some inhibition of adjuvant arthritis at 100 mg/kg p.o. [101].

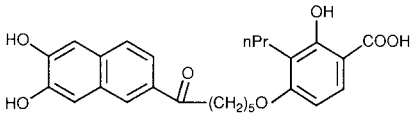
SARs for flavonoids *in vitro* have been reviewed [24]; briefly, the 3′,4′-dihydroxy substitution is required for the best activity, and alkylation of the hydroxyl group at C5 usually reduces potency [95]. Opening the pyranone ring or reducing the $\Delta^{2,3}$ double bond also causes a loss of potency [102].

A recent catecholic isoflavanoid, Zy 16372 (26), showed micromolar po-

tency in human macrophages and also inhibited AAE (5 μM/ear topically) [103]. The naphthalenediol (27) was a potent inhibitor of cRBL (4.7 nM), and inhibited both PGE_2 and LTB_4 release from rat peritoneal macrophages (2–3 μM) [104]. Oral activity was seen at 1 mg/kg in rat acetic acid-induced colitis, where cell accumulation was inhibited by 46%.

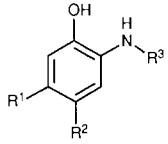


(26)

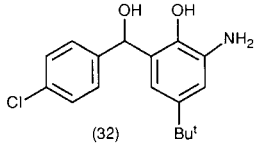


(27)

A few *ortho*-aminophenols, which are isoelectronic with catechols, have also been reported as 5-LO inhibitors. The parent is relatively weak (15 μM in crude guinea-pig neutrophil cytosol), but lipophilic 4-substitution, (28) or (29), increased the potency dramatically (0.4 μM and 0.14 μM, respectively) [105,106]. These compounds were roughly equipotent in ISN as 5-LO and CO inhibitors. A more highly substituted analogue, ONO-LP-016 (32), was somewhat less potent, but gave much greater selectivity for 5-LO over CO (3 μM and 100 μM, respectively). Two 70 mg/kg i.p. doses of (32) inhibited oedema and LTC_4 formation in gerbil brain following ischaemia/reperfusion [107].



(28) R^1 = H, R^2 = SPh, R^3 = H
(29) R^1 = H, R^2 = S-2-benzofuranyl, R^3 = H
(30) R^1 = H, R^2 = Me, R^3 = 4-Me-C_6H_4-CH_2
(31) R^1 = Me, R^2 = H, R^3 = 2-thiazolyl



(32)

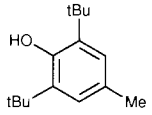
N-Substituted *ortho*-aminophenols can also inhibit 5-LO. The *N*-arylmethyl derivative (30) was a potent inhibitor of cRBL (0.029 μM), and inhibited the release of SRS-A in rat passive peritoneal anaphylaxis (75% at 200 μM i.p.; inactive p.o.) [108]. Variation of the 4-substituent, as well as the *para*-substituent on the benzyl group, indicated that overall lipophilicity was important for good potency; electronic effects in the benzyl group had a much smaller effect. CBS-113A (31) [109,110] inhibited human ISN (0.3 μM), and was about 50-fold less potent against CO and 15-LO in rabbit platelets. This compound also scavenged free radicals, inhibited the oxidative burst in neutrophils following phagocytosis, and reduced the LPS-stimulated release of interleukin 1 (IL-1) from cultured endothelial cells. Topically, CBS-113A inhibited various models of conjuctivitis and uveitis in

rabbits, rats, and guinea-pigs, as well as AAE (60% at 1 mg/ear). At 10 μM (i.p.), leukocyte influx in rat carrageenan pleurisy was inhibited, but no oral activity was seen.

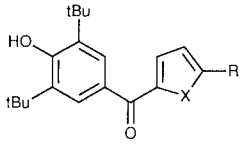
BHT DERIVATIVES AND RELATED STERICALLY HINDERED PHENOLS

Butylated hydroxytoluene (BHT) (33) is another widely used lipophilic antioxidant. Inhibition of 12-LO and 15-LO has been reported at millimolar concentrations, with somewhat more potent activity against CO [58,111,112]. More recently, BHT was found to inhibit 5-LO from potato [113] and RBL-1 cells [114] at concentrations near 1 μM. However, anti-inflammatory activity has not been reported.

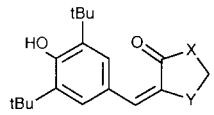
In the past decade a number of anti-inflammatory derivatives of BHT have been reported. The earliest was the 4-thenoyl analogue R-830 (prifelone) (34) from Riker, which showed a typical NSAID anti-inflammatory profile following oral dosing [115]. Additionally, prifelone inhibited the reversed passive Arthus reaction and (topically) oxazolone-contact sensitivity and UV erythema. Besides CO inhibition (0.5 μM), it also displayed activity as an antioxidant, and inhibited 5-LO in guinea-pig lung (20 μM). The 3-pyrrolyl compound (35) from Syntex had a similar profile [116]. Interestingly, attachment of a carboxylic acid group, giving (36) and the benzene analogue (37), caused loss of the CO potency (> 100 μM) while sparing the cRBL activity (20 μM); oral activity was seen in GPB for these analogues [117,118].



(33)



(34) X = S, R = H
(35) X = NH, R = H
(36) X = S, R = COOH
(37) X = CH=CH, R = COOH

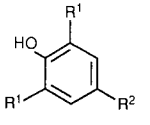


(38) X = O, Y = CH_2
(39) X = NOMe, Y = CH_2
(40) X = NH, Y = S
(41) X = NMe, Y = S

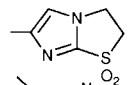
Kanegafuchi's α-benzylidene butyrolactone derivative KME-4 (38) also resembled an NSAID *in vivo*, but with a better *in vitro* balance of CO and 5-LO inhibition (cRBL and guinea-pig neutrophil sonicate: *ca.* 1 μM; seminal vesicle and rabbit platelet CO: 22.5 and 0.44 μM, respectively) [119–122]. Additionally, KME-4 inhibited carrageenan pleurisy following oral dosing [123]. At least one *ortho*-alkyl group must be *t*-butyl for good activity *in vivo* and as a CO inhibitor; replacement of the other by halogen destroyed

the activity, as did saturation of the double bond. Replacement of the lactone by a lactam or thiohydantoin also give about the same activity, but lactam *N*-methylation or enlargement of the ring reduced potency *in vivo* [124–126]. However, a closely related compound from Eisai, E-5110 (39), was very similar to KME-4 with somewhat greater *in vivo* potency [127,128]. Inhibition of the release of IL-1 from human monocytes (1.2 μM) was also seen with E-5110 [129]. LY178002 (40), from Lilly, possessed a very similar profile; methylation on nitrogen giving LY256548 (41) reduced potency slightly in this series [130–132].

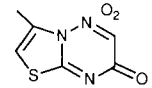
Several BHT analogues bearing heteroaryl groups at the 4-position have been described. A variety of such compounds, including indoles, benzoxazoles, and the thiazoloimidazole derivative (42) showed NSAID-like anti-inflammatory activity, but no 5-LO inhibition was reported [133,134]. A more recent compound from Hoechst, HWA-131 (43), was similar *in vivo* but was inactive against seminal vesicle CO [135]. However, rabbit mucosal CO and platelet aggregation were inhibited in the 1–2 μM range; activity in human ISN (5–25 μM) was shown. The SAR (in RAA) for this compound parallelled that of (38) and analogues in that at least one *t*-butyl group was required for activity while the best compounds had two *t*-butyls.



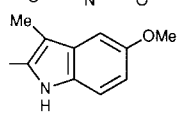
(42) R^1 = tBu, R^2 =



(43) R^1 = tBu, R^2 =



(44) R^1 = Me, R^2 =



(45) R^1 = tBu, R^2 = CH=CH

(46) R^1 = tBu, R^2 = CH=CH-3-pyridyl
(47) R^1 = tBu, R^2 = CH=CH-2-thienyl
(48) R^1 = Me , R^2 = CH=CH-2-thienyl
(49) R^1 = Me , R^2 = CH=CH-(4-F-Ph)

Replacement of the *t*-butyls by methyls, giving the related TZI-41127 (44), gave preferential inhibition of 5-LO over CO in rat ISN (2 μM and 25 μM, respectively) [136]. Inhibition of the release of SRS-A in A23187-induced pleurisy in rats was seen (30 mg/kg p.o.) [137], as was activity in coronary occlusion/reperfusion injury in beagles and rats [138]. Enlargement of the methyl or methoxy group on the heterocycle was consistent with good activity, but replacement of the alkoxy by halogens or other electron-withdrawing groups reduced potency.

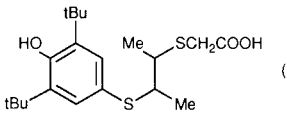
A further variation on the 4-aryl-2,6-dialkylphenols separates the two rings by an olefin; the Warner-Lambert compound PD 127443 (45) is an example [139,140]. PD 127443 was slightly more potent against 5-LO than CO

in A23187-stimulated RBL-1 cells (0.9 and 3.0 μM, respectively), and inhibited LTB_4 formation in a rat zymosan sponge model by 40–50% at 20–50 mg/kg p.o. Activity was also seen in RAA and CPE, and ulcerogenicity was reduced relative to (38) and (39). Replacement of the pyrazole by isoxazole or isothiazole gave similar activity. In contrast with the observation for (44), replacement of the *t*-butyl groups by methyls gave equal potency against CO and 5-LO, while methoxy groups increased 5-LO with respect to CO, and halogen substitution decreased the 5-LO potency.

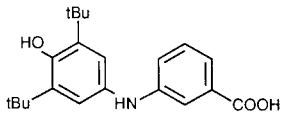
BI-L-93 (46), from Boehringer Ingelheim, was the best 5-LO inhibitor of a series of analogues which showed fairly balanced CO/5-LO inhibition in human ISN (0.67 and 2.7 μM, respectively), and looked like an NSAID *in vivo* [141]. The thienyl analogue (47) was an order of magnitude more potent against CO, and about equivalent against 5-LO. In agreement with the observation for (44), the dimethyl analogue BI-L-226 (48) showed much greater potency against 5-LO, with the CO activity essentially unchanged (0.07 and 1.3 μM, respectively) [142]. Replacement of either methyl group by a halogen decreased the 5-LO potency but increased the CO activity. Substitution on or reduction of the olefin reduced potency, while alkylation on the phenol destroyed activity. The 4-fluorophenyl analogue BI-L-239 (49) was very similar *in vitro*, but large groups replacing the fluorine of (49), or at the *ortho* position, decreased 5-LO activity. In contrast to (36) and (37), substitution by a carboxyl group destroyed both 5-LO and CO activity. Unlike the di-*t*-butyl compounds, (48) and (49) showed very poor oral activity, but by aerosol (49) blocked hyperreactivity and antigen-induced bronchoconstriction in sheep, monkeys, and guinea-pigs, and also decreased cell influx and LT production [143–145]. Release of LO products from a variety of cell types was also inhibited (0.03–0.3 μM).

Additional BHT-derived 5-LO inhibitors bear heteroatom-linked 4-substituents. Searle's SC-45662 (50) was selective (25:1) for 5-LO over CO in cRBL (3.7 μM) and in A23187-stimulated RBL-1 cells (7.1 μM) [146]. Besides NSAID-like activity in RAA (down to 10 mg/kg p.o.), SC-45662 also inhibited GPB (ED_{30} 16.7 mg/kg p.o.), and LTB_4 release from ulcerative colitis rectal mucosal biopsy samples was decreased [147]. Several patents have described similar compounds where the alkyl substituent on sulphur is varied quite widely [148–151]. Oxidation of the distal sulphur was consistent with activity, while replacement of this sulphur with oxygen gave reduced potency. Simple alkyl groups, alkylene-linked esters and amides, and disulphide-linked alkanoic esters were also active in cRBL with similar potency; free carboxylic acids were somewhat less potent. Oxidation of the sulphur attached to the phenolic ring destroyed the activity.

The aniline derivative S-26431 (51) from Riker was active in cRBL (1.2



(50)

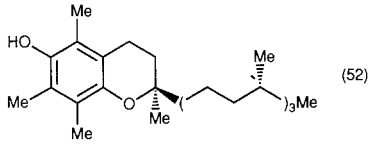


(51)

μM) and human ISN (8.3 μM), but had no effect on platelet thromboxane production below 200 μM [152]. Oral activity in GPB (20 mg/kg PO) was seen as well. A series of very similar compounds, where the carboxyl group of (51) was replaced by a wide variety of substituents at various positions of the ring, was disclosed in a patent from Otsuka [153]. In agreement with other SAR reports, replacement of the *t*-butyl groups by methyls enhanced 5-LO inhibition, but decreased oral activity in CPE.

VITAMIN E AND RELATED 4-OXY-SUBSTITUTED PHENOLS

α-Tocopherol (vitamin E) (52) represents another class of phenolic lipophilic antioxidants, para-substituted by an oxygen atom in a fused saturated ring. This compound weakly inhibited platelet 12-LO and soybean 15-LO [154,155], but not epidermal 12-LO [156]. One paper dealing with inhibition of 5-LO (from potato tubers, 5 μM) has appeared, with covalent modification reported [113].



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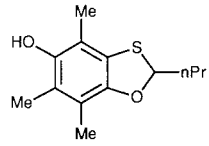
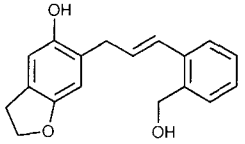


(53)

A series of related benzoxathiols, represented by (53), potently inhibited the 5-LO from sonicated guinea-pig neutrophils (0.02–0.5 μM) [157]; replacement of the propyl group by ethyl, butyl or phenyl maintained this potency. Inhibition of SRS-A release from antigen-stimulated guinea-pig lung tissue was also shown (2–6 μM), but suppression of rat liver microsomal lipid peroxidation suggested a relatively nonspecific antioxidant effect.

Merck's L-651,896 (54) was the lead compound from a series of dihydrobenzofuranols [158]. L-651,896 inhibited cRBL (1 μM) as well as platelet 12-LO (5.9 μM). In zymosan-stimulated mouse macrophages, both LTC_4 and PGE_2 release were inhibited (0.1 μM and 1.1 μM, respectively); similar potency was seen in rat and human ISN. Potent topical activity in AAE was seen, with LT production and oedema both inhibited at 20–30 nM/ear. LT levels were also reduced in mouse oxazolone contact sensitivity, but in this

case swelling was unaffected. Anti-inflammatory actions were also seen in pig skin arachidonic acid oedema (90% inhibition by 4 μg coinjected with 100 μg of AA) [159], and following topical application in A23187-induced epidermal hyperproliferation in guinea-pig skin [160]. The lack of systemic activity was attributed to rapid metabolism.



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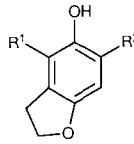
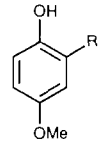


(55) R^1 = H, R^2 = H
(56) R^1 = H, R^2 = $(CH_2)_3SPh$
(57) R^1 = $(CH_2)_3SPh$, R^2 = H



(58)

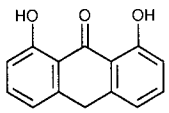
The SAR for L-651,896 and analogues was examined in human ISN [161]. Almost any substitution on the parent ring system (55) enhanced its potency (4 μM). There was shape specificity for 5-LO inhibition, demonstrated by the greater potency of 6-substituted analogues such as (56) compared to the 4-substituted compound (57) (0.019 μM and 0.12 μM, respectively). Attempts to take advantage of potential hydrogen bonding by introduction of heteroatoms at various positions in the substituent gave no improvement; carboxylic acid groups destroyed activity. Branching at the α-position also reduced activity, indicating that steric hindrance near the phenol was undesirable. Within a series (4- or 6-substituted), potency correlated very well with overall lipophilicity. This was interpreted as a reflection of partitioning into cell membranes. Expanding the dihydrofuran ring to a dihydropyran reduced the activity by 2- to 3-fold, as did replacement of the ring by a 4-methoxy substituent as in (58) [162]. Fully aromatic hydroxybenzofurans were also much less potent. Related dihydrobenzothiophene analogues were recently reported with similar potency *in vitro* [163].

NAPHTHOLS AND OTHER PHENOLIC COMPOUNDS

Anthralin (59), used clinically as an antipsoriatic, inhibited human ISN (7 μM); the oxidation products of (59) (the corresponding quinone and dimer) were not active [164]. A non-specific antioxidant effect was suggested, since ω-oxidation of LTB_4 and mouse ear 12-LO were inhibited at similar concentrations [165]. However, some other phenolic polycyclic aromatic compounds appear more specific in their actions.

In 1985, workers at Syntex described RS-43179 (lonapalene) (60) as a selective 5-LO inhibitor with topical anti-inflammatory activity [166,167]. The release of 5-LO products from human ISN was inhibited (20 μM), as was the cRBL system (0.5 μM). AAE was effectively inhibited (1 mg/ear), and

in TPA-treated mouse ears neutrophil influx (measured by myeloperoxidase levels) was decreased by 67% at 500 μg/ear, although oedema was suppressed only at higher doses (31% at 1 mg/ear) [168]. In a psoriasis clinical trial, lonapalene as a 1% gel gave responses similar to a topical corticosteroid (0.025% fluocinolone acetonide) after 4 weeks of thrice-daily application [169]. A skin-chamber technique was used to measure arachidonic acid and



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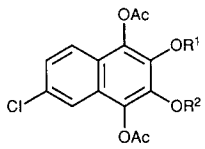
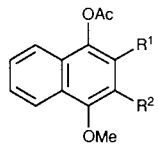
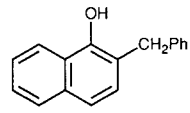


(60) R^1, R^2 = Me
(61) $R^1 + R^2 = OCH_2O$



(62) R^1 = nBu, R^2 = H
(63) R^1 = Et, R^2 = Et



(64)

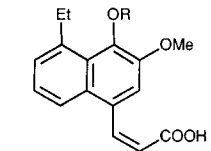
its metabolites in psoriatic plaques; a 2% ointment caused a significant decrease in levels of LTB_4, but 12-HETE and arachidonic acid levels were unchanged [170]. SAR for AAE activity showed that lipophilicity played a strong role [171], but if the compounds were too lipophilic (such as with larger alkoxy groups) activity fell off. The best substituent on the fused ring was chloro, although other groups capable of π-electron donation (other halogens, methoxy) were also effective. Hydrolysis of at least one of the ester groups appeared essential for activity, since compounds with increasing hydrolytic stability (pivalate, benzoate) were less potent. The quinones were essentially inactive. In a related series having the two alkoxy groups incorporated in a ring, the optimum compound (61) was about as potent as lonapalene [172].

A similar naphthol derivative from Upjohn is U-66,858 (bunaprolast) (62). This compound inhibited the release of LTs from rat peritoneal monocytes (0.44 μM) and reduced the synthesis of LTB_4 in ionophore-stimulated blood from rats by 61% after 50 mg/kg p.o. [173]. Neutrophil adherance and infiltration in the occluded jugular vein of cats was inhibited following intravenous administration, as was endothelial injury [174]. An oral or inhaled dose of 5–10 mg/kg inhibited bronchoconstriction caused by *Ascaris* antigen in rhesus monkeys [175]. Bunaprolast and (63) were the best representatives of the series [173]. Larger (phenyl) or electron-withdrawing (ester) groups at the 2- and/or 3-positions caused decreased potency in the monocyte assay (4–24 μM). The free phenols were also active, but were very unstable to oxidation; the quinones were about 10-fold less potent, while methyl ethers were essentially inactive. Substitution at the 5- or 6-position by methyl or methoxy is tolerated, but poly-methylation in the non-phenolic

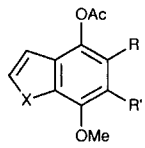
ring led to a decrease in activity. Replacement of the acetate by a variety of amino acid esters (as HCl salts) provided water soluble analogues; valine esters were about equipotent with the corresponding acetates [176].

A group at Du Pont found that simple 2-substituted 1-naphthols were both potent 5-LO inhibitors and topical anti-inflammatories. DuP 654 (64), currently in clinical trials for psoriasis, was the best of this series [177]. DuP 654 was very potent against cRBL (0.020 μM), with weaker CO inhibition (3.4 μM against bovine seminal vesicle enzyme). Topical activity was seen in AAE (11 μg/ear), A23187-induced ear oedema (7 μg/ear) and, less potently, in TPA-induced ear oedema (700 μg/ear). Levels of 5-LO products and neutrophil influx in inflamed ears were greatly decreased [178]. Structure–activity studies [179] showed that various positional isomers were significantly less potent against 5-LO than DuP 654, although the CO inhibition was less sensitive to these changes. Lipophilic phenols lacking the fused ring system, such as 2,6-dibenzylphenol, were also less potent. Lipophilic arylmethyl 2-substituents were favoured *in vitro* (0.01–0.2 μM) and topically (10–50 μg/ear), although even 2-methyl-1-naphthol was selective (but less potent) for 5-LO. The nature of the aryl group was relatively unimportant (except that carboxylic acid bearing rings were disfavoured), as was substitution in the non-phenolic ring. Substitution at the 4-position by electron-withdrawing groups reduced potency, as expected for a compound acting by a redox mechanism. In support of this, the active site iron of soybean 15-LO was reduced by DuP 654, as shown by EPR spectroscopy [180], with increasingly electron-withdrawing 4-substituents causing a progressive loss of this ability.

Another recently reported naphthol derivative is E-5090 (65) from Eisai. Oral administration gave good plasma levels of the deacetylated compound (66), which is active as a 5-LO inhibitor against cRBL and human ISN (2.5 μM) [181]. Although seminal vesicle CO was not effected, the release of PGE_2 from rat synovial cells was (14 μM). E-5090 was anti-inflammatory in CPE, RAA and collagen-induced arthritis following oral dosing at 10–100 mg/kg [182]. Compound (66) also inhibited the release of IL-1 from LPS-stimulated monocytes (5 μM), while E-5090 was similarly active in a rat air pouch system stimulated with LPS (at 50–200 mg/kg p.o.) [183].



(65) R = Ac
(66) R = H



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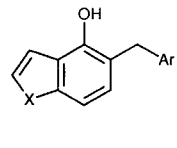
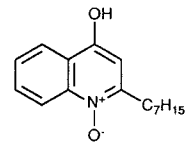


(68)



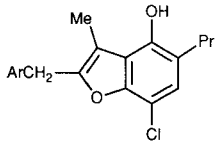
(69)

Structure–activity studies showed the hydroxyl group (or the acetate prodrug) was required for activity, as was the olefinic bond [184]. Greater lipophilicity in the substituent peri to the hydroxyl enhanced potency, but large size had a negative effect. Removal of the methyl group from the acrylic acid moiety or conversion to the trans isomer led to a loss of potency, but esters and amides were acceptable. Increasing the size of the alkoxy group was allowed for good *in vitro* activity, but potency in the LPS air pouch model was lost.

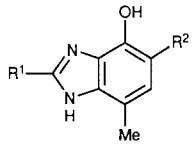
Heterocyclic naphthol isosteres have also provided potent 5-LO inhibitors. Heterocyclic analogues of (62) (67; X = O, S or NMe) were about equipotent with the isocyclic versions [173]; the same is true of heterocyclic variants of (64) (68; X = O, S or *N*-alkyl) and the corresponding acetates [185]. In 1986, the hydroxyquinoline *N*-oxide KF8940 (69), isolated from fermentation broths of *Pseudomonas methanica* KY4634, was reported as a potent inhibitor of cRBL (0.15 μM) which was quite selective with respect to inhibition of 12-LO and CO [186]. The corresponding *N*-reduced hydroxyquinoline, MY12–62c, was 500-fold less potent. The inhibition was later shown to be noncompetitive [187]. Similar concentrations also inhibited product release from rat ISN, and significant inhibition of GPB following oral dosing (10 mg/kg) was observed. Structural variations with terminally-functionalized 2-substituents (aminoalkyl, hydroxyalkyl) showed similar *in vitro* potency, but carboxyalkyl analogues and shorter-chain versions (hydroxypropyl) were 5- to 10-fold less potent; benzylation of the hydroxyl group also severely reduced activity [188].

Another heterocyclic inhibitor is L-656,224 (70) from Merck [189]. This compound was selective for 5-LO, and reduced product release from rat and human ISN (0.24 μM and 0.16 μM, respectively). Purified neutrophil enzymes were inhibited with similar potency. Although inactive in CPE, it showed oral activity in two asthma models (*Ascaris*-antigen-induced bronchoconstriction in squirrel monkeys and ovalbumin-induced asthma in rats) at doses below 1 mg/kg. Analgesic activity (yeast-induced pain) was also seen at the same dose levels. Like some of the naphthol series, an alkyl substituent (preferably methyl, ethyl or propyl) *ortho* to the hydroxyl was required for activity; the *t*-butyl analogue was less potent, as were analogues with heteroatom-containing chains at this position [190]. The chloro substituent provided oral activity, apparently inhibiting rapid oxidation to the quinone (which lacked *in vitro* activity). Substitution on the benzyl group was relatively unimportant, as long as a carboxyl group was not present.

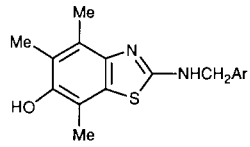
Closely related benzimidazoles such as (71) and (72) showed similar activity [191]. Besides 5-LO activity in cRBL (1–3 μM), these compounds inhibited rat passive peritoneal anaphylaxis and carrageenan pleurisy, although



(70) Ar = 4-MeOPh



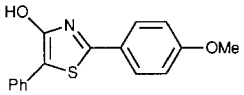
(71) R^1 = 4-MeOPhCH$_2$, R^2 = H
(72) R^1 = CF_3, R^2 = tBu



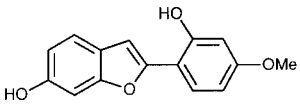
(73) Ar = 4-NH_2SO_2Ph

the *in vivo* and *in vitro* activities did not correlate. As with other related series, the free hydroxyl group was required for activity. Removal of the *para*-methyl group caused a significant drop in potency, and replacement of the benzyl group by an alkyl was acceptable (but led to reduced *in vivo* potency). Substitution on the benzyl ring was relatively unimportant, although various benzyl-substituted analogues showed enhanced *in vivo* activity in an unpredictable manner.

Other assorted phenolic heterocycles with 5-LO inhibitory activity have been reported. E-6080 (73), from Eisai, inhibited cRBL (0.2 μM) and LT release from antigen-stimulated guinea-pig lung tissue, as well as showing oral activity against GPB (30 mg/kg) and rhesus monkey bronchoconstriction (3 mg/kg) [192,193]. In guinea pigs, airway cell infiltration and LT production were suppressed. Although the phenolic ring of E-6080 shows some resemblance to vitamin E, a patent application discloses compounds with a wide variety of substituents on this ring as well as on the amine group [194]. Compound (74), from Abbott, was the most potent of a number of 4-hydroxythiazoles as a selective inhibitor of cRBL (0.037 μM) [195]. The phenolic benzofuran (75), isolated from a Chinese anti-inflammatory herb, inhibited cRBL and ISN (0.08 μM) [196]. Recently, the carbazole carbazomycin B (76), an antibiotic isolated from *Streptomyces* culture broths, was reported to inhibit cRBL (1.5 μM) [197].



(74)



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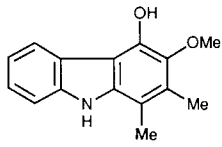
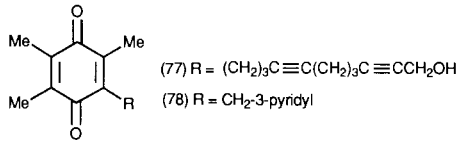


(76)

QUINONES

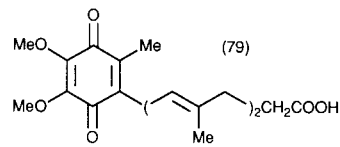
Phenolic compounds, particularly those with *para*-oxygen substituents, are

readily oxidized to quinones. Likewise, quinones are easily reduced (chemically and metabolically) to potentially 5-LO-inhibiting hydroquinones. Takeda's AA861 (docebenone) (77), which might be considered structurally related to the quinone obtained by vitamin E oxidation, has been one of the standard 5-LO inhibitors used in various physiological and pharmacological studies. Docebenone inhibited 5-LO activity from a variety of sources, such as cRBL (2.6 μM) [198], various crude leukocyte sonicates (0.12–2.3 μM) [199–201], and purified porcine leukocyte enzyme (4–10 μM) [202]. The release of LTs from antigen-stimulated guinea-pig lung tissue was inhibited with similar potency (0.8 μM) [199,200,203], as were rat peritoneal macrophages stimulated with A23187 [204] and human ISN [205]. Platelet 12-LO and CO were not affected significantly below 10 μM [205], but mouse epidermal 12-LO was inhibited (1.9 μM) [206]. Specificity for LO inhibition was questioned by a study in human neutrophils where other effects (degranulation, oxygen radical production, and chemotaxis) were altered, apparently independently of 5-LO inhibition [207].



(77) R = $(CH_2)_3C{\equiv}C(CH_2)_3C{\equiv}CCH_2OH$

(78) R = CH_2-3-pyridyl



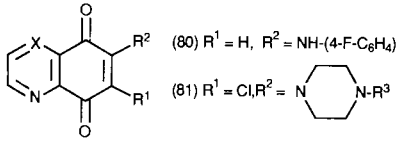
(79)

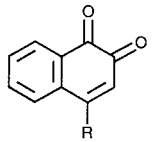
Docebenone has shown anti-inflammatory effects in several animal models following local dosing: phorbol ester-induced oedema and neutrophil influx in mouse skin [208,209], arachidonate-induced plasma extravasation in rabbit skin [210], the pleural reversed passive Arthus reaction in rats [211], and GPB [212]. An oral dose of 80 mg/kg reduced infarct size, LTB_4 levels and neutrophil infiltration in a rat myocardial infarction model [213,214], and in a rat brain ischaemia-reperfusion model oedema and LTC_4 levels were reduced at 200 mg/kg [107]. Significant, but not dramatic, improvement in nasal symptoms was seen in humans following 150 mg of docebenone twice daily for 8 weeks [215], but there was no effect on bronchial hyperresponsiveness to acetylcholine in asthmatics [216].

Only limited structure–activity information has appeared for compounds related to docebenone [199,200]. The side-chain was required for *in vitro* activity, but partial or full saturation of this group had little effect. Replacement of the methyl groups on the benzoquinone moiety by methoxyls also gave similar activity. A related compound, T22083 (79), was active but less potent against antigen-induced LT production in guinea-pig lung [203]. Recently, CV-6504 (78) was reported as a dual 5-LO/thromboxane A_2 synthe-

sis inhibitor, which is under investigation for chronic glomerular nephritis [217]. In this case, replacement of the methyls by methoxy groups abolished 5-LO activity (LTB_4 production by RBL-1 cells), as did introduction of polar substituents on the methylene carbon.

Amino-substituted naphthoquinones and heterocyclic variants have been disclosed in the patent literature as 5-LO inhibitors. Compounds represented by (80) (X = C, N) from Lilly inhibited SRS-A release from sensitized guinea-pig lung tissue [218]. Similar compounds such as (81) (R^3 = carboxylic ester, acyl, or aryl) and related naphthalene derivatives, from American Cyanamid, gave good inhibition in guinea-pig ISN (at 10 μg/ml) and in passive cutaneous anaphylaxis in mice (25–60 μM i.p.) [219,220].





(82) R = NH-C_6H_4-2-COOMe
(83) R = N(Me)-cyclohexyl
(84) R = substituted phenyl
(85) R = OH

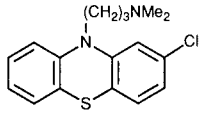
An *ortho*-naphthoquinone derivative from Ciba-Geigy, CGS 8515 (82) [221], inhibited guinea-pig ISN (0.1 μM) and LT production by ionophore-stimulated rat and human blood (4.0 and 0.8 μM, respectively) [222]. No effects on CO, 12-LO or 15-LO were seen below 100 μM. Oral inhibition of carrageenan sponge inflammation, carrageenan pleurisy, and a rat endotoxic shock model was seen down to 5 mg/kg, with significant reduction in LT levels. Inhibition of increased perfusion pressure caused by immune complex in sensitized guinea-pig hearts was accompanied by reduced LTC_4 production [223]. Although oral activity was seen, bioavailability and duration of action were poor ($T_{1/2}$ 21 min). The more water-soluble cyclohexylamine analogue (83) was similar in activity and more bioavailable [224]. The biaryl analogue (84) approached the potency of CGS 8515, with a 2- to 3-fold improvement in plasma half-life, by preventing the hydrolytic conversion to (85) seen with the amines [225]. Substituents such as halogen and methoxy on the phenyl ring of (84) gave greater potency, especially in the *ortho* position.

CONJUGATED BUT NON-AROMATIC HETEROCYCLES

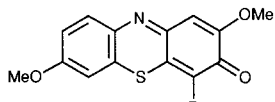
In 1983, chlorpromazine (86) and related phenothiazines were reported to inhibit cRBL (5.6 and 9.6 μM, respectively), selectively with respect to seminal vesicle CO and platelet 12-LO [226]. Heterocycles such as phenothi-

azines (which are fully conjugated but non-aromatic) are easily oxidized, indicating a possible explanation for 5-LO inhibition.

This redox activity, coupled with the lipophilicity and π-electron character (which was felt to mimic the arachidonic acid chain), led a Merck group to develop a series of substituted phenothiazinones as selective 5-LO inhibitors [227]. The lead compound, L-651,392 (87) was potent in cRBL, a puri-



(86)



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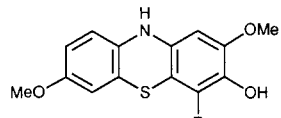
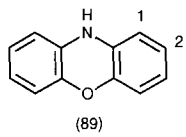


(88)

fied porcine leukocyte enzyme, and several whole-cell assays (0.06–0.26 μM) [202,228]. In cRBL, activity was seen only with added NADH or NADPH, implicating the reduced hydroxyphenothiazine (88) as the active moiety; these parent phenothiazines also possess activity both *in vitro* and *in vivo* when tested directly [229]. L-651,392 could thus be considered either as a conjugated non-aromatic or as a quinone-type inhibitor.

Other activities reported for L-651,392 include inhibition of LTC_4 (but not histamine or PGD_2) release from activated human basophils and mast cells (0.5–1.0 μM) [230], anti-asthmatic effects in various models (1–10 mg/kg p.o.) [231–233], and topical effects in A23187-induced epidermal proliferation [160] and antigen-induced conjunctival microvascular permeability [234] in guinea-pigs. Enhanced LTB_4 production was suppressed in both of the latter models. An interesting finding was the inhibition of PAF-induced hyperalgesia in rats following low (0.03–0.3 mg/kg) oral doses [235]. Structure–activity information for L-651,392 and analogues has appeared only in patent disclosures [227,229]. The reduced forms are generally more potent both *in vitro* and *in vivo*; removal or masking of the hydroxyl group in (88) does not greatly reduce potency, but oxidation of the sulphur weakens activity. Benzo-ring fusion is consistent with 5-LO inhibition [236].

A group at Lilly reported that phenoxazine (89) is a potent inhibitor of cRBL (0.02 μM) [237]. Substitution at the 1-position by carboxylic acid, ester, or hydroxamic acid groups decreased potency 10- to 30-fold. Substitution at the 2-position was less destructive of inhibition as long as the substituent was lipophilic (ester, acrylate ester); carboxylate caused a 200-fold loss of potency. Activity in rat neutrophils has been disclosed for related structures in patents from Bayer [238]. The second aromatic ring is not required, as shown by the activity of series exemplified by (90)–(92) [239–242]. Anti-asthmatic activity was indicated for these compounds, but few details were given.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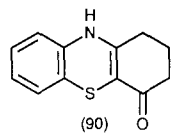
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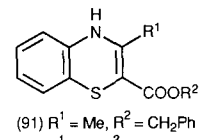
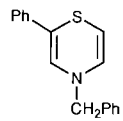

(91) R^1 = Me, R^2 = CH_2Ph
(92) R^1 = H, R^2 = nBu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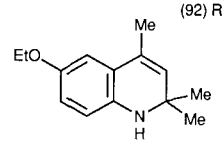

(94)

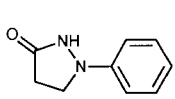
Recently, a series of substituted dihydrothiazines such as (93) was reported by workers at Merrell Dow to display potent inhibition of partially purified rat neutrophil 5-LO (0.2–2.0 μM) [243]. Substitution on the phenyl group or variation of the benzyl to other alkaryl had little effect on activity, but replacement of the benzyl by alkyl or hydroxyalkyl reduced potency about 10-fold. Replacement of the phenyl substituent by benzoyl reduced potency, while reduction of the trisubstituted double bond completely destroyed activity. No anti-inflammatory activity was reported for this series.

The antioxidant ethoxyquin (94) is conceptually similar to (88) in that it contains a benzene ring with two heteroatoms in a 1,4-relationship, one of which lies in a dihydroaromatic ring. This compound was reported to inhibit the release of LO products from rat ISN, although it was more potent against CO [244]. Orally-dosed ethoxyquin inhibited cell influx in a rabbit hydronephrotic kidney model [245], and was anti-inflammatory in guinea-pig UV erythema [246]. No relationship was drawn between these activities and 5-LO inhibition.

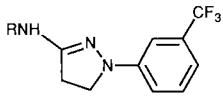
COMPOUNDS CONTAINING HETEROATOM–HETEROATOM BONDS

Perhaps the first compound to excite great interest in LO inhibition was phenidone (95), a photographic developing agent which inhibited both CO and the 12-LO from horse platelets [247]. *In vivo*, the related more potent 3-aminopyrazoline BW-755c (96) [248] inhibited not only CPE, but also leukocyte influx in carrageenan sponge oedema, an action not seen with classical NSAIDs [249]. Inhibition of 5-LO product generation was soon reported [250–252]. Since then, numerous reports of activity with a wide range of potencies in many enzyme, cell, tissue and animal models have appeared, and phenidone and BW-755c emerged as standard agents for pharmacological exploration of the LO pathways. Although clearly exhibiting

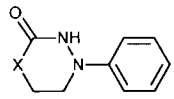
activities not typical of NSAIDs, the anti-inflammatory effects of BW-755c in some models have been attributed by some workers [253,254] largely to CO inhibition. More recently, these compounds have fallen from favour as standard 5-LO inhibitors, due to mechanistic inconsistencies and questions about their specificities. Nevertheless, phenidone and BW-755c provided an early impetus for seeking more potent and selective compounds.



(95)



(96) R = H
(97) R = Me

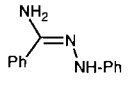


(98) X = CH_2
(99) X = NH

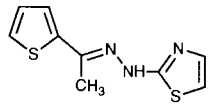
Thorough structure–activity studies for the pyrazolidinone and 3-aminopyrazoline series have not been published. Two early reports indicated that, at least for CO and 15-LO activity, separation of the aromatic ring from the nitrogen by an alkylene chain, ketone or sulphonyl group greatly decreased activity [255,256]. *N*-Methylation provided BW-540c (97), which was more potent as a 5-LO and CO inhibitor in rabbit leukocytes (0.4 μM and 1.2 μM, respectively) [257]. Potency peaked at the n-propyl derivative (0.04 μM and 0.3 μM, respectively). BW-540c was also topically effective in guinea-pig UV erythema, reducing oedema, epidermal necrosis and leukocyte infiltration [258]. Recently, derivatives of phenidone have been reported as well [259]; *C*-alkylation with lipophilic groups alpha to the carbonyl was acceptable, while *N*-methylation destroyed *in vitro* activity. A quantitative SAR study of the phenyl ring of BW-755c with respect to activity in the reverse passive Arthus reaction showed that electron-donating substituents were better than electron-withdrawing ones, and that large substituents were disfavoured, especially in the *ortho* position [260].

Ring-expanded versions of phenidone have also been reported. Abbott's A-53612 (98) was selective for 5-LO, inhibiting cRBL (4 μM), human and rat ISN (1 μM), and immune complex-induced LT biosynthesis in rats (6 mg/kg p.o.) [261]. Unfortunately, like BW-755c and its analogues, A-53612 caused methaemoglobinaemia in rats and dogs. The perhydro-1,2,4-triazin-3-one analogue A-65260 (99), however, did not have this effect [262]. A-65260 was otherwise very similar to A-53612 both *in vitro* and *in vivo*, having equal or slightly reduced potency. According to a patent disclosure, the pyridazinones were generally more potent than the triazinones; enhanced lipophilicity (by substitution on the 1-phenyl or the heterocyclic ring) increased potency, while electronic effects on the 1-aryl substituent appeared to play a minor role [263].

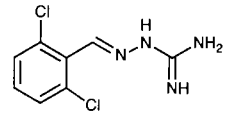
BW-755c and its congeners can be considered as reducing agents either because they are dihydroaromatic compounds, or because of the presence of a hydrazine moiety, which is easily oxidized. In the latter vein, the amidrazone CBS-1114 (100) inhibited platelet CO and 12-LO, the release of 5-LO products from human or rabbit neutrophils (1–10 μM), and leukocyte



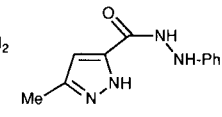
(100)



(101)



(102)

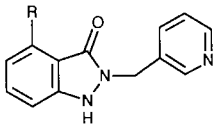


(103)

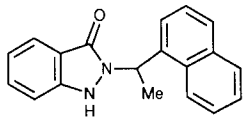
infiltration in a rat air-pouch model [264,265]. The hydrazone CBS-1108 (101) was similar, and also inhibited croton oil ear oedema [266,267]. A similar *in vitro* profile was retained by analogues with a variety or aryl groups at either end of the molecule [268]. Simple aryl hydrazones also inhibited soybean 15-LO, rat medullary CO, and human ISN [269,270]. Workers at Wyeth showed anti-inflammatory activity for guanabenz (102), an α_2-adrenoreceptor agonist and centrally-acting antihypertensive, in a variety of animal models [271]; inhibition of both PG and LT release from rat ISN and from zymosan-stimulated mouse macrophages was seen (10–30 μM). These activities were not seen in the structurally similar antihypertensive clonidine, which does not contain the N–N bond present in (102). Pyrazolecarboxylic hydrazides such as (103), which suggest acyclic analogues of phenidone, inhibited soybean 15-LO with submicromolar IC_{50} values and were inactive against CO, yet showed activity in CPE and RAA [272].

A series of indazolinones, represented by ICI 207968 (104) [273], might be considered benzo-fused analogues of phenidone. ICI 207968 inhibited cRBL (3.3 μM), and LT production by stimulated mouse macrophages and ionophore-stimulated human and rat blood with similar potency. *Ex vivo* activity in whole blood indicated oral bioavailability. Some analogues inhibited CO as well, but ICI 207968 itself was highly selective (*ca.* 300-fold) for 5-LO. Inhibition of plasma extravasation and neutrophil influx following a single oral dose of 100 mg/kg was seen in rabbit skin inflammation induced by arachidonic acid injection. Gastroprotection was also seen at this dose following oral challenge with either ethanol or indomethacin [274]. Short-lived oral activity was recently reported in rat Arthus pleurisy (2.2 mg/kg), with reduction in LTB_4 levels but not in fluid volume or cell influx [48].

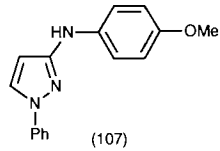
A recent SAR report [275] indicated that unsubstituted indazolinones inhibited 5-LO (2 μM), but without oral activity or selectivity (with respect to CO). Substitution on the 1-nitrogen had no effect, while substitution on



(104) R = H
(105) R = $CONHC_5H_{11}$



(106)

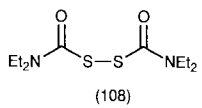


(107)

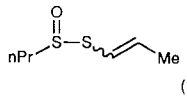
oxygen or both nitrogens destroyed activity. Phenyl and methyl groups replacing the pyridylmethyl of (104) gave greater potency, but poorer selectivity and oral activity. Benzyl and heterobenzyl groups gave the best profile; lengthening the linking group beyond one methylene had little effect. The 1-naphthylmethyl analogue was potent and selective, while the 2-naphthyl analogue had increased CO activity (and hence less selectivity). The 1-naphthylethyl analogue (106) showed good stereoselective action; the (*R*) enantiomer was very selective for 5-LO over CO, while the (*S*) enantiomer was less potent and much less selective. This is one of only two reports of stereoselective 5-LO inhibitors (the other being (146) from ICI). Compounds of this series had oxidation potentials less than 1.0 eV; although redox activity was required for 5-LO activity, it was not sufficient, since a variety of analogues having essentially the same oxidation potential had *in vitro* 5-LO IC_{50} values ranging from 0.2–14 μM. Unfortunately, methaemoglobinaemia was observed in animals; the amide derivative (105), a poorer reducing agent, was safer but also less orally active.

A fully aromatic pyrazole derivative, FPL 62064 (107), also inhibited 5-LO in the usual cellular and enzymatic systems, but was not selective with respect to CO [276]. This compound inhibited AAE (ED_{50} values against PGE_2, LTC_4, and oedema were 3.7,28.3, and 40.8 μg/ear, respectively), guinea-pig UV-induced erythema and phorbol ester ear oedema. The activity for FPL 62064 was also attributed to its antioxidant activity; presumably in this case the 4-methoxyaniline group is the redox-active moiety.

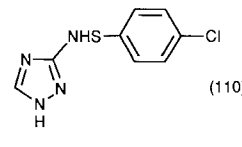
Other compounds containing heteroatom-heteroatom bonds have been reported as 5-LO inhibitors. Diphenyldisulphides and substituted analogues, as well as disulphiram (108), inhibited cRBL and LT release from zymosan-stimulated mouse macrophages (0.3–20 μM) [277, 278]. A number of thiosulphinate esters such as (109) and related compounds, isolated from onion and garlic, were likewise active in cells and cell-free enzymes over the



(108)



(109)



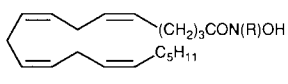
(110)

same concentration range [279, 280]. These compounds were antiasthmatic in GPB following oral dosing at 20 mg/kg. Bay 08276 (110), a sulphenamide, inhibited the production of 5-, 12-, and 15-LO products from neutrophils and platelets, but was much less potent toward CO products [281]. Topical activity was seen in a rabbit re-epithelialization model and in immunogenic keratitis [282]. A wide variety of nitrogen heterocycles replacing the triazole of (110) gave similar activity [283].

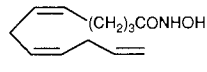
HYDROXAMIC ACIDS AND RELATED COMPOUNDS

In 1984, Corey and co-workers reported that *N*-hydroxyarachidonamides (111) were potent reversible inhibitors of cRBL (0.03–0.2 μM) [284]. Alkylation on nitrogen increased the inhibitory potency significantly, and truncation to (112) still gave activity (1.9 μM). An alternative approach at Abbott placed the hydroxamic acid moiety in the 5-position, giving analogues of 5-HPETE such as (113) which also inhibited cRBL [285].

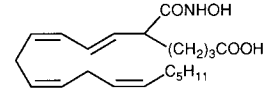
The ability of hydroxamic acids to chelate iron provided the rationale for



(111) {R = H, Me, nBu}



(112)

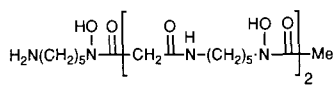


(113)

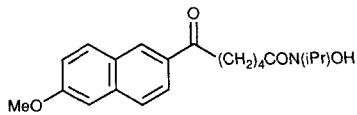
this approach, since iron is a critical part of the active site of 5-LO. In fact, desferrioxamine (114), a clinically used iron chelating agent, has been reported to inhibit reticulocyte 15-LO [286], but in rat ISN no effect was seen at low concentrations while stimulation of LTB_4 and TXB_2 release was seen at higher doses (up to 500 μM) [287]. The moderate anti-inflammatory effects reported for high doses of desferrioxamine in several animal models [288,289] may be due to sequestering of free iron, decreasing oxygen radical production, rather than to a specific effect on arachidonate metabolism.

Although the simple hydroxamate analogues of arachidonic acid showed no anti-inflammatory activity, the concept prompted a number of research groups to explore hydroxamic acids with more stable lipophilic residues. This approach has yielded some of the most interesting anti-inflammatory 5-LO inhibitors reported to date.

A series of aralkylhydroxamic acids from Bristol-Myers is represented by 9-phenylnonanohydroxamic acid (BMY 30094) [290]. This compound inhibited the production of 5-HETE by human ISN (5.8 μM), with about one third that potency against CO and 12-LO in platelets. Alkylene chains



(114)



(115)

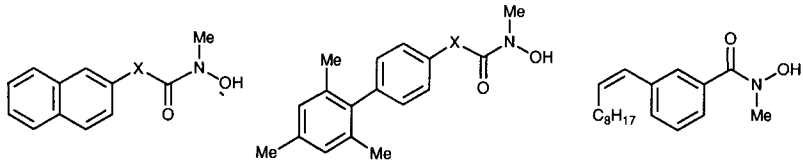
shorter than 6 or longer than 11 carbons gave inactive compounds (> 100 μM). Alkylation (methyl or isopropyl) on nitrogen greatly improved potency (< 1 μM). Small substituents on the phenyl ring (methyl, methoxy, chloro) had little effect on potency, but larger substituents (butyloxy) led to greatly decreased activity. BMY 30094 showed topical anti-inflammatory activity in AAE and phorbol ester ear oedema (550 and 375 μg/ear, respectively). In a carrageenan sponge model, a 5% solution inhibited neutrophil accumulation by 97%, but no oral activity was seen [291].

A patent from Ortho disclosed naphthoylvalerohydroxamic acids which inhibit 5-HETE release from RBL-1 cells (0.1 μM) and also showed oral activity in RAA [292]. The most potent compound (51% at 50 mg/kg) was the *N*-isopropyl analogue (115). Although only the hydroxamic acids inhibited 5-LO activity, simple acids and esters showed comparable RAA activity, suggesting that the hydroxamic acids yielded an anti-inflammatory carboxylic acid metabolite.

Recently, hydroxamic acid derivatives of common NSAIDs (meclofenamic acid, indomethacin, sulindac, and ibuprofen) were evaluated at Warner-Lambert [293]. The order of 5-LO inhibitory potency (in RBL-1 cells) for these derivatives was CON(Me)OH > CONHOH > CONH(OMe)- > COOH. The CO potency ranking was exactly opposite, although the best 5-LO inhibitors still possessed significant CO activity. Whether the observed oral activity in CPE was due to carboxylic acid metabolites or to the intrinsic activity of the hydroxamic acids was not clear. The more active 5-LO inhibitors were less ulcerogenic in fasted rats.

Hydroxamic acids have been extensively investigated at Abbott, where a hypothetical binding site hypothesis was based on examination of many simple ω-aralkylhydroxamic acids [294]. Several series of conjugated hydroxamic acids were explored based on this hypothesis, yielding potent 5-LO inhibitors (0.02–2 μM) exemplified by (116)–(119). The most potent of these (119) also inhibited purified porcine leukocyte 5-LO (0.5 μM) [202]. As other workers have found, *N*-methylation was beneficial for potency. Activity was also seen in a rat peritoneal anaphylaxis model following i.p. (0.2 mg/kg), but not oral, dosing [47].

Related benzohydroxamic acids such as (120), with similar properties, were reported from Roussel [295]. Additional cinnamohydroxamic acids, re-

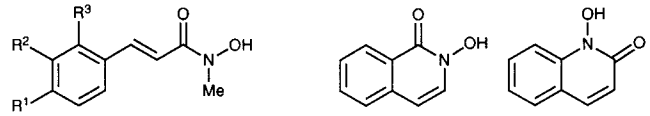


(116) X = bond
(117) X = CH=CH (trans)

(118) X = bond
(119) X = CH=CH trans

(120)

presented by (121) from Ortho [296], CGS 22745 (122) from Ciba-Geigy [297], and LY233469 (123) from Lilly [298], also showed similar *in vitro* potency. Compound (121) inhibited arachidonic acid-induced bronchospasm in guinea-pigs, but only following i.v. administration. Surprisingly, (122) showed good oral bioavailability in dogs and rats, inhibiting ionophore-stimulated LT production in whole blood (3 mg/kg 3 h after dosing) and carrageenan pleurisy (significant inhibition of cell accumulation and exudate volume at 10 and 30 mg/kg p.o.). *N*-Hydroxyquinolones such as Abbott's (124) and (125), which can be considered as cyclized versions of the benzohydroxamic or cinnamohydroxamic acids, respectively, were active but less potent (1–10 μM vs. cRBL) [299].



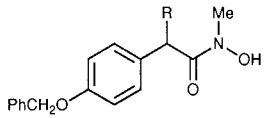
(121) R^1, R^2 = OMe, R^3 = H
(122) R^1 = 2,5-dimethyl-1-pyrrolyl, R^2, R^3 = H
(123) R^1, R^2 = H, R^3 = SMe

(124)

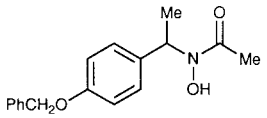
(125)

The oral inactivity of (116)–(119) was shown to result from rapid and complete metabolic hydrolysis to the inactive carboxylic acid. Non-conjugated 2-arylacetohydroxamic acid derivatives such as (126) gave better oral activity in the rat peritoneal anaphylaxis model, although *in vitro* 5-LO potency was somewhat reduced with respect to the conjugated analogues (0.3–5.0 μM) [47]. Serum level measurements indicated good absorption and metabolic stability, particularly if the α-carbon was methyl-substituted. Disubstitution or enlargement of the α-methyl caused poorer absorption. As usual, *N*-methylation enhanced potency and duration of action, but larger *N*-substituents reduced absorption. Compound (126) (*in vitro* 0.28 μM) was the best analogue reported, giving serum concentrations of 8.4 μM (20 min) and 7.9 μM (60 min) after a single oral dose of 100 mg/kg; the ED_{50} was 40 mg/kg.

A group at Rorer studied in more detail compounds lacking the α-methyl, such as (127) (RG 6866) [300,301]. Good activity was seen in crude guinea-pig neutrophil 5-LO and in guinea-pig and human ISN, as well as topical activity in AAE. *N*-Substituents as large as benzyl were compatible with good *in vitro* activity, but only *N*-methyl analogues were orally bioavailable (guinea-pig systemic anaphylaxis), in agreement with findings from Abbott. Interestingly, the SAR with respect to inhibition of 12-LO from rat platelets was different; this activity seemed much more sensitive to steric hindrance about the nitrogen atom.



(126) R = Me
(127) R = H



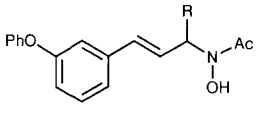
(128)

The Abbott group has published the results of a quantitative SAR study incorporating many of the series discussed above [302]. The inhibitory activity (in cRBL) was found to correlate most strongly with the molecule's overall lipophilicity, which could take a variety of forms in agreement with the results reported by others [290,296, 300]. However, hydrophobicity in the immediate vicinity of the hydroxamic acid, as well as greater than 12 Å away from this moiety, did not greatly influence potency. The adjusted lipophilicity coefficient in the correlation equation was 0.57, interpreted to indicate approximately 50% desolvation of the inhibitor on binding to the enzyme. This might imply that the active site lies either on the surface of the enzyme or in a shallow trough. Additional findings of the study supported the desirability of *N*-alkylation and conjugation of the hydroxamic acid moiety, and also indicated that electron-poor aromatic rings are desirable.

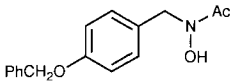
The hydroxamic acids reviewed so far have the large lipophilic group attached to the carbonyl, and a small alkyl group on nitrogen. Reversal of this pattern (dubbed 'type A' by the Abbott workers) to yield *N*-substituted acetohydroxamic acids ('type B') afforded surprising increases in oral potency without affecting *in vitro* activity. Abbott's A63162 (128) [303] was 2- to 5-fold more potent in rat peritoneal anaphylaxis than the corresponding arylacetohydroxamic acid (126) [304]. Peak serum levels for (128) and (126) were 140 μM at 2–3 h and 15 μM at 30 min, respectively, with serum half-lives following suit. While the major metabolic pathway for (126) was hydrolysis to the carboxylic acid, only glucuronic acid conjugation was seen for (128). SARs were very similar to those observed for the arylacetohydroxamic acids [305]: methyl was favoured on the carbonyl group, the best

group linking the aryl moiety to the nitrogen was CH(Me), and lipophilic substituents on the phenyl ring, preferably in the para position, were optimal. Heterocycles such as benzothiophene, benzofuran, *N*-methylindole, and dibenzofuran could also serve as the aryl group [306,307].

Closely related type B hydroxamic acids have been studied at Burroughs Wellcome also. Several reports have appeared dealing with three analogues: BW-A4C (129), BW-A137C (131), and BW-A797C (132) [308] These compounds reversibly inhibit LTB_4 synthesis by human leukocyte homogenates (0.1–0.8 μM) and in intact cells, and are quite selective for 5-LO inhibition. BW-A4C inhibited LTB_4 release from ulcerative colitis mucosal biopsy tissue [309]. These compounds are orally bioavailable, inhibiting LT release from ionophore-stimulated blood for up to 6 h after a 50 mg/kg oral dose in rats [310]. Oral anti-inflammatory effects were seen in GPB and a rat carrageenan sponge model, but not in CPE [52,311]. Inhibition of 5-LO product generation induced by ethanol in rat gastric mucosa was observed, but mucosal damage was unchanged [312]. BW-A4C and BW-A137C have also been reported to inhibit the proliferation of lymphocytes in response to IL-



(129) R = H
(130) R = Me



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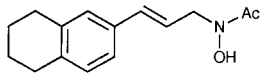
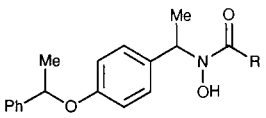


(132)

1, IL-2, mitogens and antigens [313], but this effect was probably unrelated to 5-LO inhibition, since 40-fold higher concentrations were required and excess arachidonic acid failed to reverse the effect. Recently BW-B218C (130), showing better oral bioavailability than the other analogues, was disclosed [314,315].

Conversion of the type B hydroxamic acids to hydroxyureas led to further enhancements of oral bioavailability and *in vivo* potency. Abbott's (133) [316,317] and BW-B70C (135) from Burroughs Wellcome [315,318] are examples. A direct comparison of the type B hydroxamic acid (134) and the hydroxyurea (133), as well as the type A and type B compounds (126) and (128), is shown in *Table 1.1* [304,316]. Methylation on the *N'*-position of the



(133) R = NH_2
(134) R = Me

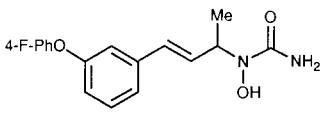


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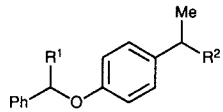
Table 1.1 COMPARISON OF TYPE A AND TYPE B HYDROXAMIC ACIDS AND A HYDROXYUREA

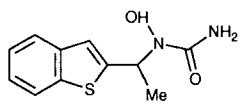


Compound	(126)[a]	(128)[a]	(134)[b]	(133)[b]
Class	type A	type B	type B	urea
R^1	H	H	Me	Me
R^2	-CON(Me)OH	-N(OH)Ac	-N(OH)Ac	$N(OH)CONH_2$
cRBL IC_{50}	0.28 μM	0.37 μM	0.50 μM	0.62 μM
In vivo ED_{50}[c]	40 mg/kg	8 mg/kg	22 mg/kg	9 mg/kg
Plasma half-life[d]	0.4 h	1.1 h	6.0 h	5.6 h
Plasma level[e]	8 μM	110 μM	126 μM	259 μM

[a] Data from Ref. [304]
[b] Data from Ref. [316]
[c] Rat peritoneal anaphylaxis, p.o.
[d] Rat, i.v.
[e] Rat, 1 h after 100 mg/kg p.o.

ureas had little effect on *in vitro* potency [306,307,317]. Heteroaryl analogues (thiophenes, benzothiazoles, carbazoles, dibenzofurans, indoles, benzofurans and benzothiophenes) were similar [306,307,319,320], and *N'*-hydroxyurea derivatives of NSAIDs such as indomethacin have also been reported [321].

The benzothiophene analogue A64077 (zileuton) (136) is one of the more interesting 5-LO inhibitors studied to date [322–324]. Zileuton inhibited cRBL (0.5 μM) and human ISN (0.7 μM), was specific for 5-LO, and was orally active in rat peritoneal anaphylaxis (3 mg/kg) and rat Arthus pleurisy [48]. In phase I studies, dose-dependent inhibition of LTB_4 synthesis in ionophore-stimulated blood was seen after oral dosing; the serum half-life was 130 min, with peak levels at 1–2 h. Promising activities have been reported in a number of diseases at daily doses of 800 mg/kg. Following antigen-induced nasal congestion, overt symptoms as well as LTB_4 and 5-HETE levels in nasal secretions were reduced [325]. The response of asthmatic patients to cold air induced bronchoconstriction was lessened [326]. Symptomatic improvements were seen in ulcerative colitis [327], with greatly decreased LTB_4 levels in rectal dialysis fluid up to 8 h after a single dose [328]. Preliminary indications of efficacy in rheumatoid arthritis have also been reported [329].



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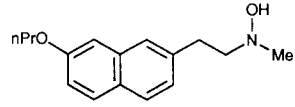


(137)

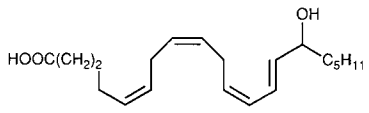
Although the original rationale for 5-LO inhibition by hydroxamic acids was iron chelation, this functional group also contains an N–O single bond which is capable of oxidation. Hydroxamic acids can act as pharmacological reducing agents [330], and some examples have been shown to reduce the iron atom in soybean 15-LO [180]. Interesting in this respect are reports that *N*-alkylhydroxylamines, which are not chelating agents, also inhibit soybean 15-LO (10–500 μM) [331]. A series of potent 5-LO-inhibitory *N*-aralkylhydroxylamines was investigated at Sandoz [332]. *N*-Alkylhydroxylamines were found to be mixed CO/5-LO inhibitors (approximately equipotent against seminal vesicle CO and LTB_4 release from human ISN). As was seen in various hydroxamic acid series, *O*-methylation increased CO potency and decreased 5-LO potency, while *N*-methylation had the opposite effect, and larger *N*-substituents decreased activity. Using an active-site binding hypothesis similar to that proposed by the Abbott group [294], analogues with 7-substituted 2-naphthyl moieties, exemplified by QA 208–199 (137), were found to give the best 5-LO potency (0.1–1 μM). Moving the substituents of (137) around the ring gave only minor reductions in potency, except for the 1,8-isomer which was much worse. QA 208–199 also significantly inhibited the formation of LTB_4 and HETEs from labelled arachidonic acid incubated with homogenized human psoriatic plaque (43% inhibition at 10 μM) [333].

MISCELLANEOUS STRUCTURES

15-HETE (138) is known to inhibit 5-LO [334]. A group at Revlon created a series of combined 5-LO inhibitors/LT antagonists derived conceptually from the structure of 15-HETE. REV 5901A (139) [335], the best of the series, inhibited 5-HETE release from rat ISN (0.12 μM) and was fairly selective with respect to CO and 12-LO inhibition. The quinoline could be replaced by another lipophilic aromatic group, but potency decreased (naphthalene was 40-fold less potent, and substituted phenyl was 5- to 20-fold less active). Pyridines were active but also less potent; 2-pyridyl was only 4-fold less active, while 3- and 4-pyridyl were 20-fold weaker. *Ortho*- and *para*-substituted phenylene groups were less active. Elimination of the side-chain hydroxyl to the olefin caused a loss of activity, as did the use of shorter alkyl chains.

REV 5901A inhibited antigen-induced LT secretion from guinea-pig lung tissue at concentrations of 1–10 μM [336,337]. LTD_4 receptor binding antagonism (K_i 0.7 μM in guinea-pig lung) was also observed, as was oral inhibition of GPB induced by antigen [335] or by LTD_4 [338] at 10 mg/kg.

However, no effect was seen in humans challenged with inhaled LTD_4 following a 1 g oral dose [339].



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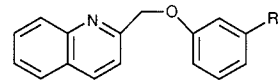


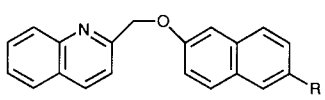
(139) R = $CHOHC_5H_{11}$
(140) R = $N(OH)COCH_2CH_2COOMe$
(141) R = $NHSO_2CF_3$

This lead has been pursued extensively at Wyeth-Ayerst, resulting in several additional series of active compounds (reviewed in [340]). Activity was retained by replacement of the quinoline by other benzo-fused heterocycles such as benzothiazole and *N*-methylbenzimidazole, but the benzoxazoles were inactive; no correlation was seen between *in vitro* and *in vivo* activities [341].

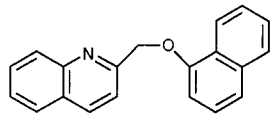
Ester- and amide-containing side-chains provided compounds such as Wy-45,911 (140) with similar activity [342–344]. In this series, the corresponding naphthalene analogue was slightly more potent as a 5-LO inhibitor *in vitro* (0.6 μM) than (140) (1.4 μM), but was weaker *in vivo* as an LTD_4 receptor antagonist. The carboxylic acid was less potent; reduction of the hydroxamic acid to the amide also ruined activity. Wy-45,911 was the most potent analogue *in vitro* and was the only one to show *in vivo* inhibition of GPB (76% inhibition at 50 mg/kg p.o.). This compound would have been developed as an anti-asthmatic except for mutagenicity in the Ames test [342].

Other compounds with acidic groups on the phenylene unit have proven interesting. Wy-48,252 (141), in clinical trials for asthma, was the most interesting of a series of sulphonamides [340,345,346]. This compound inhibited PG as well as LT biosynthesis in a variety of cell models (2–10 μM), and was very potent against GPB (0.1–0.6 mg/kg). Non-fluorinated alkylsulphonamides, which are weaker acids, were much less potent. A naphthyl analogue, Wy-48,090 (142), was somewhat less potent in the bronchoconstriction models, but showed activity in CPE (63% at 50 mg/kg p.o.) and in phorbol ester ear oedema (34% at 1 mg/ear topically); this compound was, however, mutagenic [340]. Additional related compounds bearing carboxylic acid-containing groups on the phenylene moiety have been disclosed as 5-LO inhibitors in patents from Bayer [347,348] and Leo [349].

Wy-49,232 (143), in which the NSAID naproxen is incorporated in place of the substituted phenylene moiety, was surprisingly devoid of CO activity in rat neutrophils, but was a potent 5-LO inhibitor (0.16 μM) [340]. Although both enantiomers were equipotent, the *S*-(+)-isomer of (143), Wy-



(142) R = $NHSO_2CF_3$
(143) R = CH(Me)COOH



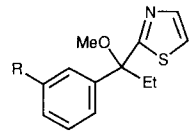
(144)

50,295, was selected for development [350]. Oral activity in CPE (75 mg/kg) and in mouse zymosan peritonitis (12 mg/kg) was seen; in the latter model peritoneal LTC_4 levels were significantly reduced. Other models where oral activity was seen include rat Arthus pleurisy [48], GPB (7.3 mg/kg), AAE (12.7 mg/kg), and ulcerative colitis in cotton top tamarin monkeys.

The unsubstituted 1-naphthyl compound Wy-47,288 (144) was inactive orally, but showed topical activity in several models (AAE, phorbol ester ear oedema, oxazolone-induced contact sensitivity, and UV erythema) [351]. In rat neutrophils, Wy-47,288 showed selectivity for 5-LO over CO (0.4 and 6.3 μM, respectively), and was even more selective in mouse macrophages.

Similar naphthalenemethyloxyphenyl compounds were recently reported from ICI as potent, selective 5-LO inhibitors which displayed no antioxidant or iron chelation properties [352]. ICI 211965 (145) was active against crude guinea-pig neutrophil 5-LO (0.13 μM) and inhibited LT release by ionophore-stimulated rat blood (0.3 μM), and was selective for 5-LO over CO in mouse macrophages (8 nM and > 50 μM, respectively). *Ex vivo* activity was seen in rat blood 1 h after oral dosing (10 mg/kg). Replacement of the ethyl group by hydrogen or methyl decreased activity, as did conversion of the methoxy to a hydroxy. *Meta* substitution on the phenylene ring was greatly preferred (by a factor of about 100), in agreement with the Wyeth-Ayerst findings. A tied-back analogue, ICI 216800 (146) showed stereospecific inhibition, with only the (+)-isomer active; this is one of only two reported examples of enantiospecific inhibition of 5-LO. ICI 216800 also inhibited arachidonic acid-induced skin inflammation in rabbits when coinjected with the inflammogen [353]. The cycloalkane analogue (147) and other related structures have also shown selective 5-LO inhibition (0.2 μM) [354–356].

Diaryl 2,3-dihydroimidazo[2,1-*b*]thiazoles in which one of the aryl groups



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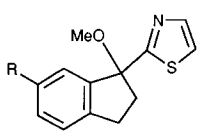

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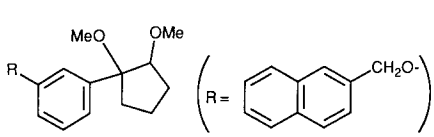
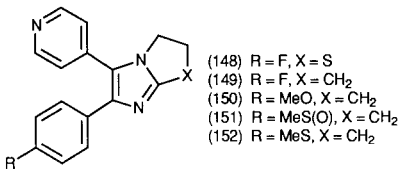
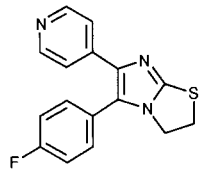


(147)

is pyridyl, from Smithkline Beecham, are dual CO/5-LO inhibitors. SK&F 86002 (148) inhibited production of LTC_4 and PGE_2 in human monocytes (20 μM and 1 μM, respectively), in cRBL, and against purified RBL-1 5-LO (35 μM) [68,357]. Oral activity (10–50 mg/kg) was seen in standard inflammation models including CPE, RAA, and collagen-induced arthritis. Inhibition of oedema and neutrophil influx in AAE and arachidonate-induced peritonitis, where classical NSAIDs are inactive [358–360], was also seen. Recently, SK&F 86002 was found to inhibit the production of IL-1 and tumour necrosis factor from human monocytes stimulated with LPS (1.3 μM and 5–8 μM, respectively) [361,362]. Inhibition of helper T-cell functions [363] and of endotoxin shock [364] was felt to be related to effects on lymphokines. The mechanism for these effects was not known, but was felt not to be a consequence of 5-LO or CO inhibition, since in human monocytes, inhibition of IL-1 production and 5-LO product release were not correlated for 16 members of the (148) series [365].



(148) R = F, X = S
(149) R = F, X = CH_2
(150) R = MeO, X = CH_2
(151) R = MeS(O), X = CH_2
(152) R = MeS, X = CH_2



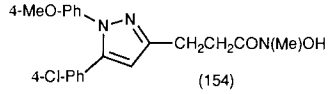
(153)

A related series of diaryl pyrrolo[1,2-*a*]imidazoles, represented by SK&F 104351 (149), 104493 (150) and 105809 (151), has been reported to show similar profiles [360,366]. In mice and rats, SK&F 105809 was a prodrug for the active methyl sulphide SK&F 105561 (152), which was about 10-fold more potent *in vitro* than SK&F 86002 [367]. The *in vivo* profile of SK&F 105809, which is reported to be in clinical trials, was similar to that of SK&F 86002.

Only sketchy structure–activity information has appeared in print for these series. For the imidazothiazoles, only the regioisomer represented by (148) was active in RAA; the alternative structure (153) was devoid of activity [368]. In the pyrroloimidazole series, alkoxy-, alkylthio-, and fluorophenyl derivatives show similar activity against the RBL-1 enzyme and in AAE [369,370]. Although these compounds are not obviously reducing agents like many other 5-LO inhibitors, they have been shown to undergo reversible oxidation, with electron-rich aryl rings having the lowest oxidation potentials; (148) oxidizes reversibly but only at higher potentials [371]. No correlation of oxidation potential with 5-LO inhibition was reported.

Tepoxalin (RWJ 20485) (154) is a hydroxamic acid derivative which in-

hibited RAA (2 mg/kg p.o.) and urate-induced synovitis in dogs, with no gastrointestinal toxicity (reviewed in [372]). Both 5-LO (cRBL-1: 0.15 μM) and CO (*ca.* 1 μM) were inhibited *in vitro*. *In vivo* activity reflecting 5-LO inhibition was seen following i.v. (GPB) or topical (phorbol ester ear oedema, AAE) administration, with biochemical verification of reduced 5-LO

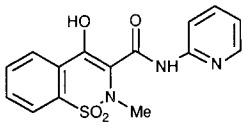


(154)

products, but oral activity in these models was very weak. According to a patent application [373], similar oral activity in RAA and dog synovitis was seen with simple carboxylic esters, implying that the hydroxamic acid moiety is not the pharmacophore in these models. Possibly the 5-LO activity seen *in vitro* and topically is due to the hydroxamic acid moiety, while metabolism to the carboxylic acid following oral dosing gives rise to the more NSAID-like activity in RAA.

Members of one major series of CO-inhibitory NSAIDs, represented by piroxicam (155), are enolic acids [374]. A few somewhat related compounds have been reported as mixed CO/5-LO inhibitors, although the CO potency was significantly greater than the 5-LO potency. For example, L-652,343 (156) is representative of a series from Merck which inhibited RBL-1 5-LO (2.0 μM) and rat and human ISN (0.62 and 1.4 μM, respectively), but was much more potent against PGE_2 release from rat neutrophils (1 nM) [375–377]. L-652,343 showed NSAID-like effects in CPE, RAA and collagen-induced arthritis (10–30 mg/kg), but also inhibited both LTC_4 and prostacyclin generation in mouse zymosan peritonitis. Curiously, in ionophore-stimulated human blood, TXB_2 formation was inhibited, but LTB_4 production was not. The same result was seen in human psoriatic plaques, where no disease improvement occurred [378].

Pfizer's tenidap (CP-66,248) (157), another enolic compound, was also more potent (500-fold) toward CO over 5-LO inhibition in human ISN (0.032 μM and 18 μM, respectively) [379–381]. Efficacy in rheumatoid arthritis clinical trials has been reported [380,382]; in patients, serum levels of acute phase proteins and synovial fluid levels of IL-1 were reduced by tenidap, in contrast to the lack of this effect with NSAIDs. Besides CO/5-LO inhibition, a variety of *in vitro* activities have been reported, including a number of effects on monocyte functions and differentiation [379], inhibition of neutrophil degranulation [382], inhibition of the activation of neutrophil collagenase [383], inhibition of leukocyte-endothelial cell adhesion [384], and inhibition of LTB_4-induced neutrophil chemotaxis [385]. Al-



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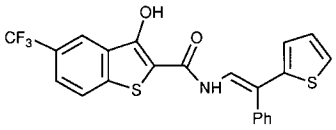

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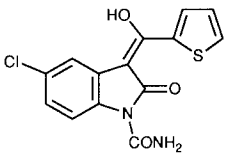

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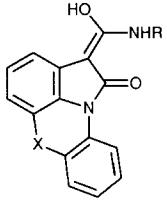

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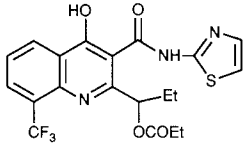


(159)

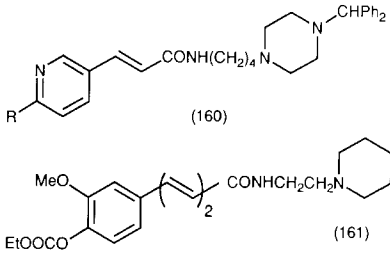
though tenidap differs from classical NSAIDs, the mechanism of the efficacy in rheumatoid arthritis, and whether 5-LO inhibition plays a role, appear at present unclear. Actions at an early stage of cellular signal transduction (G proteins, protein kinase C) have been proposed [380,383].

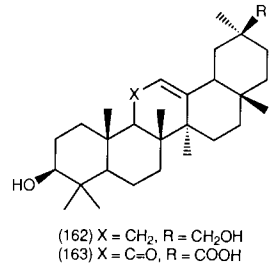
Recently, several series of tetracyclic analogues related to tenidap, represented by (158) (X = S, O, or CH_2; R = aryl), have been reported to display a better balance of 5-LO and CO inhibition [386]. However, these compounds may also be considered as modified phenothiazine, phenoxazines, and acridines, and the enhanced 5-LO inhibition may be more closely related to that of other phenothiazines such as (88).

Another compound structurally related to the oxicams is RU 43526 (159) [387], which gave approximately equipotent inhibition of seminal vesicle CO (2.1 μM) and of rat ISN (3.0 μM). Removal of the 2-substituent had no effect on the CO inhibition, but drastically reduced 5-LO potency. RU 43526 was orally active, but only in NSAID-sensitive models (CPE 4 mg/kg, RAA 0.7 mg/kg). It was unknown whether the 5-LO inhibition made any contribution to the anti-inflammatory activity of this compound.

A series of antiallergic agents from Dainippon, represented by (160), showed good activity in rat passive cutaneous anaphylaxis, and inhibited a crude guinea-pig peritoneal cell 5-LO preparation [388]. The methyl case (160, R = Me) was more potent (4.9 μM) than the unsubstituted analogue; in general, greater lipophilicity in the heteroaryl ring decreased *in vivo* activity, but enhanced 5-LO inhibition [389–391]. TMK-688 (161) showed a similar profile, with greater potency (0.35 μM) in ISN [392].

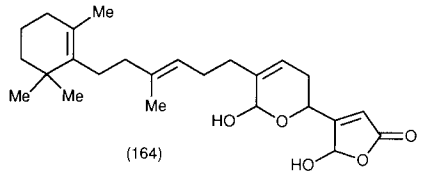
The bishemiphthalate derivative of the oleanenediol (162), related to glycyrrhetinic acid (163), inhibited 5-LO product generation by a homogenate of cloned mastocytoma cells (5.8 μM); it was about 10-fold less potent

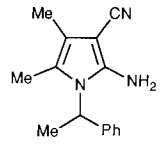


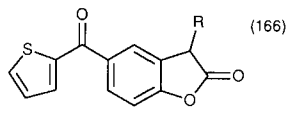


against CO [393]. The same compound also showed topical efficacy in AAE (1.9 mg/ear) [394]. Glycyrrhetinic acid itself was reported to be active in ionophore-stimulated cells, although this action was attributed to inhibition of phospholipase A_2 [395,396].

Manoalide (164), a marine natural product which inhibits the release of arachidonic acid from phospholipids by phospholipase A_2 [397,398], showed topical anti-inflammatory activity in mouse ear models [399]. Activity in ISN and cRBL (< 1 μM) have also been reported [400]. A series of analogues consisting of the furanone ring of manoalide bearing simple unsaturated 16–20 carbon chains showed similar activity in rabbit neutrophils and isolated guinea-pig neutrophil 5-LO [401]; interestingly, however, topical anti-inflammatory activity was seen in phorbol ester ear oedema but not in AAE [399]. The importance of 5-LO inhibition to the anti-inflammatory activity of manoalide is unknown; effects on phospholipase C and calcium channels have also been shown [402, 403].





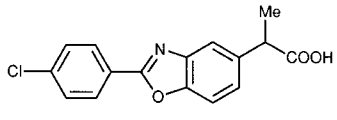


Two other series of compounds which do not fall into other structural types are (165) and (166). The pyrrole (165) showed inhibition of human ISN (3.6 μM); only a few other substituents on the ring nitrogen (benzyl, 2-bromophenyl but not 4-bromophenyl) were reported with similar activity [270]. A series of lactones (166), where R is small alkyl or benzylidene, inhib-

ited guinea-pig ISN (30 μM). CO inhibition and RAA activity were noted as well, but no correlation among these activities was apparent [404].

OTHER DRUGS REPORTED TO INHIBIT 5-LIPOXYGENASE

One of the first compounds reported to inhibit 5-LO was the NSAID benoxaprofen (167) (reviewed in [405]). This drug (marketed by Lilly as Oraflex®) was effective in rheumatoid arthritis, but was withdrawn because of phototoxicity, liver toxicity and reports of drug-related deaths [406]. The typical NSAID anti-inflammatory profile of this compound was remarkable for its very weak seminal vesicle CO activity [407]. Additional *in vivo* activities were found for benoxaprofen which were not shared by other NSAIDs, particularly inhibition of leukocyte influx in the carrageenan sponge, carrageenan pleurisy, and rat Arthus pleurisy models; monocytes were affected more than neutrophils [408–411]. More recently, benoxaprofen was reported to inhibit the adhesion of monocytes to endothelium [412].



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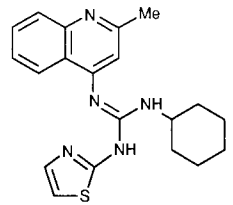


(168)

In vitro, benoxaprofen was reported to inhibit the release of 5-LO products from ISN [413,414] and RBL-1 cells [67], and from stimulated lung tissue [415–417]. However, other reports have discounted 5-LO inhibitory activity, finding fairly selective CO inhibition in mouse macrophages [418], ISN [29,419], and in the carrageenan sponge model [420]. Surprisingly, there appears to be only one report of the evaluation of benoxaprofen in cell-free 5-LO systems (from human leukocytes and RBL-1 cells), where no effects were seen below 100 μM [421]. The mechanism of action of benoxaprofen still is not clear, but its unusual pharmacological profile, along with efficacy in psoriasis [422–424], indicate that there is more to this drug than simple NSAID-like CO inhibition. (A pilot clinical trial in ulcerative colitis, however, showed no efficacy [425].)

Timegadine (168) is an atypical basic NSAID which has shown efficacy in arthritis clinical trials [426,427]. It is a potent CO inhibitor in a variety of tissues, and also inhibited the production of 12-HETE from platelet ho-

mogenates, but only at much higher concentrations (100 μM) [428]. Unlike the acidic NSAIDs, timegadine inhibited cell influx and LTC_4 release in the carrageenan sponge model at CO-inhibitory concentrations [429], and inhibited AAE and phorbol ester ear oedema at 1 mg/ear topically [51]; a tendency for improvement was noted in a small psoriasis clinical trial as well [430]. 5-LO inhibition was seen in ISN [431–433] and in immune complex-stimulated mouse macrophages [429] (15–40 μM), but CO inhibition occurred at significantly lower concentrations. Other effects on neutrophils (inhibition of degranulation and superoxide production) have also been seen [434]. It is not clear whether 5-LO inhibition is responsible for these actions, since no studies with cell-free enzymes have been reported.

A number of other drugs (shown in *Table 1.2*) have been reported to inhibit 5-LO activity. In many cases, only whole cell data supports this mechanism of action; other mechanisms may also be responsible for the cellular effects seen. A discussion of these compounds is beyond the scope of this review; some leading references are summarized in *Table 1.2*.

Table 1.2 MISCELLANEOUS DRUGS REPORTED TO INHIBIT 5-LIPOXYGENASE

Drug	*Therapeutic class*	*References*
Diclofenac	NSAID	[435]
Meclofenamate	NSAID	[436]
Tolfenamic acid	NSAID	[433, 437, 438]
Proglumetacin	NSAID	[439]
Mofebutazone	NSAID	[440]
Sulfasalazine	anti-inflammatory (arthritis, inflammatory bowel disease)	[18, 441–445]
Auranofin	anti-inflammatory (arthritis)	[446, 447]
Ebselen	anti-inflammatory	[448, 449]
Piriprost	antiallergic	[450–454]
Tiacrilast	antiallergic	[455]
Amoxanox	antiallergic	[456–458]
CI-922	antiallergic	[459–461]
Azelastine	antiallergic	[456, 462–465]
AHR-5333	antiallergic	[466, 467]
Nafazatrom	antithrombotic	[51, 212, 337, 429, 468–473]
Dipyridamole	antiangina	[474, 475]
Itraconazole, Ketoconazole	antifungal	[476]
Malotilate	hepatoprotectant	[477]

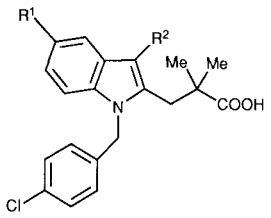
SUMMARY

A wide variety of agents have been reported as 5-LO inhibitors. The majority of the series appear to be lipophilic reducing agents, including phenols, partially saturated aromatics, and compounds containing heteroatom-heteroatom bonds. Many of these are not selective 5-LO inhibitors, but often affect CO and other LOs as well. *In vivo* systemic activity for many of these has been, in general, disappointing, probably because of poor bioavailability caused by lipophilicity and metabolic instability (oxidation, and conjugation of phenolic compounds). However, topically a number of agents have shown promise for skin inflammation, with Syntex's lonapalene the most advanced of these. Most results published to date appear more disappointing in the allergy/asthma field.

More excitingly, a few structural types are selective 5-LO inhibitors which have shown systemic activity *in vivo* and in the clinic. Abbott's zileuton (136) appears to be one of the leading compounds in this category, along with other hydroxamates such as BW-A4C (129) from Burroughs-Wellcome. Recent selective non-reducing agents such as Wyeth-Ayerst's Wy-50,295 (143) and the similar ICI compounds such as ICI 216800 (145) also hold promise. The enantiospecific effects of (106) and (145) are especially interesting for the design of new inhibitors. If compounds like these validate the hypothesis that inhibition of 5-LO will have a significant anti-inflammatory effect, a redoubling of effort throughout the industry to find second- and third-generation selective agents may be expected.

Part of the difficulty in interpreting and comparing the 5-LO literature is the plethora of test methods and activity criteria. As pointed out in the introduction, inhibition of product release from cells, often stimulated with A23187, has commonly been used to demonstrate 5-LO inhibition. However, this type of assay cannot be assumed to be diagnostic for 5-LO inhibition [28,29]. Only if specificity for 5-LO product generation and (ideally) activity in cell-free enzymes is also shown should mechanistic interpretations be made.

Recently, a new class of compounds was found at Merck which inhibited LT biosynthesis without inhibiting 5-LO, but apparently by a novel, specific mechanism. L-655,240 (169) [478] and L-663,536 (MK-886) (170) [479,480] were both active in human ISN, with IC_{50} values in the low micromolar range. Both also orally inhibited GPB (< 1 mg/kg). MK-886 was effective in *Ascaris*-induced asthma in squirrel monkeys, in rat carrageenan pleurisy, in rat Arthus pleurisy [48], and (topically) in guinea-pig ear oedema induced by A23187. Neither compound affected CO, and surprisingly both were inactive against cell-free 5-LOs (crude RBL-1, crude rat neutrophil, or puri-



(169) R^1 = F, R^2 = Me
(170) R^1 = iPr, R^2 = tBuS

fied porcine 5-LO). MK-886 was found to inhibit the translocation of cytosolic 5-LO to the cell membrane in human leukocytes [481]. This translocation occurs upon stimulation of this cell type or RBL-1 cells with A23187, and is directly correlated with the production of 5-LO products [482,483]. An 18 kDa membrane protein, dubbed 5-LO activating protein (FLAP) was recently shown to be essential for product formation in transfected osteosarcoma cells, presumably acting to anchor the cytosolic 5-LO to the cell membrane following cell activation [484]. MK-886 binds to this protein, possibly inhibiting its interaction with 5-LO and preventing the enzyme from generating 5-HETE and LTB_4.

As more is learned about the enzymology and cellular control mechanisms of LT biosynthesis, exciting new approaches to the therapeutic control of inflammatory diseases which address this pathway will certainly be developed and explored.

ACKNOWLEDGMENTS

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ADDENDUM

A number of published papers have included SAR details for several series of compounds, including the phenoxazines (89) [485], naphthoylvalerohydroxamic acids represented by (115) [486] and the ICI series represented by (145) and (146) [487]. A full paper dealing with the 4-hydroxythiazoles represented by (74) indicated that only compounds existing in the enolic form were active; 5-unsubstituted cases and also 5,5-disubstituted cases (which are ketonic) were inactive [488]. Acylation of the hydroxyl was acceptable for good activity. No strong correlation of aryl substitution with potency

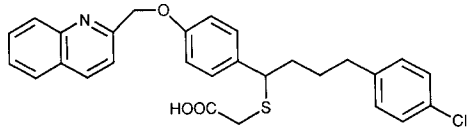
was apparent, except that ionizable groups were much less active. No oral activity was seen with this series.

Lipophilic C3-alkyl derivatives of cirsiliol (23) gave more potent inhibitors than C5-alkoxylated derivatives such as (24) [489]. QSAR studies of a series of 4- and 5-substituted analogues of phenidone (95) supported the general observation from many other series that lipophilicity enhanced potency [490]. Electron withdrawal on the phenyl ring was also desirable, while steric hindrance at the 5-position decreased potency. Using trititated L-656,224 (70), evidence was obtained for reductive inactivation of human and guinea-pig leukocyte 5-LO, accompanied by covalent modification of the enzyme and degradation of this inhibitor [491].

A summary of the *in vitro* and *in vivo* profile of zileuton (136) has appeared [492]. A review of hydroxamic acids and hydroxyureas emphasized work at Burroughs Wellcome, but included information on zileuton as well [493].

MK-886 (170), which is highly selective for inhibition of 5-LO products by virtue of its interaction with FLAP, was shown not to affect production of IL-1, implying that such effects seen with redox inhibitors such as (31), (39) and (66) are probably due to mechanisms other than 5-LO inhibition [494].

Several interesting findings dealing with 2-quinolinylmethyl esters related to Rev 5901A (139) have been reported. Workers at Wyeth-Ayerst found that attachment of this moeity to a number of classical NSAIDs endowed these cyclooxygenase inhibitors with 5-LO inhibitory activity in cell assays [495]. Wy-50,295 (143) and others of this class were found to inhibit translocation of 5-LO from the cytosol to the cell membrane with much greater potency than was the case with their inhibition of cell-free 5-LO preparations [496]. Workers at Merck [497,498] extended these findings, showing that members of one such series of compounds, represented by L-674,636 (171), are potent inhibitors of FLAP (7 nM in ISN). The *para*-substituted phenylene moiety was much better than the *meta* case. The acidic function was essential for *in vivo* activity and for best potency, while an acidic group on the terminal phenyl group gave poorer FLAP inhibition but better LTD_4 receptor antagonism. Compound (171) showed good bioavailability, and was potent in an allergic rat model and in rat Arthus pleurisy when dosed orally.



(171)

Research on FLAP has continued at a rapid pace, with gene cloning, promoter analysis and site-directed mutagenesis in progress [498,499]. This promises to be an area of great interest in the future.

REFERENCES

1 Salmon, J.A. (1986) Adv. Drug Res. 15, 111–167.
2 Lewis, R.A., Austen, K.F. and Soberman, R.J. (1990) N. Engl. J. Med. 323, 645–655.
3 Ford-Hutchinson, A.W. (1990) Crit. Rev. Immunol. 10, 1–12.
4 Brain, S.D. and Williams, T.J. (1990) Pharmacol. Ther. 46, 57–66.
5 Maycock, A.L., Pong, S., Evans, J.F. and Miller, D.K. (1989) in Leukotrienes and Lipoxygenases (Rokach, J., ed.), pp. 143–208, Elsevier, Amsterdam.
6 Corey, E.J. (1987) Pure Appl. Chem. 59, 269–278.
7 Nelson, M.J., Seitz, S.P. and Cowling, R.A. (1990) Biochemistry 29, 6897–6903.
8 Veldink, G.A. and Vliegenthart, J.F.G. (1984) Adv. Inorg. Biochem. 6, 139–161.
9 Jones, T.R., Letts, G., Chan, C. and Davies, P. (1989) in Ref. 5, pp. 309–404.
10 Ford-Hutchinson, A.W. (1989) in Ref. 5, pp. 405–426.
11 Welton, A.F., Will, P.C., Morgan, D.W., Crowley, H., O'Donnell, M., Hurley, J. and Shapiro, S. (1989) NATO ASI Ser. Ser. A 177, 195–219.
12 Masamune, H. and Melvin, L.S. (1989) Annu. Rep. Med. Chem. 24, 71–79.
13 Lewis, R.A. and Robin, J.L. (1985) J. Allergy Clin. Immunol. 76, 259–264.
14 Barnes, N.C., Evans, J., Zakrzewski, J.T., Piper, P.J. and Costello, J.F. (1989) Agents Actions Suppl. 28, 123–133.
15 Cunningham, F. (1990) J. Lipid Mediators 2, 61–74.
16 De Nucci, G. (1988) Biochem. Soc. Trans. 16, 680–682.
17 Camp, R.D.R. and Greaves, M.W. (1987) Br. Med. Bull. 43, 401–414.
18 Fretland, D.J., Djuric, S.W. and Gaginella, T.S. (1990) Prostaglandins Leukotrienes Essent. Fatty Acids 41, 215–233.
19 Kennedy, N.P. and Keeling, P.W.N. (1987) Aliment. Pharmacol. Ther. 1, 263–272.
20 Goldstein, R.E. (1990) Eur. Heart J. 11(Suppl. B), 16–26.
21 Lefer, A.M. (1988) ISI Atlas of Science: Pharmacology 2, 109–115.
22 Cashman, J.R. (1985) Pharm. Res., 253–261.
23 Gillard, J.W. and Guindon, Y. (1987) Drugs Future 12, 453–474.
24 Fitzsimmons, B.J. and Rokach, J. (1989) in Ref. 5, pp. 427–502.
25 Jakschik, B.A., Sun, F.F., Lee, L. and Steinhoff, M.M. (1980) Biochem. Biophys. Res. Commun. 95, 103–110.
26 Yamaoka, A., Sumimoto, H., Takeshige, K., Itoh, T., Isobe, R., Yoshitake, J. and Minakami, S. (1987) Biochim. Biophys. Acta 918, 284–292.
27 Reibman, J., Haines, K.A., Rich, A.M., Cristello, P., Giedd, K.N. and Weissmann, G. (1986) J. Immunol. 136, 1027–1032.
28 Reed, P.W., Bentley, J.K., Ochs, D.L., Harris, A.S. and Bokoch, G.M. (1983) in Biochemistry of Metabolic Processes (Lennon, D.L.F., Stratman, F.W. and Zahlten, R.N., ed.), pp. 55–66, Elsevier, New York.
29 Salmon, J.A., Tilling, L.C. and Moncada, S. (1985) Prostaglandins 29, 377–385.
30 Parente, L., Di Rosa, M., Flower, R.J., Ghiara, P., Meli, R., Persico, P., Salmon, J.A. and Wood, J.N. (1984) Eur. J. Pharmacol. 99, 233–239.
31 Schleimer, R.P., Davidson, D.A., Peters, S.P. and Lichtenstein, L.M. (1985) Int. Arch. Allergy Appl. Immunol. 77, 241–243.

32 Wallace, J.L. (1987) Prostaglandins 34, 311–323.
33 Bittner, S. and Wielckens, K. (1988) Endocrinology 123, 991–1000.
34 Sebaldt, R.J., Sheller, J.R., Oates, J.A., Roberts, L.J. and FitzGerald, G.A. (1990) Proc. Natl. Acad. Sci. USA 87, 6974–6978.
35 Flower, R.J. (1988) Br. J. Pharmacol. 94, 987–1015.
36 Dennis, E.A. and Davidson, F.F. (1990) Prog. Clin. Biol. Res. 349, 47–54.
37 Bailey, J.M., Makheja, A.N., Pash, J. and Verma, M. (1988) Biochem. Biophys. Res. Commun. 157, 1159–1163.
38 Koehler, L., Hass, R., DeWitt, D.L., Resch, K. and Goppelt-Struebe, M. (1990) Biochem. Pharmacol. 40, 1307–1316.
39 Masferrer, J.L., Zweifel, B.S., Seibert, K. and Needleman, P. (1990) J. Clin. Invest. 86, 1375–1379.
40 Bray, M.A. (1984) Eur. J. Pharmacol. 98, 61–67.
41 Randall, R.W., Tateson, J.E., Dawson, J. and Garland, L.G. (1987) FEBS Lett. 214, 167–170.
42 Nigam, S. (1987) Dermatologica 175 Suppl., 73–80.
43 Fiedler-Nagy, C., Wittreich, B.H. and Carey, M.A. (1989) Agents Actions 27, 313–315.
44 Orfanos, C.E., Ehlert, R. and Gollnick, H. (1987) Drugs 34, 459–503.
45 Evans, R.M. (1988) Science (Washington) 240, 889–895.
46 Otterness, I.G. and Bliven, M.L. (1985) in Nonsteroidal Anti-inflammatory Drugs (Lombardino, J.G., ed.), pp. 112–252, Wiley, New York.
47 Summers, J.B., Gunn, B.P., Mazdiyasni, H., Goetze, A.M., Young, P.R., Bouska, J.B., Dyer, R.D., Brooks, D.W. and Carter, G.W. (1987) J. Med. Chem. 30, 2121–2126.
48 Berkenkopf, J.W. and Weichman, B.M. (1991) Eur. J. Pharmacol. 193, 29–34.
49 Young, J.M. and De Young, L.M. (1989) in Pharmacological Methods in the Control of Inflammation (Chang, J.Y. and Lewis, A.J., ed.), pp. 215–231, Liss, New York.
50 Young, J.M., Spires, D.A., Bedord, C.J., Wagner, B., Ballaron, S.J. and De Young, L.M. (1984) J. Invest. Dermatol. 82, 367–371.
51 Carlson, R.P., O'Neill-Davis, L., Chang, J. and Lewis, A.J. (1985) Agents Actions 17, 197–204.
52 Payne, A.N., Garland, L.G., Lees, I.W. and Salmon, J.A. (1988) Br. J. Pharmacol. 94, 540–546.
53 Ho, C.Y., Hageman, W.E., Mohrbacher, R.J. and End, D.W. (1988) J. Pharm. Sci. 77, 149–152.
54 Buckle, D.R. and Fenwick, A.E. (1989) J. Chem. Soc., Perkin Trans. 1, 477–482.
55 Hanko, R., Hammond, M.D., Fruchtmann, R., Pfitzner, J. and Place, G.A. (1990) J. Med. Chem. 33, 1163–1170.
56 Hazato, A., Kurozumi, S., Ohta, T., Ohtsu, A., Iagyu, Y., Komoriya, K., Fujiwara, H. and Mori, R. (1989) Adv. Prostaglandin Thromboxane Leukotriene Res. 19, 639–642.
57 Malleron, J.L., Roussel, G., Gueremy, G., Ponsinet, G., Robin, J.L., Terlain, B. and Tissieres, J.M. (1990) J. Med. Chem. 33, 2744–2749.
58 Yasumoto, K., Yamamoto, A. and Mitsuda, H. (1970) Agr. Biol. Chem. 34, 1162–1168.
59 Schmidt, K.H., Steinhilber, D., Moser, U. and Roth, H.J. (1988) Int. Arch. Allergy Appl. Immunol. 85, 441–5.
60 Baader, W.J., Hatzelmann, A. and Ullrich, V. (1988) Biochem. Pharmacol. 37, 1089–98.
61 Peterman, T.K. and Siedow, J.N. (1983) Plant Physiol. 71, 55–58.
62 Kemal, C., Louis-Flamberg, P., Krupinski-Olsen, R. and Shorter, A.L. (1987) Biochemistry 26, 7064–7072.
63 Van der Zee, J., Eling, T.E. and Mason, R.P. (1989) Biochemistry 28, 8363–8367.

64 Nelson, M.J. (1988) Biochemistry 27, 4273–4278.
65 Walenga, R.W., Showell, H.J., Feinstein, M.B. and Becker, E.L. (1980) Life Sci. 27, 1047–1053.
66 Bokoch, G.M. and Reed, P.W. (1981) J. Biol. Chem. 256, 4156–4159.
67 Levine, L. (1983) Biochem. Pharmacol. 32, 3023–3026.
68 Hogaboom, G.K., Cook, M., Newton, J.F., Varrichio, A., Shorr, R.G.L., Sarau, H.M. and Crooke, S.T. (1986) Mol. Pharmacol. 30, 510–519.
69 Sabata, S., Moshonov, S., Zor, U., Floman, Y. and Naor, Z. (1986) Prostaglandins Leukotrienes Med. 23, 95–102.
70 Opas, E.E., Bonney, R.J. and Humes, J.L. (1985) J. Invest. Dermatol. 84, 253–256.
71 Della Loggia, R., Ragazzi, E., Tubaro, A., Fassina, G. and Vertua, R. (1988) Pharmacol. Res. Commun. 20, 91–94.
72 Newton, J.A., Boodle, K.M., Dowd, P.M. and Greaves, M.W. (1988) Br. J. Dermatol. 119, 404–406.
73 Hamasaki, Y. and Tai, H.H. (1985) Biochim. Biophys. Acta 834, 37–41.
74 Koshihara, Y., Neichi, T., Murota, S., Lao, A.N., Fujimoto, Y. and Tatsuno, T. (1984) Biochim. Biophys. Acta 792, 92–97.
75 Murota, S. and Koshihara, Y. (1985) Drugs Exp. Clin. Res. 11, 641–644.
76 Iwakami, S., Shibuya, M., Tseng, C.F., Hanaoka, F. and Sankawa, U. (1986) Chem. Pharm. Bull. 34, 3960–3963.
77 Kimura, Y., Okuda, H., Nishibe, S. and Arichi, S. (1987) Planta Med. 53, 148–153.
78 Kimura, Y., Okuda, H., Okuda, T., Hatano, T. and Arichi, S. (1987) J. Nat. Prod. 50, 392–399.
79 Nishizawa, M., Izuhara, R., Kaneko, K., Koshihara, Y. and Fujimoto, Y. (1988) Chem. Pharm. Bull. 36, 87–95.
80 Neichi, T., Koshihara, Y. and Murota, S. (1983) Biochim. Biophys. Acta 753, 130–132.
81 Kuhl, P., Shiloh, R., Jha, H., Murawski, U. and Zilliken, F. (1984) Prostaglandins 28, 783–804.
82 Panossian, A.G. (1984) Biomed. Biochim. Acta 43, 1351–1355.
83 Kimura, Y., Okuda, H., Arichi, S., Baba, K. and Kozawa, M. (1985) Biochim. Biophys. Acta 834, 224–229.
84 Flynn, D.L., Rafferty, M.F. and Boctor, A.M. (1986) Prostaglandins Leukotrienes Med. 22, 357–360.
85 Koshihara, Y., Fujimoto, Y. and Inoue, H. (1988) Biochem. Pharmacol. 37, 2161–2165.
86 Kimura, Y., Okuda, H., Okuda, T. and Arichi, S. (1988) Phytother. Res. 2, 140–145.
87 Wong, S.M., Antus, S., Gottsegen, A., Fessler, B., Rao, G.S., Sonnenbichler, J. and Wagner, H. (1988) Arzneim.-Forsch. 38, 661–665.
88 Aihara, K., Higuchi, T. and Hirobe, M. (1990) Biochem. Biophys. Res. Commun. 168, 169–175.
89 Reddy, G.R., Ueda, N., Hada, T., Sackeyfio, A.C., Yamamoto, S., Hano, Y., Aida, M. and Nomura, T. (1991) Biochem. Pharmacol. 41, 115–118.
90 Hope, W.C., Welton, A.F., Fiedler-Nagy, C., Batula-Bernardo, C. and Coffey, J.W. (1983) Biochem. Pharmacol. 32, 367–371.
91 Welton, A.F., Tobias, L.D., Fiedler-Nagy, C., Anderson, W., Hope, W., Meyers, K. and Coffey, J.W. (1986) Prog. Clin. Biol. Res. 213, 231–242.
92 Takahama, U. (1985) Phytochemistry 24, 1443–1446.
93 Welton, A.F., Hurley, J. and Will, P. (1988) Prog. Clin. Biol. Res. 280, 301–312.
94 Mascolo, N., Pinto, A. and Capasso, F. (1988) J. Pharm. Pharmacol. 40, 293–295.
95 Yoshimoto, T., Furukawa, M., Yamamoto, S., Horie, T. and Watanabe-Kohno, S. (1983) Biochem. Biophys. Res. Commun. 116, 612–618.

96 Horie, T., Tsukayama, M., Kourai, H., Yokoyama, C., Furukawa, M., Yoshimoto, T., Yamamoto, S., Watanabe-Kohno, S. and Ohata, K. (1986) J. Med. Chem. 29, 2256–2262.
97 Yamamoto, S., Ueda, N., Kaneko, S., Yokoyama, C., Tonai, T., Yoshimoto, T., Ban, M., Matsumoto, K. and Horie, T. (1987) Int. Congr. Ser. Excerpta Med. 750, 325–328.
98 Kimura, Y., Okuda, H. and Arichi, S. (1985) Planta Med., 132–136.
99 Kimura, Y., Okuda, H. and Arichi, S. (1986) Chem. Pharm. Bull. 34, 2279–2281.
100 Kimura, Y., Okuda, H. and Arichi, S. (1987) Phytother. Res. 1, 48–49.
101 Kubo, M., Matsuda, H., Tanaka, M., Kimura, Y., Okuda, H., Higashino, M., Tani, T., Namba, K. and Arichi, S. (1984) Chem. Pharm. Bull. 32, 2724–2729.
102 Alcaraz, M.J., Ferrandiz, M.L. and Villar del Fresno, A. (1986) Pharmazie 41, 299–300.
103 Montandon, J.B., Zijlstra, F.J., Wilson, J.H.P., Grandjean, E.M. and Cicurel, L. (1989) Int. J. Tissue React. 11, 107–112.
104 Carson, M., Han, R.L. and LeMahieu, R.A. (1990) U.S. Patent 4,937,371; (1990) Chem. Abstr. 113, 211598.
105 Miyamoto, T. and Obata, T. (1983) Int. Congr. Ser. Excerpta Med. 623, 78–80.
106 Arai, Y., Wakatsuka, H., Mohri, T., Obata, T. and Miyamoto, T. (1985) Adv. Prostaglandin Thromboxane Leukotriene Res. 15, 313.
107 Minamisawa, H., Terashi, A., Katayama, Y., Kanda, Y., Shimizu, J., Shiratori, T., Inamura, K., Kaseki, H. and Yoshino, Y. (1988) Stroke 19, 372–377.
108 Buckle, D.R., Bumstead, J., Clarke, G.D., Foster, K.A., Parr, H., Taylor, J.F., Thody, V.E. and Webster, R.A.B. (1988) Prostaglandins Leukotrienes Essent. Fatty Acids 33, 29–33.
109 Bonne, C., Muller, A., Latour, E., Tissie, G., Emerit, I., Modat, G., Dornand, J., Roch, M. and Giroud, J.P. (1989) Arzneim.-Forsch. 39, 1242–1245.
110 Bonne, C., Latour, E., Muller, A., De Kozak, Y., Faure, J.P., Malet, F., Colin, J., Tissot, M. and Giroud, J.P. (1989) Arzneim.-Forsch. 39, 1246–1250.
111 Vanderhoek, J.Y. and Lands, W.E.M. (1973) Biochim. Biophys. Acta 296, 382–385.
112 Slapke, J., Schewe, T., Hummel, S., Winkler, J. and Kopf, M. (1983) Biomed. Biochim. Acta 42, 1309–1318.
113 Reddanna, P., Rao, M.K. and Reddy, C.C. (1985) FEBS Lett. 193, 39–43.
114 Thody, V.E., Buckle, D.R. and Foster, K.A. (1987) Biochem. Soc. Trans. 15, 416–417.
115 Moore, G.G.I. and Swingle, K.F. (1982) Agents Actions 12, 674–683.
116 Muchowski, J.M., Greenhouse, R., Young, J. and Krishna Murthy, D.V. (1989) U.S. Patent 4,833,155.
117 Bell, R.L. and Moore, G.G.I. (1987) U.S. Patent 4,677,113; (1987) Chem. Abstr. 107, 134191.
118 Bell, R.L. and Moore, G.G.I. (1987) U.S. Patent 4,714,776.
119 Hidaka, T., Hosoe, K., Ariki, Y., Takeo, K., Yamashita, T., Katsumi, I., Kondo, H., Yamashita, K. and Watanabe, K. (1984) Jpn. J. Pharmacol. 36, 77–85.
120 Hidaka, T., Takeo, K., Hosoe, K., Katsumi, I., Yamashita, T. and Watanabe, K. (1985) Jpn. J. Pharmacol. 38, 267–272.
121 Hidaka, T., Hosoe, K., Yamashita, T., Watanabe, K., Hiramatsu, Y. and Fujimura, H. (1986) J. Pharm. Pharmacol. 38, 748–753.
122 Hidaka, T., Hosoe, K., Yamashita, T. and Watanabe, K. (1986) Jpn. J. Pharmacol. 42, 181–187.
123 Hidaka, T., Hosoe, K., Katsumi, I., Yamashita, T. and Watanabe, K. (1986) J. Pharm. Pharmacol. 38, 244–247.
124 Katsumi, I., Kondo, H., Yamashita, K., Hidaka, T., Hosoe, K., Yamashita, T. and Watanabe, K. (1986) Chem. Pharm. Bull. 34, 121–129.

125 Katsumi, I., Kondo, H., Fuse, Y., Yamashita, K., Hidaka, T., Hosoe, K., Takeo, K., Yamashita, T. and Watanabe, K. (1986) Chem. Pharm. Bull. 34, 1619–1627.
126 Ikuta, H., Shirota, H., Kobayashi, S., Yamagishi, Y., Yamada, K., Yamatsu, I. and Katayama, K. (1987) J. Med. Chem. 30, 1995–1998.
127 Shirota, H., Chiba, K., Ono, H., Yamamoto, H., Kobayashi, S., Terato, K., Ikuta, H., Yamatsu, I. and Katayama, K. (1987) Arzneim.-Forsch. 37, 930–936.
128 Shirota, H., Kobayashi, S., Terato, K., Sakuma, Y., Yamada, K., Ikuta, H., Yamagishi, Y., Yamatsu, I. and Katayama, K. (1987) Arzneim.-Forsch. 37, 936–940.
129 Shirota, H., Goto, M., Hashida, R., Yamatsu, I. and Katayama, K. (1989) Agents Actions 27, 322–324.
130 Phillips, M.L., Shadle, J.K., Ho, P.P.K., Bertsch, B.A., Walters, C.P., Towner, R.D., Wang, L.Y., Hunden, D. and Panetta, J.A. (1988) Presented at American Chemical Society 196th National Meeting, Los Angeles, CA; abstract MEDI 125.
131 Phillips, M.L., Ho, P.P.K., Benslay, D.N., Bertsch, B., Towner, R.D., Wang, L.Y. and Panetta, J.A. (1989) Presented at American Chemical Society 197th National Meeting, Dallas, TX; abstract MEDI 26.
132 Panetta, J.A., Benslay, D.N., Phillips, M.L., Towner, R.D., Bertsch, B., Wang, L. and Ho, P.P.K. (1989) Agents Actions 27, 300–302.
133 Isomura, Y., Ito, N., Homma, H., Abe, T. and Kubo, K. (1983) Chem. Pharm. Bull. 31, 3168–3178.
134 Isomura, Y., Ito, N., Sakamoto, S., Homma, H., Abe, T. and Kubo, K. (1983) Chem. Pharm. Bull. 31, 3179–3185.
135 Bartlett, R.R., Gebert, U., Von Kerekjarto, B., Schleyerbach, R., Thorwart, W. and Weithmann, K.U. (1989) Drugs Exp. Clin. Res. 15, 521–526.
136 Miyasaka, K., Mikami, T., Miyazawa, K. and Hagiwara, M. (1987) Jpn. J. Pharmacol. 43, 118P.
137 Suzuki, Y., Hasegawa, Y., Sato, M., Saito, M., Yamamoto, N., Miyasaka, K., Mikami, T. and Miyazawa, K. (1987) U.S. Patent 4,695,581.
138 Hashimoto, H., Miyazawa, K., Hagiwara, M., Miyasaka, K. and Nakashima, M. (1990) Arzneim.-Forsch. 40, 126–129.
139 Schrier, D.J., Adamchak, M.A., Boctor, A.M., Kostlan, C.R., Flynn, D.L., Jordan, J.H., Jr., Lesch, M.E. and Okonkwo, G.C.N. (1989) Agents Actions 27, 391–394.
140 Flynn, D.L., Belliotti, T.R., Boctor, A.M., Connor, D.T., Kostlan, C.R., Nies, D.E., Ortwine, D.F., Schrier, D.J. and Sircar, J.C. (1991) J. Med. Chem. 34, 518–525.
141 Lazer, E.S., Wong, H.C., Possanza, G.J., Graham, A.G. and Farina, P.R. (1989) J. Med. Chem. 32, 100–104.
142 Lazer, E.S., Wong, H.C., Wegner, C.D., Graham, A.G. and Farina, P.R. (1990) J. Med. Chem. 33, 1892–1898.
143 Lazer, E.S., Farina, P.R., Gundel, R.H. and Wegner, C.D. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 152.
144 Wolyniec, W.W., LaPlante, A.M., Kontny, M.J., Lazer, E.S., Letts, L.G. and Wegner, C.D. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 70.
145 Wegner, C.D., Lazer, E.S., Abraham, W.M. and Gundel, R.H. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 156.
146 Smith, W., Mueller, R., Nakao, A., Partis, R., Thompson, J., Yang, D.C., Jung, G., Port, C. and Fuller, G. (1988) FASEB J. 2, A1109.
147 Gertner, D.J., Lennard-Jones, J.E. and Rampton, D.S. (1990) Gastroenterology 98, A450.

148 Mueller, R.A., Partis, R.A. and Deason, J.R. (1987) U.S. Patent 4,711,903.
149 Mueller, R.A. and Partis, R.A. (1988) Eur. Pat. Appl. 293,900; (1990) Chem. Abstr. 112, 3575.
150 Mueller, R.A. and Partis, R.A. (1989) U.S. Patent 4,835,189.
151 Mueller, R.A. and Partis, R.A. (1989) U.S. Patent 4,835,190.
152 Hammerbeck, D.M., Bell, R.L., Stelzer, V.L., Heghinian, K., Reiter, M.J., Scherrer, R.A., Rustad, M.A., Hupperts, A.M. and Swingle, K.F. (1988) Ann. N.Y. Acad. Sci. 524, 398–401.
153 Hashimoto, K., Kanai, K. and Tsuda, Y. (1990) U.S. Patent 4,906,662.
154 Gwebu, E.T., Trewyn, R.W., Cornwell, D.G. and Panganamala, R.V. (1980) Res. Commun. Chem. Pathol. Pharmacol. 28, 361–376.
155 Grossman, S. and Waksman, E.G. (1984) Int. J. Biochem. 16, 281–289.
156 Nakadate, T., Yamamoto, S., Aizu, E. and Kato, R. (1984) Gann 75, 214–222.
157 Aizawa, Y., Kanai, T., Hasegawa, K., Yamaguchi, T., Iizuka, Y. and Iwaoka, T. (1990) J. Med. Chem. 33, 1491–1496.
158 Bonney, R.J., Davies, P., Dougherty, H., Egan, R.W., Gale, P.H., Chang, M., Hammond, M., Jensen, N., MacDonald, J., Thompson, K., Zambias, R., Opas, E.E., Meurer, R., Pacholok, S. and Humes, J.L. (1987) Biochem. Pharmacol. 36, 3885–3891.
159 Argenbright, L.W., Opas, E.E., Meurer, R.D., Feeney, W.P., Bonney, R.J. and Humes, J.L. (1986) J. Invest. Dermatol. 86, 460.
160 Chan, C.C., Dubois, L. and Young, V. (1987) Eur. J. Pharmacol. 139, 11–18.
161 Hammond, M.L., Kopka, I.E., Zambias, R.A., Caldwell, C.G., Boger, J., Baker, F., Bach, T., Luell, S. and MacIntyre, D.E. (1989) J. Med. Chem. 32, 1006–1020.
162 Hammond, M.L., Zambias, R.A., Chang, M.N., Jensen, N.P., McDonald, J., Thompson, K., Boulton, D.A., Kopka, I.E., Hand, K.M., Opas, E.E., Luell, S., Bach, T., Davies, P., MacIntyre, D.E., Bonney, R.J. and Humes, J.L. (1990) J. Med. Chem. 33, 908–918.
163 Zambias, R.A. and Hammond, M.L. (1990) U.S. Patent 4,963,580.
164 Schroeder, J.M. (1986) J. Invest. Dermatol. 87, 624–629.
165 Bedord, C.J., Young, J.M. and Wagner, B.M. (1983) J. Invest. Dermatol. 81, 566–71.
166 Krishna Murthy, D.V., Kruseman-Aretz, M., Rouhafza-Fard, S., Bedord, C.J., Young, J.M., Jones, G. and Venuti, M. (1985) Fed. Proc. 44, 886.
167 Young, J.M., Bedord, C.J., Murthy, D.V.K., Venuti, M.C., Jones, G.H., De Young, L., Akers, W.A. and Scholtz, J.R. (1985) J. Invest. Dermatol. 84, 358.
168 De Young, L.M., Kheifets, J.B., Ballaron, S.J. and Young, J.M. (1989) Agents Actions 26, 335–341.
169 Lassus, A. and Forsstrom, S. (1985) Br. J. Dermatol. 113, 103–106.
170 Kobza Black, A., Camp, R.D.R., Mallet, A.I., Cunningham, F.M., Hofbauer, M. and Greaves, M.W. (1990) J. Invest. Dermatol. 95, 50–54.
171 Jones, G.H., Venuti, M.C., Young, J.M., Murthy, D.V.K., Loe, B.E., Simpson, R.A., Berks, A.H., Spires, D.A., Maloney, P.J., Kruseman, M., Rouhafza, S., Kappas, K.C., Beard, C.C., Unger, S.H. and Cheung, P.S. (1986) J. Med. Chem. 29, 1504–1511.
172 Venuti, M.C., Loe, B.E., Jones, G.H. and Young, J.M. (1988) J. Med. Chem. 31, 2132–2136.
173 Yamashita, A., Schaub, R.G., Bach, M.K., White, G.J., Toy, A.G., Nabil, B., Burdick, M.D., Brashler, J.R. and Holm, M.S. (1990) J. Med. Chem. 33, 775–781.
174 Schaub, R.G. and Yamashita, A. (1986) Exp. Mol. Pathol. 45, 343–353.
175 Johnson, H.G. and Stout, B.K. (1988) Int. Arch. Allergy Appl. Immunol. 87, 204–207.
176 Schaub, R.G., Yamashita, A., Bach, M.K., White, G.J., Toy, A., Ghazal, N.B., Simmons, C.A., Burdick, M.D., Brashler, J.R. and Holm, M.S. (1990) Prostaglandins Leukotrienes Essent. Fatty Acids 39, 255–259.

177 Harris, R.R., Batt, D.G., Galbraith, W. and Ackerman, N.R. (1989) Agents Actions 27, 297–299.
178 Harris, R.R., Mackin, W.M., Batt, D.G., Rakich, S.M., Collins, R.J., Bruin, E.M. and Ackerman, N.R. (1990) Skin Pharmacol. 3, 29–40.
179 Batt, D.G., Maynard, G.D., Petraitis, J.J., Shaw, J.E., Galbraith, W. and Harris, R.R. (1990) J. Med. Chem. 33, 360–370.
180 Nelson, M.J., Batt, D.G., Thompson, J.S. and Wright, S.W. (1991) J. Biol. Chem. 266, 8225–8229.
181 Goto, M., Chiba, K., Hashida, R. and Shirota, H. (1990) Presented at IAIS Symposium on Drugs in Inflammation, Noordwijk, The Netherlands; abstract P3.
182 Shirota, H., Chiba, K., Goto, M., Hashida, R. and Ono, H. (1990) Presented at IAIS Symposium on Drugs in Inflammation, Noordwijk, The Netherlands; abstract O14.
183 Chiba, K., Goto, M. and Shirota, H. (1990) Presented at IAIS Symposium on Drugs in Inflammation, Noordwijk, The Netherlands; abstract P4.
184 Okita, M., Tanaka, M., Kaneko, T., Tagami, K., Hibi, S., Okamoto, Y., Chiba, K., Goto, M., Sakurai, H. and Shirota, H. (1990) Presented at IAIS Symposium on Drugs in Inflammation, Noordwijk, The Netherlands; abstract P1.
185 Batt, D.G. (1990) Eur. Pat. Appl. 377,450; (1991) Chem. Abstr. 114, 61921.
186 Kitamura, S., Hashizume, K., Iida, T., Miyashita, E., Shirahata, K. and Kase, H. (1986) J. Antibiot. 39, 1160–1166.
187 Kitamura, S., Hashizume, K., Ohmori, K. and Kase, H. (1988) Agr. Biol. Chem. 52, 3131–3136.
188 Teranishi, M., Suzuki, K., Kase, H., Kitamura, S., Shuto, K. and Omori, K. (1986) Eur. Pat. Appl. 177,764; (1986) Chem. Abstr. 105, 97343.
189 Belanger, P., Maycock, A., Guindon, Y., Bach, T., Dollob, A.L., Dufresne, C., Ford-Hutchinson, A.W., Gale, P.H., Hopple, S., Lau, C.K., Letts, L.G., Luell, S., McFarlane, C.S., MacIntyre, E., Meurer, R., Miller, D.K., Piechuta, H., Riendeau, D., Rokach, J., Rouzer, C. and Scheigetz, J. (1987) Can. J. Physiol. Pharmacol. 65, 2441–2448.
190 Lau, C.K., Belanger, P.C., Scheigetz, J., Dufresne, C., Williams, H.W.R., Maycock, A.L., Guindon, Y., Bach, T., Dallob, A.L., Denis, D., Ford-Hutchinson, A.W., Gale, P.H., Hopple, S.L., Letts, L.G., Luell, S., McFarlane, C.S., MacIntyre, E., Meurer, R., Miller, D.K., Piechuta, H., Riendeau, D., Rokach, J. and Rouzer, C. (1989) J. Med. Chem. 32, 1190–1197.
191 Buckle, D.R., Foster, K.A., Taylor, J.F., Tedder, J.M., Thody, V.E., Webster, R.A.B., Bermudez, J., Markwell, R.E. and Smith, S.A. (1987) J. Med. Chem. 30, 2216–2221.
192 Abe, S., Katayama, S., Tsunoda, H., Sakuma, Y., Yoshimura, T., Machida, Y., Katayama, K., Miyamoto, M., Okano, K. and Yamatsu, I. (1989) Presented at American Chemical Society 198th National Meeting, Miami Beach, FL; abstract MEDI 51.
193 Tsunoda, H., Katayama, S., Sakuma, Y., Tanaka, S., Tsukidate, K., Abe, S., Yamatsu, I. and Katayama, K. (1991) Prostaglandins Leukotrienes Essent. Fatty Acids 42, 51–56.
194 Abe, S., Miyamoto, M., Tanaka, M., Akasaka, K., Hayashi, K., Kawahara, T., Katayama, T., Sakuma, Y., Suzuki, T. and Yamatsu, I. (1988) Eur. Pat. Appl. 295,656; (1989) Chem. Abstr. 110, 192808.
195 Kerdesky, F.A.J., Holms, J.H., Moore, J.L., Dyer, R.D., Carter, G.W. and Brooks, D.W. (1989) Presented at American Chemical Society 197th National Meeting, Dallas, TX; abstract MEDI 23.
196 Miller, D.K., Sadowski, S., Han, G.Q. and Joshua, H. (1989) Prostaglandins Leukotrienes Essent. Fatty Acids 38, 137–143.
197 Hook, D.J., Yacobucci, J.J., O'Connor, S., Lee, M., Kerns, E., Krishnan, B., Matson, J. and Hesler, G. (1990) J. Antibiotics 43, 1347–1348.

198 Furukawa, M., Yoshimoto, T., Ochi, K. and Yamamoto, S. (1984) Biochim. Biophys. Acta 795, 458–465.
199 Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S. and Shiraishi, M. (1982) Biochim. Biophys. Acta 713, 470–473.
200 Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S. and Shiraishi, M. (1983) Int. Congr. Ser. Excerpta Med. 623, 72–77.
201 Okumura, M., Obata, N., Yamamura, H., Kohno, S. and Ohata, K. (1989) Jpn. J. Pharmacol. 50, 37–46.
202 Riendeau, D., Falgueyret, J.P., Nathaniel, D.J., Rokach, J., Ueda, N. and Yamamoto, S. (1989) Biochem. Pharmacol. 38, 2313–2321.
203 Ishihara, Y., Kitamura, S. and Takaku, F. (1983) Prostaglandins 26, 623–629.
204 Ashida, Y., Saijo, T., Kuriki, H., Makino, H., Terao, S. and Maki, Y. (1983) Prostaglandins 26, 955–972.
205 Mita, H., Yui, Y. and Shida, T. (1986) Allergy (Copenhagen) 41, 493–498.
206 Nakadate, T., Yamamoto, S., Aizu, E. and Kato, R. (1985) J. Pharm. Pharmacol. 37, 71–73.
207 Ozaki, Y., Ohashi, T. and Niwa, Y. (1986) Biochem. Pharmacol. 35, 3481–3488.
208 Nakadate, T., Yamamoto, S., Aizu, E. and Kato, R. (1985) Jpn. J. Pharmacol. 38, 161–168.
209 Griffiths, R.J., Wood, B.E., Li, S. and Blackham, A. (1988) Agents Actions 25, 344–351.
210 Aked, D., Foster, S.J., Howarth, A., McCormick, M.E. and Potts, H.C. (1986) Br. J. Pharmacol. 89, 431–438.
211 Makino, H., Ashida, Y., Saijo, T., Kuriki, H., Terao, S. and Maki, Y. (1986) Int. Arch. Allergy Appl. Immunol. 79, 38–44.
212 Kreutner, W., Sherwood, J. and Rizzo, C. (1989) Agents Actions 28, 173–184.
213 Sasaki, K., Ueno, A., Kawamura, M., Katori, M., Shigehiro, S. and Kikawada, R. (1987) Adv. Prostaglandin Thromboxane Leukotriene Res. 17, 381–383.
214 Sasaki, K., Ueno, A., Katori, M. and Kikawada, R. (1988) Cardiovasc. Res. 22, 142–148.
215 Ancill, R.J., Takahashi, Y., Kibune, Y., Campbell, R. and Smith, J.R. (1990) J. Int. Med. Res. 18, 75–88.
216 Fujimura, M., Sasaki, F., Nakatsumi, Y., Takahashi, Y., Hifumi, S., Taga, K., Mifune, J., Tanaka, T. and Matsuda, T. (1986) Thorax 41, 955–959.
217 Ohkawa, S., Terao, S., Terashita, Z., Shibouta, Y. and Nishikawa, K. (1991) J. Med. Chem. 34, 267–276.
218 Marshall, W.S., Fleisch, J.H. and Cullinan, G.J. (1984) UK Pat. Appl. 2,127,815; (1984) Chem. Abstr. 101, 191968.
219 Medwid, J.B., Torley, L.W. and Tomcufcik, A.S. (1987) Eur. Pat. Appl. 226,842; (1987) Chem. Abstr. 107, 236736.
220 Medwid, J.B. and Torley, L.W. (1987) U.S. Patent 4,692,449; (1988) Chem. Abstr. 108, 6049.
221 Ku, E.C., Raychaudhuri, A., Ghai, G., Kimble, E.F., Lee, W.H., Colombo, C., Dotson, R., Oglesby, T.D. and Wasley, J.W.F. (1988) Biochim. Biophys. Acta 959, 332–342.
222 Matera, G., Cook, J.A., Hennigar, R.A., Tempel, G.E., Wise, W.C., Oglesby, T.D. and Halushka, P.V. (1988) J. Pharmacol. Exp. Ther. 247, 363–371.
223 Yaacob, H.B. and Piper, P.J. (1988) Br. J. Pharmacol. 95, 1322–1328.
224 Rakhit, A., Kuwahara, S.K., Jones, D.R., Soliman, V.F., Kotake, A.N., Oglesby, T.D., Wasley, J.W.F., Tripp, S.L. and L., D.F. (1990) Pharm. Res. 7, 1071–1076.
225 Roland, D.M., Macchia, W.M., Grim, K.S., Kimble, E.F., White, D.H. and Kowalski,

T.J. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 118.
226 Fiedler-Nagy, C., Welton, A.F., Batula-Bernardo, C., Sauchelli, K. and Coffey, J.W. (1983) Fed. Proc. 42, 2091.
227 Lau, C.K., Yoakim, C., Rokach, J. and Guindon, Y. (1987) U.S. Patent 4,667,032.
228 Guindon, Y., Girard, Y., Maycock, A., Ford-Hutchinson, A.W., Atkinson, J.G., Belanger, P.C., Dallob, A., DeSousa, D., Dougherty, H., Egan, R., Goldenberg, M.M., Ham, E., Fortin, R., Hamel, P., Hamel, R., Lau, C.K., Leblanc, Y., McFarlane, C.S., Piechuta, H., Therien, M., Yoakim, C. and Rokach, J. (1987) Adv. Prostaglandin Thromboxane Leukotriene Res. 17, 554–557.
229 Fortin, R., Lau, C.K., Guindon, Y., Rokach, J. and Yoakim, C. (1987) U.S. Patent 4,666,907.
230 Warner, J.A., Lichtenstein, L.M. and MacGlashan, D.W., Jr. (1988) J. Pharmacol. Exp. Ther. 247, 218–222.
231 Piechuta, H., Ford-Hutchinson, A.W. and Letts, L.G. (1987) Agents Actions 22, 69–74.
232 Leikauf, G.D., Doupnik, C.A., Leming, L.M. and Wey, H.E. (1989) J. Appl. Physiol. 66, 1838–1845.
233 McFarlane, C.S., Hamel, R. and Ford-Hutchinson, A.W. (1987) Agents Actions 22, 63–68.
234 Garceau, D., Ford-Hutchinson, A.W. and Charleson, S. (1987) Eur. J. Pharmacol. 143, 1–7.
235 Dallob, A., Guindon, Y. and Goldenberg, M.M. (1987) Biochem. Pharmacol. 36, 3201–3204.
236 Guindon, Y., Girard, Y., Lau, C.K., Fortin, R., Rokach, J. and Yoakim, C. (1986) U.S. Patent 4,611,056; (1987) Chem. Abstr. 106, 33082.
237 Yu, M.J., McCowan, J.R., Bertsch, B. and Ho, P.P.K. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 75.
238 Mardin, M., Sundermann, R., Hoffmeister, F., Busse, W., Horstmann, H. and Raddatz, S. (1985) U.S. Patent 4,552,874.
239 Niemers, E., Gruetzmann, R., Mardin, M., Busse, W. and Meyer, H. (1987) U.S. Patent 4,636,497.
240 Benz, G., Fengler, G., Meyer, H., Niemers, E., Fiedler, V., Mardin, M., Mayer, D., Perzborn, E., Seuter, F., Hammond, M. and Taylor, W. (1985) Austral. Pat. Abstr. 44,773/85.
241 Klausener, A., Fengler, G., Buysch, H. and Pelster, B. (1988) U.S. Patent 4,784,997.
242 Fengler, G., Klausener, A., Buysch, H., Mardin, M. and Pelster, B. (1989) U.S. Patent 4,861,878.
243 Weintraub, P.M., Skoog, M.T., Nichols, J.S., Wiseman, J.S., Huber, E.W., Baugh, L.E. and Farrell, A.M. (1989) J. Pharm. Sci. 78, 937–943.
244 Miyazawa, K., Iimori, Y., Makino, M., Mikami, T. and Miyasaka, K. (1985) Jpn. J. Pharmacol. 38, 199–205.
245 Spaethe, S.M., Hsueh, W. and Needleman, P. (1989) J. Pharmacol. Exp. Ther. 248, 1308–1316.
246 Dillard, R.D., Pavey, D.E. and Benslay, D.N. (1973) J. Med. Chem. 16, 251–253.
247 Blackwell, G.J. and Flower, R.J. (1978) Prostaglandins 16, 417–425.
248 Higgs, G.A., Flower, R.J. and Vane, J.R. (1979) Biochem. Pharmacol. 28, 1959–1961.
249 Higgs, G.A., Eakins, K.E., Mugridge, K.G., Moncada, S. and Vane, J.R. (1980) Eur. J. Pharmacol. 66, 81–86.
250 Burka, J.F. and Flower, R.J. (1979) Br. J. Pharmacol. 65, 35–41.
251 Raadmark, O., Malmsten, C. and Samuelsson, B. (1980) FEBS Lett. 110, 213–215.

252 Randall, R.W., Eakins, K.E., Higgs, G.A., Salmon, J.A. and Tateson, J.E. (1980) Agents Actions 10, 553–555.
253 Foster, S.J., McCormick, M.E. and Howarth, A. (1985) Agents Actions 17, 358–359.
254 Burka, J.F. (1985) Br. J. Pharmacol. 85, 411–420.
255 Robak, J. and Duniec, Z. (1982) Biochem. Pharmacol. 31, 1955–1959.
256 Frigola, J., Colombo, A., Pares, J., Martinez, L., Sagarra, R. and Roser, R. (1989) Eur. J. Med. Chem. 24, 435–445.
257 Copp, F.C., Islip, P.J. and Tateson, J.E. (1984) Biochem. Pharmacol. 33, 339–340.
258 Dawson, B., Follenfant, R.L., Higgs, G.A., Schneider, C. and Gibson, J.R. (1985) Br. J. Dermatol. 113 Suppl., 137–140.
259 Hlasta, D.J., Gordon, R.J., Casey, F.B., Ferguson, E.W., Gangell, S.J. and Heimann, M.R. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 77.
260 Kim, K.H., Martin, Y.C., Norris, B., Young, P.R., Carter, G.W., Haviv, F. and Walters, R.L. (1990) J. Pharm. Sci. 79, 609–613.
261 Albert, D.H., Dyer, R., Young, P.R., Barlow, J., Bornemeier, D., Bouska, J., Roberts, E., Sonsalla, J., Brooks, D.W. and Carter, G.W. (1988) FASEB J. 2, A369.
262 Brooks, D.W., Basha, A., Bhatia, P.A., Ratajczyk, J.D., Schmidt, S.P., Albert, D.H., Young, P.R., Bouska, J.B., Dyer, R.D., Bell, R.L. and Carter, G.W. (1989) Presented at American Chemical Society 198th National Meeting, Miami Beach, FL; abstract MEDI 87.
263 Brooks, D.W., Carter, G.W., Basha, A., Dyer, R.D., Gunn, B.P. and Bhatia, P.A. (1989) Eur. Pat. Appl. 299,449; (1989) Chem. Abstr. 111, 134201.
264 Bertez, C., Conduzorgues, J.P., Sincholle, D., Coquelet, C. and Bonne, C. (1986) Adv. Inflammation Res. 10, 414–416.
265 Coquelet, C., Conduzorgues, J.P., Bertez, C., Bonne, C. and Sincholle, D. (1984) Drugs Future 9, 102–103.
266 Bertez, C., Miquel, M., Coquelet, C., Sincholle, D. and Bonne, C. (1984) Biochem. Pharmacol. 33, 1757–1762.
267 Sincholle, D., Bertez, C., Legrand, A., Conduzorgues, J.P. and Bonne, C. (1985) Arzneim.-Forsch. 35, 1260–1263.
268 Ghiglieri-Bertez, C., Coquelet, C., Alazet, A. and Bonne, C. (1987) Eur. J. Med. Chem. 22, 147–152.
269 Baumann, J. and Wurm, G. (1982) Agents Actions 12, 360–364.
270 Steinhilber, D., Schmidt, K., Eger, K. and Roth, H.J. (1986) Pharm. Res. 3, 271–277.
271 Chang, J., Blazak, E., Skowronek, M., Marinari, L. and Carlson, R.P. (1987) Eur. J. Pharmacol. 142, 197–205.
272 Tihanyi, E., Feher, O., Gal, M., Janaky, J., Tolnay, P. and Sebestyen, L. (1984) Eur. J. Med. Chem.--Chim. Ther. 19, 433–439.
273 Foster, S.J., Bruneau, P., Walker, E.R.H. and McMillan, R.M. (1990) Br. J. Pharmacol. 99, 113–118.
274 Foster, S.J., Aked, D.M., McCormick, M.E. and Potts, H.C. (1989) Br. J. Pharmacol. 96, 38P.
275 Bruneau, P., Delvare, C., Edwards, M.P. and McMillan, R.M. (1991) J. Med. Chem. 34, 1028–1036.
276 Blackham, A., Griffiths, R.J., Hallam, C., Mann, J., Mitchell, P.D., Norris, A.A. and Simpson, W.T. (1990) Agents Actions 30, 432–442.
277 Egan, R.W. and Gale, P.H. (1985) J. Biol. Chem. 260, 11554–11559.
278 Choo, L.Y. and Riendeau, D. (1987) Can. J. Physiol. Pharmacol. 65, 2503–2506.
279 Dorsch, W., Wagner, H., Bayer, T., Fessler, B., Hein, G., Ring, J., Scheftner, P., Sieber, W., Strasser, T. and Weiss, E. (1988) Biochem. Pharmacol. 37, 4479–4486.

280 Wagner, H., Dorsch, W., Bayer, T., Breu, W. and Willer, F. (1990) Prostaglandins Leukotrienes Essent. Fatty Acids 39, 59–62.
281 Mardin, M., Busse, W., Gruetzmann, R. and Rochels, R. (1983) Arch. Pharmacol. 322 suppl, R114.
282 Verbey, N.L.J., Van Haeringen, N.J. and De Jong, P.T.V.M. (1988) Curr. Eye Res. 7, 361–368.
283 Busse, W.-D., Krauthausen, E. and Mardin, M. (1982) German Patent 3,118,126; (1983) Chem. Abstr. 98, 143447.
284 Corey, E.J., Cashman, J.R., Kantner, S.S. and Wright, S.W. (1984) J. Am. Chem. Soc. 106, 1503–1504.
285 Kerdesky, F.A.J., Holms, J.H., Schmidt, S.P., Dyer, R.D. and Carter, G.W. (1985) Tetrahedron Lett. 26, 2143–2146.
286 Hirschelmann, R., Kuhn, C., Zarnack, S., Funke, T., Bekemeier, H., Giessler, A.J., Phuoc, L.T. and Buege, A. (1989) Biomed. Biochim. Acta 47, S256-S259.
287 Laughton, M.J., Moroney, M.A., Hoult, J.R.S. and Halliwell, B. (1989) Biochem. Pharmacol. 38, 189–193.
288 Blake, D.R., Hall, N.D., Bacon, P.A., Dieppe, P.A., Halliwell, B. and Gutteridge, J.M.C. (1983) Ann. Rheumatic Dis. 42, 89–93.
289 Andrews, F.J., Morris, C.J., Kondratowicz, G. and Blake, D.R. (1987) Ann. Rheumatic Dis. 46, 327–333.
290 Zusi, F.C., Marathe, S.A. and Tramposch, K.M. (1988) U.S. Patent 4,731,382; (1988) Chem. Abstr. 109, 6233.
291 Tramposch, K.M., Zusi, F.C., Marathe, S.A., Stanley, P.L., Nair, X., Steiner, S.A. and Quigley, J.W. (1990) Agents Actions 30, 443–450.
292 Murray, W.V. and Wachter, M.P. (1987) U.S. Patent 4,681,894; (1987) Chem. Abstr. 107, 175687.
293 Flynn, D.L., Capiris, T., Cetenko, W.J., Conner, D.T., Dyer, R.D., Kostlan, C.R., Nies, D.E., Schrier, D.J. and Sircar, J.C. (1990) J. Med. Chem. 33, 2070–2072.
294 Summers, J.B., Mazdiyasni, H., Holms, J.H., Ratajczyk, J.D., Dyer, R.D. and Carter, G.W. (1987) J. Med. Chem. 30, 574–580.
295 Hedgecock, C.J.R. and Rowlands, D.A. (1987) UK Pat. Appl. 2,189,486.
296 Demers, J.P., Murray, W.V. and Sulsky, R.B. (1989) U.S. Patent 4,820,828; (1989) Chem. Abstr. 111, 133796.
297 Kimble, E., Kowalski, T., White, D., Raychauduri, A., Pastor, G., Chertock, H., Lee, W., Neale, R., Hamdan, A. and Wasley, J. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 100.
298 Hahn, R.A., MacDonald, B.R., Simpson, P.J., Wang, L., Towner, R.D., Ho, P.P.K., Goodwin, R.M., Breau, A.P., Suarez, T. and Mihelich, E.D. (1991) J. Pharmacol. Exper. Ther. 256, 94–102.
299 Gunn, B.P. and Summers, J.B. (1987) Eur. Pat. Appl. 240,859; (1988) Chem. Abstr. 108, 75233.
300 Huang, F.C., Shoupe, T.S., Lin, C.J., Lee, T.D.Y., Chan, W.K., Tan, J., Schnapper, M., Suh, J.T., Gordon, R.J., Sonnino, P.A., Sutherland, C.A., Van Inwegen, R.G. and Coutts, S.M. (1989) J. Med. Chem. 32, 1836–1842.
301 Carnathan, G.W., Sweeney, D.M., Travis, J.J., Gordon, R.J., Sutherland, C.A., Jariwala, N., Clearfield, M., O'Rourke, S., Huang, F.C. and Van Inwegen, R.G. (1989) Agents Actions 28, 204–211.
302 Summers, J.B., Kim, K.H., Mazdiyasni, H., Holms, J.H., Ratajczyk, J.D., Stewart, A.O., Dyer, R.D. and Carter, G.W. (1990) J. Med. Chem. 33, 992–998.
303 Carter, G.W., Young, P.R., Dyer, R., Albert, D.H., Bouska, J., Barlow, J., Roberts, E., Sonsalla, J., Summers, J. and Brooks, D. (1988) FASEB J. 2, 5623.

304 Summers, J.B., Gunn, B.P., Martin, J.G., Mazdiyasni, H., Stewart, A.O., Young, P.R., Goetze, A.M., Bouska, J.B., Dyer, R.D., Brooks, D.W. and Carter, G.W. (1988) J. Med. Chem. 31, 3–5.
305 Summers, J.B., Gunn, B.P., Martin, J.G., Martin, M.B., Mazdiyasni, H., Stewart, A.O., Young, P.R., Bouska, J.B. and Goetze, A.M. (1988) J. Med. Chem. 31, 1960–1964.
306 Summers, J.B., Gunn, B.P. and Brooks, D.W. (1988) Eur. Pat. Appl. 279,263; (1989) Chem. Abstr. 110, 57509.
307 Summers, J.B. (1989) U.S. Patent 4,822,811; (1989) Chem. Abstr. 111, 109039.
308 Bhattacherjee, P., Boughton-Smith, N.K., Follenfant, R.L., Garland, L.G., Higgs, G.A., Hodson, H.F., Islip, P.J., Jackson, W.P., Moncada, S., Payne, A.N., Randall, R.W., Reynolds, C.H., Salmon, J.A., Tateson, J.E. and Whittle, B.J.R. (1988) Ann. N.Y. Acad. Sci. 524, 307–320.
309 Hawthorne, A.B., Boughton Smith, N., Kurlak, L.O., Whittle, B.J.R. and Hawkey, C.J. (1989) Gut 30, A1501.
310 Tateson, J.E., Randall, R.W., Reynolds, C.H., Jackson, W.P., Bhattacherjee, P., Salmon, J.A. and Garland, L.G. (1988) Br. J. Pharmacol. 94, 528–539.
311 Higgs, G.A., Follenfant, R.L. and Garland, L.G. (1988) Br. J. Pharmacol. 94, 547–551.
312 Boughton-Smith, N.K. and Whittle, B.J.R. (1988) Br. J. Pharmacol. 95, 155–162.
313 Liu, D.S., Liew, F.Y. and Rhodes, J. (1989) Immunopharmacology 17, 1–9.
314 Salmon, J.A. and Jackson, W.P. (1989) Eur. Pat. Appl. 299,761; (1989) Chem. Abstr. 111, 77652.
315 Garland, L.G., Jackson, W.P., Salmon, J.A. and Nicholls, A. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 149.
316 Summers, J.B. (1988) Eur. Pat. Appl. 279,281; (1988) Chem. Abstr. 109, 210739.
317 Summers, J.B., Stewart, A.O. and Brooks, D.W. (1988) Eur. Pat. Appl. 292,699; (1989) Chem. Abstr. 110, 172898.
318 Anderson, G. (1990) Trends Pharmacol. Sci. 11, 348–349.
319 Dellaria, J.F., Jr., Brooks, D.W., Summers, J.B., DeNinno, S.L., Martin, M.B., Martin, J.G., Stewart, A.O., Sallin, K.J., Bouska, J.B., Young, P.R., Bell, R.L. and Carter, G.W. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 76.
320 Summers, J.B. and Stewart, A.O. (1989) U.S. Patent 4,822,809; (1989) Chem. Abstr. 111, 115179.
321 Brooks, D.W. and Rodriques, K.E. (1990) Eur. Pat. Appl. 374,602; (1991) Chem. Abstr. 114, 81580.
322 Carter, G.W., Young, P.R., Albert, D.H., Bouska, J.B., Dyer, R.D., Bell, R.L., Summers, J.B., Brooks, D.W., Gunn, B.P., Rubin, P. and Kesterson, J. (1989) New Trends Lipid Mediators Res. 3, 50–55.
323 Brooks, D.W., Summers, J.B., Gunn, B.P., Dellaria, J.F., Holmes, J.H., Maki, R.G., Martin, J.G., Martin, M.B., Moore, J.L., Rodriques, K.E., Stewart, A.O., Albert, D.H., Bell, R.L., Bouska, J.B., Dyer, R.D., Malo, P.E., Young, P.R., Rubin, P., Kesterson, J. and Carter, G.W. (1990) Presented at American Chemical Society 199th National Meeting, Boston, MA; abstract MEDI 148.
324 Rubin, P. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA.
325 Knapp, H.R. (1990) N. Engl. J. Med. 323, 1745–1748.
326 Israel, E., Dermarkarian, R., Rosenberg, M., Sperling, R., Taylor, G., Rubin, P. and Drazen, J.M. (1990) N. Engl. J. Med. 323, 1740–1744.
327 Collawn, C., Rubin, P., Perez, N., Reyes, E., Bobadilla, J., Cabrera, G., Moran, M.A. and Kerschenobich, D. (1989) Am. J. Gastroenterol. 184, 1187A.

328 Laursen, L.S., Naesdal, J., Bukhave, K., Lauritsen, K. and Rask-Madsen, J. (1990) Lancet 335, 683–685.
329 Weinblatt, M., Kremer, J., Helfgott, S., Coblyn, J., Maier, A., Sperling, R., Petrillo, G., Kesterson, J., Dube, L., Henson, B., Teoh, N. and Rubin, P. (1990) Arthritis Rheum. 33 Suppl., S152.
330 Blobstein, S.H., Grady, R.W., Meshnick, S.R. and Ceram, A. (1978) Biochem. Pharmacol. 27, 2939–2945.
331 Clapp, C.H., Banerjee, A. and Rotenberg, S.A. (1985) Biochemistry 24, 1826–1830.
332 Strasser, M., Cooper, P., Dewald, B. and Payne, T. (1988) Helv. Chim. Acta 71, 1156–1176.
333 Schnyder, J., Hunziker, T., Strasser, M., Richardson, B. and Krebs, A. (1986) Arch. Dermatol. Res. 278, 494–6.
334 Vanderhoek, J.Y., Bryant, R.W. and Bailey, J.M. (1980) J. Biol. Chem. 255, 10064–10066.
335 Musser, J.H., Chakraborty, U.R., Sciortino, S., Gordon, R.J., Khandwala, A., Neiss, E.S., Pruss, T.P., Van Inwegen, R., Weinryb, I. and Coutts, S.M. (1987) J. Med. Chem. 30, 96–104.
336 Tennant, C.M., Seale, J.P. and Temple, D.M. (1987) J. Pharm. Pharmacol. 39, 309–311.
337 Kusner, E.J., Marks, R.L., Aharony, D. and Krell, R.D. (1989) Biochem. Pharmacol. 38, 4183–4189.
338 Van Inwegen, R.G., Khandwala, A., Gordon, R., Sonnino, P., Coutts, S. and Jolly, S. (1987) J. Pharmacol. Exp. Ther. 241, 117–124.
339 Evans, J.M., Barnes, N.C., Piper, P.J. and Costello, J.F. (1987) Proc. Br. Pharmacol. Soc., 111P.
340 Musser, J.H. and Kreft, A.F. (1990) Drugs Future 15, 73–80.
341 Musser, J.H., Kubrak, D.M., Chang, J., DiZio, S.M., Hite, M., Hand, J.M. and Lewis, A.J. (1987) J. Med. Chem. 30, 400–405.
342 Musser, J.H., Kubrak, D.M., Chang, J. and Lewis, A.J. (1986) J. Med. Chem. 29, 1429–1435.
343 Kees, K.L., Musser, J.H., Chang, J., Skowronek, M. and Lewis, A.J. (1986) J. Med. Chem. 29, 2329–2334.
344 Chang, J., Musser, J.H. and Hand, J.M. (1988) Int. Arch. Allergy Appl. Immunol. 86, 48–54.
345 Chang, J., Borgeat, P., Schleimer, R.P., Musser, J.H., Marshall, L.A. and Hand, J.M. (1988) Eur. J. Pharmacol. 148, 131–141.
346 Musser, J.H., Kreft, A.F., Bender, R.H.W., Kubrak, D.M., Carlson, R.P., Chang, J. and Hand, J.M. (1989) J. Med. Chem. 32, 1176–1183.
347 Mohrs, K., Raddatz, S., Fruchtmann, R., Kohlsdorfer, C. and Mueller-Peddinghaus, R. (1989) Eur. Pat. Appl. 339,416; (1990) Chem. Abstr. 112, 216719.
348 Mohrs, K., Raddatz, S., Perzborn, E., Fruchtmann, R., Kohlsdorfer, C., Muller-Peddinghaus, R. and Theisen, P. (1989) Eur. Pat. Appl. 344,519; (1990) Chem. Abstr. 113, 58959.
349 Ahnfelt-Roenne, I., Torngaard Hansen, E., Kirstein, D., Tvaermose Nielsen, O.B. and Rachlin, S. (1989) PCT Int. Pat. Appl. 8905294; (1990) Chem. Abstr. 112, 7392.
350 Kreft, A., Musser, J., Marshall, L. and Grimes, D. (1990) Drugs Future 15, 805–807.
351 Carlson, R.P., O'Neill-Davis, L., Calhoun, W., Datko, L., Musser, J.H., Kreft, A.F. and Chang, J.Y. (1989) Agents Actions 26, 319–328.
352 Bird, T.G.C., Crawley, G.C., Edwards, M.P., Foster, S.J., Girodeau, J.M., Kingston, J.F. and McMillan, R.M. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 85.

353 McMillan, R.M., Girodeau, J.M. and Foster, S.J. (1990) Br. J. Pharmacol. 101, 501–503.
354 Girodeau, J.M.M.M. (1990) Eur. Pat. Appl. 375,368; (1991) Chem. Abstr. 114, 42285.
355 Girodeau, J.M.M.M. (1990) Eur. Pat. Appl. 375,452; (1991) Chem. Abstr. 114, 6044.
356 Hammon, A. (1990) Eur. Pat. Appl. 375,457; (1991) Chem. Abstr. 114, 42774.
357 Griswold, D.E., Marshall, P.J., Webb, E.F., Godfrey, R., Newton, J., Jr., DiMartino, M.J., Sarau, H.M., Gleason, J.G., Poste, G. and Hanna, N. (1987) Biochem. Pharmacol. 36, 3463–3470.
358 Griswold, D.E., Webb, E., Schwartz, L. and Hanna, N. (1987) Inflammation (N.Y.) 11, 189–199.
359 DiMartino, M.J., Griswold, D.E., Berkowitz, B.A., Poste, G. and Hanna, N. (1987) Agents Actions 20, 113–123.
360 Griswold, D.E., Hillegass, L.M., Meunier, P.C., DiMartino, M.J. and Hanna, N. (1988) Arthritis Rheum. 31, 1406–1412.
361 Lee, J.C., Griswold, D.E., Votta, B. and Hanna, N. (1988) Int. J. Immunopharmacol. 10, 835–843.
362 Lee, J.C., Rebar, L. and Laydon, J.T. (1989) Agents Actions 27, 277–279.
363 Barton, B.E. and James, L.C. (1990) Immunopharmacol. Immunotoxicol. 12, 105–121.
364 Badger, A.M., Olivera, D., Talmadge, J.E. and Hanna, N. (1989) Circ. Shock 27, 51–61.
365 Lee, J.C., Votta, B., Dalton, B.J., Griswold, D.E., Bender, P.E. and Hanna, N. (1990) Int. J. Immunother. 6, 1–12.
366 Griswold, D.E., Hoffstein, S., Marshall, P.J., Webb, E.F., Hillegass, L., Bender, P.E. and Hanna, N. (1989) Inflammation (N.Y.) 13, 727–739.
367 Hanna, N., Marshall, P.J., Newton, J., Schwartz, L., Kirsh, R., Dimartino, M.J., Adams, J., Bender, P. and Griswold, D.E. (1990) Drugs Exp. Clin. Res. 16, 137–147.
368 Lantos, I., Bender, P.E., Razgaitis, K.A., Sutton, B.M., DiMartino, M.J., Griswold, D.E. and Walz, D.T. (1984) J. Med. Chem. 27, 72–75.
369 Adams, J.L., Hanna, N., Razgaitis, K., Bender, P.E., Newton, J.F., Gleason, J.G. and Perchonock, C.D. (1989) Eur. Pat. Appl. 306,300; (1989) Chem. Abstr. 111, 115176.
370 Bender, P.E. and Hanna, N. (1988) U.S. Patent 4,719,218.
371 Cheng, H.Y., Davis, L.L., Bender, P.E. and Gleason, J. (1989) J. Electrochem. Soc. 136, 3679–3683.
372 Robinson, C.P. (1990) Drugs Future 15, 902–904.
373 Murray, W.V. and Wachter, M.P. (1988) Eur. Pat. Appl. 293,220; (1989) Chem. Abstr. 111, 23508.
374 Lombardino, J.G. (1985) in Ref. 46, pp. 379–391.
375 Tischler, A., Bailey, P., Dallob, A., Witzel, B., Durette, P., Rupprecht, K., Allison, D., Dougherty, H. and Humes, J. (1986) Adv. Prostaglandin Thromboxane Leukotriene Res. 16, 63–66.
376 Gresele, P., Arnout, J., Deckmyn, H. and Vermylen, J. (1987) Biochem. Pharmacol. 36, 3529–3531.
377 Bailey, P.J., Dallob, A.L., Allison, D.L., Anderson, R.L., Bach, T., Durette, P.L., Hand, K.M., Hopple, S.L. and Luell, S. (1988) Arzneim.-Forsch. 38, 372–378.
378 Barr, R.M., Kobza Black, A., Dowd, P.M., Koro, O., Mistry, K., Isaacs, J.L. and Greaves, M.W. (1988) Br. J. Clin. Pharmacol. 25, 23–26.
379 Robinson, C. (1990) Drugs Future 15, 898–901.
380 Smith, D.M., Johnson, J.A., Loeser, R. and Turner, R.A. (1990) Agents Actions 31, 102–109.
381 Moilanen, E., Alanko, J., Asmawi, M.Z. and Vapaatalo, H. (1988) Eicosanoids 1, 35–39.

382 Blackburn, W.D., Heck, L.W., Loose, L.D., Eskra, J.D. and Carty, T.J. (1991) Arthritis Rheum. 34, 204–210.
383 Blackburn, W.D., Loose, L.D., Heck, L.W. and Chatham, W.W. (1991) Arthritis Rheum. 34, 211–216.
384 Kyan-Aung, U., Thornhill, M.H., Lee, T.H. and Haskard, D.O. (1990) Presented at International Congress on Inflammation, Barcelona, Spain; abstract 243.
385 Showell, H.J., Breslow, R., Conklyn, M.J., Farrell, C.A. and Kadin, S.B. (1990) Presented at Inflammation Research Association Fifth International Conference, White Haven, PA; abstract 12.
386 Mylari, B.L., Carty, T.J., Moore, P.F. and Zembrowski, W.J. (1990) J. Med. Chem. 33, 2019–2024.
387 Clemence, F., Le Martret, O., Delevallee, F., Benzoni, J., Jouanen, A., Jouquey, S., Mouren, M. and Deraedt, R. (1988) J. Med. Chem. 31, 1453–1462.
388 Nishikawa, Y., Shindo, T., Ishii, K., Nakamura, H., Kon, T. and Uno, H. (1989) Chem. Pharm. Bull. 37, 100–105.
389 Nishikawa, Y., Shindo, T., Ishii, K., Nakamura, H., Kon, T. and Uno, H. (1989) J. Med. Chem. 32, 583–593.
390 Nishikawa, Y., Shindo, T., Ishii, K., Nakamura, H., Kon, T. and Uno, H. (1989) Chem. Pharm. Bull. 37, 684–687.
391 Nishikawa, Y., Shindo, T., Ishii, K., Nakamura, H., Kon, T., Uno, H. and Matsumoto, J. (1989) Chem. Pharm. Bull. 37, 1256–1259.
392 Tasaka, K. (1988) Drugs Future 13, 1056–1059.
393 Inoue, H., Saito, H., Koshihara, Y. and Murota, S. (1986) Chem. Pharm. Bull. 34, 897–901.
394 Inoue, H., Mori, T., Shibata, S. and Koshihara, Y. (1988) J. Pharm. Pharmacol. 40, 272–277.
395 Koda, N., Mizoguchi, Y., Kobayashi, J., Kondo, Y., Morisawa, S., Monna, T. and Yamamoto, H. (1988) Wakan Iyaku Gakkaishi 5, 508–509; (1989) Chem. Abstr. 111, 89972.
396 Kawada, N., Hasegawa, I., Sakagami, Y., Mizoguchi, Y., Kobayashi, K., Kondou, H., Morisawa, S., Monna, T. and Yamamoto, S. (1989) Ensho 9, 29–32; (1990) Chem. Abstr. 112, 69579.
397 Lombardo, D. and Dennis, E.A. (1985) J. Biol. Chem. 260, 7234–7240.
398 Glaser, K.B. and Jacobs, R.S. (1986) Biochem. Pharmacol. 35, 449–453.
399 Blankemeier, L.A. and Jacobs, R.S. (1983) Fed. Proc. 42, 374.
400 De Vries, G.W., Amdahl, L., Mobasser, A., Wenzel, M. and Wheeler, L.A. (1988) Biochem. Pharmacol. 37, 2899–2905.
401 Mihelich, E.D., Morgan, B.P., Ho, P.P.K., Walters, C.P. and Bertsch, B.A. (1988) Ann. N.Y. Acad. Sci. 524, 445–447.
402 Wheeler, L.A., Sachs, G., De, V.G., Goodrum, D., Woldemussie, E. and Muallem, S. (1987) J. Biol. Chem. 262, 6531–6538.
403 Bennett, C.F., Mong, S., Wu, H.L.W., Clark, M.A., Wheeler, L. and Crooke, S.T. (1987) Mol. Pharmacol. 32, 587–593.
404 Chakrabarti, J.K., Eggleton, R.J., Gallagher, P.T., Harvey, J., Hicks, T.A., Kitchen, E.A. and Smith, C.W. (1987) J. Med. Chem. 30, 1663–1668.
405 Dahl, S.L. and Ward, J.R. (1982) Pharmacotherapy 2, 354–366.
406 Allen, B.R. (1983) Br. J. Dermatol. 109, 361–364.
407 Cashin, C.H., Dawson, W. and Kitchen, E.A. (1977) J. Pharm. Pharmacol. 29, 330–336.
408 Ford-Hutchinson, A.W., Walker, J.R., Connor, N.S., Oliver, A.M. and Smith, M.J.H. (1977) J. Pharm. Pharmacol. 29, 372–373.

409 Meacock, S.C.R., Kitchen, E.A. and Dawson, W. (1979) Eur. J. Rheumatol. Inflammation 3, 23–28.
410 Meacock, S.C.R. and Kitchen, E.A. (1979) J. Pharm. Pharmacol. 31, 366–370.
411 Willoughby, D.A., Dawson, W., Kitchen, E.A. and Walker, J.R. (1981) Rhumatologie (Paris) 11, 431–433.
412 Brown, K.A., Ferrie, J., Wilbourn, B. and Dumonde, D.C. (1989) Agents Actions 28, 115–120.
413 Walker, J.R. and Dawson, W. (1979) J. Pharm. Pharmacol. 31, 778–780.
414 Harvey, J., Parish, H., Ho, P.P.K., Boot, J.R. and Dawson, W. (1983) J. Pharm. Pharmacol. 35, 44–45.
415 Walker, J.R., Boot, J.R., Cox, B. and Dawson, W. (1980) J. Pharm. Pharmacol. 32, 866–867.
416 Dawson, W., Boot, J.R., Harvey, J. and Walker, J.R. (1982) Eur. J. Rheumatol. Inflammation 5, 61–68.
417 Lee, V.Y., Hughes, J.M., Seale, J.P. and Temple, D.M. (1982) Clin. Sci. 63, 219–221.
418 Humes, J.L., Sadowski, S., Galavage, M., Goldenberg, M., Subers, E., Kuehl, F.A. and Bonney, R.J. (1983) Biochem. Pharmacol. 32, 2319–2322.
419 Salmon, J.A., Higgs, G.A., Tilling, L., Moncada, S. and Vane, J.R. (1984) Lancet i, 848.
420 Salmon, J.A., Tilling, L.C. and Moncada, S. (1984) Biochem. Pharmacol. 33, 2928–2930.
421 Masters, D.J. and McMillan, R.M. (1984) Br. J. Pharmacol. 81, 70P.
422 Allen, B.R. and Littlewood, S.M. (1982) Br. Med. J. 285, 1241.
423 Kragballe, K. and Herlin, T. (1983) Arch. Dermatol. 119, 548–552.
424 Voorhees, J.J. (1983) Arch. Dermatol. 119, 541–547.
425 Hawkey, C.J. and Rampton, D.S. (1983) Prostaglandins Leukotrienes Med. 10, 405–409.
426 O'Sullivan, M. and Molloy, M.G. (1985) Clin. Rheumatol. 4, 362–363.
427 Egsmose, C., Lund, B. and Andersen, R.B. (1988) Scand. J. Rheumatol. 17, 103–111.
428 Ahnfelt-Roenne, I. and Arrigoni-Martelli, E. (1980) Biochem. Pharmacol. 29, 3265–3269.
429 Blackham, A., Norris, A.A. and Woods, F.A.M. (1985) J. Pharm. Pharmacol. 37, 787–793.
430 Kassis, V. (1985) IRCS Med. Sci. 13, 182–183.
431 Ahnfelt-Roenne, I. and Arrigoni-Martelli, E. (1982) Biochem. Pharmacol. 31, 2619–2624.
432 Nielsen, O.H., Ahnfelt-Roenne, I. and Elmgreen, J. (1988) Pharmacol. Toxicol. (Copenhagen) 62, 322–325.
433 Moilanen, E., Alanko, J., Seppala, E. and Vapaatalo, H. (1988) Agents Actions 24, 387–394.
434 Laghi Pasini, F., Pasqui, A.L., Ceccatelli, L. and Di Perri, T. (1984) Int. J. Tissue React. 6, 9–15.
435 Ku, E.C., Lee, W., Kothari, H.V. and Scholer, D.W. (1986) Am. J. Med. 80, 18–23.
436 Boctor, A.M., Eickholt, M. and Pugsley, T.A. (1986) Prostaglandins Leukotrienes Med. 23, 229–238.
437 Moilanen, E., Alanko, J., Juhakoski, A. and Vapaatalo, H. (1989) Agents Actions 28, 83–88.
438 Alanko, J., Moilanen, E., Opas, J. and Vapaatalo, H. (1989) Agents Actions 26, 254–255.
439 Ono, N., Yamasaki, Y., Yamamoto, N., Sunami, A. and Miyake, H. (1986) Jpn. J. Pharmacol. 42, 431–439.

440 Paradies, H.H., Ziedrich, H.K. and Flamig, H.H. (1990) Eur. J. Med. Chem. 25, 143–156.
441 Carlin, G., Djursaeter, R. and Smedegaard, G. (1989) Pharmacol. Toxicol. (Copenhagen) 65, 121–127.
442 Pullar, T. (1989) Pharmacol. Ther. 42, 459–468.
443 Williams, J.G. and Hallett, M.B. (1989) Gut 30, 1581–1587.
444 Ahnfelt-Roenne, I. and Nielsen, O.H. (1987) Agents Actions 21, 191–194.
445 Aruoma, O.I., Wasil, M., Halliwell, B., Hoey, B.M. and Butler, J. (1987) Biochem. Pharmacol. 36, 3739–3742.
446 Peters-Golden, M. and Shelly, C. (1989) Biochem. Pharmacol. 38, 1589–1595.
447 Betts, W.H., Hurst, N.P., Murphy, G.A. and Cleland, L.G. (1990) Biochem. Pharmacol. 39, 1233–1237.
448 Kuhl, P., Borbe, H.O., Fischer, H., Roemer, A. and Safayhi, H. (1986) Prostaglandins 31, 1029–1248.
449 Kuhl, P., Borbe, H.O., Roemer, A., Fischer, H. and Parnham, M.J. (1985) Agents Actions 17, 366–367.
450 Nakaguma, H. and Takahashi, H. (1990) Inflammation (N.Y.) 14, 195–203.
451 Bach, M.K., Brashler, J.R., White, G.J. and Galli, S.J. (1987) Biochem. Pharmacol. 36, 1461–1466.
452 Mann, J.S., Robinson, C., Sheridan, A.Q., Clement, P., Bach, M.K. and Holgate, S.T. (1986) Thorax 41, 746–752.
453 Bach, M.K. (1985) NATO ASI Ser. Ser. A 95, 73–86.
454 Cromwell, O., Shaw, R.J., Walsh, G.M., Mallet, A.I. and Kay, A.B. (1985) Int. J. Immunopharmacol. 7, 775–781.
455 Welton, A.F., Dunton, A.W. and McGhee, B. (1986) Agents Actions 18, 313–317.
456 Taniguchi, K., Masuda, Y. and Takanaka, K. (1990) Jpn. J. Pharmacol. 52, 101–108.
457 Makino, H., Saijo, T., Ashida, Y., Kuriki, H. and Maki, Y. (1987) Int. Arch. Allergy Appl. Immunol. 82, 66–71.
458 Saijo, T., Makino, H., Tamura, S., Kuriki, H., Ashida, Y., Terao, S. and Maki, Y. (1986) Int. Arch. Allergy Appl. Immunol. 79, 231–237.
459 Robichaud, L.J., Stewart, S.F. and Adolphson, R.L. (1987) Int. J. Immunopharmacol. 9, 41–49.
460 Wright, C.D., Hoffman, M.D., Thueson, D.O. and Conroy, M.C. (1987) J. Leukocyte Biol. 42, 30–35.
461 Boctor, A.M. and Pugsley, T.A. (1986) Inflammation (N.Y.) 10, 435–441.
462 Achterrath-Tuckermann, U., Simmet, T., Luck, W., Szelenyi, I. and Peskar, B.A. (1988) Agents Actions 24, 217–223.
463 Katayama, S., Tsunoda, H., Sakuma, Y., Kai, H., Tanaka, I. and Katayama, K. (1987) Int. Arch. Allergy Appl. Immunol. 83, 284–289.
464 Nishihira, J., Hayakawa, T., Suzuki, K., Kato, K. and Ishibashi, T. (1989) Int. Arch. Allergy Appl. Immunol. 90, 285–290.
465 Chand, N., Pillar, J., Nolan, K., Diamantis, W. and Sofia, R.D. (1989) Int. Arch. Allergy Appl. Immunol. 90, 67–70.
466 Graff, G. and Anderson, L.A. (1989) Prostaglandins 38, 473–496.
467 Walsh, D.A. and Yanni, J.M. (1988) Drugs Future 13, 605–609.
468 Janero, D.R., Burghardt, B., Lopez, R. and Cardell, M. (1989) Biochem. Pharmacol. 38, 4381–4387.
469 Fuller, R.W., Maltby, N., Richmond, R., Dollery, C.T., Taylor, G.W., Ritter, W. and Philipp, E. (1987) Br. J. Clin. Pharmacol. 23, 677–681.
470 Sweeney, F.J., Eskra, J.D. and Carty, T.J. (1987) Prostaglandins Leukotrienes Med. 28, 73–93.

471 Sweeney, F.J., Eskra, J.D., Ernest, M.J. and Carty, T.J. (1987) Agents Actions 21, 393–396.
472 Seuter, F., Fiedler, V.B. and Philipp, E. (1986) New Cardiovasc. Drugs, 163–183.
473 Brune, K. and Peskar, B.A. (1985) Int. J. Tissue React. 7, 97–103.
474 Ardekani, A.M., English, D. and Van Dyke, K. (1986) Microchem. J. 33, 309–315.
475 Gresele, P., Arnout, J. and Vermylen, J. (1987) Thromb. Haemostasis 57, 235.
476 Steinhilber, D., Jaschonek, K., Knospe, J., Morof, O. and Roth, H.J. (1990) Arzneim. Forsch. 40–2, 1260–1263.
477 Zijlstra, F.J., Wilson, J.H.P., Vermeer, M.A., Ouwendijk, R.J.T. and Vincent, J.E. (1989) Eur. J. Pharmacol. 159, 291–295.
478 Hall, R.A., Gillard, J., Guindon, Y., Letts, G., Champion, E., Ethier, D., Evans, J., Ford-Hutchinson, A.W., Fortin, R., Jones, T.R., Lord, A., Morton, H.E., Rokach, J. and Yoakim, C. (1987) Eur. J. Pharmacol. 135, 193–201.
479 Gillard, J., Ford-Hutchinson, A.W., Chan, C., Charleson, S., Denis, D., Foster, A., Fortin, R., Leger, S., McFarlane, C.S., Morton, H., Piechuta, H., Riendeau, D., Rouzer, C.A., Rokach, J., Young, R., MacIntyre, D.E., Peterson, L., Bach, T., Eiermann, G., Hopple, S., Humes, J., Hupe, L., Luell, S., Metzger, J., Meurer, R., Miller, D.K., Opas, E. and Pacholok, S. (1989) Can. J. Physiol. Pharmacol. 67, 456–464.
480 Menard, L., Pilote, S., Naccache, P.H., Laviolette, M. and Borgeat, P. (1990) Br. J. Pharmacol. 100, 15–20.
481 Rouzer, C.A., Ford-Hutchinson, A.W., Morton, H.E. and Gillard, J.W. (1990) J. Biol. Chem. 265, 1436–1442.
482 Rouzer, C.A. and Kargman, S. (1988) J. Biol. Chem. 263, 10980–10988.
483 Wong, A., Hwang, S.M., Cook, M.N., Hogaboom, G.K. and Crooke, S.T. (1988) Biochemistry 27, 6763–6769.
484 Ford-Hutchinson, A.W. (1991) Trends Pharmacol. Sci. 12, 68–70.
485 Yu, M.J., McCowan, J.R., Bertsch, B. and Ho, P.P.K. (1991) Bioorg. Med. Chem. Lett. 1, 107–110.
486 Murray, W.V., Wachter, M.P., Kasper, A.M., Argentieri, D.C., Capetola, R.J. and Ritchie, D.M. (1991) Eur. J. Med. Chem. 26, 159–166.
487 Bird, T.G.C., Bruneau, P., Crawley, G.C., Edwards, M.P., Foster, S.J., Girodeau, J., Kingston, J.F. and McMillan, R.M. (1991) J. Med. Chem. 34, 2176–2186.
488 Kerdesky, F.A.J., Holms, J.H., Moore, J.L., Bell, R.L., Dyer, R.D., Carter, G.W. and Brooks, D.W. (1991) J. Med. Chem. 34, 2158–2165.
489 Horie, T., Tominaga, H., Kawamura, Y., Hada, T., Ueda, N., Amano, Y. and Yamamoto, S. (1991) J. Med. Chem. 34, 2169–2176.
490 Hlasta, D.J., Casey, F.B., Ferguson, E.W., Gangell, S.J., Heimann, M.R., Jaeger, E.P., Kullnig, R.K. and Gordon, R.J. (1991) J. Med. Chem. 34, 1560–1570.
491 Rouzer, C.A., Riendeau, D., Falgueyret, J.-P., Lau, C.K. and Gresser, M.J. (1991) Biochem. Pharmacol. 41, 1365–1373.
492 Carter, G.W., Young, P.R., Albert, D.H., Bouska, J., Dyer, R., Bell, R.L., Summers, J.B. and Brooks, D.W., (1991) J. Pharmacol. Exp. Ther. 256, 929–937.
493 Garland, L.G. and Salmon, J.A. (1991) Drugs Future 16, 547–558.
494 Hoffman, T., Lee, Y.L., Lizzio, E.F., Tripathi, A.K., Jessop, J.J., Taplits, M., Abrahamsen, T.G., Carter, C.S. and Puri, J. (1991) Clin. Immunol. Immunopathol. 58, 399–408.
495 Musser, J.H., Kreft, A., Kubrak, D., Banker, A., Failli, A., Steffan, R., Demerson, C., Nelson, J., Shah, U., Marshall, L., Sturm, R., Carlson, R., Berkenkopf, J., Grimes, D., Weichman, B. and Chang, J. (1991) Presented at the XIth Washington International Spring Symposium (Prostaglandins, Leukotrienes, Lipoxins and PAF), Washington DC; Abstr. 335.

496 Wong, A., Hwang, S.M., Kreft, A. and Marshall, L. (1991) Presented at the XIth Washington International Spring Symposium (Prostaglandins, Leukotrienes, Lipoxins and PAF), Washington DC; Abstr. 307.

497 Zamboni, R., Prasit, P., Labelle, M., Leblanc, Y., Belley, M., Gauthier, J.Y., Therien, M., Riendeau, D., Jones, T., Evans, J., Young, R.N. and Ford-Hutchinson, T. (1991) Presented at the XIth Washington International Spring Symposium (Prostaglandins, Leukotrienes, Lipoxins and PAF), Washington DC; Abstr. 84.

498 Gillard, J.W., Prasit, P., Hutchinson, J., Leger, S., Charleson, S., Evans, J., Chan, C., Riendeau, D., Young, R.N. and Ford-Hutchinson, A.W. (1991) Presented at the XIth Washington International Spring Symposium (Prostaglandins, Leukotrienes, Lipoxins and PAF), Washington DC; Abstr. 125.

499 Kennedy, B.P., Diehl, R.E., Boie, Y., Adam, M. and Dixon, R.A.F. (1991) J. Biol. Chem. 266, 8511–8516.

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2 An Overview of Class III Electrophysiological Agents: A New Generation of Antiarrhythmic Therapy

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INTRODUCTION

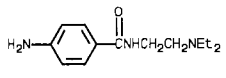
Approximately 400,000 lives in the United States are claimed each year due to sudden cardiac death (SCD) [1]. Most of these deaths are due to an electri-

cal malfunction (ventricular fibrillation (VF)) [2,3] and not to a failure of the contractile function of the heart muscle itself (mechanical malfunction). Thus, the treatment and prevention of life-threatening arrhythmias is a major focus of the health care industry today. During the last decade, the emphasis of drug discovery efforts in this area has evolved from the preparation of Class I antiarrhythmic agents (see below) to the design and synthesis of Class III agents. This review will discuss recent developments in the Class III area.

CLASSIFICATION OF ANTIARRHYTHMIC AGENTS

For many years physicians had relatively few drugs from which to choose for the treatment of arrhythmias. These consisted primarily of agents which had local anaesthetic activity such as procainamide (1) or quinidine (2). As the number of potential therapeutic agents increased and a better understanding of cardiac electrophysiology developed, a classification system based on the agent's predominant electrophysiological activity was proposed by Vaughan Williams [4]. This system divides antiarrhythmic agents into four classes.

Class I drugs act predominantly by slowing conduction in cardiac tissue. This results from a decrease in sodium conduction during the rapid depolarization phase (Phase 0) of the action potential of the cardiac cell. Class I agents have been further subdivided based on their effect on action potential duration [5] or rate of onset of frequency-dependent block [6]. The two subclassifications generally yield the same groupings of drugs. Examples of Class IA drugs, which have an intermediate rate of onset, are procainamide (1), quinidine (2) and disopyramide (3). Because Class IA drugs increase action potential duration (APD) and refractory periods in some tissues, these



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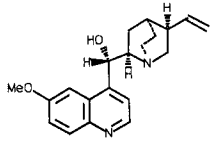

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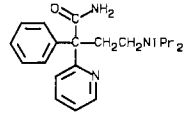
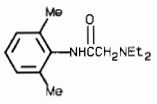


(3)

agents have been described as having varying degrees of Class III electrophysiological activity in addition to Class I activity. Drugs in which the inhibition of phase 0 depolarization (V_{max}) occurs rapidly only at fast stimulation rates (fast onset of frequency-dependent block) are termed Class IB

agents and are typified by lidocaine (lignocaine, (4), mexiletine (5) and tocainide (6)). Class IC compounds are exemplified by flecainide (7) and encainide (8) and have a very slow onset rate for frequency-dependent block.



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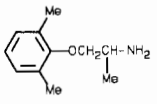

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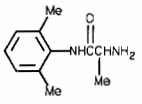

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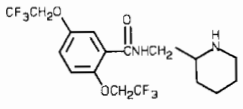

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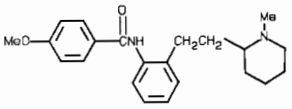

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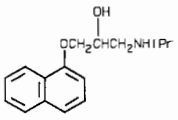
Class IC agents are extremely effective at preventing premature ventricular contractions (PVC's). The second grouping of compounds in the Vaughan Williams classification are sympatholytic agents. These are designated Class II and are predominantly exemplified by β-adrenergic blocking agents such as propranolol (9) and atenolol (10). Class III agents are those compounds which increase action potential duration (and hence refractory period) with-



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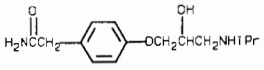

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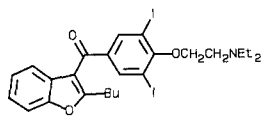
out significantly affecting the rate of depolarization of cardiac cells. Early examples of Class III agents which have been studied clinically are amiodarone (11), bretylium (12), sotalol (13) and clofilium (14). Calcium channel blocking agents, such as nifedepine (15), diltiazem (16) and verapamil (17) comprise the fourth class of antiarrhythmic drugs.

MECHANISMS OF LETHAL ARRHYTHMIAS

Arrhythmias can range from isolated PVC's, coupled beats, non-sustained ventricular tachyarrhythmias (NSVT), sustained ventricular tachyarrhythmias (SVT) and, ultimately, ventricular fibrillation (VF). It is generally



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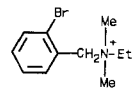

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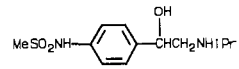

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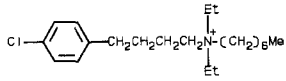

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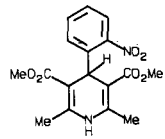

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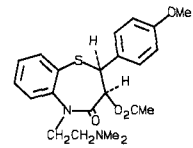

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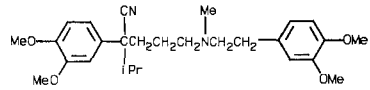

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agreed that the most serious arrhythmias, SVT and VF, arise by re-entrant mechanisms [7]. That is, a propagated impulse follows a pathway which returns to its origin and re-excites the original tissue, thus forming a continuous loop for the aberrant impulse. The re-entrant pathway is dependent on conduction velocity, path length and the refractory period of the tissue. If these parameters remain constant, then an ordered re-entrant mechanism results (for example, NSVT, SVT); if multiple pathways exist and continuously change with time, then random re-entry results (VF) [7]. Sufficient change in any one of the parameters will break the loop and terminate the arrhythmia. Similarly, these parameters (for example, conduction velocity or refractoriness) can be altered in a beneficial manner to prevent a specific re-entrant route from forming.

It has been shown that patients who have a significant number of PVC's are at increased risk for sudden cardiac death [8]. It is thought that the aberrant beat can act as a trigger for a re-entrant circuit. Recently, the Cardiac Arrhythmia Suppression Trial (CAST Study) was designed to test the hypothesis that suppression of PVC's would decrease mortality in a post-myocardial infarction patient population [9]. Two of the agents employed in the study were the Class IC agents, flecainide (7) and encainide (8). While these two agents were effective in suppressing PVC's, they did not decrease mortality. In fact, the trial of the two drugs was terminated prior to completion because a significant increase (about 2-fold) in mortality was found in the treatment group compared to the control group [9]. Thus, suppression of

PVC's alone with Class IC drugs was not sufficient to reduce mortality in the specific post-infarction population chosen for the CAST study. The failure of Class IC agents in the CAST study and their low efficacy in preventing induction of SVT during electrophysiological testing has increased the interest in alternative approaches to antiarrhythmic therapy, particularly towards Class III agents, a number of which are in Phase II clinical evaluation.

MECHANISM OF ACTION OF CLASS III AGENTS

Class III agents increase the refractoriness of cardiac tissue, thus preventing an aberrant impulse from propagating. A selective Class III agent has little or no effect on simple PVC's. At the cellular level, the increased refractoriness is manifest by a delay in the repolarization phase (Phase 3) of the cardiac action potential (*Figure 2.1*), thereby increasing action potential duration. During the action potential cycle a complex series of ionic currents,

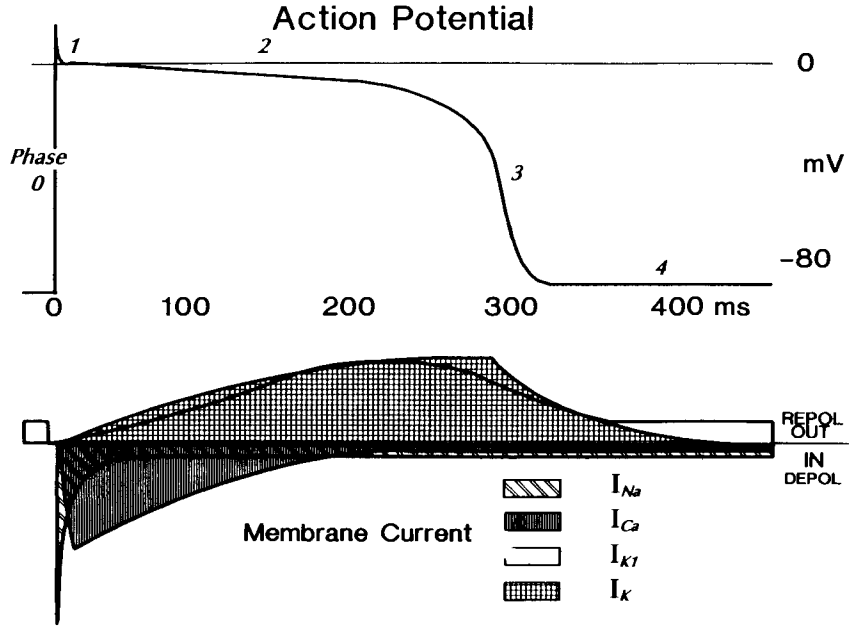


Figure 2.1. Idealized action potential showing current densities of major currents which flow during the action potential.

which are both time and voltage dependent, flow into and out of the cell. Depolarization (Phase 0) is initiated by a rapid influx of sodium ions (I_{Na}) which changes the cell potential from about -90 mV to about $+30$ mV. During the plateau phase (Phase 2), the cell potential is maintained by the influx of calcium ions (I_{Ca}). In the repolarization phase (Phase 3) the predominant outward currents, (I_K) and (I_{K1}), are carried by potassium ions and restore the cellular potential to its initial resting potential. In addition to these major ionic currents that flow during the cycle, a number of background currents are flowing which modulate the cellular potential, for example, the sodium and calcium window currents. There is a complex interrelationship among all these processes such that activation of a specific ion channel may be dependent not only on time and membrane potential but also on the concentration of another ion (for example, the calcium-dependent potassium-channel). From this brief description of the action potential cycle, it is obvious that there are a variety of ways by which action potential duration can be increased. The most obvious mechanism to increase action potential duration is inhibition of the repolarizing potassium currents. More than one potassium current (for example, delayed rectifier (I_K) and inward rectifier (I_{K1})) contribute to repolarization and inhibition of any one of them may be sufficient to prolong APD [10]. Alternatively, delay in the inactivation of the rapid sodium influx during depolarization will maintain the cell at more positive potentials and delay repolarization. Additionally, prolongation of slow inward sodium and calcium currents will also increase APD. Many of the Class III agents which will be described have been shown to have as their dominant mechanism the inhibition of potassium currents. The exceptions will be discussed under the individual agents.

While the action potential is a measurement of the electrical activity of a single cardiac cell, the surface electrocardiogram (ECG) (*Figure 2.2*) provides a measurement of the electrical activity of the whole heart. In essence, the ECG is the time-dependent sum of action potentials from all cardiac cells. The P-wave results from the depolarization of atrial tissue at the beginning of the cardiac cycle. The large deflection [QRS-complex] in the ECG is indicative of the depolarization of the larger mass of ventricular tissue. The width of the QRS-complex provides an indication of ventricular conduction. Class I antiarrhythmic agents, because they slow ventricular conduction, will broaden the width of the QRS-complex. The T-wave marks the repolarization phase of the ventricular tissue. The QT-interval gives a measurement of ventricular repolarization time. In general, selective Class III agents will increase the QT interval without broadening the QRS-complex. When the QT measurement is corrected for heart rate it is

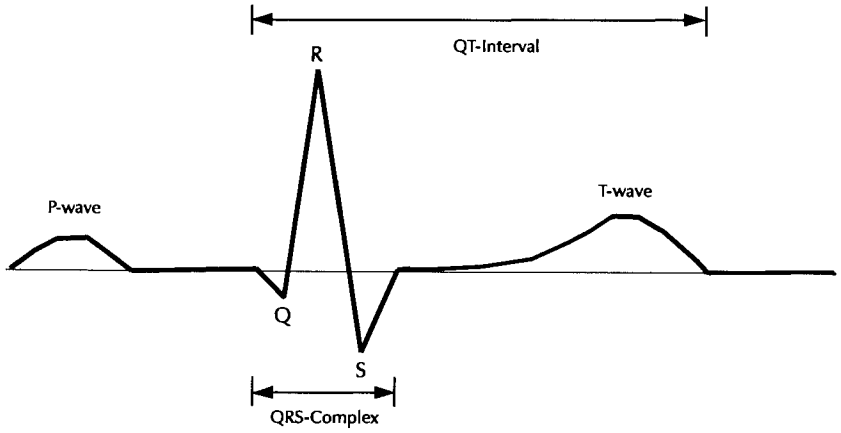


Figure 2.2. Idealized Lead II surface electrocardiogram

known as QT_c. The correction for QT often uses Bazett's formula [QT_c = = $QT/\sqrt{\text{preceding RR-interval}}$].

PROTOTYPE CLASS III ANTIARRHYTHMIC AGENTS

As stated above, amiodarone (11), bretylium (12), sotalol (13) and clofilium (14) are prototype Class III electrophysiological agents. With the exception of clofilium, which was designed to be a Class III agent, the Class III activity of the other agents was discovered after the compounds had been in use for other indications.

AMIODARONE

Amiodarone (11), a benzofuran derivative, was initially developed as a coronary vasodilator in the early 1960's [11,12]. Several years later, the efficacy of the compound as an antiarrhythmic agent began to be exploited. The first clinical trials with amiodarone were reported in 1974 [13]. Amiodarone was effective in controlling the tachyarrhythmias of eleven patients with Wolff-Parkinson-White syndrome. Since that time the compound has been studied extensively [14,15]. Recently, in the Canadian Amiodarone Myocardial Infarction Arrhythmia Trial (CAMIAT), amiodarone was shown to reduce mortality during a mean 18 month period following myocardial infarction (13.8% deaths in placebo group vs. 2.1% deaths in the treatment group) [16].

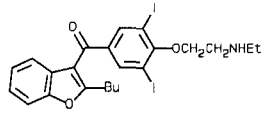
These results support two other studies which suggested beneficial effects for amiodarone treatment after myocardial infarction and may have major implications for the efficacy of Class III agents.

The pharmacological profile of amiodarone is extremely complex. On acute exposure the compound slows conduction (indicative of Class I electrophysiological activity) [17], exhibits both α- and β-adrenergic antagonism [18], and may have an effect on calcium channels [19]. The Class III electrophysiological activity is generally not manifest on acute exposure but may take several days to weeks to reach maximal effect in patients [20,21].

At the cellular level, the major electrophysiological effect appears to be rate-dependent blockade of sodium channels [22]. The onset for this Class I effect (64 $\pm$ 9% of the final depression of V_{max} between the first and second beat of the train) was similar to that for Class IB agents [23]. The offset rate (recovery of V_{max} from rate-dependent depression) for amiodarone was 1.48 s. This value falls between those seen for Class IB agents (200–500 ms) and IA agents (2.3–12.2 s) [23]. Amiodarone inhibited the binding of [^{3}H]batrochotoxinin A 20α-benzoate to the sodium channel, suggesting that it binds to inactivated sodium channels [24].

The Class III effects of amiodarone develop over several weeks. This time-course is similar to that seen in thyroid gland ablation [25]. It is well known that patients with hypothyroidism have long QT intervals which are indicative of prolonged action potentials. Amiodarone has been shown to inhibit the conversion of thyroxine (T_4) to triiodothyronine (T_3) both in human subjects [26] and *in vitro* [27]. It has been argued that the Class III effects of amiodarone are due to its effects on thyroid hormones [28]. Others, however, argue that there is no relationship between prolongation of ventricular refractory period by amiodarone and thyroid state [29].

In addition to the slow onset of action of amiodarone, the half-life of the biological effects of the drug are extremely long [30]. This may be due in part to the principal metabolite of (11), which is desethylamiodarone (18).



(18)

This metabolite has been shown to reach serum levels approaching that of the parent compound during therapy, to have similar effects on levels of thyroid hormones [31] and to have a comparable electrophysiological profile

[23]. Desethylamiodarone (18), however, appears to be more toxic than the parent compound [32].

BRETYLIUM

Bretylium (12) is an adrenergic neuronal blocking agent that was initially developed for use in hypertension [33]. Due to its quaternary ammonium structure, the compound had erratic oral bioavailability; in addition, patients developed tolerance and thus it found only limited utility as an antihypertensive agent [34]. Later the anitarrhythmic efficacy of bretylium was discovered. The compound was shown to prevent acetylcholine-induced atrial fibrillation in hypokalaemic dogs [35] and to cause spontaneous defibrillation of fibrillating canine ventricles [36]. This unique antifibrillatory activity, which could not be demonstrated with other antiarrhythmic agents or β-blocking agents, stimulated research toward elucidation of the antifibrillatory mechanism of action of bretylium. Wit and co-workers [37] showed that in addition to antiadrenergic activity, bretylium increased the action potential duration without affecting V_{max} (that is, Class III activity). Until the approval of amiodarone (11), bretylium, approved in 1978 for parenteral use, was the only Class III agent available for clinical use in the United States.

More recent studies continue to support the unique antifibrillatory activity of bretylium. Kowey et al. [38] have shown that bretylium prevented spontaneous VF and decreased the effects on VF threshold in a feline myocardial infarction model. They attributed this beneficial effect to a decrease in the dispersion of refractoriness between normal and ischaemic regions of the heart. In contrast, clofilium (14, see below), which had little effect on dispersion of refractoriness after coronary occlusion, was unable to prevent spontaneous VF. Similar results were seen in isolated tissue studies with canine subendocardial Purkinje fibres and ventricular muscle which contained both normal and ischaemic regions [39]. In these studies bretylium caused a smaller increase in dispersion of refractoriness in subendocardial Purkinje fibres than either sotalol or clofilium. In ventricular muscle tissue, bretylium decreased dispersion while sotalol and clofilium increased dispersion of refractoriness.

SOTALOL

As for amiodarone and bretylium, the Class III activity of sotalol (13) was discovered only after its initial development as a β-blocking agent. The initial hypothesis, which led to the synthesis of sotalol as a β-blocking agent,

was the use of a methylsulphonylamino group to replace the phenolic hydroxy group in analogues of the catecholamine sympathetic hormones [40]. This fortuitous replacement inadvertently provided a critical structural lead for later synthetic efforts in the Class III antiarrhythmic field. In 1970, Singh and Vaughan Williams showed that sotalol selectively increased APD in rabbit atrial tissue at concentrations approximately one-fourth of that required to reduce the rate of depolarization (Class I activity) [41]. Since that time the compound has been studied extensively as a Class III agent [42].

In view of the two electrophysiological effects manifested by sotalol, it is of interest to understand the relative potency of the compound in producing its β-blocking and Class III activities. In receptor affinity studies, the concentration producing a 50% inhibition of binding of β_1- and β_2-receptors by sotalol was 9 μM and 5 μM, respectively. The concentration of sotalol required to increase APD by 20% at 95% repolarization ($EC_{20}APD_{95}$) was 18 μM [43]. Thus sotalol is slightly more potent as a β-blocking agent than as a Class III agent. These results are supported by studies by Nattel and coworkers in anaesthetized dogs which show that substantially higher plasma concentrations of sotalol were needed to increase atrial and ventricular refractory periods (about 7 mg/l) than to block the chronotropic actions of isoproterenol (about 1 mg/l) [44]. A study in patients with chronic stable ventricular premature complexes suggests that the plasma concentration of sotalol required for significant QT_c prolongation (Class III effect) is approximately 3-times higher than that needed for a 50% reduction of the maximal exercise-induced increase in heart rate (2.55 μg/ml vs. 0.8 μg/ml, respectively) [45]. In contrast, a second study in patients with Wolff-Parkinson-White syndrome showed that significant Class III activity was present at the β-blocking dose [46]. However, in this study Class III activity, as measured by increases in refractory periods and QT-intervals, became more marked at a dose higher than the β-blocking dose. In general, β-blocking activity is manifest at lower concentrations than the concentrations necessary to maximize Class III activity.

Sotalol (13) is a racemic mixture, and its two enantiomers have been studied extensively [47–51]. As expected from studies with other β-blocking agents, the Class II activity resides predominantly in only one enantiomer [(−)-sotalol] while the Class III activity can be found equally in both enantiomers. Therefore, the (+)-enantiomer, which is essentially devoid of β-blocking activity, has been studied as one of the few early examples of a selective Class III agent. Both sotalol [52–55] and its enantiomer, (+)-sotalol [56–58], have been shown to be clinically effective in the prevention of ventricular arrhythmias. The efficacy rate for suppression of inducible ventricular tachycardia during programmed electrical stimulation for both

compounds is in the range of 20–40%. The key question that remains to be addressed at the clinical level, however, is whether there is an advantage of one agent over the other. It is not known whether the β-blocking activity of the racemic compound provides an added benefit over selective Class III activity alone.

CLOFILIUM

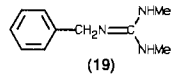
In contrast to compounds (11)–(13), which were initially developed for other uses, the Lilly compound, clofilium (14), was the first prospectively developed Class III antiarrhythmic agent [59,60]. The Lilly group began with the knowledge that small quaternary ammonium ions such as tetraethylammonium (TEA), inhibited potassium currents and prolonged APD [10]. They then explored structure–activity relationships in order to improve potency and the ability of the compounds to penetrate the cell membrane (problems with TEA). Clofilium was the most interesting compound from this study and has been shown to be effective in isolated cardiac tissue preparations, intact animal models [61] and clinically [62,63]. Although the poor absorption of clofilium, due to the quaternary ammonium centre, made the compound unsuitable for development as an oral antiarrhythmic agent, it still remains a useful tool for the investigation of the cardiac effects of Class III agents.

In a more recent study, Echt and co-workers examined the effects of clofilium and other compounds on defibrillation energy requirements in dogs [64]. In contrast to the Class I agent, lidocaine, which increased defibrillation energy requirements, clofilium caused a modest decrease in the defibrillation energy required. The authors discuss the apparent antagonistic effect of sodium channel blockade and the beneficial effects of APD prolongation on defibrillation energy requirements. Others have used clofilium to study the potential arrhythmogenic effects of Class III agents. Gough et al. showed that clofilium was more likely to produce early after depolarizations (EADs) in ischaemic Purkinje fibres (10 of 11 fibres) than in normal fibres (2 of 10) [65]. Similar results were reported for the effect of clofilium in ventricular muscle tissue [39]. The effects of α-adrenoreceptor stimulation and action potential prolongation were studied in chlorolase-anaesthetized rabbits [66]. Administration of the α_1-agonist methoxamine, either concomitantly or following administration of clofilium, produced EADs and Torsades de Pointes. Pretreatment with prazosin blunted the arrhythmic response. Thus, stimulation of α_1-receptors may aggravate the potential for proarrhythmic activity by Class III agents. The authors caution that this effect may be species-dependent.

OTHER COMPOUNDS WITH CLASS III ACTIVITY

The observation that agents which prolonged APD possessed unique antiarrhythmic properties, together with the preliminary clinical efficacy of the prototype agents, stimulated the search for additional compounds with Class III activity. Several compounds, ranging from other cardiovascular drugs to marine toxins, were shown to possess varying degrees of Class III activity. However, few if any of these compounds could be classified as selective Class III agents.

Among other cardiovascular drugs there are two non-quaternary analogues of bretylium, bethanidine (19) and meobentine (20), that exhibit indications of Class III activity. Both compounds were developed by Burroughs



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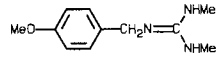
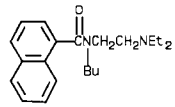


(20)

Welcome [67,68]. Bethanidine was initially patented as a hypotensive agent but was found to have antifibrillatory activity [69]. Attempts to limit the hypotensive effects of bethanidine led to meobentine. At a concentration of 44 μM, bethanidine has minimal effects on APD_{100} in propranolol-treated and untreated canine Purkinje fibres (+2% and −5%, respectively) [70,71]. At 350 μM in untreated fibres, bethanidine increased APD_{100} by about 6%. In contrast, meobentine, which has less sympatholytic activity, increased APD_{100} by 12% at 37 μM in untreated fibres during the same study. Both compounds showed modest decreases of V_{max} at the stated concentrations. The Class I activity and the sympatholytic activity (for bethanidine) may act to decrease the apparent Class III effects of the two compounds (see below).

Bunaftine (21) is a naphthalenecarboxamide derivative, that has been developed as an antiarrhythmic agent. The compound exhibits both Class I and Class III electrophysiological effects. Fenici and co-workers studied bunaftine in patients with paroxysmal atrial tachyarrhythmia and recorded right atrial monophasic action potentials [72]. A mean increase of 18% in atrial repolarization time and an increase in monophasic APD during pac-



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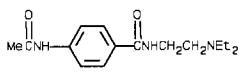
ing were observed after drug administration. The investigators concluded that bunaftine possessed Class III activity in atrial tissues. Earlier studies [73] had demonstrated the membrane stabilizing activity of bunaftine. More recently the Class I and Class III actions of bunaftine have been demonstrated in isolated guinea-pig ventricular tissue [74,75]. At concentrations below 3–5 μM, bunaftine produced a modest (about 8–10%) increase in APD, while at higher concentrations (10–15 μM), a pronounced decrease in V_{max} was observed. In this respect bunaftine is similar to the Class IA agents (2) and (3).

Acetylation of the amino substituent in the Class IA agent procainamide affords acecainide (22). The acetylated compound, in contrast to the parent compound, exhibits Class III electrophysiological action. Since acecainide is a major metabolite of procainamide, in some patients it may contribute to the overall efficacy of that compound. Acecainide is being developed by Medco and Warner-Lambert in the U.S., and the pharmacodynamic and potential therapeutic utility have been recently reviewed [76]. The drug is effective in the suppression of atrial flutter in both dogs [77–79] and patients [80]. In patients with PVC's and ventricular tachycardia, acecainide is also moderately effective [76]. One of the major advantages of acecainide over the parent compound, procainamide, may be the decreased propensity for producing systemic lupus erythematosus [76].



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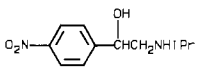
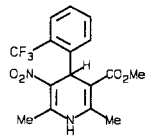


(23)

Nifenalol (23) is the nitro analogue of sotalol. Singh and Vaughan Williams showed that nifenalol increased APD_{90} of rabbit atrial tissue by 24% at 48 μM [81]. This was the first indication that a nitro group may be comparable to a methylsulphonylamino moiety when substituted in the *para* position on the phenyl ring to produce Class III activity. Other studies have shown that nifenalol and sotalol both exerted a positive inotropic effect in cardiac tissue from kittens and guinea-pigs which was independent of β-receptors [82]. This effect has been observed for several Class III agents [10]. It is thought that the prolonged APD allows increased entry of calcium ions into the cells and the increased calcium ion concentration mediates the positive inotropic activity.

Just as Class III agents can exhibit positive inotropy, some positive inotropic agents demonstrate Class III electrophysiologic activity. The increase in intracellular calcium-ion concentration produced by the inotropic

agent helps to prolong the plateau phase of the action potential. The Ca^{2+}-channel agonist, BAY K8644 (24), increased APD_{90} of sheep cardiac Purkinje fibres by 30% at 1 μM [83]. The Otsuka phosphodiesterase inhibitors, OPC-8212 (25a) and OPC-8490 (25b), also show Class III electrophysiological activity. In canine cardiac Purkinje fibres, OPC-8212 at a concentration of 6 μM increased APD_{90} by 22% [84]. Voltage clamp studies have shown,



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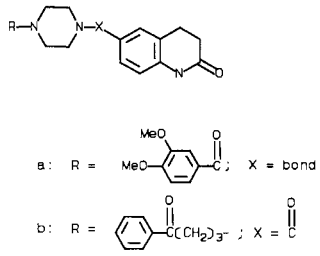
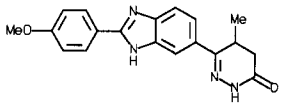


(25)

however, that in addition to the increased inward calcium current, which would be expected from a phosphodiesterase inhibitor, OPC-8212 also decreases the delayed outward and inward rectifying potassium currents in guinea-pig ventricular myocytes [85]. In a study of OPC-8490 in isolated guinea-pig atrial myocytes, the compound increased APD_{90} by 55% at 30 μM and selectively inhibited the delayed rectifier [86]. Another phosphodiesterase inhibitor, pimobendan (26, UD-CG 115), also increased APD_{90} of guinea-pig papillary muscle (22% increase), but this effect is seen only at high concentration (30 μM) [87]. As would be expected from these results, pimobendan at doses which are devoid of Class III activity (0.3 mg/kg) was not effective in preventing programmed electrical stimulation (PES)-induced ventricular tachycardia in dogs [88]. Two additional phosphodiesterase inhibitors, milrinone (27) and enoximone (28), shortened APD during *in vitro* studies [89,90]. Thus, it appears that Class III activity is not necessarily related to phosphodiesterase inhibition. The novel cardiotonic agent, DPI 201-106 (29), under investigation by Sandoz, also increases APD. In isolated rabbit atria, the Sandoz compound increased APD_{70} by 120% at 3 μM concentration, while V_{max} decreased by about 20% [91]. Similar results were observed in guinea-pig papillary muscles. The electrophysiological effects occur in the same range as the positive inotropic effects. The positive inotropic and APD-prolonging activities were found to reside in the (*S*)-enantiomer. Further studies showed that DPI 201-106 did not act via cAMP-dependent processes or inhibition of Na^+/K^+-ATPase; rather, the compound



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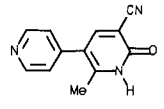

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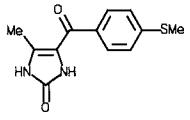

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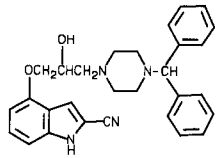
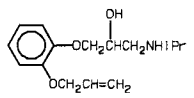


(29)

delays inactivation of Na^+ channels [92]. At a dose of 0.3 mg/kg, DPI 201–106 prevented both occlusion- and reperfusion-induced arrhythmias in the rat [93]. The compound was effective (1 mg/kg) in delaying aconitine-induced arrhythmias, but was essentially ineffective (10 mg/kg) against ouabain-induced arrhythmias.

In addition to sotalol and nifenalol, which were initially developed as β-receptor antagonists then later were found to possess Class III electrophysiological activity, several other receptor blocking agents have exhibited varying degrees of Class III activity. These include not only β-blockers, but also α-blockers, 5-HT antagonists, and drugs that bind to opioid receptors. In most cases, the Class III effect is believed to be produced by direct action; however, receptor-mediated activity cannot be dismissed.

While most β-blocking agents on acute administration have little direct electrophysiological effects, studies in rabbits [94] and man [95] have shown that chronic administration of β-blockers increases APD. This increase in APD (and hence refractory period) has been postulated to contribute to the effectiveness of β-receptor blocking agents in the prevention of sudden cardiac death [94]. Direct Class III action has been claimed for the β-blockers oxprenolol (30) [96,97], nadolol (31) [96] and atenolol (10) [98] in addition



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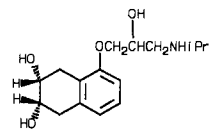


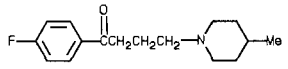
(31)

to sotalol and nifenalol; however, there are conflicting results for atenolol and nadolol [97]. The Class III activity of oxprenolol appears only at high

concentrations, at which a decrease in V_{max} is also manifest [97]. Only sotalol and nifenalol appear to exert selective Class III effects in addition to β-blockade.

Increase of refractory period and prolongation of APD appear to be a common property of α-receptor blocking agents [99]. In many cases, the increase in APD is coupled with a decrease in V_{max}. There is a question as to whether the APD-prolonging properties of α-blocking agents are due to receptor-mediated effects or direct electrophysiological action. Low (α_1-stimulating) doses of epinephrine (adrenaline) and norepinephrine (noradrenaline) increase APD in a variety of *in vitro* preparations [99]; however, under ischaemia-reperfusion conditions, stimulation of α-receptors with methoxamine shortens refractory period [100]. Whether direct or receptor-mediated effects of α-blockers dominate in the modulation of APD may depend on the condition (for example, normal, ischaemic, reperfused) of the cardiac tissue.

Of all the α-receptor antagonists studied, the compounds receiving the most attention in terms of Class III activity are melperone (32) (initially developed as a neuroleptic agent), indoramin (33), and amosulalol (34). In electrophysiological studies conducted in rabbit cardiac tissue, melperone at a concentration of 8.3 μM increased APD_{90} of atrial and ventricular muscle



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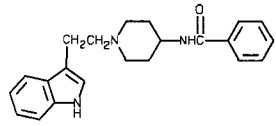

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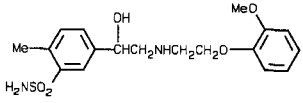
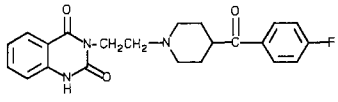


(34)

by an equal amount (32%); however, V_{max} was decreased in the atrial preparation by only 5%, while V_{max} was decreased in the ventricle preparation by 15% [101]. At higher concentrations (16 μM), melperone produced a significant inhibition of V_{max} (about 33%) in both atrial and ventricular tissues. In patients, melperone at doses of at least 240 mg/day increased both QT intervals (8%) and ventricular refractory periods (8–10%) and was moderately effective (23%) in preventing PES-induced re-entrant ventricular arrhythmias. In this study, the major side-effects of melperone were due to its neuroleptic activity which could limit use as an antiarrhythmic agent [102].

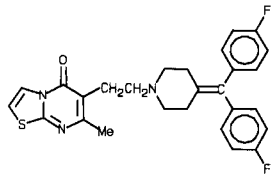
The tryptamine derivative, indoramin, at 1 μg/ml increased the ST interval (repolarization) without affecting the QRS interval (conduction) in isolated guinea-pig heart [103]. Slightly higher concentrations (3 μg/ml) also increased the QRS interval. Similar effects were seen in anaesthetized cats. The threshold for Class I activity of indoramin is very close to the effective Class III dose. The QT and QT_c intervals (indicative of an increase in refractory period) were increased after administration of 100 mg/day of indoramin in healthy volunteers with no significant changes in QRS interval [104]. The α- and β-blocker, amosulalol, is purported to possess some Class III activity (that is, prolongation of APD_{90} in rabbit papillary muscle); however, this effect is concurrent with a decrease in V_{max} of similar magnitude [105].

Similar to the adrenergic blocking agents, Class III electrophysiological activity has been observed for a number of serotonergic-blocking agents. Ketanserin (35), a 5-HT_2 blocking quinazoline derivative, increased APD_{90}



(35)

by 25% at 1 μM with little effect on V_{max}; however, at 10 μM the APD_{90} decreased (10%) from the maximum observed at 1 μM and V_{max} also began to decrease (6%) [106]. This is indicative of the onset of a Class I effect. In ventricular muscle, APD was prolonged even at 10 μM. Saman and coworkers found similar results for ketanserin in guinea-pig papillary muscle and state that the APD prolongation appears to be a direct effect of the drug, since the concentration required to increase APD is 2–3 orders of magnitude higher than that required to block serotonergic receptors [107]. Acute effects of ketanserin at 10 μM in rabbit ventricular muscle were similar to those above in guinea-pig (APD_{90} increased by 20%); however, on chronic administration (40 mg/kg per day for 7 days) the drug increased APD_{90} by 106% [108]. These investigators also attribute the Class III action of ketanserin to direct effects. In patients, low intravenous doses (sufficient to decrease blood pressure) of ketanserin produced a modest increase (3%) in QT_c indicative of Class III activity [109]. A similar increase (9%) in QT_c, with no effect on QRS interval, was observed for patients treated chronically (20 mg/day for 4 weeks) with retanserin (36), a 5-HT_2-antagonist that is structurally similar to (35) [110]. Amperozide (37) is a psychotropic compound which also blocks 5-HT_2 receptors. This compound, which is pur-



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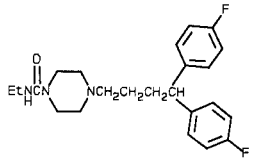
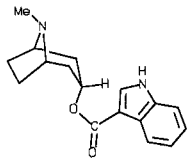


(37)

ported to have Class III antiarrhythmic properties, was studied in the digoxin-induced arrhythmia model in guinea-pigs [111]. In this study, amperozide, as well as bretylium and melperone, statistically increased the time to arrhythmia. In contrast, lidocaine, a Class IB agent, did not statistically increase the time to arrhythmia. This result is interesting because we have observed in our laboratories that selective Class III agents are generally less effective than Class I agents in this model in which the arrhythmias are thought to be due to enhanced automaticity and not re-entry. A 5-HT_3-receptor antagonist, ICS 205-930 (38), has also exhibited Class III electrophysiological activity. Studies with ICS 205-930 using whole-cell patch clamp techniques showed that the drug inhibits I_K and I_{K1} at about 10 μM while at higher concentrations (about 100 μM) I_{Na} and to a lesser extent I_{Ca} are also depressed [112]. These results support earlier studies in guinea-pig papillary muscle in which ICS 205-930 at 3 μM increased APD_{70} by 14% and decreased V_{max} by 15% [113]. In contrast, the related 5-HT_3 antagonist, MDL 73147 (39), has been identified as a Class I antiarrhythmic agent [114]; however, there are some indications that the compound also has Class III activity [115]. Thus, the electrophysiological profiles of both (38) and (39) are similar to Class IA antiarrhythmic agents.



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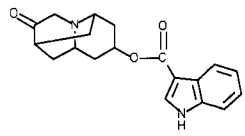
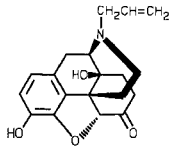


(39)

From a structural viewpoint, it is of interest to note that (35)–(37) as well as (32) all contain a 4-fluorophenyl moiety. This suggests that the 4-fluorophenyl group may play a role in determining the Class III electrophysiological profile of these compounds.

The opioid receptor antagonist, naloxone (40), has been reported to show Class III activity. The (+)- and (−)-isomers of naloxone were studied in isolated guinea-pig cardiac tissue in order to determine whether the electrophysiological actions of the compound were due to receptor (usually enantioselective) or direct effects [116]. In guinea-pig atrial strips, both enantiomers (120 μM) increased APD_{80} by about 45% with minimal negative effects on V_{max} (about 5%). In papillary muscle, (+)- and (−)-naloxone increased APD_{80} by 22% and 13%, respectively, with modest negative effects on V_{max} (11% and 6%, respectively). The comparable electrophysiological effects of the two enantiomers and the relatively high concentrations of the drug required to increase APD indicate that the activity of the compounds



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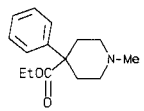
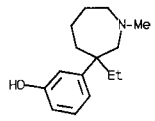


(41)

is direct and is not receptor-mediated. In contrast to the above results, naloxone produced significant conduction depression in sheep Purkinje fibres at lower concentrations (10 μM), while producing only modest increases in APD [117]. Naloxone decreased V_{max} by 24% while increasing APD_{90} by only 11%. Thus, like most of the receptor antagonists described above, naloxone appears not to possess selective Class III actions, but rather mixed Class I and Class III activities. Surprisingly, the narcotic analgesic pethidine ((41), meperidine) is reported to exhibit Class III electrophysiological effects in contrast to other narcotic agents such as morphine and piritramide [118]. At concentrations of 10 μM and 50 μM pethidine increased the effective refractory period of rat atrial tissue by about 10% and 35%, respectively. Similarly, contractile force was increased by about 5% and 20%. Morphine and piritramide produce little effect on refractory period and negative effects on contractile force. Modest but significant increases in monophasic APD_{90} were observed after administration of 2 mg/kg of pethidine to anaesthetized guinea-pigs [119]. A second narcotic analgesic, meptazinol (42), also increases action potential duration [120]. In isolated

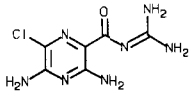


(42)

rat papillary muscle, meptazinol at 17 μM and 34 μM increased APD_{90} by 26% and 55%, respectively, while decreasing V_{max} by 11% and 16%. The drug was effective in suppressing arrhythmias in rats which had been subjected to coronary occlusion. It has been suggested that the antiarrhythmic effects of meptazinol are due to direct cardiac action but receptor-mediated activity cannot be excluded.

Several structural types, for example, compounds (30)–(42) which are active at a number of receptors, have demonstrated Class III electrophysiological activity in a variety of models. The majority of compounds are not selective Class III agents, but also exhibit substantial conduction-slowing activity (Class I). This factor together with the observation that the concentration ranges for receptor affinity and electrophysiological activity do not always coincide suggests that the drugs are exhibiting secondary direct cardiac actions in addition to their intended receptor-mediated activity.

The potassium sparing diuretic, amiloride (43), also produces a Class III effect in cardiac tissue. In canine Purkinje fibres APD is increased by 35% after prolonged exposure to 5 μM of the drug [121]. The authors suggest two potential mechanisms for this effect: (1) delay of inactivation of Na^+ channels, or (2) inhibition of Na^+/Ca^{2+} exchange. In infarcted dogs which were subjected to a PES protocol to produce re-entrant ventricular arrhyth-



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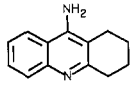


(44)

mias, amiloride was effective in the prevention of ventricular tachyarrhythmias in six of twelve animals [122]. In those animals which responded to amiloride, repolarization time of the border zone was increased; in non-responding animals, border zone repolarization time was shortened.

Tacrine (44), a centrally acting cholinesterase inhibitor, is being investigated for the treatment of Alzheimer's disease [123]. Recent studies have shown that (44) inhibits both the delayed rectifier (I_K) and inward rectifier (I_{K1}) potassium currents in guinea-pig ventricular myocytes [124]. The compound also blocked calcium channels. At 1 μM in isolated guinea-pig atria, tacrine increased APD_{50} by 14% but also decreased V_{max} by 17% [125], suggesting blockade of Na^+ channels as well. A related compound, 4-aminoquinoline (45), increased APD_{90} by 15% at a concentration of 0.5 mM in frog sinus venosus cells (atrial strips) [126]. These results are not surprising, since both compounds are structurally related to aminopyridines (46) and (47) which have been used for several years as potassium channel blocking agents [127,128].

(45)

(46)

(47)

A number of naturally occurring compounds have been reported to possess Class III electrophysiological activity. These will not be discussed in detail in this review but are listed in *Table 2.1.*

Table 2.1. NATURALLY OCCURRING COMPOUNDS WITH CLASS III ACTIVITY

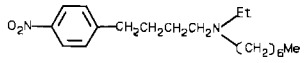
Compound	*Reference*
Goniopora toxin	129, 130
Prostaglandin-I_2 (PGI_2), 7-oxo-PgI_2	131–133
ATX-II	134
Cycloprotobuxine A	135
Anthopleurin A	10
Veratine	136
Neferine	137
Benzyltetrahydropalmitine	138, 139
Sophocarpine	140
Dauricine	141, 142
Berberine	143
Berbamine	144
Pantethine[a]	145
Inosine[a]	146
Coenzyme Q10[a]	147

[a]APD-prolonging activity was observed in hypoxic tissue.

NEWER CLASS III AGENTS

During their developmental work on clofilium, the Lilly group discovered LY097241 (48), a tertiary amine analogue of clofilium, which was an order of magnitude more potent than the parent compound. The $EC_{20}APD_{95}$ for LY097241 was 2.4 nM [10]. This result, along with the Class III activity of nifenalol, supports the observation that a nitro group in the 4-position on the phenyl ring is a useful Class III pharmacophore. Further work by the

Lilly group led to LY190147 (49) [148,149]. LY190147 exhibited Class III electrophysiological activity in canine Purkinje fibres at concentrations below 1 μM. At concentrations above 1 μM, Class I activity was manifested.



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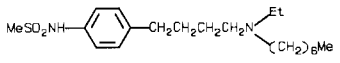
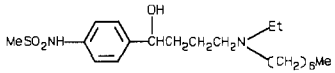


(49)

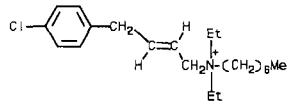
The Upjohn Company is developing a Class III agent very similar to LY190147 [150]. The Upjohn compound, ibutilide (50), possesses an electrophysiological profile that is very similar to LY190147. Ibutilide produces a maximum increase (35%) of APD at 0.1 μM and a shortening of APD at higher concentrations [151–153]. The Upjohn investigators attribute this biphasic effect to an initial activation of an inward Na^+ current at low concentrations and activation of a K^+ current (I_K) at higher concentrations, although ibutilide is also reported to inhibit the fast inward Na^+ current at 10 μM [153].



(50)

At Berlex Laboratories, we initially explored the effects of structural modifications to clofilium on electrophysiological activity. We examined the effects of constraints in flexibility of the connecting chain between the phenyl ring and the quaternary centre of clofilium, as in (51). Unsaturation (*cis* or *trans* double bonds and triple bonds) produced only marginal change in electrophysiological activity [154]. During this work, we also demonstrated the utility of methylsulphonylamino and nitro moieties as pharmacophores in related tertiary amines, (52) and (53), respectively. Further work led to incorporation of the quaternary centre of clofilium into a quinuclidinium framework. Attachment of the phenyl moiety at either the 2- or the 3-position of the quinuclidine, as in (54) or (55), again afforded potent compounds with electrophysiological activity comparable to clofilium [155]. Thus, it appears that the binding site for 'clofilium-like' agents can tolerate a variety of modifications provided the aromatic ring and ammonium centre remain intact.

Finally, we examined replacement of the quaternary ammonium centre of clofilium with a positive centre derived from an imidazolium cation, for example, (56) [156]. Although these types of compound possessed Class III



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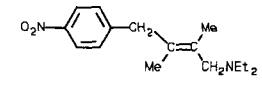

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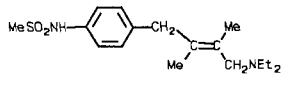

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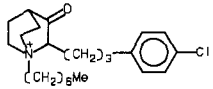

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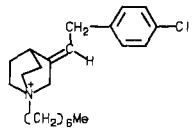
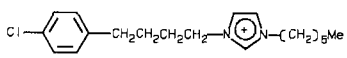


(55)

electrophysiological activity, they were generally less potent ($EC_{20}APD_{95}$ > 10 μM) than the parent quaternary ammonium agents. However, replacement of the isopropylamino group in sotalol by the imidazolium moiety gave CK-1649 (57) a potent Class III agent [157,158]. CK-1649 ($EC_{20}APD_{95}$ = 1.6 μM) was almost an order of magnitude more potent than sotalol and



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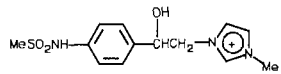
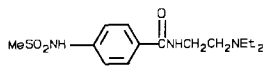


(57)

devoid of β-blocking activity. Development of CK-1649 was suspended due to erratic oral bioavailability, which is typical for many permanently charged compounds.

The research that led to the Berlex compound in clinical investigation, sematilide (58), began with the observation that, although acecainide was a Class III agent it could be metabolized to procainamide, a Class I drug which in some patients induced lupus. Replacement of the acetyl group in

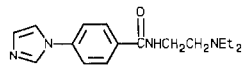


(58)

acecainide with a more metabolically stable amide could, in principle, provide a better Class III agent. This hypothesis, coupled with the successful use of the methylsulphonylamino group in a variety of compounds, led us to sematilide [159]. Sematilide ($EC_{20}APD_{95}$ = 3.5 μM) was much more potent than acecainide (10% increase in APD_{95} at about 30 μM). Sematilide

was the first orally available selective Class III agent in clinical trials and is now entering Phase III studies.

Continuing research at Berlex has addressed questions of alternative Class III pharmacophores. The methylsulphonylamino substitutent has been used successfully in a number of compounds to produce Class III activity. We have shown that the methylsulphonylamino group can be replaced by the 1*H*-imidazol-1-yl moiety [160]. Indeed, replacement of the methylsulphonyl moiety in sematilide affords (59). Compound (59) is equipotent to sematilide and possesses a similar cardiovascular profile. The ac-

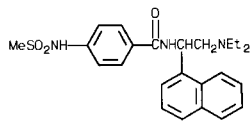


(59)

tivity of these compounds is sensitive to point of attachment and degree of substitution of the imidazole substituent. Attachment of the imidazole to the phenyl ring via the 2-position (that is, 1*H*-imidazol-2-yl) or addition of a methyl group at the 2-position (that is, 2-methyl-1*H*-imidazol-1-yl) greatly reduces activity [160].

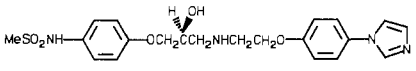
Although Class III agents show promise in the prevention of life-threatening re-entrant ventricular arrhythmias, they are not generally effective against PVC's. Further, elevated catecholamine levels can blunt their overall efficacy [161]. We have explored the combination of Class III pharmacophores with either Class I or Class II pharmacophores in a single molecule in an attempt to obtain compounds with broader therapeutic utility. Modification of sematilide by the addition a second aromatic group on the connecting chain produced compounds with both Class III and Class I activities [162]. In general, however, Class III activity appeared at lower concentrations and was greatly diminished at higher concentrations when significant Class I activity appeared. This is similar to the electrophysiological profiles seen for (49) and (50). We have also observed that the action potential prolonging activity of sematilide is decreased in the presence of increasing concentrations of flecainide (unpublished results). This suggests that Class I/III agents act by blocking potassium channels at lower concentrations and sodium channels at higher concentrations. The most interesting compound from the Class I/III studies was compound (60), which was efficacious against both re-entrant (PES-induced) and automatic (Harris [163] type) arrhythmias. Compound (60) also produced emesis after intravenous administration, which is suggestive of undesirable CNS activity.

In contrast to the apparent incompatibility of Class III and Class I activi-



(60)

ties, combination of Class III and Class II activities is apparently complementary. Administration of propranalol to dogs that had received subtherapeutic doses of sematilide resulted in effective prevention of PES-induced arrhythmias and an enhanced prolongation of refractoriness [164]. These results, together with the success of Class II agents in the prevention of mortality after myocardial infarction [165], stimulated research toward agents with Class III and Class II activity combined within a single molecule. Sotalol can be considered a prototype Class III/II agent, but it lacks potency as a Class III agent relative to its Class II activity and is a non-selective β-blocker. Examination of the combination of several Class III and Class II pharmacophores linked through a common nitrogen resulted in the selection of (61) for further development [43,166]. Compound (61) is effective against both PES-induced arrhythmias in infarcted conscious dogs and catecholamine-induced arrhythmias in halothane-anaesthetized dogs. The compound has a more favourable haemodynamic profile than sotalol. Compound (61) is continuing in preclinical development.



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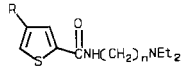
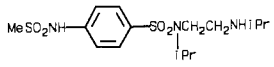


a: R = -$NHSO_2Me$, n=2,
b: R = -NO_2, n=3,
c: R = -NO_2, n=2,

(62)

The successful preclinical use of sematilide as a Class III antiarrhythmic agent has stimulated the synthesis of related compounds. Chemists at Kotobuki Seiyaku have prepared the thiophene analogues (62) [167]. Compound (62a) increases APD_{95} by 20% at 9.6 μM. The most potent compounds, however, are nitro-substituted thiophenes (62b and c), which increase APD_{95} by 20% at 1 and 2.3 μM, respectively. The Wyeth-Ayerst group examined analogues of sematilide in which the carboxamide group was replaced by a sulphonamide group [168,169]. The most interesting compound from this series is risotilide (63, WY-48986), which increased APD (at -60 mV) by 31% at a concentration of 3 μM in canine Purkinje fibres. Studies



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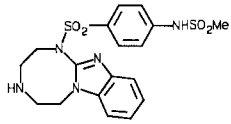
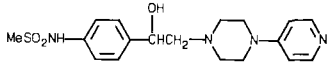


(64)

in feline ventricular myocytes using voltage clamp techniques showed that risotilide selectively blocked the delayed rectifier current (I_K) at 3 μM [170]. No effects on the inward rectifier current (I_{K1}) were seen until 100 μM concentrations of the drug. Risotilide is effective against PES-induced arrhythmias in dogs (about 5 mg/kg), and in decreasing mortality in anaesthetized pigs after coronary occlusion [171]. The drug is not effective, as would be expected, in the Harris [163] dog arrhythmia model. Risotilide is in Phase II clinical trials. In addition to their work with analogues of risotilide, scientists in the Wyeth-Ayerst group have recently patented a series of novel heterocyclic Class III agents [172] of which (64) is an example.

The Pfizer group in the United Kingdom has been actively involved in the search for new Class III antiarrhythmic agents. It has patented a variety of structural types that make extensive use of the methylsulphonylamino moiety. These include indanes [173,174], pyridines [175,176], piperazines [177–179], benzazepines [180], bisarylalkylamines [181] and diazabicyclic compounds [182]. From this extensive work, two compounds, UK-66914 (65) and UK-68798 (66), have entered clinical trials. Compound (65) increases the effective refractory period (ERP) of ferret papillary muscle by 25% at a concentration of 0.5 μM [183]. Whole-cell patch-clamp studies



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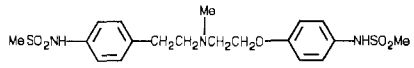
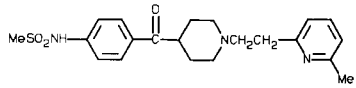


(66)

showed that UK-66914 reduced the delayed rectifier current (I_K) without affecting the inward rectifier current (I_{K1}) [184]. As is expected, UK-66914 shows modest positive inotropic activity both in ferret papillary muscle (27% increase in developed tension at 10 μM) [185] and in anaesthetized dogs (35% increase in left ventricular dP/dt at 1 mg/kg) [184,186]. Studies in healthy volunteers indicate that UK-66914 is well tolerated, with no adverse haemodynamic or proarrhythmic effects [187]. The bisarylalkylamine, UK-68798, has a profile similar to UK-66914, but is 10–20-fold more potent. In guinea-pig papillary muscle UK-66914, prolongs APD_{90} by 22% at

a concentration of 50 nM [188]. Like UK-66914, compound UK-68798 selectively blocks I_K without affecting I_{K1} [189]. UK-68798 is in Phase II clinical trials.

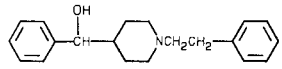
The Class III antiarrhythmic agent under development by Eisai is a methylsulphonylamino-substituted benzoyl piperidine, E-4031 (67). In guinea-pig papillary muscle E-4031 increased APD_{90} by 25% at a concentration of 10 μM [190] and increased ERP in ferret papillary muscle by 25% at 58 nM



(67)

[183]. Like other selective Class III agents E-4031 exhibits positive inotropic activity [191]. The mechanism by which E-4031 prolongs APD is under some dispute. The Eisai group reports that E-4031 depresses I_K [192], while a group from Merck using similar methodology suggests that it does not block I_K but may block another K^+ current [193]. In studies in dogs, E-4031 was effective in preventing PES-induced arrhythmias in at least 70% of the animals [194,195] and demonstrated a cardioprotective as well as an antiarrhythmic effect in a model of ischaemia-induced arrhythmia (amiodarone and sotalol were also effective) [196]. E-4031 is proceeding in Phase II clinical trials.

During the past few years, a number of novel structural types have been developed which possess class III activity. Several of the new compounds do not utilize the methylsulphonylamino moiety and provide new leads for the design of future Class III agents. The group at Merrell Dow Research Institute is developing a piperidine-4-methanol derivative, MDL 11,939 (68), as a potential Class III antiarrhythmic agent. The MDL compound prolongs APD_{90} in canine Purkinje fibres, but is less effective than bretylium, sotalol and clofilium in this preparation [197]. It was suggested that the decreased efficacy in Purkinje fibres may produce a smaller dispersion of refractoriness and therefore be less proarrhythmic than other Class III agents. In anaesthetized dogs, MDL 11,939 increased QT_c and was effective in the prevention of PES-induced re-entrant ventricular arrhythmias (50%



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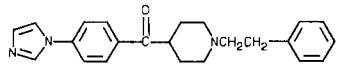
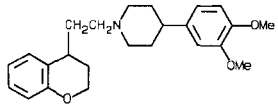


(69)

of animals at 10 mg/kg) [198]. The Merrell Dow group is also examining some analogues of MDL 11,939 that utilize the 1*H*-imidazolyl moiety as a class III pharmacophore [199]. These compounds are exemplified by (69).

A benzopyran derivative, RP-58866 (70) is under development by Rhone-Poulenc. Patch clamp studies in guinea-pig ventricular myocytes indicate that RP-58866 inhibits the inward rectifying potassium current (I_{K1}) with no effects on the delayed rectifier, ATP-sensitive potassium currents or calcium currents [200]. RP-58866 increases APD_{90} of Purkinje fibres by about 45% at 0.3 μM without affecting V_{max} [201]. The compound was effective



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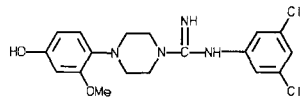
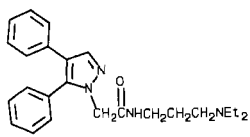


(71)

in models of electrically induced atrial fibrillation and occlusion/reperfusion-induced ventricular fibrillation in dogs at 0.1–0.3 mg/kg; however, it was not effective in models of enhanced automaticity which are typically used to identify Class I agents (for example, aconitine rat, ouabain dog or Harris dog models). Further, RP-58866 has little effect on haemodynamics at therapeutic doses.

RS-87337 (71), which is being developed at Syntex France, is claimed to have an electrophysiological profile of both Class III and Class IA agents [202]. At low concentrations (< 10 μM) in guinea-pig papillary muscles, RS-87337 increased APD; at higher concentrations (10–30 μM) V_{max} was reduced. Although the compound did not affect either atrial or ventricular conduction in dogs at doses up to 10 mg/kg [203], it was effective in reducing ectopy in dogs 24 h after coronary ligation (Class I model) [202]. RS-87337 appears to have a profile more indicative of a Class IA agent than a selective Class III agent.

Like RS-87337, Win 54177-4 (72) is reported to be a combined Class I/III antiarrhythmic agent. At a dose of 3 mg/kg, the compound increased effective refractory period by 21% in anaesthetized guinea-pigs [204]. The com-



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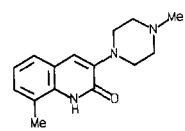
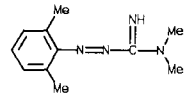


(73)

pound was effective in Class I models such as the aconitine rat and Harris dog. The compound was also effective in a PES-model of sudden death at doses of 5–10 mg/kg, i.v. [205]. In this model, Win 54177-4 was superior to disopyramide and comparable to sotalol.

The quinolinone derivative, OPC-88117 (73), is yet another compound described as possessing both Class I and Class III electrophysiological activities. Studies in guinea-pig papillary muscle showed that OPC-88117 at 30 μM increased APD_{80} by about 15% and decreased V_{max} by only 4%; however, at 100 μM APD_{80} was prolonged by 23% and V_{max} was decreased by 23% [206]. Further experiments in isolated rabbit hearts demonstrated that OPC-88117 increased atrio-His bundle (A-H) and His bundle-ventricle (H-V) conduction times and refractory periods with a profile that was similar to, but more potent than that of lidocaine [207].

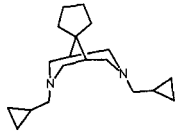
TYB-3823 (74), an antiarrhythmic agent synthesized at the University of Hawaii, exhibits an electrophysiological profile similar to the Class I/III agents discussed above, but there is a broader separation of the two effects.



(74)

At 0.1 and 0.3 μM the compound significantly prolonged APD of guinea-pig papillary muscle with little effect on V_{max} [208]. At a much higher concentration (100 μM), V_{max} was significantly inhibited. Voltage-clamp studies with single ventricular myocytes showed that TYB-3823 blocked outward potassium currents. Further studies in vascular tissue indicated that it also blocks α- and β-receptors [209]. This may also contribute to its antiarrhythmic profile.

Two 3,7-diazabicyclo[3.3.1]nonane derivatives are being evaluated as Class III electrophysiological agents. Tedisamil (75, KC-8857) is being developed by Kali-Chemie; Knoll is developing ambasilide (76, LU-47,710). Tedisamil is being developed primarily as an antianginal-bradycardiac



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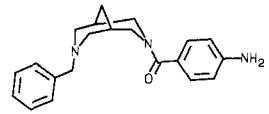
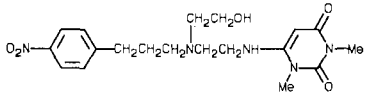


(76)

agent; however, its unique profile of positive inotropy and bradycardiac action provided an indication of Class III activity. In the isolated working rat heart, tedisamil at 1.8 μM increased dP/dt_{max} by 16%, decreased heart rate by 27% and resulted in an overall increase of cardiac contractile efficiency of 36% [210]. At 4 μM in the isolated guinea-pig heart, the compound prolonged QT_c 12.5% and at 3 μM it prolonged AV-conduction 25%. In guinea-pig papillary muscle, APD was increased modestly below 10 μM, while above 10 μM, APD was shortened (Class I effect) [211]. In rat ventricular myocytes, tedisamil increased the rate of inactivation of the transient outward potassium current (I_{to}. In guinea-pig myocytes the compound blocked I_K [212]. Inhibition of these currents would increase APD. At higher concentrations, tedisamil inhibited I_{Na} which is the dominant current blocked by Class I agents. Tedisamil was effective in ischaemia-reperfusion induced arrhythmias in the rat [213]. Ambasilide, in contrast, appears to selectively block the delayed rectifier current (I_K) with little affect on the inward rectifier (I_{K1}) [214].

Mitsui Pharmaceuticals is pursuing the development of MS-551 (77), which utilizes the 4-nitrophenyl Class III pharmacophore. Studies in canine Purkinje fibre have shown that 10 μM of MS-551 increases APD_{90} by 36% with no effects on V_{max} [215]. Further, MS-551 at 0.03–0.3 mg/kg given intravenously converted atrial flutter to sinus rhythm in 7 of 8 dogs [216].



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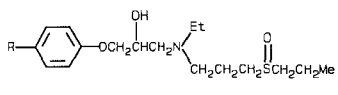


(78)

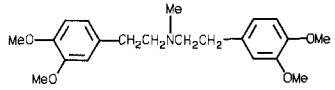
a: R = -CN
b: R = -NO_2
c: R = -$NHSO_2Me$

There was no effect on interatrial conduction and negligible effects on haemodynamic parameters.

A novel agent, H 234/09 (78a), which makes the first use of the 4-cyanophenyl moiety as a Class III pharmacophore, has been described by the group from AB Hässle. Ventricular monophasic action potentials determined in anaesthetized guinea-pigs were increased by 20% at a dose of 5×10^{-8} mol/kg [217]. The compound was equipotent with the corresponding nitro analogue (78b) and an order of magnitude more potent than the methylsulphonylamino analogue (78c) as well as sotalol and sematilide [218]. At therapeutic concentrations, the Hässle compound had no effect on conduction (isolated human papillary muscle), no significant β-blockade (rat atria)

and exhibited positive inotropic activity (cat papillary muscle). During acute left ventricular failure in the dog, H 234/09 increased left ventricular dP/dt (5%) and QT (13%) without changing left ventricular systolic pressure, diastolic pressure or cardiac output [219]. These results suggest that this compound, like most selective Class III agents, should be safe for use in the heart failure population.

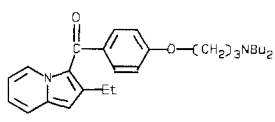
YS-035 (79), a calcium channel antagonist derivative of verapamil [220], has been shown to possess Class III electrophysiological activity in isolated



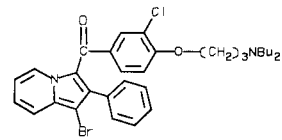
(79)

tissues. At concentrations up to 10 μM, it significantly prolongs APD in atrial and ventricular muscle (guinea-pig) and Purkinje fibres (sheep) without affecting V_{max} [221]. Two-microelectrode voltage clamp studies in sheep Purkinje fibres demonstrated that (79) inhibited several membrane currents. The inward rectifier (I_{K1}) and instantaneous outward current (I_{inst}) were reduced by 25% at 10 μM. The transient outward current (I_{to}) and pacemaker current (I_f) were reduced by 50% and 65%, respectively, at the same concentration. There was, however, little effect on the time-dependent potassium current (I_K) at concentrations up to 100 μM. YS-035 appears to have a unique profile in that it not only is a calcium antagonist, but also inhibits a variety of potassium currents with little apparent effect on the fast inward sodium current.

Although amiodarone was one of the first compounds identified as a Class III antiarrhythmic agent, relatively little has been done to explore the structural requirements for Class III activity around this nucleus. This may be due to the complex pharmacological profile exhibited by the compound. Two indolizine analogues of amiodarone, butoprozine (80) and rilozarone (81), have been evaluated by researchers at Sanofi. A comparison of butoprozine with amiodarone and verapamil using voltage-clamp techniques in frog atrial fibres showed that butoprozine was more potent than amioda-



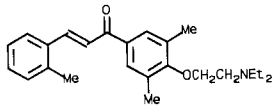
(80)



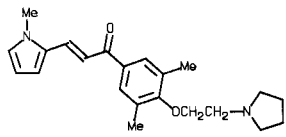
(81)

rone but less potent than verapamil at inhibiting the fast inward sodium current [222]. A similar order of potency was observed for effects on the slow inward calcium current. The effects of butoprozine were comparable to those of amiodarone on the total outward current (predominantly potassium current). In sheep Purkinje fibres, butoprozine had more pronounced negative effects on V_{max} and action potential amplitude (APA) and APD_{60} than amiodarone; however, the compound still produced an increase in APD_{60} comparable to that of the parent compound [223]. Thus, like amiodarone, butoprozine possesses multiple activities on ionic currents. While the Class III effects of both compounds are comparable, the Class I and Class IV actions of butoprozine are greater than those of amiodarone. Rilozarone was initially investigated as both a coronary vasodilator (antianginal) and as an antiarrhythmic agent [224,225]. There is a suggestion in the literature that rilozarone possesses Class III activity [226]; however, no data were found. Based on the structural similarity to butoprozine and amiodarone, rilozarone probably possesses a variety of cardiovascular effects which may include Class III activity.

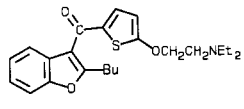
More recently, a BASF group has been evaluating analogues of amiodarone in which the benzofuran moiety has been replaced by 2-arylethenyl and heteroarylethenyl moieties. Compounds (82) [227] and (83) [228] are two of the more potent agents from this work. Class III potency was assessed in anaesthetized guinea-pigs by determining the dose that increased QT interval by 20%. In this model (+)-sotalol was effective at 3.6 mg/kg i.v. Com-



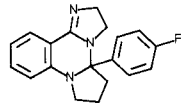
(82)



(83)



(84)



(85)

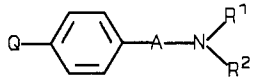
pounds (82) and (83) were effective at 0.65 mg/kg and 1.1 mg/kg, respectively. Finally, Ebewe Arzneimittel has patented a series of thienyl analogues of amiodarone, exemplified by (84), as antiarrhythmics [229]. No data are available for these compounds.

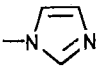
In addition to the amiodarone-related compounds, (81) and (82), described above, BASF has been exploring some novel heterocyclic compounds as Class III antiarrhythmic agents. A series of imidazo[1,2-*c*]pyrrolo[1,2-*a*]quinazoline derivatives have been patented which are several times more potent than (+)-sotalol in lengthening QT interval of the electrocardiogram in the anaesthetized guinea-pig model [230]. One of the most potent compounds is (85), which was 17-times more potent than the standard. These compounds represent one of the unique Class III structural types described to date.

STRUCTURE–ACTIVITY CONSIDERATIONS

From the discussion of compounds above, it can be seen that Class III electrophysiological activity can be elicited with a wide variety of structural types. At first glance there do not seem to be specific requirements to produce active compounds. When the structures of the selective Class III compounds (that is, concentration-dependent prolongation of APD with minimal (< 5%) inhibition of V_{max} are compared, a pattern emerges which can be used to define structure activity requirements. A general structure for a selective Class III agent is presented in *Figure 2.3.*

The phenyl substituent Q is comprised of a limited number of groups to date. The most effective of these are nitro, cyano, methylsulphonylamino



Q = $-NO_2$, -CN, $-NHSO_2Me$, 

A = 1-4 atoms, linking chain may contain heteroatoms, polar groups or be part of a ring

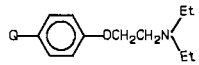
R^1 = H, alkyl, phenylalkyl

R^2 = Alkyl, arylalkyl, heteroarylakyl
(including a second Class III pharmacophore)

Figure 2.3. General structural requirements for a selective Class III electrophysiological agent.

and 1*H*-imidazol-1-yl (see *Table 2.2* for a representative sample). Homologous extensions of the methylsulphonylamino substituent (for example, butylsulphonylamino) produce a significant fall in activity. Substituents that are much less effective are acetylamino and fluoro. The chloro substituent (as found in clofilium) is not a generally effective substituent. For the most part, attachment of Q at the *para* position on the phenyl ring appears to be critical for selective Class III activity. The only exception noted is the *meta* isomer of (58), which maintains selective Class III activity [160]. The phenyl ring seems to be preferred over other aromatic or heteroaromatic rings (with the exception of the Kotobuki compounds) based on the selective compounds in development. Additional substituents on the phenyl ring often lead to a significant decrease in activity. The linking group A which connects the phenyl ring and the basic amine is one of the more variable regions of the molecule. The linking group may contain from one to four atoms that need not be carbon. Heteroatoms, as well as polar groups, such as amides and hydroxyl substituents, are well tolerated. Further, the linkage and amino group may be part of a ring. The nitrogen substituents, R^1 and R^2 (*Figure 2.2*) can play a major role in determining potency of the Class III agents. The R^1 group can be hydrogen, alkyl (for example, C_1–C_{10}) or phenylalkyl. For the case when R^1 is a hydrogen, additional lipophilic character must be built into R^2 or elsewhere in the molecule (for example, in the linking group A) in order to maintain activity. The second nitrogen substituent R^2 is generally an alkyl, arylalkyl or heteroarylalkyl group. Based

Table 2.2 EFFECT OF THE PHENYL SUBSTITUENT ON CLASS III ELECTROPHYSIOLOGICAL ACTIVITY IN CANINE CARDIAC PURKINJE FIBRES[a]



Q	C_{20} APD_{95}[b] μM (range)	n[c]
-NO_2	0.2 (0.1-0.3)	4
-CN	1.0 (0.6-1.3)	4
-$NHSO_2Me$	1.1 (0.7-2.1)	4
-(1*H*-imidazol-1-yl)	2.1 (0.6-4.1)	4

[a]The experimental procedure has been described [154].
[b]C_{20} APD_{95} = concentration of drug which caused a 20% increase in action potential duration at 95% repolarization (APD_{95}). Reported is the log mean average of n determinations with the lowest and highest values given in parentheses.
[c]n = number of experiments.

on the potency of selected Class III agents (for example, (60), (65), (66)) there appears to be an auxillary binding site for an aromatic ring connected by one to three atoms to the basic nitrogen. The Berlex [43] and Pfizer groups [188] both have noted the increased activity when two Class III pharmacophores are linked via the basic nitrogen. This suggests the possibility of adjacent binding domains for Class III agents within potassium channels [188]. In designing Class III agents derived from the above scheme, care must be taken in maintaining an appropriate balance in the lipophilic character of R^1 and R^2. In general, increasing the lipophilic character of R^1 and R^2 leads to more potent Class III compounds; however, past a certain point increasing lipophilicity results in agents with additional Class I activity. The basic nitrogen can also be replaced by a quaternary centre (for example, (14) and (56)). This suggests that the active form of the basic drug is the protonated species, which would be expected for most of these amines at physiological pH.

The obvious exceptions to the general requirements for Class III activity described above are (68) and (70). These two compounds appear to be selective Class III agents; however, they lack an appropriate Q moiety. It is interesting to speculate whether these compounds bind to an alternate domain in the potassium channel or, possibly for (68), an entirely different site (for example, sodium or calcium channels) to effect their Class III activity.

CONCLUSIONS

Since the postulation by Vaughan Williams in 1970 that action potential prolongation might constitute a unique mechanism of antiarrhythmic action, a considerable research effort has been expended in pursuit of Class III antiarrhythmic agents. Several selective Class III agents (for example, (57), (65) and (66)) have shown promising results in clinical trials. No selective Class III agent has reached the market-place and the extensive clinical experience that comes with a marketed product is lacking. Thus, the effectiveness of selective Class III antiarrhythmic therapy still remains to be proven.

For the most part the selective Class III agents appear to act by inhibition of one or more repolarizing potassiuim currents, especially the delayed rectifier (I_K) and/or the inward rectifier (I_{K1}). Less selective agents generally affect sodium currents, as well as potassium currents, which translates to Class I (conduction-slowing) activity. Additional work still needs to be done to increase our understanding of the currents and channels involved in regulation of APD. Care must be taken to evaluate species differences, tissue dif-

ferences (for example, atria vs. ventricles), differences within a species and the differences between normal and diseased (ischaemic) tissues in order to develop models that appropriately reflect the mechanisms involved in the human heart.

Although numerous structural types have been synthesized to date, we expect research to continue in this area. Several companies, not mentioned here, have ongoing Class III discovery programmes. As medicinal chemists begin to take advantage of information from new pharmacological studies and clinical experience, we expect to see agents with increased selectivity (for example, normal vs. ischaemic tissue), improved efficacy and improved safety.

ACKNOWLEDGEMENTS

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REFERENCES

1 Kaplan, H.R. (1986) Fed. Proc. 45, 2184–2185.
2 Savage, D.D., Castelli, W.P., Anderson, S.J. and Kannel, W.B. (1983) Am. J. Med. 74, 148–152.
3 Lown, B., Fakhro, A.M., Hood, W.B., Jr. and Thorn, G.W. (1967) J. Am. Med. Assoc. 199, 188–198.
4 Vaughan Williams, E.M. (1970) in Symposium on Cardiac Arrhythmias (Sandoe, E., Flensted-Jansen, E. and Olesen, K.H., eds.), pp. 449–472, AB Astra, Sodertalje.
5 Hill. J.L. (1985) in Mechanism and Treatment of Cardiac Arrhythmias; Relevance of Basic Studies to Clinical Management (Reiser, H.J. and Horowitz, L.N., eds.), pp. 173–182, Urban & Schwarzenberg, Baltimore.
6 Vaughan Williams, E.M. in Ref. 5, pp. 165–172.
7 Wit, A.L. in Ref. 5, pp. 11–37.
8 Mukharje, J., Rude, R.E., Poole, W.K., Gustafson, N., Thomas, L.J., Jr., Strauss, H.W., Jaffe, A.S., Muller, J.E., Roberts, R., Raabe, O.S., Jr., Croft,. C.H., Passamani, E., Braunwald, E., Willerson, J.T. and the MILIS Study Group (1984) Am. J. Cardiol. 54, 31–36.
9 The Cardiac Arrhythmia Supression Trial Investigators (1989) N. Engl. J. Med. 321, 406–412.
10 Steinberg, M.I. and Michelson, E.L. in Ref. 5, pp. 263–281.

11 Tondeur, R. and Binon, F. (1963) Fr. Patent 1,339,389; (1964) Chem. Abstr. 60, P2893b; (1966) U.S. Patent 3,248,401.
12 Charlier, R., Deltour, G., Tondeur, R. and Binon, F. (1962) Arch. Int. Pharmacodyn. 139, 255–264.
13 Rosenbaum, M.B., Chiale, P.A., Ryba, D. and Elizari, M.V. (1974) Am. J. Cardiol. 34, 215–223.
14 Haffajee, C.I. in Ref. 5, pp. 283–294.
15 Proceedings of the Symposium: Amiodarone: Basic Concepts and Clinical Applications (Singh, B.N. and Zipes, D.P., eds.), (1983) Am. Heart J. 106, 787–964.
16 Scrip No. 1555, October 5, 1990, p. 26.
17 Mason, J.W. (1987) N. Engl. J. Med. 316, 455–466.
18 Charlier, R., Deltour, G., Baudine, A. and Chaillet, F. (1968) Arzneim.-Forsch. 18, 1408–1417.
19 Gloor, H.O., Urthaler, F. and James, T.N. (1983) J. Clin. Invest. 71, 1457–1466.
20 Mitchell, L.B., Wyse, G., Gillis, A.M. and Duff, H.J. (1989) Circulation 80, 34–42.
21 Somani, P. (1989) J. Clin. Pharmacol. 29, 405–412.
22 Aomine, M. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 589–599.
23 Pallandi, R.T. and Campbell, T.J. (1987) Br. J. Pharmacol. 92, 97–103.
24 Sheldon, R.S., Hill, R.J., Cannon, N.J. and Duff, H.J. (1989) Circ. Res. 65, 477–482.
25 Freedberg, A.S., Papp, J.G. and Vaughan Williams, E.M. (1970) J. Physiol. 207, 357–369.
26 Nademanee, K., Singh, B.N., Hendrickson, J.A., Reed, A.W., Melmed, S. and Hershman, J. (1982) Circulation 66, 202–211.
27 Aanderud, S., Sundsfjord, J. and Aarbakke, J. (1984) Endocrinology 115, 1605–1608.
28 Talajic, M., Nattel, S., Davies, M. and McCans, J. (1989) J. Cardiovasc. Pharmacol. 13, 447–450.
29 Lambert, C., Cardinal, R., Vermeulen, M., Lamontagne, D., Nadeau, R., Paradis, P. and Rouleau, J.-L. (1987) J. Pharmacol. Exp. Ther. 242, 320–325.
30 Gillis, A.M. and Kates, R.E. (1984) Clin. Pharmacokinet. 9, 375–403.
31 Venkatesh, N., Al-Sarraf, L., Hershman, J.M. and Singh, B.N. (1986) Proc. Soc. Exp. Biol. Med. 181, 233–236.
32 Gross, S.A., Bandyopadhyay, S., Klaunig, J.E. and Somani, P. (1989) Proc. Soc. Exp. Biol. Med. 190, 163–169.
33 Heissenbuttel, R.H. and Bigger, T.J., Jr. (1979) Ann. Int. Med. 91, 229–238.
34 Anderson, J.L. (1985) Clin. Ther. 205–224.
35 Leveque, P.E. (1965) Nature 207, 203–204.
36 Bacaner, M.B. (1968) Am. J. Cardiol. 21, 504–512.
37 Wit, A.L., Steiner, C. and Damato, A.N. (1970) J. Pharmacol. Exp. Ther. 173, 344–356.
38 Kowey, P.R., Friehling, T.D., O'Connor, K.M., Wetstein, L. and Kelliher, G.J. (1985) Am. Heart J. 110, 363–370.
39 Argentieri, T.M., Ambelas, E.M., Sullivan, M.E. and Wiggins, J. (1988) FASEB J., Abstr. 7317.
40 Uloth, R.H., Kirk, J.R., Gould, W.A. and Larsen, A.A. (1966) J. Med. Chem. 9, 88–97.
41 Singh, B.N. and Vaughan Williams, E.M. (1970) Br. J. Pharmacol. 39, 675–687.
42 Singh, B.N. (1990) in A Symposium: Controlling Cardiac Arrhythmias with Sotalol, a Broad-Spectrum Antiarrhythmic with Beta-Blocking Effects and Class III Activity (Singh, B.N., ed.), Am. J. Cardiol. 65 No. 2 pp. 3A–11A.
43 Morgan, T.K., Jr., Lis, R., Lumma, W.C., Jr., Wohl, R.A., Nickisch, K., Phillips, G.B., Lind, J.M., Lampe, J.W., Di Meo, S.V., Reiser, H.J., Argentieri, T.M., Sullivan, M.E. and Cantor, E. (1990) J. Med. Chem. 33, 1087–1090.

44 Nattel, S., Feder-Elituv, R., Matthews, C., Nayebpour, M. and Talajic, M. (1989) J. Am. Coll. Cardiol. 13, 1190–1194.
45 Wang, T., Bergstrand, R.H., Thompson, K.A., Siddoway, L.A., Duff, H.J., Woosley, R.L. and Roden, D.M. (1986) Am. J. Cardiol. 57, 1160–1165.
46 Mitchell, L.B., Wyse, D.G. and Huff, H.J. (1987) Circulation 76, 810–818.
47 Lathrop, D.A. (1985) Can. J. Physiol. Pharmacol. 53, 1506–1512.
48 Lynch, J.J., Wilber, D.J., Montgomery, D.G., Hsieh, T.M., Patterson, E. and Lucchesi, B.R. (1984) J. Cardiovasc. Pharmacol. 6, 1132–1141.
49 Gomoll, A.W. and Bartek, M.J. (1986) Eur. J. Pharmacol. 132, 123–136.
50 Hafner, D., Berger, F., Borchard, U., Kallman, A. and Scherlitz, A. (1988) Arzneim.-Forsch. 38, 231–236.
51 Tande, P.M. and Refsum, H. (1988) Pharmacol. Toxicol. 62, 272–277.
52 Janssen, J., Loomans, L., Harink, J., Taams, M., Brunninkhuis, L., Van der Starre, P. and Kootstra, G. (1986) Angiology 37, 601–609.
53 Touboul, P., Atallah, G., Kirkorian, G., Lavaud, P., Mathieu, M.P. and Dellinger, A. (1987) Am. Heart J. 114, 545–550.
54 Anderson, J.L., Askins, J.C., Gilbert, E.M., Miller, R.H., Keefe, D.L., Somberg, J.C., Freedman, R.A., Haft, L.R., Mason, J.W. and Lessem, J.N. (1986) J. Am. Coll. Cardiol. 8, 752–762.
55 Kienzle, M.G., Martins, J.B., Wendt, D.J., Constantin, L., Hopson, R. and McCue, M.L. (1988) Am. J. Cardiol. 61, 1012–1017.
56 Bourke, J.P., McComb, J., Cowan, J.C., Tansuphaswadikul, S. and Campbell, R.W.F. (1988) Br. Heart J. 59, 114.
57 Brachmann, J., Schoels, W., Waldecker, B., Senges, J., Schmitt, C. and Kuebler, W. (1987) J. Am. Coll. Cardiol. 9 (2 Suppl. A), 229A.
58 Sahar, D.I., Reiffel, J.A., Bigger, J.T., Jr., Squatrito, A. and Kidwell, G.A. (1989) Am. Heart J. 117, 562–568.
59 Steinberg, M.I. and Malloy, B.B. (1979) Life Sci. 25, 1397–1406.
60 Malloy, B.B. and Steinberg, M.I. (1981) U.S. Patent 4,289,787; (1982) Chem. Abstr. 96, 68564.
61 Steinberg, M.I., Sullivan, M.E., Wiest, S.A., Rockhold, F.W. and Malloy, B.B. (1981) J. Cardiovasc. Pharmacol. 3, 881–895.
62 Platia, E.V. and Reid, P.R. (1984) Clin. Pharmacol. Ther. 35, 193–202.
63 Greene, H.L., Werner, J.A., Gross, B.W., Sears, G.K., Trobaugh, G.B. and Cobb, L.A. (1983) Am. Heart J. 106, 492–501.
64 Echt, D.S., Black, J.N., Barbey, J.T., Coxe, D.R. and Cato, E. (1989) Circulation 79, 1106–1117.
65 Gough, W.B., Hu, D. and el-Sherif, N. (1988) J. Am. Coll. Cardiol. 11, 431–437.
66 Carlsson, L., Almgren, O. and Duker, G. (1989) Circulation 80 (4 Suppl 2), II–139.
67 Walton, E., Ruffell, G.K., Grivsky, E. and Wood, J.R. (1965) U.S. Patent 3, 168,562; (1965) Chem. Abstr. 62, 1604c.
68 Maxwell, R.A. and Walton, E. (1976) U.S. Patent 3,949,089; (1971) Chem. Abstr. 74, 87660q; (1971) Ger. Offen. DE 2030693.
69 Leveque, P.E. (1966) Arch. Int. Pharmacodyn. 163, 422–426.
70 Dangman, K.H., Wang, H.H. and Reynolds, R.D. (1986) J. Cardiovasc. Pharmacol. 8, 1185–94.
71 Dangman, K.H. and Miura, D.S. (1985) J. Cardiovasc. Pharmacol. 7, 50–58.
72 Fenici, R., Marchei, M., Bellocci, F. and Zecchi, P. (1977) Br. Heart J. 39, 787–794.
73 Ferroni, A. and Monticelli, G. (1973) Pharmacol. Res. Commun. 5, 151–171.
74 Tamargo, J. (1979) Br. J. Pharmacol. 67, 410P.

75 Kimura, T., Nakazawa, M. and Imai, S. (1986) Folia Pharmacol. Jpn. 88, 1–8.
76 Harron, D.W.G. and Brogden, R.N. (1990) Drugs 39, 720–740.
77 Wu, K.M. and Hoffman, B.F. (1987) Circulation 76, 1397–1408.
78 Okumura, K. and Waldo, A.L. (1987) J. Am. Coll. Cardiol. 9, 1332–1338.
79 Feld, G.K., Venkatesh, N. and Singh, B.N. (1988) J. Cardiovasc. Pharmacol. 11, 573–580.
80 Feld, G.K., Nademanee, K., Noll, E. and Singh, B.N. (1989) Cardiovasc. Drugs Ther. 3, 191–198.
81 Singh, B.N. and Vaughan Williams, E.M. (1971) Br. J. Pharmacol. 43, 10–22.
82 Kaumann, A.J. and Blinks, J.R. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 311, 205–218.
83 January, C.T., Riddle, J.M. and Salata, J.J. (1988) Circ. Res. 62, 563–571.
84 Lathrop, D.A., Varro, A. and Schwartz, A. (1989) Eur. J. Pharmacol. 164, 487–496.
85 Ijima, T. and Taira, N. (1987) J. Pharm. Exp. Ther. 240, 657–662.
86 Kurachi, Y., Asano, Y., Ito, H. and Sugimoto, T. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 324–330.
87 Honerjäger, P., Heiss, A., Schäfer-Korting, M., Schönsteiner, G. and Reiter, M. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 325, 259–269.
88 Lynch, J.J., Kitzen, J.M., Hoff, P.T. and Lucchesi, B.R. (1988) J. Cardiovasc. Pharmacol. 12, 547–554.
89 Canniff, P.C., Farah, A.E., Sperelakis, N. and Wahler, G.M. (1985) J. Cardiovasc. Pharmacol. 7, 813.
90 Vaughan Williams, E.M. (1987) J. Clin. Pharmacol. 27, 91–100.
91 Scholtysik, G., Salzmann, R., Berthold, R., Herzig, J.W., Quast, U. and Markstein, R. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 316–325.
92 Buggisch, D., Isenberg, G., Ravens, U. and G. Scholtysik (1985) Eur. J. Pharmacol. 118, 303–311.
93 Scholtysik, G. and Williams, F.M. (1986) Br. J. Pharmacol. 89, 287–292.
94 Raine, A.E.G. and Vaughan Williams, E.M. (1981) Circ. Res. 48, 804–812.
95 Edvardson, N. and Olsson, S.B. (1981) Br. Heart J. 45, 628–636.
96 Taggart, P., Donaldson, R., Abed, J. and Nashat, F. (1984) Cardiovasc. Res. 18, 683–689.
97 Manley, B.S., Alexopoulos, D., Robinson, G.J. and Cobbe, S.M. (1986), Cardiovasc. Res. 20, 705–709.
98 Ren, X.-L., Lin, C.-X. and Wang, X.-S. (1989) Zhongua Xinxueguanbing Zazhi 17, 73–76; (1989) Biol. Abstr. 88, 133977.
99 Benfey, B.G. (1990) Life Sci. 46, 743–757.
100 Sheridan, D.J. and Culling, W. (1985) J. Cardiovasc. Pharmacol. 7 (Suppl. 5), S55–S60.
101 Ikeguchi, S., Hashimoto, S., Horie, M., Kadoya, M., Konishi, T. and Kawai, C. (1988) Br. J. Pharmacol. 94, 1063–1068.
102 Hui, W.K., Mitchell, L.B., Kavanagh, K.M., Gillis, A.M., Wyse, D.G., Manyari, D.E. and Duff, H.J. (1990) J. Cardiovasc. Pharmacol. 15, 144–149.
103 Lillie, C., Harron, D.W. and Kobinger, W. (1985) Arzneim.-Forsch. 35, 301–305.
104 Harron, D.W., Nicholls, D.P. and Shanks, R.G. (1985) Br. J. Clin. Pharmacol. 19, 266–271.
105 Tohse, N., Nakaya, H. and Kanno M. (1986) Eur. J. Pharmacol. 125, 411–419.
106 Zaza, A., Malfatto, G. and Rosen, M.R. (1989) J. Pharmacol. Exp. Ther. 250, 397–404.
107 Saman, S., Thandroyen, F. and Opie, L.H. (1985) J. Cardiovasc. Pharmacol. 7 (Suppl. 7), S70–S75.
108 Kamiya, K., Venkatesh, N. and Singh, B.N. (1988) Am. Heart J. 115, 1222–1228.

109 Nademanee, K., Lockhart, E., Pruitt, C. and Singh, B.N. (1987) J. Cardiovasc. Pharmacol. 10 (Suppl. 3), S81–S85.
110 Stott, D.J., Saniabadi, A.R., Hosie, J., Lowie, G.D.O. and Ball, S.G. (1988) Eur. J. Clin. Pharmacol. 35, 123–129.
111 Höglund, P., Eriksson, M. and Christenson, E.G. (1986) J. Pharm. Pharmacol. 38, 861–863.
112 Scholtysik, G., Imoto, Y., Yatani, A. and Brown, A.M. (1988) J. Pharmacol. Exp. Ther. 245, 773–778.
113 Scholtysik, G., (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. 335, 692–696.
114 Gittos, M.W. (1989) Eur. Pat. Appl. 330,788; (1990) Chem. Abstr. 112, 178714.
115 Petty, M.A., DiFrancesco, G.F., Chaurvin, M. and Gittos, M.W. (1990) Eur. J. Pharmacol. 183, 1159.
116 Brasch, H. (1986) Br. J. Pharmacol. 88, 733–740.
117 Cerbai, E., Cavalcabó, P. de B., Masini, I., Porciatti, F. and Mugelli, A. (1989) Eur. J. Pharmacol. 162, 491–500.
118 Helgesen, K.G. and Refsum, H. (1987) Pharmacology 35, 121–129.
119 Helgesen, K.G. and Refsum, H. (1990) Pharmacol. Toxicol. 66, 217–220.
120 Fagbemi, O., Kane, K.A., Leprán, I., Parratt, J.R. and Szekeres, L. (1983) Br. J. Pharmacol. 78, 455–460.
121 Marchese, A.C., Hill, J.A., Jr., Xie, P.-D., and Strauss, H.C. (1985) J. Pharmacol. Exp. Ther. 232, 485–491.
122 Duff, H.J., Lester, W.M. and Rahmberg, M. (1988) Circulation 78, 1469–1477.
123 Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K. and Kling, A. (1986) N. Engl. J. Med. 315, 1241–1245.
124 Osterreider, W. (1987) Br. J. Pharmacol. 92, 521–525.
125 Freeman, S.E., Lau, W.-M. and Szilagyi, M. (1988) Eur. J. Pharmacol. 154, 59–65.
126 Guerrero, S. (1982) Arch. Int. Pharmacodyn. 259, 271–283.
127 Kenyon, J.L. and Gibbons, W.R. (1979) J. Gen. Physiol. 73, 139–157.
128 Kirsch, G.E. and Narahashi, T. (1978) Biophys. J. 22, 507–512.
129 Ikushima, S., Muramatsu, I., Fujiwara, M. and Ashida, K. (1981) Jpn. J. Pharmacol. 31, 1051–1060.
130 Noda, M., Muramatsu, I., Fujiwara, M. and Ashida, K. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 330, 59–66.
131 Szekeres, L., Szilvassy, Z., Udvary, E. and Vegh, A. (1989) J. Mol. Cell. Cardiol. 21 545–554.
132 Szekeres, L., Nemeth, M., Papp, J.G. and Udvary, E. (1990) Cardiovasc. Res. 24, 37–41.
133 Szekeres, L., Nemeth, M., Szilvassy, Z., Tosaki, A., Udvary, E. and Vegh, E. (1988) Biomed. Biochim. Acta 47, S6–S11.
134 Platou, E.S., Refsum, H. and Hotvedt, R. (1986) J. Cardiovasc. Pharmacol. 8, 459–465.
135 Wang, Y.-X., Liu, J.-W., Tan, Y.-H. and Sheng, B.-H. (1989) Acta Pharmacol. Sin. 10, 389–393; (1989) Biol. Abstr. 88, 122870.
136 Brooks, C.McC., Hoffman, B.F., Suckling, E.E. and Orias, O. (1955) Excitability of the Heart, p. 263, Grune & Stratton, New York.
137 Li, G.R., Qian, J.-Q., and Lu, F.-H. (1990) Acta Pharmacol. Sin. (China) 11, 158–161.
138 Zeng, W.-Z., Xia, G.-J., Yao, W.-X., Zong, X.-G. and Jiang, M.-X. (1990) Acta Pharmacol. Sin. (China) 11, 314–317.
139 Yao, W.-X., Xia, G.-J., Zhang, J.-S., Zeng, W.Z., Zhang, S.-D. and Jiang, M.-X. (1990) J. Tongji Med. Univ. (China) 10, 1–4; (1991) Chem. Abstr. 113, 126258k.
140 Zhu, P.-J., Liu, Y.-C., Li, Z.-Y. and Yu, D.-Z. (1989) Chung Kuo Yao Li Hsueh Pao (China) 10, 227–229.

141 Zhu, J.-Q., Zeng, F.-D. and Ha, C.-J. (1990) Acta Pharmacol. Sin. (China) 11, 220–225.
142 Zong, X.-G. and Jiang, M.-X. (1990) Acta Pharmacol. Sin. (China) 11, 241–245.
143 Huang, W., Zhang, Z. and Xu, Y. (1990) J. Electrocardiol. 23, 231–234.
144 Li, G.-Z., Wu, X.-C. and Li, W.-H. (1990) Asia Pac. J. Pharmacol. (Singapore) 5, 61–64.
145 Hayashi, H., Kobayashi, A., Terada, H., Nagao, B., Nishiyama, T., Kamikawa, T. and Yamazaki, N. (1985) Jpn. Heart J. 26, 289–96.
146 Chu, P.-C., Li, Z.-Y. and Yu, T.-C. (1987) Acta Physiol. Sin. 39, 343–349; (1987) Biol. Abstr. 85, 61043.
147 Tsutsumi, T., Sekiya, S., Okazaki, O. and Harumi, K. (1988) Arzneim.-Forsch. 38, 21–24.
148 Smallwood, J.K., Wiest, S.A., Malloy, B.B. and Steinberg, M.I. (1986) Fed. Proc. 45, 804.
149 Malloy, B.B. and Steinberg, M.I. (1990) U.S. Patent 4,569,801; (1986) Chem. Abstr. 105, 60417.
150 Hester, J.B. (1985) Eur. Patent Application 164,865A; (1986) Chem. Abstr. 104, 109269m; (1985) Jpn. Kokai Tokyo Koho JP 60239458.
151 Hester, J.B., Gibson, J.K., Cimini, M.G., Emmert, D.E., Locker, P.K., Percicone, S.C., Skaletsky, L.L., Sykes, J.K. and West, B.E. (1991) J. Med. Chem. 34, 308–315.
152 McKay, M.C., Sykes, J.S. and Lee, K.S. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. I) S15.
153 Lee, E.W., McKay, M.C. and Lee, K.S. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. I) S15.
154 Morgan, T.K., Jr., Wohl, R.A., Lumma, W.C., Jr., Wan, C.-N., Davey, D.D., Gomez, R.P., Marisca, A.J., Briggs, M., Sullivan, M.E. and Wong, S.S. (1986) J. Med. Chem. 29, 1398–1405.
155 Morgan, T.K., Jr., Lis, R., Marisca, A.J., Argentieri, T.M., Sullivan, M.E. and Wong, S.S. (1987) J. Med. Chem. 30, 2259–2269.
156 Diamond, J., Lumma, W.C., Jr., Morgan, T.K., Jr. and Wohl, R.A. (1986) U.S. Patent 4,581,370; (1985) Chem. Abstr. 103, 37475e; (1985) Eur. Pat. Appl. EP 131302.
157 Lis, R., Morgan, T.K., Jr., DeVita, R.J., Davey, D.D., Lumma, W.C., Jr., Wohl, R.A., Diamond, J., Wong, S.S. and Sullivan, M.E. (1987) J. Med. Chem. 30, 696–704.
158 Lis, R., Davey, D.D., Morgan, T.K., Jr., Lumma, W.C., Jr., Wohl, R.A., Jain, V.K., Wan, C.-N., Argentieri, T.M., Sullivan, M.E. and Cantor, E.H. (1987) J. Med. Chem. 30, 2303.
159 Lumma, W.C., Jr., Wohl, R.A., Davey, D.D., Argentieri, T.M., DeVita, R.J., Gomez, R.P., Jain, V.K., Marisca, A.J., Morgan, T.K., Jr., Reiser, H.J., Sullivan, M.E., Wiggins, J. and Wong, S.S. (1987) J. Med. Chem. 30, 755–758.
160 Morgan, T.K., Jr., Lis, R., Lumma, W.C., Jr., Nickisch, K., Wohl, R.A., Phillips, G.B., Gomez, R.P., Lampe, J.W., Di Meo, S.V., Marisca, A.J. and Forst, J. (1990) J. Med. Chem. 33, 1091–1097.
161 Steinberg, M.I. and Smallwood, J.K. (1989) in Handbook of Experimental Pharmacology, Vol. 89, Antiarrhythmic Drugs (Vaughan Williams, E.M., ed.) pp. 389–412, Springer, Berlin.
162 Phillips, G.B., Morgan, T.K., Jr., Nickisch, K., Lind, J.M., Gomez, R.P., Wohl, R.A., Argentieri, T.M. and Sullivan, M.E. (1990) J. Med. Chem. 33, 627–633.
163 Harris, A.S. (1950) Circulation 1, 1318–1328.
164 Doroshuk, C.M. and Sullivan, M.E. (1988) Pharmacologist 30, A177.
165 Frishman, W.H. and Laifer, L.I. in Ref. 5, pp. 207–248.
166 Lis, R., Morgan, T.K., Jr., Marisca, A.J., Gomez, R.P., Lind, J.M., Davey, D.D., Phillips, G.B. and Sullivan, M.E. (1990) J. Med. Chem. 33, 2883–2891.
167 Tomiyama, T., Tomiyama, A., Wakabayashi, S., Sajiki, H. and Sekiguchi, J. (1990) Ger. Offen. DE 3,931,402; (1990) Chem. Abstr. 113, 131987.

168 Buzby, G.C., Jr. and Colatsky, T.J. (1988) U.S. Patent 4,721,809; (1989) Chem. Abstr. 110, 212382.
169 Colatsky, T.J. and Buzby, G.C., Jr. (1987) U.S. Patent 4,665,227; (1987) Chem. Abstr. 107, 96434.
170 Follmer, C.H., Poczobutt, M.T. and Colatsky, T.J. (1989) J. Mol. Cell. Cardiol. 21 (Supplement II), S185.
171 Colatsky, T.J., Jurkiewicz, N.K., Follmer, C.H., Bird, L.B. (1989) J. Mol. Cell. Cardiol. 21 (Supplement II), S10.
172 Wenkley, M.W. and Diebold, J.L. (1989) World Patent WO8908111; (1990) Chem. Abstr. 112, 118867.
173 Cross, P.E. and Arrowsmith, J.E. (1988) Eur. Pat. Appl. EP 286,277; (1989) Chem. Abstr. 110, 75290.
174 Cross, P.E. and Arrowsmith, J.E. (1988) Eur. Pat. Appl. EP 286,278; (1989) Chem. Abstr. 110, 57291.
175 Cross, P.E. and Dickinson, R.P. (1990) U.S. Patent 4,968,704; (1990) Chem. Abstr. 113, 231211; (1990) Eur. Pat. Appl. EP 359389.
176 Cross, P.E. and Dickinson, R.P. (1988) Eur. Pat. Appl. EP 291,210; (1989) Chem. Abstr. 110, 154160.
177 Kemp, J.E. and Cross, P.E. (1989) U.S. Patent 4,797,401; (1988) Chem. Abstr. 109, 6547; (1988) Eur. Pat. Appl. EP257864.
178 Arrowsmith, J.E. and Cross, P.E. (1989) Eur. pat. Appl. EP 307,121; (1990) Chem. Abstr. 112, 7494.
179 Cross, P.E. and Dickinson, R.P. (1987) U.S. Patent 4,788,196; (1988) Chem. Abstr. 108, 94595; (1987) Eur. Pat. Appl. EP 274115.
180 Cross, P.E. and Arrowsmith, J.E. (1988) Eur. Pat. Appl. EP 284,384; (1989) Chem. Abstr. 110, 75074.
181 Arrowsmith, J.E., Cross, P.E. and Thomas, G.N. (1987) Eur. Pat. Appl. EP 245,997; (1988) Chem. Abstr. 109, 37600.
182 Arrowsmith, J.E., Campbell, S.F., Cross, P.E., Dickinson, R.P. and MacKenzie, A.R. (1989) Eur. Pat. Appl. EP 324,543; (1990) Chem. Abstr. 112, 77222.
183 Baskin, E., Serik, C., Wallace, A., Brookes, L., Claremon, D., Selnick, H., Stein, R. and Lynch, J. (1990) FASEB J. 4, A455 (Abstr. 1092).
184 Gwilt, M., Dalrymple, H.W., Blackburn, K.J., Burges, R.A. and Higgins, A.J. (1988) Circulation 78, II–150.
185 Serik, C., Baskin, E., Wiedmann, R., Brookes, L., Claremon, D., Selnik, H., Stein, R. and Lynch, J. (1990) FASEB J. 4, A455 (Abstr. 1094).
186 Stupienski, R., Wallace, A., Brookes, L., Claremon, D., Selnik, H., Stein, R. and Lynch, J. (1990) FASEB J. 4, A455 (Abstr. 1093).
187 Hardcastle, J. (ed.) (1989) Pharmaprojects, Vol. 10 (May, 1990 update).
188 Cross, P.E., Arrowsmith, J.E., Thomas, G.N., Gwilt, M., Burges, R.A. and Higgins, A.J. (1990) J. Med. Chem. 33, 1151–1155.
189 Gwilt, M., Dalrymple, H.W., Burges, R.A., Blackburn, K.J., Arrowsmith, J.E., Cross, P.E., and Higgins, A.J. (1989) J. Mol. Cell. Cardiol. 21 (Suppl. II), S11.
190 Oinuma, H., Miyake, K., Yamanaka, M., Nomoto, K.-I., Katoh, H., Sawada, K., Shino, M. and Hamano, S. (1990) J. Med. Chem. 33, 905–908.
191 Lynch, J.J., Wiedmann, R.T., Baskin, E.P., Heaney, L.A., Stein, R.B. and Cingolani, H.E. (1989) Circulation 80 (Suppl. II) II–16.
192 Sawada, K. (1989) J. Mol. Cell. Cardiol. 21 (Suppl. II), S20.
193 Sanguinetti, M.C., Siegl, P.K.S., Zingaro, G.J. (1989) J. Mol. Cell. Cardiol. 21 (Suppl. II), S21.

194 Katoh, H., Ogawa, S., Furuno, I., Sato, Y., Yoh, S., Saeki, K. and Nakamura, Y. (1988) Circulation 78 (Suppl. II), II–148.
195 Lynch, J.J., Jr., Heaney, L.A., Wallace, A.A., Gehret, J.R., Selnick, H.G. and Stein, R.B. (1990) J. Cardiovasc. Pharmacol. 15, 764–775.
196 Sano, T., Sugizama, S., Taki, K., Hanaki, Y., Shemada, Y. and Ozawa, T. (1990) Br. J. Pharmacol. 99, 577–581.
197 Li, T. and Dage, R.C. (1989) FASEB J 3, A1204 (Abstr. 5677).
198 Koerner, J.E. and Dage, R.C. (1990) J. Cardiovasc. Pharmacol. 16, 383–393.
199 Carr, A.A., Koerner, J.E., Dage, R.C., Li, T., Hay, D.A. (1989) U.S. Patent 4,868,194; (1990) Chem. Abstr. 112, 98533e.
200 Escande, D., Mestre, M., Hardy, J.-C. and Cavero, I. (1989) Circulation 80 (Suppl. II), II–607.
201 Mestre, M., Hardy, J.-C., Escande, D. and Cavero, I. (1989) Circulation 80 (Suppl. II), II–139.
202 Dumez, D., Patmore, L., Ferrandon, P., Allely, M. and Armstrong, J.M. (1989) J. Cardiovasc. Pharmacol. 14, 184–193.
203 Allely, M.C. and Alps, B.J. (1988) Arch. Int. Pharmacodyn. Ther. 295, 138–146.
204 Ezrin, A., Stankus, G., Croote, S., Volberg, W., Weldon, M., Rosi, D., D'Ambra, T., Wentland, M. and Bailey, D. (1988) FASEB J. 2, A1557 (Abstr. 7324).
205 Stankus, G., Eisenberg, W., Bailey, D. and Ezrin, A. (1988) FASEB J. 2, A1557 (Abstr. 7325).
206 Toyama, J., Kodama, I., Honjo, H. and Kamiya, K. (1989) Br. J. Pharmacol. 98, 177–185.
207 Nezasa, Y., Kodama, I., and Toyama, J. (1989) Br. J. Pharmacol. 98, 186–191.
208 Ohmura, T., Nishio, M., Kigoshi, S., Muramatsu, I. and Momose, Y. (1990) Gen. Pharmacol. 21, 493–497.
209 Ito, M., Satake, N. and Shibata, S. (1988) FASEB J. 2, A608 (Abstr. 1815).
210 Duchosal, F. and Opie, L.H. (1987) J. Mol. Cell. Cardiol. 19 (Suppl. III), S19.
211 Oexle, B., Weirich, J. and Antioni, H. (1987) J. Mol. Cell. Cardiol. 19 (Suppl. III), S65.
212 Dukes, I.D., Cleemann, L. and Morad, M. (1990) J. Pharmacol. Exp. Ther. 254, 560–569.
213 Beatch, G.N., Courtice, I.D. and Salari, H. (1989) Proc. West Pharmacol. Soc. 32, 285–289.
214 Zhang, Z.-H., Sarma, J.S.M., Chen, F. and Singh, B.N. (1990) Circulation 82 (Suppl. III) III–527.
215 Hashimoto, K., Ishii, M. and Kamiya, J. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. III) S83.
216 Kamiya, J., Hirayama, M., Ishii, M., Yokoyama, T. and Katakami, T. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. III) S70.
217 Duker, G.D. and Almgren, O.S. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. III) S82.
218 Strandlund, G., Andersson, K., Frantsi, M., Gaarder, J., Hansson, I., Malm, B., Nilsson, G., Almgren, O. and Duker, G. XIth International Symposium on Medicinal Chemistry, Jerusalem, Israel, September 2–7, 1990.
219 Mortenson, E., Yang, T. and Refsum, H. (1990) J. Mol. Cell. Cardiol. 22 (Suppl. III), S87.
220 Deana, R., Panato, L., Cancellotti, F.M., Quadro, G., Galzigna, L. (1984) Biochem. J. 218, 899–905.
221 Borchard, U., Berger, F., Hafner, D. and Kammer, T. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 337 (Suppl.), R59.

222 Neliat, G., Ducouret, P., Moreau, M. and Gargouïl, Y.M. (1982) Arch. Int. Pharmacodyn. 255, 237–255.
223 Neliat, G., Moreau, M., Ducouret, P. and Gargouïl, Y.M. (1982) Arch. Int. Pharmacodyn. 255, 220–236.
224 Rosseels, G. and Inion, H. (1983) U.S. Patent 4,378,362, (1983) Chem. Abstr. 99, 22316.
225 Rosseels, G., Peiren, M., Cornil, R., Inion, H., Prost, M., Descamps, M., Bauthier, J., Tornay, C., Collette, J.F., Colot, M. and De Claviere, M. (1983) Eur. J. Med. Chem. - Chim. Ther. 18, 339–346.
226 Gagnol, J.P., Brice, G., Schwartz, P.J. and Stone, H.L. (1985) Fed. Proc. 44, 713 (Abstr. 1767).
227 Lubisch, W., Raschack, M. and Von Philipsborn, G. (1990) Eur. Pat. Appl. EP 352,639; (1990) Chem. Abstr. 113, 58925; (1990) Ger. Offen. DE 3825559.
228 Lubisch, W. and Von Philipsborn, G. (1990) Eur. Pat. Appl. EP 352,640; (1990) Chem. Abstr. 113, 58926; (1990) Ger. Offen. DE 3825561.
229 Binder, D., Greier, G., Rovenszky, F. and Hillebrand, F. (1990) PCT Int. Appl. WO 90 02,743; (1990) Chem. Abstr. 113, 97445.
230 Franke, A., Ostersehlt, B., Schlecher, R., Rendenbach, B. and Von Philipsborn, G. (1989) Eur. Pat. Appl. EP 307,814; (1989) Chem. Abstr. 111, 151199; (1989) Ger. Offen. DE 3730718.

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3 Chemical Structures and Biological Activities of Non-Peptide Selective Kappa Opioid Ligands

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PREFACE

It is now widely accepted that there are at least three opioid receptor subtypes, mu kappa and delta. During the last decade increasing evidence has accumulated to support the hypothesis that a selective kappa opioid agonist will be a powerful analgesic without the clinically limiting side-effects that characterise morphine (e.g., respiratory depression, constipation, addiction)

and all other mu opioid selective analgesic drugs. This has provided the impetus for the discovery and preclinical development of selective non-peptide kappa opioid agonists. Some of these compounds have recently been progressed into clinical trials, although to date results describing their analgesic properties in humans have not been published. Potential side-effects include diuresis, sedation and possibly dysphoria.

The discovery of highly selective non-peptide kappa agonists has assisted the pharmacological study of this system and it has become apparent that these compounds also behave as neuroprotective agents in animal models of stroke/cerebral ischaemia. This is of particular current interest because of the lack of any effective medicine for these patients. Further developments in the kappa field have led to patents and publications disclosing selective antagonists and also the identification of agonists with limited access to the central nervous system. The potential therapeutic utility of these agents is currently being investigated.

The primary purpose of this chapter is to review the structure–activity relationships (SAR) of non-peptide kappa opioid agonists and antagonists from the viewpoint of a medicinal chemist. It is intended to present an account of work in this area published in journals and in patents from 1985 up to the end of 1990. During the late 1980's there was a significant increase in the literature on kappa opioids and this has resulted in several publications which, at the time of writing, have not been previously reviewed. Three pharmaceutical companies, Upjohn, Parke-Davis and Zambeletti (SB-Italy), have progressed kappa agonists into clinical trials, so it seems an appropriate and opportune time to review the preclinical data.

There have been several reviews covering other aspects of the opioid literature and the early work on kappa opioids. These are mentioned below partly to put the kappa work in context and partly to aid the reader who is less familiar with opioids.

OTHER REVIEWS OF THE OPIOID LITERATURE

The chemical structures and biological activities of hundreds of opioid analgesics derived from the prototype opioid drug morphine are most comprehensively described in two books published in 1986, one entitled 'Opioid Analgesics, Chemistry and Receptors' by Casy and Parfitt [1] and the other entitled 'Opiates' by Lenz et al. [2]. Follow-up articles include those by: Casy in 1989, entitled 'Opioid Receptors and their Ligands: Recent Developments' [3] which also includes sections on opioid peptides, affinity labelling and opioid receptor subtypes; Rees and Hunter in 1990 [4] covering the

medicinal chemistry and biological characterization of opioid receptors and Zimmerman and Leander in 1990 [5] entitled 'Selective Opioid Receptor Agonists and Antagonists: Research Tools and Potential Therapeutic Agents'. Furthermore, the literature on analgesics has been covered in a section of the Annual Reports in Medicinal Chemistry [6].

It should be noted that much of the early SAR data on opioids was generated using whole-animal analgesic assays rather than with isolated tissue preparations rich in receptor subtypes.

A comprehensive review of receptor selective opioid peptide analogues by Schiller [7] appeared in the previous volume of this Series and for this reason the present chapter describes only non-peptide structures. A leading review which introduces kappa opioid analgesics was written by Horwell in 1988 [8] and a subsequent article focusing on kappa receptors and analgesia by Millan appeared in 1990 [9].

The biological methods used for *in vitro* and *in vivo* study of opioid receptors are described by Leslie [10] in a review which includes useful tables summarizing the opioid receptor subtypes present in various biological tissues, and the binding selectivity of several standard reference opioid ligands.

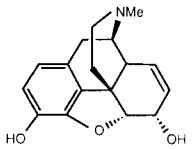
Progress in the molecular characterization of opioid receptors has been slower than for other cell-surface receptors and, to date, none has been sequenced or cloned and the second messengers mediating opioid actions are still unknown. The literature in this area has been reviewed in 1990 by Lo and Smith [11], who cite three main problems with the opioid receptor; it is difficult to solubilize, there are no simple biochemical assays to test the functional integrity of an isolated receptor extract and there are at least three receptor subtypes (designated as mu, kappa and delta).

Another area of opioid research which is still not fully understood is the molecular basis for agonist verses antagonist activity. Various hypotheses have been suggested and these are reviewed in the major articles cited above and also by Kolb [12] (see later).

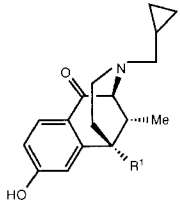
INTRODUCTION: SELECTIVE KAPPA OPIOID LIGANDS

In 1976 Martin proposed the theory that there are three subtypes of opioid receptor on the basis of behavioural studies using a chronic spinal dog model which revealed that the opioids morphine (mu) (1), ketazocine (kappa) (2) and *N*-allylnormetazocine (SKF 10047) (4) (sigma) had different effects on respiration, heart rate and locomotor activity [13]. Furthermore, these ligands were unable to replace each other to prevent withdrawal symptoms in dogs that had been chronically treated with one of the compounds.

Although Martin's original hypothesis has been modified (the sigma site is no longer regarded as an opioid receptor [14]), there is widespread agreement for the existence of three major subtypes of opioid receptor, designated mu, kappa and delta. (Some of the data suggesting further subtypes within this classification will be presented later.)

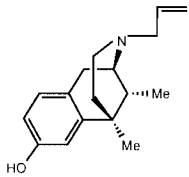


(1) morphine



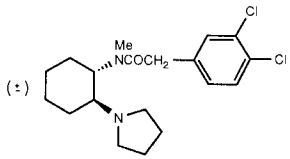
(2) ketazocine R^1 = Me

(3) ethylketazocine (EKC) R^1 = Et

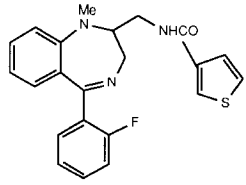


(4) SKF 10047

Both ketazocine (2), which was used in these pioneering experiments to suggest the existence of a kappa opioid receptor, and ethylketazocine (EKC) (3) are actually only very poorly selective for this site. EKC has kappa IC_{50} = 1.9 nM, mu/kappa ratio = 1.8 (for comparison, morphine mu/kappa ratio = 0.08) [15] and a fuller biochemical and pharmacological characterization of kappa opioid receptors was not possible without a more selective compound. Thus, the report in 1982 that the Upjohn compound U-50488 (5) is selective for the kappa receptor [16] represents a milestone in opioid research. U-50488 has been designated a selective kappa opioid agonist on the basis of behavioural tests and *in vitro* biochemical assays. Thus it behaves as a naloxone reversible analgesic in rodents (mouse tail flick test: U-50488 ED_{50} = 2.5 mg/kg s.c. and 16 mg/kg p.o.; morphine ED_{50} = 1.5 mg/kg s.c.) without displaying mu opioid behavioural properties (Straub tail and arched back) [16a]. Monkeys trained to discriminate ethylketazocine (kappa-like) from saline show a complete generalization to U-50488 but not



(5) U-50488



(6) tifluadom

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg

(7) dynorphin (1-9)

to morphine [16b, 16c] and in a radiolabelled receptor binding assay it has a higher affinity for the kappa receptor (K_i = 114 nM vs. [^{3}H]-EKC) than for the mu receptor (K_i = 6100 nM; mu/kappa ratio = 53 for U-50488 and 0.08 for morphine). This compound rapidly became accepted as an excellent kappa agonist and has been used widely to study the properties of the kappa receptor. It also became the chemical lead for the design of still more selective and potent kappa agonists (see later).

It is of historical interest to note that the disclosure of U-50488 occurred at roughly the same time as two chemically unrelated compounds which behave as poorly selective kappa agonist analgesics. These are the benzodiazepine, tifluadom (6) [17] and the amino-terminal nonapeptide fragment of dynorphin, Dyn (1–9), (7) (kappa K_i = 0.21 nM, mu K_i = 3.6 nM; delta K_i = 3.2 nM) [18], which is thought to be the endogenous kappa opioid agonist.

The biochemical and pharmacological properties of the kappa receptor and the differences between the kappa, mu and delta receptors have been reviewed elsewhere. The reader is directed to the opioid review articles by Rees and Hunter (1990) [4], Casy (1989) [3] and Leslie (1987) [10] and also to two shorter reviews which deal specifically with kappa agonists; the review by Horwell published in 1988 entitled 'Kappa Opioid Analgesics' [8] and the review by Millan in 1990 on kappa opioid receptors and analgesia [9]. An account of the medicinal chemistry of selective opioid agonists and antagonists was published in 1990 by Zimmerman and Leander [5].

Biological characterization of kappa ligands primarily involves *in vitro* receptor binding and smooth muscle assays and *in vivo* analgesia tests. The radiolabelled receptor binding assay is frequently performed using rodent brain homogenates, which are rich in all three opioid receptors with the mu and delta sites saturated with selective ligands, (for example, [D-Ala^2,Me-Phe^4,Glyol^5]enkephalin (DAGOL) and [D-Pen^2,D-Pen^5]enkephalin (DPDPE), respectively). These binding assays allow the receptor selectivity to be quantified more reproducibly than behavioural assays do. Intrinsic activity can be measured using the isolated rabbit vas deferens (LVD) (rich in kappa receptors) or guinea-pig ileum (GPI) (rich in mu and kappa receptors) preparations. (See Casy [3] and Leslie [10] for a list of the receptor subtypes present in various smooth muscle/CNS tissues.)

The most commonly used analgesic tests utilize a chemical, thermal or mechanical nociceptive stimulus, for example, rodent paw pressure, tail flick or hot plate assays.

KAPPA RECEPTOR SUBTYPES

There is evidence to suggest further subtypes of the originally proposed kappa opioid receptor. A brief overview of some of the data is presented here. One of the early kappa ligands, [^{3}H]EKC (3) labels two populations of non-mu, non-delta binding sites in rat brain which are partially differentiated by the Upjohn ligand U-69593 (9) and EKC (3) [19]. There is evidence for a similar situation in dog brain [20]. The U-69593 site for which U-50488 (4), ICI 197067 (38) and nor-BNI (81) are selective is regarded as a kappa site, but the EKC site has been designated a kappa-2 opioid receptor by some authors [21] and an epsilon opioid receptor (the beta-endorphin site) by other authors [19]. One of the complications is that, to date, no ligands which are selective for the EKC (a benzomorphan) site are known. Other reports suggest that there may be three [22] or even four [23] types of kappa opioid binding site, but currently it may be premature to regard these as fully characterized receptor subtypes.

POTENTIAL THERAPEUTIC USES OF KAPPA OPIOID AGONISTS

ANALGESIA

Analgesic activity is associated with agonism of kappa or mu or delta receptors, but there is a qualitative difference in other behavioural effects observed with selective ligands. In most animal species where a direct comparison is possible, kappa agonists cause sedation at a lower multiple of the analgesic dose than do mu agonists [24]. Kappa agonists are distinguished from mu agonists by causing diuresis in both animals and humans [24, 25] and selective kappa agonists (U-50488) cannot suppress the characteristic withdrawal symptoms which develop in monkeys that have become dependent upon the mu agonist morphine [26]. Monkeys chronically treated with U-50488 do develop a withdrawal syndrome, but this is qualitatively different from, and less severe than, that observed with morphine addicted animals [26]. Kappa agonists do not cause respiratory depression or constipation, which is characteristic of mu analgesics in animal models.

These differences in the behavioural properties of the opioid receptor subtypes are of considerable interest because the clinical use of currently marketed opioid analgesic drugs is limited by their undesirable side-effects, which include respiratory depression, constipation and an abuse or dependence liability. These side-effects have been associated with mu receptor ac-

tivation and would not be expected to occur with a kappa-selective analgesic.

It has been proposed, on the basis of observations made using the benzomorphan derivative (MR 2034) (8), that all kappa agonists may cause undesired dysophoria and even psychotomimesis in man [27]. Benzomorphans such as MR 2034 with affinity for sigma receptors are well-known to be associated with dysphoria. To date, there has been no report of a dose-ranging clinical study with a truly kappa selective agonist which describes the analgesic effects and the onset of dysphoric symptoms.

NEUROPROTECTION

There is increasing evidence from animal studies to suggest that opioid ligands may have a beneficial effect in the treatment of cerebral ischaemic injury. This has led to speculation that they might be useful drugs to treat stroke patients.

During the early 1980's, a number of investigators implicated the involvement of opioid receptors in the pathophysiology of circulatory shock, and at this time it appeared that the non-specific antagonist naloxone (75) was beneficial [28]. More recently, the kappa selective antagonist nor-BNI (81) has been reported to improve neurological recovery and motor co-ordination after traumatic spinal-cord injury in rats [29]. However, the development by Upjohn of selective kappa agonists has led to discrepant findings and there are now publications from several laboratories suggesting that a kappa agonist may have neuroprotective properties. In 1985, Upjohn reported that their prototype kappa agonist U-50488 (5) protects against the lethal effects of bilateral carotid artery occlusion (BCO) in gerbils and rats [30]. This may be related in part to the diuretic effects of U-50488 which reduces oedema in the forebrain after BCO [31]. Furthermore, U-50488 can enhance the early neurological recovery and post-traumatic cerebral blood flow of head-injured mice and retard the development of post-traumatic spinal cord ischaemia [32]. In a subsequent study Parke-Davis showed that their kappa agonist CI-977 (11) decreases the infarct size in a model of focal cerebral ischaemia in rats [33a, b].

The mechanism of action of this kappa mediated neuroprotective effect is under investigation. In addition to the diuretic effects mentioned above, it has been shown that CI-977 (11) is inactive in a model of focal brain ischaemia in Brattleboro rats which lack vasopressin and do not exhibit kappa-mediated water diureses [33c]. An alternative mechanism for which there is increasing evidence is an inhibition of excitatory amino-acid release. U-50488 (5) and PD 117302 (12) have been shown to block convulsions in-

duced by the excitatory amino-acid receptor agonist *N*-methyl-D-aspartic acid (NMDA) in rats [34, 35]. Subsequently, a similar effect has been shown with CI-977 (11) in mice and this was antagonized by a putative glycine agonist at the allosteric site of the NMDA receptor, D-serine, indicating that there may be an indirect interaction between the kappa receptor and the glycine site of the NDMA receptor [36]. Furthermore, in neonatal rats U-50488, (5) attenuates NMDA-induced brain injury [37].

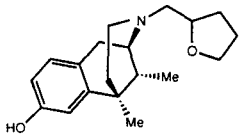
In electrophysiological studies aimed at elucidating the mechanism of action of kappa agonists, U-50488 has been shown to depress excitatory postsynaptic potentials in a rat locus coeruleus preparation, which indicates that it acts presynaptically to inhibit transmitter release [38]. Also, in spinal cord slice preparations from the 9–16-day-old rat, U-69593 (9) produced a naloxone-reversible depression of spontaneous and electrically evoked activity in dorsal horn neurones [39].

Further evidence which indicates that kappa agonists may be neuroprotective and gives insight into the possible mechanism of action is that PD 117302 (12) protects cultured rat neurons from glutamate-induced cell death [40].

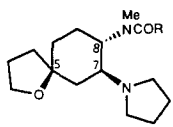
STRUCTURE–ACTIVITY RELATIONSHIPS OF SELECTIVE KAPPA AGONISTS BASED ON THE 1,2-AMINOAMIDE, U-50488 (5)

As discussed above, the discovery by the Upjohn Company in 1982 of U-50488 (5) was a milestone achievement in opioid research. This compound has significantly greater selectivity for the kappa opioid receptor than the previously used ketazocine (2) or EKC (3) and its widespread use in opioid research to study the properties of the kappa receptor has led to its being generally regarded as the prototype non-peptide kappa selective agonist.

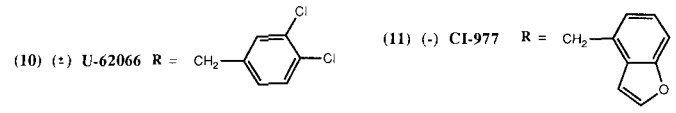
U-50488 has not only been a useful biological lead – its chemical structure, which is apparently unrelated to any of the classical morphine derivatives, has been used as the starting point for several drug discovery programmes. This has led to several publications and patents, particularly during the period 1989–1990, and these are discussed in this section.



(8) MR 2034

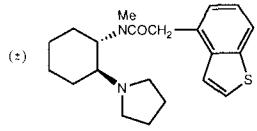


(9) (-) U-69593 R = CH_2



The first report of the properties of U-50488 was made in 1982 [16] and at this time relatively little was known about the structure–activity relationships (SAR) within this chemical class.

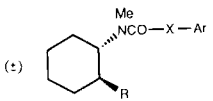
A significant development came in 1985 with the incorporation of a spiro ether group onto the cyclohexane ring, giving U-69593 (9) and U-62066 (spiradoline) (10). U-69593 is a single enantiomer with very high selectivity for the kappa receptor *in vitro* (mu/kappa ratio = 484) [15]. It was radiolabelled by a catalytic exchange of tritium for the two aromatic chlorine substituents of the precursor. This ligand is commercially available and has been widely used as a radiolabel for the kappa receptor. An X-ray analysis of the crystal structures of U-50488 and U-69593 have been determined [41, 42]. The cyclohexane ring adopts a chair conformation with both nitrogen substituents attached equatorially The amide bond is in a *trans* conformation and the absolute stereochemistry of the kappa opioid active enantiomer of U-50488 is [*S*,*S*]. There appears to be some confusion concerning the absolute stereochemistry of U-69593. The structure in the publication describing the X-ray crystallography is drawn as 5*R*,7*S*,8*S*, but the title of this paper, which gives the full chemical name of U-69593, indicates 5*S*,7*S*,8*S* [42].



(12) PD 117302

Scientists at the Parke-Davis Laboratories in Cambridge adopted the Upjohn structures as chemical leads for a drug discovery programme and extensively developed the SAR of this chemical class. The aromatic ring of U-50488, which is optimally separated by a single methylene from the amide carbonyl, was replaced with a substituted phenoxy group (13) [43–46] or with a bicyclic 10-pi aromatic, optimally a 4-benzo[*b*]thienyl or 4-benzofuranyl system (*Table 3.1*). This gives, for example, 117302 (12) [43], which behaves as a selective kappa opioid *in vitro* (receptor binding using guinea-pig brain: kappa K_i = 3.7 nM, mu K_i = 408 nM; causes naloxone reversible inhibition of electrically evoked contractions of guinea-pig ileum with IC_{50}

Table 3.1. MODIFICATIONS TO THE AMINO GROUP AND THE AROMATIC GROUP IN THE U-50488/PD 117302 SERIES



				Opioid receptor binding K_i *(nM)*			
Compd.	*R*	*X*	*Ar*	*kappa*	*mu*	*mu/kappa*	*Ref.*
5(U-50488)	1-pyrrolidinyl	CH_2	3,4-Cl_2-phenyl	10	880	88	43
12(PD117302)	1-pyrrolidinyl	CH_2	4-benzo[*b*]thienyl	3.7	410	110	43
13	1-pyrrolidinyl	CH_20	3,4,5-Cl_3-phenyl	20	140	12	43
14	NEt_2	CH_2	4-benzo[*b*]thienyl	1800	350	0.19	43
15	1-piperidinyl	CH_2	4-benzo[*b*]thienyl	250	2400	9.6	43
16	1-homopiperidinyl	CH_2	4-benzo[*b*]thienyl	40	810	20	43
17	$MeNCH_2CH=CH_2$	CH_2	4-benzo[*b*]thienyl	52	210	4.0	43
18	$MeNCH_2CH(CH_2)_2$	CH_2	4-benzo[*b*]thienyl	200	190	0.95	43
19	$MeNCH_2CH_2Ph$	CH_2	4-benzo[*b*]thienyl	30	26	0.87	43
20	1-[(*RS*)-3-(CH_2OH)pyrrolidinyl]	CH_2	4-benzo[*b*]thienyl	7	560	80	52
21	1-pyrrolidinyl	CH_2	3-benzo[*b*]thienyl	69	1400	21	52
22	1-pyrrolidinyl	CH_2	7-benzo[*b*]thienyl	17	650	39	52
23	1-pyrrolidinyl	CH_2	5-benzo[*b*]thienyl	94	475	5	52
24	1-pyrrolidinyl	CH_2	6-benzo[*b*]thienyl	146	425	3	52
25	1-pyrrolidinyl	CHMe	4-benzo[*b*]thienyl	172	2460	14	53

= 1.1 nM) [47] and an agonist *in vivo* with a similar profile of activity to U-50488. It behaves as a naloxone reversible analgesic in a variety of rodent models, for example, mouse tail clip MPE_{50} = 2.2 mg/kg s.c. (PD 117302) and MPE_{50} = 6.4 mg/kg s.c. (U-50488). In the rat it is approximately 10-times more potent by i.v. than p.o. administration [48]. Other behavioural effects were characteristic of a kappa agonist, that is, naloxone reversible diuresis and locomotor impairment but no sign of mu receptor mediated respiratory depression, inhibition of gastrointestinal motility or naloxone-precipitated withdrawal jumping in mice.

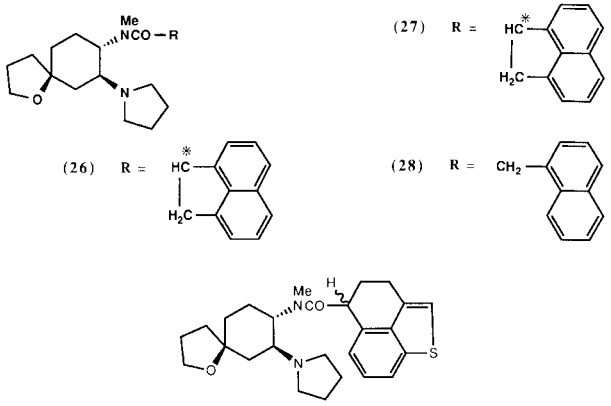
Both U-62066 (spiradoline) (10) and PD 117302 (12) are racemic mixtures of two enantiomers. The kappa opioid activity has been shown to reside in the (−) enantiomer, and in the case of U62066 the (+) enantiomer is a weak mu receptor agonist [49, 50]. (See above for discussion on absolute stereochemistry.)

Attaching an isothiocyanate group to the aromatic ring of U-50488 has been reported to produce the first site-directed alkylating agent to bind irreversibly to kappa receptors in guinea-pig membranes [51].

Other conclusions about the SAR of this chemical series that have been developed at Parke-Davis can be summarized as follows. The kappa receptor is highly sensitive to steric changes in the substituents on the basic nitrogen atom. Thus, the ring-opened diethylamino compound (14) corresponding to PD 117302 (12) has 500-fold weaker affinity for the kappa receptor (kappa K_i = 1800 compared with 3.7 nM, respectively) [43] (*Table 3.1*). Ring homologation of the pyrrolidine gives the piperidine (15) and the homopiperidine (16) which possess at least an order of magnitude weaker affinity than the parent (12) (kappa K_i = 250, 40, 3.7 nM, respectively). Attaching an *N*-allyl, *N*-cyclopropylmethyl or *N*-phenethyl group, (17–19) which are well-known modifications in the morphine congeners [1–3], removes the kappa selectivity (kappa K_i = 52, 200, 30 nM; mu/kappa ratio = 4.0, 0.95, 0.87, respectively) [43]. The only reported alternative to the pyrrolidine which preserves the receptor selectivity in this series is the 3-hydroxymethylpyrrolidine (20) (kappa K_i = 7 nM, mu/kappa ratio = 80) [52].

Variation in the binding affinity obtained for the regioisomerically substituted benzothiophenes (12, 21–24), kappa K_i = 3.7, 69, 17, 94, 146 nM, respectively (*Table 3.1*), led to the proposal that the kappa receptor is sensitive to the steric properties of the 10 pi aromatic ring. This rationale has been used to explain the significantly reduced binding affinity of the methyl substituted isomers (25) (K_i = 172 nM) [53]. Consideration of these data has led to the suggestion that on binding to the kappa receptor the 10-pi aromatic ring is held in a conformation that is mimicked by the corresponding

acenaphthene ring system. To test this hypothesis the acenaphthene derivatives (26, 27) of spiradoline were prepared and one of the diastereoisomers was shown to have 10-fold greater affinity than the corresponding conformationally flexible naphthalene analogue (28) (kappa K_i = 0.37, 1.2, 3.35 nM, respectively) and was a potent analgesic agent in the rat paw pressure assay (MPE_{50} = 0.014 mg/kg i.v.) [53]. This study was extended to include the synthesis of the rigid [6.6.5]-tricyclic benzothiophene derivatives (29, 30), one of which is kappa-selective, and further supports the hypothesis regarding the orientation of the aromatic ring (kappa K_i = 4.6 nM, mu/kappa ratio = 109) [53].



(29) (-) isomer

(30) (+) isomer

The compound which Parke-Davis selected for clinical evaluation was CI-977 (11), a single enantiomer of the 4-benzofuranyl derivative of spiradoline (10). CI-977 (11) is an exceptionally selective and potent kappa agonist (kappa K_i = 0.83 nM, mu/kappa ratio = 1520, delta/kappa ratio = 1250, rat paw pressure test MPE_{50} = 0.024 mg/kg i.v.) [54]. Its pharmacological profile in rodents is qualitatively similar to, although at lower doses than that of the typical kappa agonists described above [55]. The spiro ether group, which was first disclosed by Upjohn, appears to enhance the analgesic potency when attached to the C-4 position of the cyclohexane ring. The reason for this is not known, but a subsequent study showed that the oxygen atom of a methyl ether has a similar effect when joined to the equatorial or axial C-4 position but not to the C-5 position [54].

The *N*-methylacetamide moiety of U-50488/PD117302 has been subjected to extensive modification. Shortening the bridging chain by one methylene

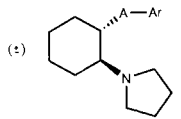
group to an *N*-methylformamide produces a selective mu rather than kappa ligand [16a], while classical amide 'isosteres' in the PD 117302 series, such as the NH amide (31), the reversed amide (32), the ester (33), the thioamide (34) and the *N*-methylamine (35) all have significantly reduced kappa receptor affinity and selectivity (*Table 3.2*) [52]. The only modification known to be tolerated is the lactam (36). This compound and the diastereoisomer (37) were prepared in order to explore the kappa opioid-active conformation of U-50488 [56]. Taken together with the acenaphthene compound (26), this lactam suggests a rigid kappa opioid-active conformation for the entire arylacetamide side-chain of the U-50488 series.

The cyclohexane ring of U-50488 (5) has been replaced with a substituted ethane by the ICI group to give a series of compounds (38–40) which are amongst the most selective agonists reported to date (*Table 3.3*). Compared with U-50488, compound (38) (ICI 197067) has 13-fold greater mu/kappa selectivity, while (39) (ICI 199441) is 275-times more potent as an analgesic [57, 58]. A conformational analysis of these compounds has led to the proposal that only those capable of occupying an energy minimum close to the more rigid U-50488 structure possess kappa agonist properties [59].

A group at E.I. du Pont de Nemours have disclosed compounds which constitute a hybrid of the ICI and the Upjohn chemical structures. DuP 747 (41), for example, contains the aromatic ring of the ICI series fused onto the cyclohexane of U-50488. It has been characterized as a kappa selective analgesic approximately equipotent with morphine in dog and rodent antinociceptive assays displaying a side-effect profile consistent with the proposed mechanism of action [60–62]. When considering the potential therapeutic use of all of these 1,2-aminoamides, it is worth noting that where both oral and parenteral analgesic doses are reported in the same species there is generally at least an order of magnitude difference between them; this indicates possible problems with an oral formulation in man. For example, in the mouse phenylquinone writhing test DuP 747 (41) has ED_{50} = 0.46 mg/kg s.c. and 6.2 mg/kg p.o. [62].

A further modification of the ICI/Upjohn structures which has been extensively studied by Zambeletti (SB, Italy) and independently by Glaxo involves cyclizing the amide *N*-methyl onto the carbon atom which was previously the C-6 position of the U-50488 cyclohexane ring. This led to the compound ZT 52656A (42) which, in the binding assays, is as selective as U-69593 or ICI 197067 (*Table 3.4*) [63] and behaves as a full agonist in the electrically-stimulated rabbit vas deferens assay (IC_{50} = 12 nM). A molecular mechanics computational study and a proton NMR spectroscopic study of the Zambeletti compounds has shown that the active conformation has a torsional angle ($N_2C_2C_7N_8$) of 60° [64]. This is in close agreement with

Table 3.2. RECEPTOR BINDING OF AMIDE REPLACEMENTS IN THE PD 117302/U-50488 SERIES



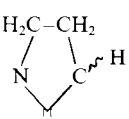
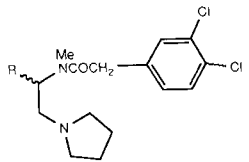
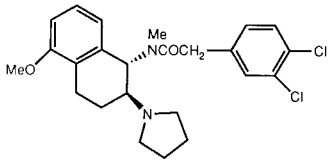
			Opioid receptor binding K_i *(nM)*			
Compd.	*A*	*Ar*	*kappa*	*mu*	*mu/kappa*	*Ref.*
12(PD117302)	$NMeCOCH_2$	4-benzo[*b*]thienyl	3.7	410	110	52
31	$NHCOCH_2$	4-benzo[*b*]thienyl	333	4800	14	52
32	$CONMeCH_2$	4-benzofuranyl	37000	20300	0.5	52
33	$OCOCH_2$	4-benzo[*b*]thienyl	261	3340	13	52
34	$NMeCSCH_2$	4-benzo[*b*]thienyl	53	700	13	52
35	$NMeCH_2CH_2$	4-benzo[*b*]thienyl	8100	14300	20	52
36		3,4-dichlorophenyl	10	225	23	56
37		3,4-dichlorophenyl	92	864	9.4	56
5(U-50488)	$NMeCOCH_2$	3,4-dichlorophenyl	15	825	55	56

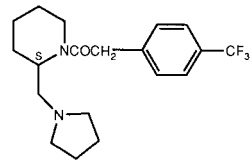
Table 3.3. RECEPTOR BINDING AND ANALGESIC ACTIVITY OF THE ICI KAPPA AGONISTS [57,58].



Compd.	*Stereochem.*	*R*	*Opioid receptor binding* K_i *(nM)*			*Mouse abdominal constriction analgesia assay* ED_{50} *(mg/kg s.c.)*
			kappa	*mu*	*mu/kappa*	
ICI 197067(38)	*S*	isopropyl	6.3	11800	1900	0.05
ICI 199441(39)	*S*	phenyl	6.9	4500	650	0.004
ICI 204879(40)	*R,S*	3,4 $(MeO)_2C_6H_3$	32.8	6400	200	0.05
U-50488 (5)	–	–	95.5	14200	150	1.1



(41) Dup 747



(42) ZT-52656A

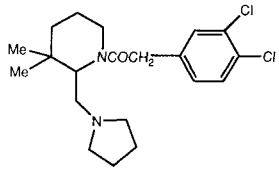
the results obtained in the ICI study and is similar to the corresponding angle between the two nitrogen atoms in the Upjohn and Parke-Davis ligands.

In view of the psychotomimetic effects in man that have been attributed to the kappa receptor, Zambeletti studied the EEG and behavioural profile in rats of compound (42). SKF 10047 and PCP (phencyclidine) showed marked similarities to each other but not to ZT 52656A while U-62066 showed certain features in common with both [65]. Further work is required to assess whether these data might have any clinical significance. Modifications to the structure of ZT 52656A which have been reported in the patent literature to produce kappa-selective agonists include methylation of the piperidine ring (43) [66] and ring fusion of an aromatic group as in compound (44) which appears to be claimed in patents by Zambeletti, Glaxo and Merck [67–69].

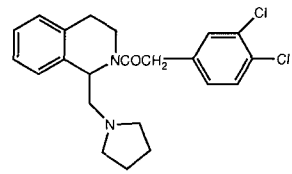
A research group at Glaxo has independently pursued a chemical strategy very similar to that of Zambeletti, and their series of kappa agonists includes further oxygen substituents on the pyrrolidine and the piperidine rings (45–47). A very potent compound is the piperazine derivative GR 103545 (48) [70]. [N.B. The orientation of the pyrrolidine with respect to the chiral carbon atom is the same as in ZT 52656A (42), despite the change

Table 3.4. OPIOID RECEPTOR BINDING SELECTIVITY OF ZT-52656A (42) COMPARED WITH OTHER KAPPA-SELECTIVE AGONISTS [63]

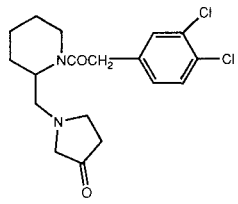
	Opioid receptor binding K_i *(nM)*			
Compd.	*kappa*	*mu*	*delta*	*mu/kappa*
ZT-52656A (42)	0.57	2341	> 10000	4107
U-62066 (10)	0.57	45.1	5979	79
U-69593 (9)	1.89	2694	> 10000	1425
U-50488 (5)	0.97	616	> 10000	635
ICI 197067 (38)	0.14	347	> 10000	2479



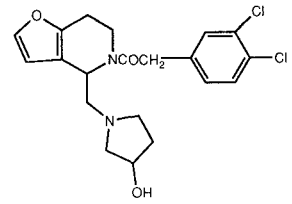
(43)



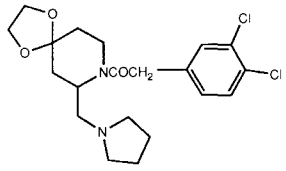
(44)



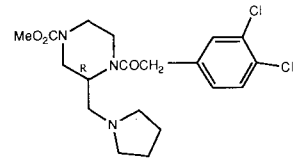
(45) GR 44821



(46) GR 91272



(47) GR 45809

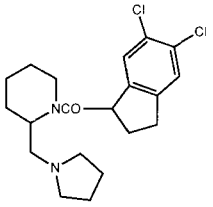


(48) GR 103545

in *RS* nomenclature.] The Glaxo compounds have been characterized as kappa agonists using isolated tissue experiments. Thus, GR 103545 behaves as a full agonist for inhibiting twitch responses in the rabbit vas deferens assay (IC_{50} = 0.02 nM) which contains opioid receptors mainly of the kappa type but has very little activity in the delta-rich hampster vas deferens assay (IC_{50} > 160 nM) and has no effect (up to 10 nM) in the mu-rich rat vas deferens. Furthermore, the effects of one of the compounds (GR 89696) in the rabbit vas deferens were antagonized by the antagonist naloxone (75) with a mean pK_B value of 7.7 and in the mouse vas deferens were antagonized by the kappa selective antagonist norBNI (81) with a mean pA_2 value of 9.9, data which support the kappa selectivity of this compound [70]. The pharmacology of GR 103545 is typical of that expected for a kappa agonist: antinociceptive in the mouse abdominal constriction test (ED_{50} = 0.00025 mg/kg s.c., 0.012 mg/kg p.o.); sedative in the mouse rotarod test (ED_{50} = 0.022 mg/kg s.c.); diuretic in water-loaded rats (at 0.008–0.12 mg/kg s.c.) and very little effect on respiration rate or gastrointestinal motility [70]. The

patent that claims GR 103545 covers both pain and cerebral ischaemia [71], which are now typical claims for a kappa agonist.

The Sankyo company have shown that conformational restriction of the arylacetamide fragment of the Zambeletti/Glaxo series as in the indane derivative (49) preserves kappa selectivity (kappa K_i = 0.67 nM, mu K_i = 698 nM, mouse phenylquinone-induced writhing ED_{50} = 1.3 mg/kg s.c.) [72]. This aromatic group contains two of the rings found in the acenaphthene derivatives (26, 27) described above.



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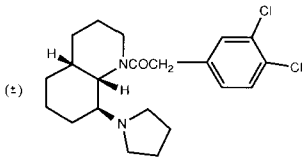
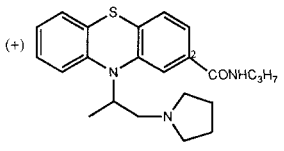


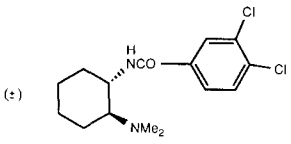
(50)

An interesting hybrid-type structure which contains both the cyclohexyl ring of the Upjohn and Parke-Davis series, and the piperidine ring of the Zambeletti and Glaxo series is the decahydroisoquinoline (50). This compound was first disclosed in a Roussell-UCLAF patent published in 1987 [73]. The *in vivo* data are not compared directly with reference compounds, but (50) appears to be a somewhat weaker analgesic than those discussed above (mouse hot plate test AD_{100} = 20 mg/kg s.c.; mouse acetic acid-induced stretching AD_{50} 1.8 mg/kg s.c.) [74]. The anti-ischaemic properties were investigated with an asphyxic anoxia test in anaesthetized rats. Analysis of the EEG activity showed that compound (50) at a dose of 5 mg/kg i.v. caused a significantly more effective and more rapid recovery of the electro-cortical activity than the controls [74].

Rhone-Poulenc have synthesized some kappa agonists which are of chemical interest because the arylacetamide fragment, which characterizes all of



(51) RP 60180



(52)

the selective compounds discussed so far, is replaced with a tricyclic phenothiazine group. However, the pyrrolidine amine is still present in RP 60180 (51) (kappa K_i = 0.55 nM, delta K_i = 1.6 nM, mu K_i = 57.1 nM) which behaves as a kappa agonist in the rabbit vas deferens (IC_{50} = 15 nM) but is without agonist activity in the delta-rich hampster vas deferens up to a concentration of 300 nM [75]. Chemical changes which have been made to RP 60180 (51) include halogenation of the C-7 position of the phenothiazine ring [76] and replacement of the C-2 amide with an amidine group which gives anti-diarrhoeal agents with no effect on the CNS [77].

Mu and kappa receptor agonists have been shown to affect central dopaminergic activity in rodents *in vivo* and *in vitro* [78, 79]. This has been investigated with RP 60180 (51), which decreases dopamine (DA) utilization in rat prefrontal cortex and in the striatum (by 30–35 % and 10 % respectively at 1–2.5 mg/kg s.c.) whereas the mu agonist morphine causes a significant increase (90–150 % and 30–40 % respectively at 5 mg/kg), an effect which is abolished by RP 60180 (1–2.5 mg/kg) [80].

NON-KAPPA OPIOID BIOLOGICAL ACTIVITY OF RELATED 1,2-AMINOAMIDES

The 1,2-aminoamides are now established as a chemical series with several highly selective kappa opioid receptor agonists. However, the biological activity of 1,2-aminoamides is not restricted to kappa analgesics. Several related structures exhibit biological activity in other systems of importance and interest. In order to appraise the significance of this chemical class and to put the SAR for kappa receptor activity into context, a selection of these compounds is discussed here. This is not a comprehensive literature review but rather a selection of a few compounds to illustrate the broad range of medicinal activity exhibited by these somewhat similar chemical structures.

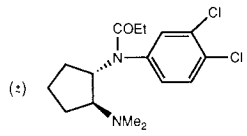
MU OPIOIDS

In the above discussion on the mu/kappa receptor selectivity of the U-50488 (5) series, the steric properties of the tertiary amine and the distance between the amide and the aromatic ring were cited as important factors. This has been exploited by the Upjohn company to give the arylformamide-dimethylamine derivative (52) which is an analgesic in the mouse tail flick test (ED_{50} = 0.2 mg/kg s.c.) and causes mu-opioid like side-effects such as Straub tail, arched back and increased locomotor activity [81]. These behavioural effects and the association constant for the morphine receptor of compound

(52) (receptor affinity, $K_i = 1.1 \times 10^{-7}$ M) indicate that it is a selective mu-opioid receptor agonist (opioid receptor subtype binding not reported).

ANTIDEPRESSANT ACTIVITY

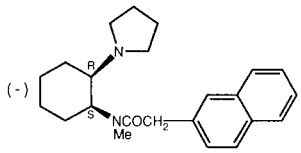
Changing the substitution pattern at the amide nitrogen of the opioid agonist (52) gives U-48753, (eclanamine, (53)) which behaves as a potent antidepressant agent. In a study aimed at elucidating the mechanism of action of this effect, it was shown that (53) does not inhibit monoamine oxidase activity but it does block biogenic amine uptake (noradrenaline, adrenaline and 5HT) and it desensitizes α-2 adrenergic receptors (as shown by its antagonism of the hypothermia induced by the α-2 agonist, clonidine) [82]. It is interesting to note and to acknowledge that the chemist who invented this U-48753 antidepressant series, Jacob Szmuszkovicz [83, 84], also discovered U-50488 [16a].



(53) U-48753
(eclanamine)

SIGMA RECEPTOR LIGANDS

Changing the stereochemistry of the 1,2-aminoamide of U-50488 from *trans* to *cis* produces potent ligands for the sigma receptor [85]. The aromatic group of this *cis*-fused series has been modified to improve the sigma receptor selectivity and the naphthyl derivative (54) has high affinity for the [^{3}H](+)-3-PPP-labelled sigma site (K_i = 8.66 nM) and greater than 100-fold weaker affinity for the dopamine D_2 receptor ([^{3}H](−)-sulpiride K_i > 10,000 nM), the kappa opioid receptor ([^{3}H]bremazocine K_i > 10 000 nM; [^{3}H]U-69593 K_i = 1667 nM) and the phencyclidine receptor ([^{3}H]TCP K_i > 10,000 nM) [86]. Further chemical modifications included changes to the amide group which gave the most selective compound from this study, the diamine (55) (sigma K_i = 1.3 nM) [86]. These ligands should provide useful pharmacological tools with which to study the properties of the sigma receptor.



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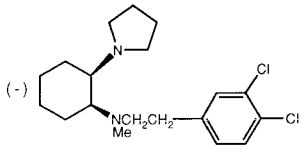
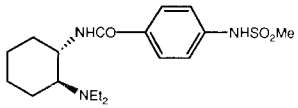


(55)

ANTIARRHYTHMIC AGENTS

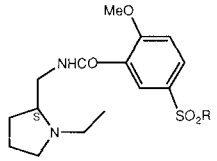
Scientists at the Berlex laboratories, working on the design of antiarrhythmic agents based on the chemical lead of procainamide, have reported compound (56) which can be thought of as a secondary amide derivative of U-50488 (5). This is active as a class III antiarrhythmic agent in isolated canine Purkinje fibres and blocks sustained ventricular tachycardia (rate > 250 bpm) elicited by programmed electrical stimulation in anaesthetized and conscious dogs (dose 1 mg/kg i.v.) [87]. Acyclic derivatives (which lack the cyclohexane ring) are also active.



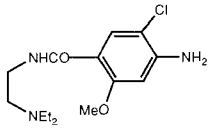
(56)

DOPAMINE LIGANDS

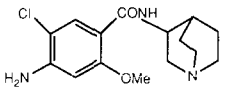
Other related secondary amides include an important class of dopamine (D_2) antagonists used as antipsychotic drugs. Two examples are sulpiride (57) and sultopride (58) which are chemical derivatives of the mixed dopamine/$5HT_3$ ligand, metoclopramide (59) [88, 89]. Modifying the amino group of metoclopramide has produced the antiemetic $5HT_3$ receptor agonist zacopride (60) [90]. The similarity between the SAR of opioid and D_2 receptors is also illustrated by the dextrorotatary isomer of the 4-phenyl piperidine (61). This compound is a mu opioid analgesic (receptor binding IC_{50} = 6.1 nM vs. [^{3}H]dihydromorphine; mouse tail flick analgesic assay ED_{50} = 0.8 μmol/kg s.c.) and also a D_2 neuroleptic (receptor binding IC_{50} = 43.3 nM vs. [^{3}H]spiroperidol; antagonism of apomorphine-induced circling behaviour ED_{50} = 1.6 μmol/kg s.c.). The *laevo* isomer is significantly less active at both of these sites [91].



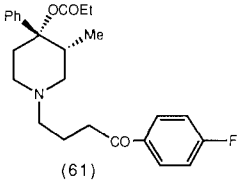
(57) sulpiride R = NH_2
(58) sultopride R= Et



(59) metoclopramide



(60)



(61)

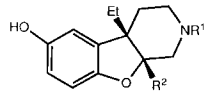
ATTEMPTS TO DEVELOP KAPPA-SELECTIVE LIGANDS FROM OTHER CHEMICAL LEADS

The majority of studies aimed at preparing kappa-selective opioids have used U-50488 (5) as the chemical lead and, as the above discussion shows, this has proved to be a highly productive approach. However, as was pointed out above, there are other structures [EKC (3), tifluadom (6) and the peptide dynorphin (7)] which have been reported to bind to the kappa receptor, albeit with poor opioid receptor subtype selectivity. Some attempts have been made to develop kappa-selective ligands from these structures and they are summarized here.

A modelling study using Dreiding models of EKC (3) has revealed similarities between the twist-boat benzomorphan conformer and the chair conformer of the benzofuro[2,3-*c*]pyridin-6-ol template (62). The basic *N*-methyl compound (62) is a mu-selective analgesic (mouse phenylquinone abdominal stretching assay ED_{50} = 5 mg/kg s.c.) (*Table 3.5*). Changing the nitrogen substituent to an *n*-propyl (63) or cyclopropylmethyl (64) group significantly increases the kappa affinity, but incorporation of other side-chains which produce analgesics in the morphine series (for example, phenylethyl) decreased kappa affinity. Substitution at the C-9a carbon with a methyl retained kappa affinity (compound (65)), but none of the reported compounds had any selectivity for the kappa receptor [92].

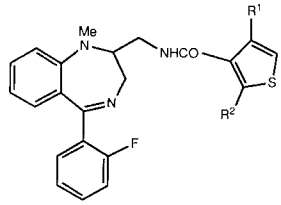
In an attempt to prepare a ligand suitable for use in an affinity column for kappa opioids, a haloacetamide group was attached to the thiophene ring of tifluadom to give compounds (66, 67). Unfortunately, neither of these derivatives was suitable because of their poor selectivity with consider-

Table 3.5. OPIOID RECEPTOR AFFINITIES OF BENZOFUROPYRIDINE DERIVATIVES [92]



			Opioid receptor binding (K_i nM)		
No.	*R^1*	*R^2*	*kappa*	*mu*	*delta*
62	Me	H	> 5000	19	212
63	n-Pr	H	335	30	231
64	$CH_2cC_3H_5$	H	34	4	53
65	$CH_2cC_3H_5$	Me	35	8	37
3 (EKC)			6	1.3	2.3

Table 3.6. OPIOID RECEPTOR AFFINITIES OF TIFLUADOM DERIVATIVES [93]



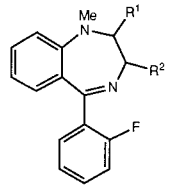
			Opioid receptor binding (IC_{50} nM)		
No.	*R^1*	*R^2*	*mu*	*kappa (bovine)*	*kappa (guinea-pig)*
66	H	$NHCOCH_2Br$	183	> 1000	193
67	$NHCOCH_2Br$	H	10	120	89

able mu receptor affinity [93] (*Table 3.6*). In addition to kappa binding tifluadom also has affinity for the pancreatic cholecystokinin (CCK-A) receptor and various 2- and 3-substituted-1,4,-benzodiazepines (68, 69) have been prepared in an attempt to separate these activities. None of the compounds improved CCK-A affinity, but (68) and (69) have a slightly increased opioid/CCK-A receptor selectivity [94] (*Table 3.7*). No *in vivo* data or opioid receptor subtype data were reported in this study.

The SAR of the putative endogenous dynorphin kappa agonists was re-

Table 3.7. OPIOID AND CCK-AFFINITY OF TIFLUADOM DERIVATIVES [94]

	CCK-receptor binding (IC_{50} nM) ([^{125}I]CCK-8)		*Opioid binding (IC_{50} nM)*
No.	*pancreas*	*brain*	*[^{3}H] naloxone*
6 [(−)-Tifluadom]	29	> 10^5	1.1
68	160	15500	1.1
69	4000	34000	8.6



(68) R^1 = CH_2NHCO-2-indolyl , R^2 = H
(69) R^1 = H , R^2 = CH_2NHCO-2-indolyl

viewed by Schiller in the previous issue of this Series [7]. Since then, studies aimed at identifying the biologically active conformation of dynorphin or synthesizing metabolically stable derivatives have continued to be published. For example, Hruby and co-workers have prepared conformationally restricted dynorphin derivatives by cyclization via two cysteine residues. The peptides (70) and (71), which are cyclized through residues 5 and 11, have high affinity but, compared with dynorphin, reduced selectivity for kappa receptors in the guinea-pig brain and they are less active in the guinea-pig ileum smooth muscle assay (*Table 3.8*). The dynorphin (1–13) derivatives (72–74) retain activity in the GPI but are of variable selectivity in the central receptor binding assay (*Table 3.8*), indicating the importance of the configuration of the Cys residues. In the mouse hot-plate test for analgesia, (74) behaves as an agonist (A_{50} = 10 μg, i.c.v.) [95].

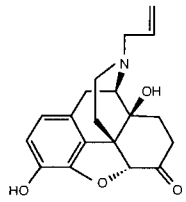
KAPPA ANTAGONISTS

Naloxone (75) is an opioid antagonist with slightly higher affinity for the mu subtype than kappa (K_i in guinea-pig brain: mu = 1.78 nM; kappa = 27 nM; delta = 1.72 nM) [96] and is widely used as a pharmacological tool to demonstrate the mechanism of action of opioid agonists. Despite much

Table 3.8. RECEPTOR BINDING AND SMOOTH MUSCLE ACTIVITY OF CYCLIC DYNORPHIN DERIVATIVES [95]

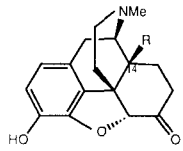
Compound	*No.*	*Opioid receptor binding (IC_{50} nM)*			*Smooth muscle assay (IC_{50} nM)*	
		kappa	*mu*	*delta*	*GPI*	*MVD*
Dyn A(1–17)-OH		0.087	3.40	2.74	2.15	22.5
[$Cys^{5,11}$]Dyn A(1–11)-NH_2	(70)	0.285	0.270	1.63	1080	421
[$Cys^{5,11}$,D-Ala^{8}]Dyn A(1–11)-NH_2	(71)	0.391	2.30	18.6	4406	1660
[$Cys^{8,13}$]Dyn A(1–13)-NH_2	(72)	0.076	0.986	3.97	1.3	20.1
[D-Cys^{8},Cys^{13}]Dyn A(1–13)-NH_2	(73)	1.76	10.3	104	2.27	24.5
[D-$Cys^{8,13}$]Dyn A(1–13)-NH_2	(74)	0.110	0.362	14.3	1.75	19.5

speculation on how the chemical differences between morphine (1) and naloxone (75) might cause their agonist/antagonist activity, there is still no satisfactory explanation. The alkyl group on the basic nitrogen atom and the C-14 substituent (-OH in naloxone) are crucial in determining opioid efficacy. The literature in this area has already been reviewed elsewhere and the reader is directed to the major reviews on opioid ligands cited above [1–5] and also to a review by Kolb on the stereoelectronic properties of opioid agonists and antagonists [12]. Several authors have proposed that there are separate binding sites or conformations of the opioid receptors for agonists and antagonists. The relative population of these two entities has been related to (i) the equatorial-axial equilibrium of the substituents on the basic nitrogen atom, (ii) the orientation of the electron lone pair on nitrogen and (iii) the existence of the skew-boat or flexible conformations of the piperidine ring [12, 97]. A recent study has shown that *N*-cyclopropylmethylmorphine derivatives with a C-14 beta alkyl substituent (instead of the C-14 OH found in naloxone (75) and naltrexone (80)) are antagonists (76–79, *Table 3.9*) [98]. The compound with greatest kappa receptor activity is the ethyl derivative (77).



(75) naloxone

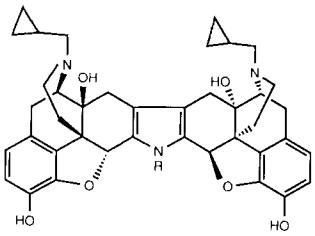
Table 3.9. ANTAGONIST ACTIVITY OF C-14 BETA-SUBSTITUTED MORPHINE DERIVATIVES [98]



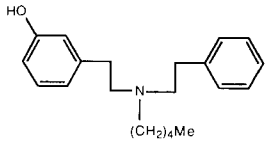
		Receptor binding (IC_{50} nM)		*Antagonism of mouse tail flick analgesia (AD_{50} mg/kg i.v) induced by*	
No.	*R*	*mu*	*kappa*	*morphine (5.6 mg/kg s.c.)*	*EKC (0.56 mg/kg s.c.)*
(76)	Me	0.2	4.0	0.005	>0.05
(77)	Et	<1	0.2	0.005	0.010
(78)	n-Pr	<0.1	0.7	0.004	0.020
(79)	CH_2Ph	1.1	14.0	0.120	0.460
(80)	OH	naltrexone		0.006	0.070

Until 1987 there were no known antagonists which demonstrated a useful selectivity for the kappa site. Then Portoghese's group reported that the bivalent ligands norbinaltorphimine (nor-BNI) (80) and BNI (81) are potent, selective kappa antagonists. These ligands were characterized in the guinea-pig ileum smooth muscle assay (rich in mu and kappa receptors), where they antagonize the effects of kappa selective agonists such as U-50488 much more effectively than they antagonize the effects of the mu agonist morphine, and in the mouse vas deferens (rich in delta receptors), where they are poor antagonists of the delta agonist [D-Ala^2-D-Leu^5]enkephalin (DADLE) (*Table 3.10*) [99]. Furthermore, in the mouse writhing assay, nor-BNI (81) (s.c. or i.c.v.) antagonizes the antinociceptive effects of EKC and U-50488 (kappa) at doses that have no effect on the antinociceptice actions of morphine (mu) or the delta agonist DPDPE ([D-Pen^2-D-Pen^5]enkephalin) [100]. An improved chemical synthesis of nor-BNI has been reported [101]. This route allowed the corresponding *N*-allyl derivatives to be prepared but they were found to be less selective.

In 1990, Roussell-UCLAF published a patent disclosing that the tertiary amine (83) is a kappa antagonist [*in vitro* receptor binding IC_{50}: kappa = 57 nM, mu = 840 nM, dopamine (spiroperidol) = 10,600 nM] which when given to rats at 20 mg/kg s.c. antagonizes the diuresis and antinociception induced by U-50488 (5 mg/kg p.o.) [102]. It remains to be seen whether this



(81) nor-BNI, R=H
(82) BNI, R=Me



(83)

Table 3.10. ANTAGONIST EFFECT OF BNI AND NOR-BNI ON VARIOUS AGONISTS [99]

	IC_{50} agonist/IC_{50} control			
	20 nM BNI (82)		*20 nM nor-BNI (81)*	
Agonist	*GPI*	*MVD*	*GPI*	*MVD*
Morphine	2.8	1.8	2.6	1.3
EKC	147.8	–	49.8	–
U-50488	167.0	–	129.3	–
Dyn(1–17)	26.7	–	44.4	–
DADLE	–	4.5	–	2.0

novel chemical structure will lead to a series of kappa antagonists and to the evaluation of the therapeutic potential of such ligands.

REFERENCES

1 Casy, A.F. and Parfitt, R.T. (1986) Opioid Analgesics, Chemistry and Receptors, Plenum Press, New York.
2 Lenz, G.R., Evans, S.M., Walters, D.E., Hopfinger, A.J. and Hammond, D.L. (1986) Opiates, Academic Press, London.
3 Casy, A.F. (1989) Adv. Drug Res. 18, 177–289.
4 Rees, D.C. and Hunter, J.C. (1990) in Comprehensive Medicinal Chemistry, Volume 3 (Emmett, J.C., ed.), Ch. 13.2, pp. 805–846, Pergamon, Oxford.
5 Zimmerman, D.M. and Leander, J.D. (1990) J. Med. Chem. 33, 895–902.
6 Evans, S.M., Lenz, G.R. and Lessor, R.A. (1990) in Annu. Rep. Med. Chem. 25, 11–20; Chipkin, R.E. and Gaeta, F.C.A. (1988) in Annu. Rep. Med. Chem. 23, 11–18; James, R. (1986) in Annu. Rep. Med. Chem. 21, 21–30.
7 Schiller, P.W. (1991) Prog. Med. Chem. 28, 301–340.

8 Horwell, D.C. (1988) Drugs Future 13, 1061–1071.
9 Millan, M.J. (1990) Trends Pharmacol. Sci. 11, 70–76.
10 Leslie, F.M. (1987) Pharmacol. Rev. 39, 197–249.
11 Lo, H.H. and Smith, A.P. (1990) Annu. Rev. Pharmacol. Toxicol. 30, 123–147.
12 Kolb, V.M. (1987) Adv. Drug Res. 16, 281–307.
13 (a) Martin, W.R., Eades, G.C., Thompson, J.A., Huppler, R.E. and Gilbert, P.E. (1976); J. Pharmacol. Exp. Ther. 197, 517. (b) Martin, W.R. (1983) Pharmacol. Rev. 35, 283.
14 Zukin, R.S. and Zukin, S.R. (1981) Mol. Pharmacol. 20, 246.
15 Lahti, R.A., Mickelson, M.M., McCall, J.M. and Von Voigtlander, P.F. (1985) Eur. J. Pharmacol. 109, 281–284.
16 (a) Szmuszkovicz, J. and Von Voigtlander, P.F. (1982) J. Med. Chem. 25, 1126–1127; (b) Lahti, R.A., Von Voigtlander, P.F. and Barsuhn, C. (1982) Life Sci. 31, 2257–2260; (c) Katz, J.L., Woods, J.H., Winger, G.D., Jacobson, A.E. (1982) Life Sci. 31, 2375–2378.
17 Romer, D., Buscher, H.H., Hill, R.C., Maurer, R., Petcher, T.J., Zeugner, H., Benson, W., Finner, E., Milkowski, W. and Thies, P.W. (1982) Nature (London) 298, 759–760.
18 Corbett, A.D., Paterson, S.J., McKnight, A.T., Magnan, J. and Kosterlitz, H. (1982) Nature (London) 299, 79–81.
19 Nock, B., Giordano, A.L., Cicero, T.J. and O'Connor, L.H. (1990) J. Pharmacol. Exp. Ther. 254, 412–419 and references cited therein.
20 Hunter, J.C., Birchmore, B., Woodruff, R. and Hughes, J. (1989) Neuroscience 31, 735–743.
21 Zukin, R.S., Eghbali, M., Olive, D., Unterwald, E.M. and Tempel, A. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 4061–4065.
22 Pesce, G.O., Gruciani, R.A., Munson, P.J. and Rodbard, D. (1990) Eur. J. Pharmacol. 183, 429–440.
23 Rothman, R.B., Bykov, V., De Costa, B.R., Jacobson, A.E., Rice, K.C. and Brady, L.S. (1990) in The International Narcotics Research Conference 1989 (Quirion, R., Jamandas, K. and Gianoulakis, C., eds.), pp. 9–12, Alan Liss, New York.
24 Dykstra, L., Gmerek, D.E., Winger, G. and Woods (1987) J. Pharmacol. Exp. Ther. 242, 413–420; Leighton, G.E., Johnson, M.A., Meecham, K.G., Hill, R.G. and Hughes, J. (1987) Br. J. Pharmacol. 92, 915–922; Tang, A.H. and Collins, R.J. (1985) Psychopharmacology (Berlin) 85, 309. Steinfels, G.F. and Cook, L. (1986) J. Pharmacol. Exp. Ther. 236, 111.
25 Peters, G.R., Ward, N.J., Antal, E.G., Lai, P.Y. and DeMaar, E.W. (1987) J. Pharmacol. Exp. Ther. 240, 128.
26 Gmerek, D.E., Dykstra, L.A. and Woods, J.H. (1987) J. Pharmacol. Exp. Ther. 242, 428–436; Gmerek, D.E. and Woods, J.H. (1986) Life Sci. 39, 987.
27 Pfeiffer, P.F., Brantl, V., Herz, A. and Emrich, H.M. (1986) Science (Washington, DC) 233, 774–776.
28 (a) Faden, A.I., Jacobs, T.P. and Holaday, J.W. (1981) Science (Washington, DC) 211, 493–494; (b) Holaday, J.W. (1983) Biochem. Pharmacol. 32, 573–585.
29 Faden, A.I., Takemore, A.E. and Portoghese, P.S. (1987) Cent. Nerv. Syst. Trauma, 4, 227.
30 Tang, A.H. (1985) Life Sci. 37, 1475–1482.
31 Silvia, R.C., Slizgi, G.R., Ludens, J.H. and Tang, A.H. (1987) Brain Res. 403, 52–57.
32 Hall, E.D., Wolf, D.L., Althans, J.S. and Von Voigtlander, P.F. (1987) Brain Res. 435, 174–180.

33 (a) Cordon, J.J., Boxer, P.A., Dominick, M.A. and Marcoux, F.W. (1990) 20th Ann. Meeting Soc. Neurosci., Soc. Neurosci. Abstr. 16 (2), 934; (b) Horwell, D.C. and Rees, D.C. (1990) Eur. Patent 393, 696; (c) Boxer, P.A., Cordon, J.J., Rodolosi, L.C. and Marcoux, F.W. (1991) Proc. XV Int. Symp. Cerebral Blood Flow and Metabolism-Brain 91, in press.
34 Tortella, F.C., Robels, L., Echevaria, E., Hunter, J.C. and Hughes, J. (1990) Life Sci. 46, PL-1–PL-7.
35 Von Voigtlander, P.F., Hall, E.D., Camacho, M., Ochoa, M., Lewis, R.A. and Triezenburg, H.J. (1987) J. Pharmacol. Exp. Ther. 243, 542.
36 Singh, L. Vass, C.A., Hunter, J.C., Woodruff, G.N. and Hughes, J. (1990) Eur. J. Pharmacol. 191, 477–480.
37 Hudson, C.J., Scherch, H.M. and Means, E.D. (1989) 19th Annu. Meeting Soc. Neurosci., Soc. Neurosci. Abstr. 15 (1), 762.
38 McFadzean, I., Lacey, M.G., Hill, R.G. and Henderson, G. (1987) Neuroscience 20, 231–239.
39 Allerton, C.A., Smith, J.A.M., Hunter, J.C., Hill, R.G. and Hughes, J. (1989) Brain Res. 502, 149–157.
40 DeCoster, M.A., Hunter, J.C., Hughes, J. and Tortella, F.C. (1990) 20th Annu. Meeting Soc. Neurosci., Soc. Neurosci. Abstr. 16 (1), 463.
41 Doi, M., Ishida, T. and Inoue, M. (1990) Acta Cryst. C46, 676–678.
42 Doi, M., Ishida, T. and Inoue, M. (1990) Chem. Pharm. Bull. 38, 1815–1818.
43 Clark, C.R., Halfpenny, P.R., Hill, R.G., Horwell, D.C., Hughes, J., Jarvis, T.C., Rees, D.C. and Schofield, D. (1988) J. Med. Chem. 31, 831–836.
44 Horwell, D.C. (1987) U.S. Patent 4, 656, 182; (1987) Chem. Abstr. 106, 131727.
45 Horwell, D.C. (1986) U.S. Patent 4, 598,087; (1986) Chem. Abstr. 104, 68752.
46 Horwell, D.C. and Schofield, D. (1987) U.S. Patent 4, 663,343; (1987) Chem. Abstr. 107, 77620.
47 Clark, C.R., Birchmore, B., Sharif, N.A., Hunter, J.C., Hill, R.G. and Hughes, H. (1988) Br. J. Pharmacol. 93, 618–626.
48 Leighton, G.E., Johnson, M.A., Meecham, K.G., Hill, R.G. and Hughes, J. (1987) Br. J. Pharmacol. 92, 915–922.
49 Meecham, K.G., Boyle, S.J., Hunter, J. and Hughes, J. (1989) Eur. J. Pharmacol. 173, 151–158.
50 VonVoigtlander, P.F. and Lewis, R.A. (1988) J. Pharmacol. Exp. Ther. 246, 259–262.
51 De Costa, B.R., Rothman, R.B., Bykov, V., Jacobson, A.E. and Rice, K.C. (1989) J. Med. Chem. 33, 281–283.
52 Halfpenny, P.R., Hill, R.G., Horwell, D.C., Hughes, J., Hunter, J.C., Johnson, S. and Rees, D.C. (1989) J. Med. Chem. 32, 1620–1626.
53 Halfpenny, P.R., Horwell, D.C., Hughes, J., Humblet, C., Hunter, J.C., Neuhaus, D. and Rees, D.C. (1991) J. Med. Chem. 34, 190–194.
54 Halfpenny, P.R., Horwell, D.C., Hughes, J., Hunter, J.C. and Rees, D.C. (1990) J. Med. Chem. 33, 286–291.
55 Hunter, J.C., Leighton, G.E., Meecham, K.G., Boyle, S.J., Horwell, D.C., Rees, D.C. and Hughes, J. (1990) Br. J. Pharmacol. 101, 183–189.
56 Cheng, C.-Y., Lu, H.-Y., Lee, F.-M. and Tam, S.W. (1990) J. Pharm. Sci. 79, 758–762.
57 Costello, G.F., Main, B.G., Barlow, J.J., Carroll, J.A. and Shaw, J.S. (1988) Eur. J. Pharmacol. 151, 475–478.
58 Costello, G.F. (1988) Eur. Patent 254, 545; Chem. Abstr. 109, 37733.
59 Costello, G.F., James, R., Shaw, J.S., Slater, A.M. and Stutchbury, N.C.J. (1991) J. Med. Chem. 34, 181–189.

60 (a) Pennev, P., Rajagopalan, P. and Scribner, R.M. (1990) U.S. Patent 4,929,627; (b) Pennev, P., Rajagopalan, P. and Scribner, R.M. (1988) Eur. Patent 260555; Chem. Abstr. 110, 8036.

61 Rajagopalan, P., Scribner, R.M., Pennev, P., Maltel, P.L., Kezar, H.S., Cheng, C.Y., Cheesman, R.S., Gangtl, V.R., Johnson, A.L., Schmidt, W.K., Tam, S.W., Steinfels, G. and Cook, L. (1990) 20th Am. Chem. Soc. Nat. Mtg., Washington D.C., Aug. 26–31, Abstr. 58.

62 Ref. 61, *ibid*, Abstr. 59.

63 Clark, G.D., Sbacchi, M. and Misiano, P. (1990) Eur. J. Pharmacol. 183, 2325–2326.

64 Vecchietti, V., Giordani, A., Giardina, G., Colle, R. and Clarke, G.D. (1991) J. Med. Chem. 34, 397–403.

65 Campi, C. and Clark, G.D. (1990) Eur. J. Pharmacol. 183, 2326.

66 Vecchietti, V. (1990) Eur. Patent 361,791.

67 Colle, R. and Giardina, G. (1990) Eur. Patent 370,732; Chem. Abstr. 113, 191325.

68 Gottschlich, R. (1990) Eur. Patent 374,756; Chem. Abstr. 113, 211862.

69 Merch GmbH (1990) Australian Patent 8,947,143.

70 Hayes, A.G., Birch, P.J., Hayward, N.J., Sheehan, M.J., Rogers, H., Tyers, M.B., Judd, D.B., Scopes, D.I.C. and Naylor, A. (1990) Br. J. Pharmacol. 101, 944–948.

71 Naylor, A., Judd, D.B. and Brown, D.S. (1990) U.S. Patent 4, 943, 578; Chem. Abstr. 112, 198421.

72 Terada, A., Iizuka, Y., Wachi, K. and Fuibayashi, K. (1990) Eur. Patent 356,247; Chem. Abstr. 113, 78419.

73 (a) Clemence, F., Le Martret, O., Fortin, M. and Delevallee, F. (1987) Eur. Patent 233,793; (b) Clemence, F., Le Martret, O., Fortin, M. and Delevallee, F. (1988) Eur. Patent 258,095; Chem. Abstr. 109, 73351.

74 Clemence F., Le Martret, O. and Delevallee, F. (1989) U.S. Patent 4,816,465;

75 Jolly, F.V., Carruette, F.O., Laduron, P.-M. and Garret, C. (1990) Eur. J. Pharmacol. 183, 2332.

76 Garret, C., Guyon, C., Plan, B. and Taurand, G. (1989) Eur. Patent 346,239; Chem. Abstr. 113, 40702.

77 Garret, C., Guyon, C., Plan, B. and Taurand, G. (1989) Eur. Patent 346,240; Chem. Abstr. 113, 23933.

78 DiChiara, C. and Imerato, A. (1988) J. Pharmacol. Exp. Ther. 244, 1067.

79 Kim, H.S., Iyengar, S. and Wood, P.L. (1987) Life Sci. 41, 1711.

80 Boireau, A., Dubedat, B.A., Laduron, D.P., Blanchard, J.-C., Doble, A. and Garret, C. (1990) Eur. J. Pharmacol. 183, 2330.

81 Cheney, V., Szmuszkovicz, J., Lahti, R.A. and Zichi, D.A. (1985) J. Med. Chem. 28, 1853–1864.

82 VonVoigtlander, P.F., Triezenberg, H.J., Losey, E.G. and Gay, D.D. (1983) Drug Dev. Res. 3, 545–554.

83 Szmuszkovicz, J. (1979) U.S. Patent 4,159,340; (1979) Chem. Abstr. 91, 157342.

84 Szmuszkovicz, J. (1979) U.S. Patent 4,156,015; (1979) Chem. Abstr. 91, 74259.

85 De Costa, B.R., Bowen, W.D., Hellewell, S.B., George, C., Rothman, R.B., Reid, A.A., Walker, J.M., Jacobson, A.E. and Rice, K.C. (1989) J. Med. Chem. 32, 1996–2002.

86 De Costa, B.R., Rice, K.C., Bowen, W.D., Thurkauf, A., Rothman, R.B., Band, L., Jacobson, A.E., Radesca, L., Contreras, P.C., Gray, N.M., Daly, I., Iyengar, S., Finn, D.T., Vazirani, S. and Walker, J.M. (1990) J. Med. Chem. 33, 3100–3110.

87 Lumma W.C., Wohl, R.A., Davey, D.D., Argentieri, T.M., De Vita, R.J., Gomes, R.P., Jain, V.K., Marisca, A.J., Morgan, T.K., Reiser, H.J., Sullivan, M.E., Wiggins, J. and Wong, S.S. (1987) J. Med. Chem. 30, 755–758.

88 Horn, A.S. (1990) in Comprehensive Medicinal Chemistry (Hansch, C., Sammes, P.G. and Taylor, J.B., eds.), Vol. 3, pp. 229–290, Pergamon, Oxford.
89 Ince, F. (1990) in Comprehensive Medicinal Chemistry (Hansch, C., Sammes, P.G. and Taylor, J.B., eds.), Vol. 3, pp. 291–321, Pergamon, Oxford.
90 Anon. (1986) Clin. Pharmacol. Ther. 40, 597.
91 Iorio, M.A., Frigeni, V., Bowman, E.R., Harris, L.S., May, E.L. and Aceto, M.D. (1991) J. Med. Chem. 34, 194–197.
92 Hutchinson, A.J., De Jesus, R., Williams, M., Simke, J.P., Neale, R.F., Jackson, R.H., Ambrose, F., Barbaz, B.J. and Sills, M.A. (1989) J. Med. Chem. 32, 2221–2226.
93 Archer, S., Seyed-Mozaffari, A., Simon, E.J. and Gioannini, T.L. (1989) Eur. J. Med. Chem. 24, 569–572.
94 Bock, M.G., DiPardo, R.M., Evans, B.E., Rittle, K.E., Whitter, W.L., Veber, D.F., Freidinger, R.M., Chang, R.S.L., Chen, T.B. and Lotti, V.J. (1990) J. Med. Chem. 33, 450–455.
95 Kawasaki, A.M., Knapp, R., Wire, W.S., Kramer, T., Yamamura, H.I., Burks, T.F. and Hruby, V.J. (1990) in Peptides Chemistry, Structure and Biology, Proc. 11th American Peptide Symposium (Rivier, J.E. and Marshall, G.R., eds.), pp. 337–338, Escom, Leiden.
96 Magnan, J., Paterson, S.J., Tavani, A. and Kosterlitz, H.W. (1982) Naunyn-Schmiedebergs Arch. Pharmakol. 319, 197–205.
97 Maurer, P.J. and Rappoport, H. (1987) J. Med. Chem. 30, 2016–2026.
98 Revesz, L., Siegel, R.A., Buescher, H.-H., Marko, M., Maurer, R. and Meigel, H. (1990) Helv. Chim. Acta 73, 326–336.
99 Portoghese, P.S., Lipkowski, A.W. and Takemori, A.E. (1987) Life Sci. 40, 1287–1292.
100 Takemore, A.E., Ho, B.Y., Naeseth, J.S. and Portoghese, P.S. (1987) J. Pharmacol. Exp. Ther. 246, 255–258.
101 Schmidhammer, H. and Smith, C.F.C. (1989) Helv. Chim. Acta 72, 675–677.
102 Cosquer, P., Delevallee, F., Droux, S., Fortin, M. and Petit, F. (1990) Eur. Patent 395, 529.

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4 Pharmacologically Active Pyridazine Derivatives. Part 2

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INTRODUCTION

The increasing interest in bio-active pyridazine derivatives, as observed within the past two decades, is well reflected by the fact that a review like the present one, which is an attempt to summarize the literature as covered by *Chemical Abstracts* from 1975 (Vol. 82) through 1988 (Vol. 108), had to be divided into two parts.

Part 1, dealing mainly with pyridazines as chemotherapeutic, antithrombotic, antisecretory and anti-ulcer agents, as well as with various central nervous system stimulants and depressants, has been published in a preceding volume of Progress in Medicinal Chemistry [1]. The present Part 2 will be devoted primarily to cardiovascular active compounds and to a compilation of miscellaneous additional pharmacological activities of pyridazine derivatives. In this part, the literature cited in *Chemical Abstracts* up to Volume 111 is included, too. Moreover, selected papers published in 1990 will be considered. For general remarks regarding the scope and limitations of the present review and for reviews on the chemistry of pyridazines see [1]. It should be mentioned that a recent update of a series devoted to the advances in pyridazine chemistry [2] also provides valuable information regarding biological activities of 1,2-diazine derivatives.

CARDIOVASCULAR AGENTS

The fact that cardiovascular diseases occupy first place as *causa mortis* in civilized countries continues to influence strongly research in the field of bioactive pyridazine derivatives also, as shown by the enormous number of patents and papers dealing with pyridazines useful as cardiovascular drugs which have appeared within the period covered by this review.

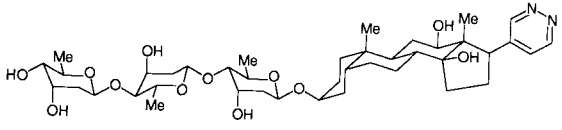
In this chapter, the agents to be discussed are classified according to their pharmacological action. Thus, cardiovascular active pyridazines are divided into the following classes: cardiotonics (including pyridazines exhibiting both positive inotropic and systemic vasodilator activity, that is, inodilators), coronary vasodilators, antiarrhythmics, cardioselective β-blockers, antihypertensive agents, antihypotensive agents, and hypocholesterolaemic agents. A compilation of pyridazines as blood platelet aggregation inhibitors and antithrombotics is given in Part 1 [1].

CARDIOTONICS

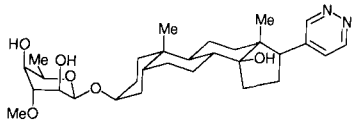
For a long period, a butenolide or a 2-pyrone ring in the 17β-position of the steroid system has been considered an essential feature for digitalis-like cardiotonic activity. However, it turned out that the unsaturated lactone ring can be replaced without loss of activity by some other moieties, including the 4-pyridazinyl group.

Thus, compound SC 4453 (1), a pyridazine analogue of digoxin studied in Belgium, has been shown to have cardioactive properties similar to those of the natural cardiac glycoside [3]. 17β-(4-Pyridazinyl)androstane deriva-

tives of type (2) have been claimed as useful agents in the treatment of tachycardia [4]. Within a series of 3β-aminocardenolide aglycones, investigated in England with respect to inotropic and chronotropic activity (isolated guinea-pig atrial preparations), the 17β-(4-pyridazinyl) analogue (3) has been studied [5]. Weak digitalis-like activities have been observed. The same authors also reported on the positive inotropic activity of the 4-pyridazinyl cardenolide congener (4) and the related *N*-oxides (5), (6) [6]. The N^1-oxide (5) turned out to be more active than the parent compound (4), whereas the N^2-oxide (6) was less active.



(1)



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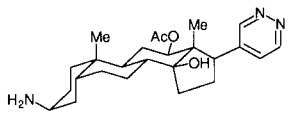
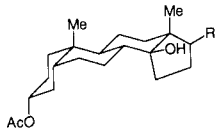
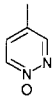


(3)



(4) - (6)

(4) R = 

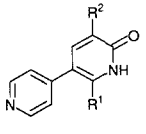
(5) R = 

(6) R = 

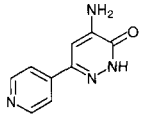
The discovery of the positive inotropic and systemic vasodilator activities of bipyridine-derived compounds, like amrinone (7) and milrinone (8), has markedly stimulated research aimed at the development of structurally related non-steroidal, non-catecholamine cardiotonics. In this context, a wide variety of pyridazinone derivatives have been prepared and investigated in search for novel agents useful for the chronic management of congestive heart failure.

It has been claimed that cardiotonic activity is retained upon replacement of the pyridone moiety in the amrinone molecule by a 3(2*H*)-pyridazinone system (9) [7]. Moreover, the patent literature covers various cardiotonic 6-(pyridyl)-3(2*H*)-pyridazinones and 4,5-dihydro congeners as exemplified by (10), which have been developed in the United States [8–11]. Also 3-amino-

and 3-hydrazino-6-(pyridyl)pyridazines as represented by formula (11) have been claimed as cardiotonic agents [12–14]. Thus, for instance, compound (11, $R^1 = NH_2$; $R^2 = H$) has been reported to exhibit significant cardiotonic activity at 0.1 mg/kg (dogs; i.v. administration) [13].



(7) R^1=H,R^2=NH_2
(8) R^1=Me,R^2=CN



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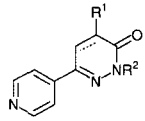
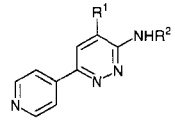
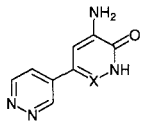


R^1=H,Me; R^2=H,alkyl
(10)

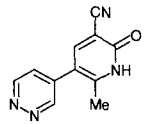


R^1=H,CN,$CONH_2$; R^2=NH_2,alkyl
(11)

Quite recently, another type of 1,2-diazine-derived compounds structurally closely related to amrinone and milrinone, namely aza-isosters characterized by the replacement of the 4-pyridyl substituent by a 4-pyridazinyl group (12), (13), (14), became available in Austria [15]. Preliminary *in vitro* investigations (guinea-pig atria; spontaneously beating isolated guinea-pig heart) indicate the azaamrinone (12) and the azamilrinone (14) to be up to 3-times more effective with regard to their ability to increase myocardial contractility than the lead compounds (7), (8) at a concentration of 10^{-5} g/ml. Similar observations have been made upon replacement of the pyridyl substituent in the cardiotonic pyridazinone (9) by a 4-pyridazinyl core (13) [15].



(12) X=CH
(13) X=N

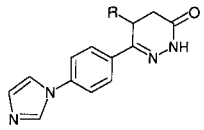


(14)

The main interest, however, so far has been focused on attempts to find effective cardiotonics in the 6-aryl-4,5-dihydro-3(2*H*)-pyridazinone series and eventually in the past decade several very interesting novel agents presently in clinical trials emerged from these efforts.

In the United States, two potent orally active positive inotropic agents, CI-914 [imazodan hydrochloride; CAS 89198-09-4, (15)] and CI-930 [CAS 86798-59-6, (16)], both characterized by a 4-(1*H*-imidazol-1-yl)phenyl substituent attached to C-6 of a 4,5-dihydro-3(2*H*)-pyridazinone system, have been developed [16]. Patents exist on compounds of this type and related unsaturated pyridazinones [17–21] and also on homologues in which the

imidazolylphenyl substituent is separated from the pyridazine system by an alkylene or alkylidene group [22]. The insertion of such a spacer group recently has been reported to result in retention of the inotropic and vasodilator activity and in enhancement of the platelet aggregation inhibitory potency [23]. On the other hand, the inotropic potency of imazodan was found to be significantly reduced upon (formal) opening of the dihydropyridazinone ring [24].

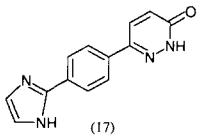


(15) R=H
(16) R=Me

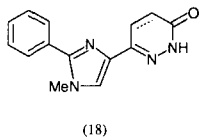
Most members of the imidazolylphenylpyridazinone series have been found to produce dose-related increases in myocardial contractility that were associated with minor increases in heart rate and decreases in systemic arterial blood pressure. Studies of structure–activity relationships in this class have been published [25–27].

Positive inotropic properties have been observed also with compounds bearing an imidazol-2-ylphenyl group (17) and with compounds in which the pyridazinone core is attached to C-4 of a 2-phenylimidazole system (18) [27].

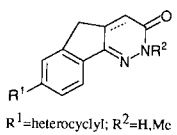
A variety of tricylic compounds [indenopyridazinones (19)] have been prepared as rigid structural modifications of compounds like CI-930 (16) [28,29]. Most of them have been found to retain the positive inotropic and direct vasodilator activity of the freely rotating pyridazinones [28]. Also, hydrazinopyridazines of type (20) have been investigated as structural analogues of CI-914 and CI-930, respectively. Whereas considerable inotropic activity has been observed in this series as well, ring closure to triazolo[4,3-*b*]pyridazines resulted in significantly less potent compounds [30].



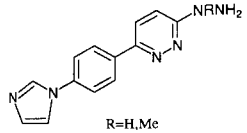
(17)



(18)



R^1=heterocyclyl; R^2=H,Me
(19)



R=H,Me
(20)

Compounds CI-914 and CI-930 have been reported to produce dose-related increases in cardiac contractile force at doses of 0.01–1.0 mg/kg and

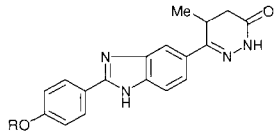
0.001–0.1 mg/kg, respectively (i.v.; anaesthetized dogs), being thus more potent than amrinone [25]. Compound CI-930 has been claimed as the most effective agent in this series [31], being 3-times more potent (i.v. administration) and up to 10-times more potent (oral administration) than milrinone (dog model) [25]. Coronary vasodilator and peripheral vasodilator properties of compound CI-914 have been described [32,33]; a review on the animal and early clinical pharmacology is available [34].

The inotropic effects of these agents are not mediated via direct stimulation of β-adrenergic receptors or indirectly by release of catecholamines, but by selective inhibition of cardiac cAMP phosphodiesterase (PDE) type III [25,35–40]. Recently, it has been demonstrated that the imidazole core is primarily responsible for PDE isozyme specificity, whereas the dihydropyridazinone moiety is responsible for inhibitory potency; the phenylene moiety obviously acts mainly as a spacer [26]. A five-point model for positive inotropic activity of PDE III inhibitors has been elaborated [41].

Using ^{14}C-labelled CI-914, the disposition in dogs and rats has been studied. Unchanged CI-914 disappeared rapidly from plasma after i.v. administration [terminal half-lives 1.6 h (dogs) and 2.2 h (rats)] [42]. Oral absorption (dogs) has been found to be rapid and complete; the major route of elimination is urinary excretion [43]. Evaluation of CI-914 in patients suffering from congestive heart failure has been reported [44] as also has the determination of CI-914 in human plasma [45]. The synthesis of carbon-14 labelled CI-930 has been reported as well [46]. Recently, related pyrazolone derivatives have been prepared as ring contracted analogues of imazodan [47].

Pharmaceutical preparations containing imidazolylphenylpyridazinones have been claimed as medicaments for the treatment of congestive heart failure [48,49]. Moreover, it should be mentioned that deaza-analogues, namely, pyrrol-1-ylphenyldihydropyridazinones, have also been claimed as cardiotonics [50,51].

Another pyridazinone-derived inotropic agent with cAMP phosphodiesterase inhibitory activity is pimobendan [UD-CG-115-BS; CAS 74150-27-9, (21)], which has been developed in Germany. In contrast to the imidazolylphenylpyridazinone CI-930 (16) discussed above, in pimobendan the imidazole nucleus (substituted at C-2 by a 4-methoxyphenyl group) is fused to the benzene moiety attached to C-6 of the 4,5-dihydro-5-methyl-3(2*H*)-pyridazinone system.

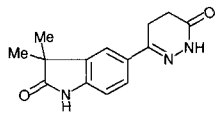


(21) R=Me
(22) R=H

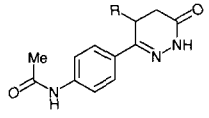
This agent emerged from investigations of a variety of benzimidazolylpyridazinones exhibiting also antihypertensive and antithrombotic activity [52–55]. Additional patents are available on cardioactive compounds of this class [56–58]. Recently, 4,5-cyclopropano-pyridazinone congeners have been claimed as cardiovascular agents [59]. There is also a patent on structurally related compounds in which the methoxyphenylbenzimidazolyl moiety has been replaced by a 2-(substituted)-aminobenzoxazolyl substituent [60].

Pimobendan is an orally effective cardiotonic agent which, in addition, produces arterial and venous dilatation. *In vivo* experiments (guinea-pigs, cats, dogs) indicated pimobendan to be 5–6-times more active than sulmazole. A dose of 1 mg/kg (p.o.; conscious dog) of pimobendan induced a marked positive inotropic effect which lasted for more than 8 h [61]. The mechanism of the positive inotropic effect has been analyzed [62,63]. There are additional reports on the pharmacology of (21) [64–67]; and its use in traumatic shock (rat model) has been described [68]. Pimobendan is rapidly absorbed and excreted in man (half-life 0.9 ± 0.5 h) [63]. The major metabolite (formed by *O*-demethylation) is UD-CG 212 Cl (CAS 77469-70-6) (22) which has been found to be more potent at increasing myocardial contractile force than (21) [69]. Recent studies on the pharmacology [70–72] and clinical trials of (22) have been published [73].

Within a series of 4,5-dihydro-3(2*H*)-pyridazinones bearing a substituted indole system at C-6, which have been prepared in the United States [74,75], indolidan (LY195115; CAS 100643-96-7) (23) has been found to possess the most promising cardiovascular profile [76]. Indolidan exhibits vasodilator and inotropic effects as well and appears to be one of the most potent and long-acting oral non-glycoside, non-catecholamine cardiotonic described so far. It has been developed as a lactam analogue of acetamidophenyl-substituted dihydropyridazinones of type (24), which are potent inotropic agents as well [76]. It is also of interest to note that an imidazole substituent, as present in compounds CI-914 and CI-930 (see above), can serve as an acetamido surrogate. Structure–activity relationships in the indolylpyridazinone series have been discussed [76]. The three-dimensional structure of (23) has been mapped using X-ray crystallography [77].



(23)



R=H,Me

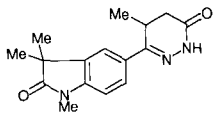
(24)

A dose of 7.0 μg/kg of indolidan (i.v. administration) resulted in a 50% increase in contractility in anaesthetized dogs. A selective inotropic response has been observed with conscious dogs upon oral administration of 25 μg/kg of (23). The haemodynamic profile of (23) has been evaluated in anaesthetized dogs [78]. The acute and subchronic toxicology of indolidan has been studied (rats, dogs) [79].

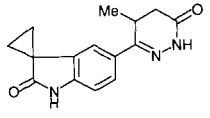
Indolidan has been shown to be a potent highly selective inhibitor of cyclic nucleotide phosphodiesterase (PDE) located in the sarcoplasmic reticulum [80], being bound more avidly by PDE III than cAMP ($K_i = 80$ nM) [80]. Quite recently, tritium-labelled LY186126 (25), a compound that mimics indolidan pharmacologically and biochemically as well, has been prepared as a tool for studying the binding site for PDE-inhibitor cardiotonics [81].

Pharmacokinetic and metabolic investigations of indolidan [82] and the preparation of carbon-14- and deuterium-labelled indolidan have been described [83]. The major metabolite isolated from monkey urine has been shown to result from oxidation of the dihydropyridazinone core to the pyridazinone system.

The effects caused by replacement of the geminal dimethyl grouping in the indole part of indolidan by a spiroalkane ring system have been investigated recently [84]. It has been found that ring size and inotropic potency are inversely related and that potency within this series can be further increased by incorporation of a methyl substituent into the pyridazine nucleus. The most potent compound is represented by formula (26). It has an ED_{50} of 1.5 μg/kg (i.v. administration, anaesthetized dogs).



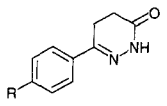
(25)



(26)

The studies have been extended also to pyridylphenyl substituted dihydropyridazinones of type (27), (28) based on the hypothesis that a hydrogen-bond acceptor moiety three atoms removed from the phenyl ring [as present in (15), (24), (23)] may be crucial for high cardiotonic activity [85,86]. The 3-pyridyl derivative (27) has been found to be a potent inotrope ($ED_{50} = 20$ μg/kg, i.v.) being 2-fold more active than the isosteric imidazolyl derivative (15), whereas the 4-pyridyl congener (28) and a compound having the 3-pyridyl moiety replaced by a phenyl group were significantly less active [86].

Despite the fact that cardiotonics of the above-mentioned class (PDE III

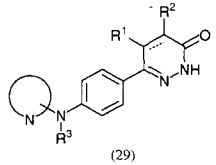


(27) R=3-pyridyl
(28) R=4-pyridyl

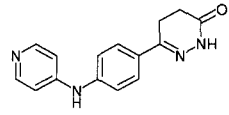
inhibitors) have been shown to produce haemodynamic and functional improvements when administered to severely ill CHF patients who were refractory to established therapy, there is some concern that these agents may increase mortality. Accordingly, there is still controversy regarding their therapeutic use [87,88].

In Japan, a variety of heterocyclylaminophenylpyridazinones as represented by the general formula (29) have been prepared and investigated [89–92]. Structure–activity relationships have been discussed [92]; additional classes of compounds in which the heterocyclic substituent is separated from the aminophenyl ring by a carbon chain have been patented [93,94]. From these studies, a novel class of pyridazine-derived cardiotonics and vasodilators has emerged.

From this series, compound MCI-154 (CAS 98326-33-1) (30) has been investigated in detail [95,96]. *In vivo* studies (anaesthetized dogs) revealed that doses of 0.3–100 μg/kg (i.v. administration) of MCI-154 produce dose-dependent increases in dP/dt_{max} and cardiac output, and decreases in arterial blood pressure and total peripheral resistance. The positive inotropic effect of (30) has been found to be superior to that exhibited by amrinone and milrinone [97,98]. It has been stated that MCI-154 exerts its activity probably by increasing the calcium-ion sensitivity of the contractile protein system of the cardiac skinned fibres [99,100]. A recent investigation suggests that inhibition of phosphodiesterase III is an important component of its cardiotonic activity [101].



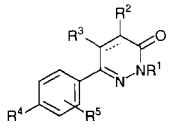
(29)



(30)

In addition to the dihydropyridazinones discussed above, the patent literature covers a wide variety of 6-phenyl-substituted derivatives in which the benzene moiety is either further substituted or annulated to a heterocyclic

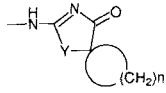
system. Some selected examples of such compounds exhibiting cardiotonic activity are given in formulae (31)–(34) [102–120], (35)–(37) [121–123], (38,39) [124,125], (40)–(44) [126–131]. A number of other recent patents cover this field [132–157].



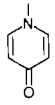
R^1,R^2,R^3=H,alkyl

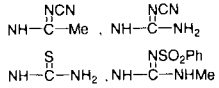
(31) - (34)

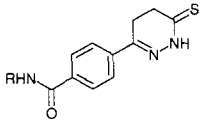
(31) R^4,R^5=H,halo,alkyl,OH OPh,alkoxy,NH_2,CN polyfluoroalkoxy acylamino,NO_2,F_3C

(32) R^5=H, R^4= 

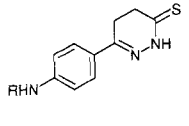
Y=S,subst.N

(33) R^5=H, R^4= 

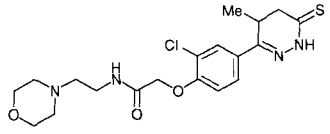
(34) R^5=H, R^4= 



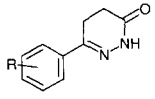
(35) R=H,alkyl



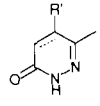
(36) R=H,alkyl,acyl



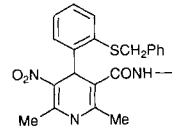
(37)

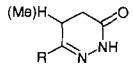


(38),(39)

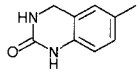
(38) R = 

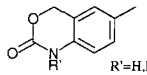
R'=H,Me

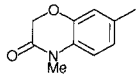
(39) R = 

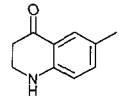


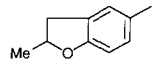
(40) - (44)

(40) R = 

(41) R = 
R'=H,Me

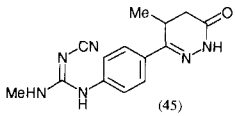
(42) R = 

(43) R = 

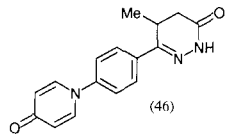
(44) R = 

As development candidates, two (substituted)phenyldihydropyridazinones, namely SK&F 94836 (45) and SK&F 95654 (46) which have been prepared in the United Kingdom, should be mentioned [158].

A recent report from the U. K. deals with 1,4-bis(oxodihydropyridazinyl)benzenes and congeners which are also potent phosphodiesterase inhibitors and inodilators [159]. This investigation, together with computer-aided modelling studies on various phosphodiesterase III inhibitors [160], may well stimulate the rational design of additional pyridazine-derived inodilators.

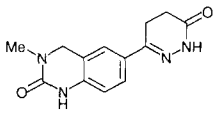


(45)

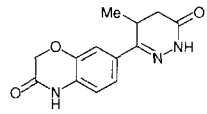


(46)

In the United States, compound RGW-2938 (47) has been developed as a selective positive inotropic agent; its synthesis and pharmacological actions have been reviewed [161]. Related compounds, in which the dihydropyridazinone system is replaced by a benzoxazinyl system instead of the quinazoline system, have recently been studied in India and in the United States [162,163]. From this series, bemoradan (48) was found to be a very potent orally active, long-acting inotropic vasodilator agent in canine models (i.v. $ED_{50} = 5.4$ μg/kg; 24h duration of action) [163].

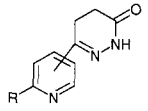


(47)



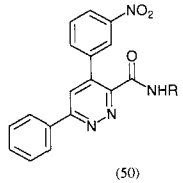
(48)

Moreover, there are patents on pyridazinone-derived cardiotonic compounds in which the phenyl substituent is separated from the pyridazinone system by an alkylene or alkylidene bridge [164,165] and on various azolyl-substituted pyridylpyridazinones of type (49) [166]. Cardiovascular 6-phenylpyridazines of type (50) have been patented [167]. In Taiwan, cyclohexylammonium 3,6-pyridazinediolate (51) has been reported to show positive inotropic activity [168].

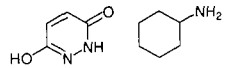


R=1,2,4-triazol-1-yl, imidazol-1-yl

(49)



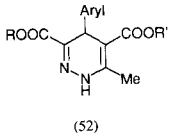
(50)



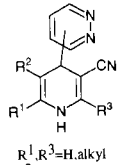
(51)

CORONARY VASODILATORS

Most of the cardiotonic pyridazine derivatives discussed in the preceding chapter also exhibit vasodilator effects. In addition, various dihydropyridazine derivatives like (52), structurally closely related to calcium-antagonistic Hantzsch-type dihydroypyridines, have been patented as coronary vasodilators [169–171]. Another type of pyridazine analogue of the previously mentioned dihydropyridines, namely compounds of type (53), in which the aryl substituent at C-4 is represented by a pyridazine nucleus, has been claimed in a patent [172]. Quite recently, pyridazinyldihydropyridine-3,5-dicarboxylates (54,55) have been prepared in Austria [173].

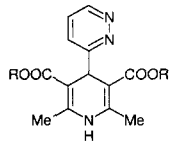


(52)



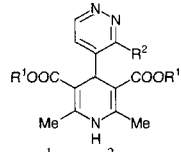
R^1,R^3=H,alkyl
R^2=SO_2R,COR

(53)



R=alkyl

(54)

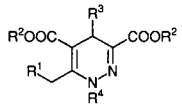


R^1=alkyl; R^2=H,Me

(55)

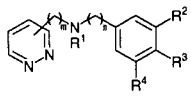
ANTIARRHYTHMICS

Dihydropyridazines of type (56) have been claimed as antiarrhythmic and cardiotonic agents [170], while compounds of type (57) and (58) show antiarrhythmic activity [174,175].



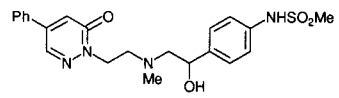
R^1=halo; R^2=alkyl
R^3=aryl,heteroaryl
R^4=H,alkyl

(56)



R^1=H,alkyl,aryl
R^2=subst.aminomethyl
R^3=H,NH_2,OH,OR
R^4=H,subst.aminomethyl
n,m = 1-5

(57)

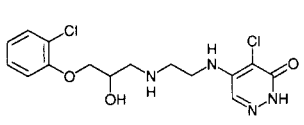


(58)

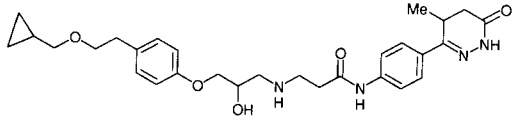
CARDIOSELECTIVE β-BLOCKERS

From a variety of basic substituted pyridazinones investigated in Germany [176], ridazolol (59) (CAS 83395-21-5) has been selected for further evaluation as a cardioselective β-blocker, since *in vivo* studies (anaesthetized dogs) had revealed that (59) is characterized by a high degree of cardioselectivity. The haemodynamic effects of ridazolol have been studied [177,178]. Doses of 20–80 mg of ridazolol have been reported to cause dose-related decrease in exercise-induced tachycardia (for 8 h) and in systolic blood pressure (for 4 h) [179].

In the U. K., it has been found quite recently that substituted dihydropyridazinones (60) act as combined β-adrenoceptor antagonists and vasodilators. The effect of stereochemistry on biological activity has been discussed [180]. Other dihydropyridazinone derivatives which show β-sympatholytic and cardiac stimulant actions are described in a patent [181].



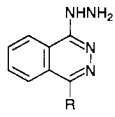
(59)



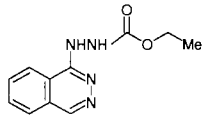
(60)

ANTIHYPERTENSIVE AGENTS

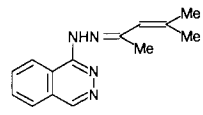
The potent and long-lasting antihypertensive effect of the peripherally acting vasodilator hydralazine (61) and of related hydrazinophthalazines like compounds of type (62)–(64) has prompted extensive research aimed at the development of novel antihypertensive agents containing the pyridazine nucleus. In the following discussion, which is devoted to compounds characterized by a 3-hydrazinopyridazine substructure, only mononuclear pyridazine derivatives will be presented; antihypertensive agents derived from phthalazine, cinnoline and other condensed pyridazines will not be included.



(61) R=H (Hydralazine)
(62) R=$NHNH_2$ (Dihydralazine)

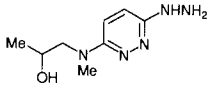


(Ecarazine)
(63)

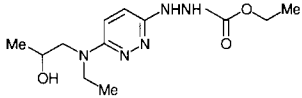


(Budralazine)
(64)

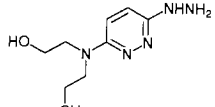
A wide variety of C-6-substituted 3-hydrazinopyridazines has been investigated in Italy. A review covers some of the results obtained up to 1980 [182]. Most of these compounds, prepared as potential antihypertensive agents, bear a mono- or di-substituted amino group at C-6; the terminal NH_2-function of the hydrazino group also has been varied [183–193]. Structure–activity relationships have been discussed [183]. Compounds with a free hydrazino group have been found to be strong inhibitors of pyridoxalphosphate-dependent enzymes (diamine oxidase, benzylamine oxidase, aspartate aminotransferase, dopa decarboxylase) *in vitro*. *In vivo*, inactive products obviously are transformed into active metabolites with a free hydrazino moiety [194–196]. From these studies of C-6 aminosubstituted 3-hydrazinopyridazines several interesting antihypertensive agents emerged: pildralazine [propildazine, atensil, ISF 2123 ; CAS 64000-73-3, (65)], cadralazine [ISF 2469, DC 826; CAS 64241-34-5, (66)], and oxdralazine [minoten, DL 150 IT, L 6150, CAS 17259-75-5, (67)].



(65)



(66)



(67)

Pildralazine (65), when administered i.v. or orally, displays a high antihypertensive activity in conscious hypertensive animals (rats, dogs). The hypotensive effect, caused by oral doses of 0.1 mg/kg to 1 mg/kg, has been reported to last more than 24 h. The therapeutic index (LD_{50}/ED_{25}; rats; oral administration) has been found to be 70-times higher than that of hydralazine due to both higher potency and reduced toxicity [197]. It has been concluded that (65), like hydralazine, acts on peripheral smooth muscle on specific receptors which are physiologically affected by ATP released from

sympathetic nerve terminals [198–201]. Renal function studies (dogs) with (65) alone and in combination with propranolol have been published [202]; the efficacy of long-term treatments of spontaneously hypertensive rats with (65) or combinations thereof with propranolol and dihydrochlorothiazide has been evaluated [203]. Results of additional pharmacological investigations have been published [204,205]. The metabolism and degradation of (65) in aqueous solution have been investigated [206,207]. Whereas a lack of carcinogenic activity has been reported for pildralazine in 1983 [208], a report published in Germany in 1985 states the drug to be mutagenic with auxotrophic mutants of *S. typhimurium* and *E. coli* [209].

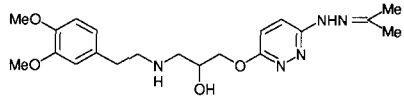
As in compound (65), the amino group in cadralazine (66) bears a 2-hydroxypropyl substituent. Cadralazine differs from pildralazine by an ethoxycarbonyl group attached to the terminal N-atom of the hydrazino moiety and by replacement of the methyl group at the amino nitrogen by an ethyl group. Masking the hydrazino moiety protects this group from acetylation, a reaction which is a capacity-limiting step in the metabolism of hydralazine. Extensive pharmacological studies [210,211] revealed (66) to be an orally effective, direct vasodilator with marked activity on the renal vascular bed. The antihypertensive effect is slow in onset and long-lasting. The effects of (66) have been shown not to be due to a blockade of α-adrenoceptors, sympathetic neurons or ganglionic transmission [210]. Toxicological studies indicate a high degree of safety [212, 213], and mutagenic or carcinogenic effects have not been detected [212]. Pharmacokinetic studies [214–219] and reports on the analytical profile of (66) are available [216,220]. Cadralazine after oral administration is rapidly and almost completely absorbed. Metabolic studies [221,222] revealed (66) to be mainly excreted in urine as unchanged drug. To a small extent (0.3–1.7% of the dose administered), a metabolite which is 60-times more potent than (66) is formed by removal of the ethoxycarbonyl group.

Cadralazine has been launched under the trade names cadraten and cadrilan. Pharmacological, pharmacokinetic and clinical studies on it have been reviewed [212]. A once-daily dosage of 15 mg cadralazine has been shown to be effective in the treatment of mild to severe hypertension and to be well tolerated. The therapeutic efficacy and tolerability can be improved by combining cadralazine with a β-adrenoceptor antagonist [212].

Oxdralazine (67) is characterized by a bis(2-hydroxyethyl)amino group attached to position 6 of 3-hydrazinopyridazine. The antihypertensive effect of (67) has been studied in rats (spontaneous, desoxycorticosteron-induced, and renal hypertension) [223]. Its effect in the long-term treatment of hypertensive rats on plasma and kidney renin activities has been reported [224]. The effects of (67) on the general and cardiac haemodynamics of anaesthe-

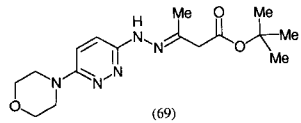
tized dogs also have been investigated [225]. From comparative studies of oxdralazine and hydralazine in hypertensive patients (i.v. administration), it has been concluded that (67) is at least 5-times more potent than hydrazinophthalazine [226,227]. In patients with renal or essential hypertension, oxdralazine (67), administered intravenously at a dose of 2–9 mg, has been found to cause marked hypotension and increase of cardiac output [228]. Its disposition and metabolism in rats [229] as well as in man [230] have been studied. Ring closure of the hydrazinopyridazine moiety to afford a 1,2,4-triazolo[4,3-*b*]pyridazine system has been reported [229,231].

In addition, various antihypertensive 3-hydrazinopyridazine derivatives bearing an alkoxy group at C-6 have been studied in Italy [183,232,233]. In particular, compound ISF 3382 (68) appears to be an interesting vasodilating β-adrenergic blocking agent. This compound has been developed in an attempt to overcome the reflex tachycardia typical of hydralazine-like compounds by attaching a 3-alkylamino-2-hydroxypropoxy substituent (characteristic of various β-adrenergic blocking agents) to a 3-hydrazinopyridazine system. Compound (68) has been reported to be highly β_1-selective and considerably less toxic than hydralazine [232,233].



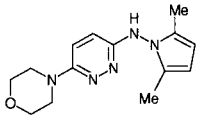
(68)

In Hungary, much effort has been devoted to the development of antihypertensive hydrazinopyridazine derivatives with higher activity and lower toxicity than hydralazine [234–236]. Among a wide variety of 6-substituted-3-pyridazinylhydrazones prepared and screened for hypotensive activity in normotensive cats and spontaneously hypertensive rats [236], compound (69) (GYKI 11679, RGH 5526; CAS 69579-13-1) appears to be the most interesting agent, being 5-times more active than hydralazine but being markedly less toxic [236]. Additional reports on pharmacological investigations [236–240], and structure–activity relationships in this series have been published [236]. Metabolic studies in rats and dogs revealed compound (69) to be mainly transformed into the corresponding 1,2,4-triazolopyridazine [241].

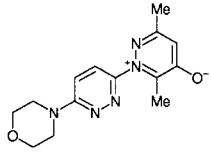


(69)

In mopidralazine [MDL 899; CAS 75841-82-6, (70)], a hydrazinopyridazine derivative prepared in Italy, carbon atom 6 bears a morpholino substituent. In this case, the terminal nitrogen atom of the hydrazino group, however, is incorporated into a pyrrole ring. Such compounds have been patented [242,243]. In developing these compounds, again the idea was to prevent toxic and mutagenic effects as observed with hydralazine by masking the free hydrazino group. Out of this series, mopidralazine has been selected for further development due to its vasodilator antihypertensive action (equipotent to hydralazine after p.o. administration), its low toxicity and the lack of mutagenic activity [244]. Mopidralazine is slower in onset and longer lasting than hydralazine and is devoid of adrenergic system stimulation [245]. Its antihypertensive action is mediated by arteriolar dilatation [246, 247]. Studies of the pharmacokinetics in rats and dogs have been reported [248]. In the rat, (70) is rapidly metabolized [249], mainly by pyrrole-ring opening and subsequent formation of a mesoionic 3-(1-pyridazinyl)pyridazine (71) [249,250]. In dogs, oxidative cleavage of the morpholine ring has been found to be the primary metabolic attack [251]. Various diaza-isosters of mopidralazine-type compounds in which the pyrrole ring is replaced by a 1,2,4-triazole system have been prepared. These compounds, however, have been found to be devoid of antihypertensive activity [252].



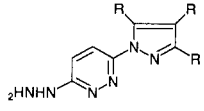
(70)



(71)

A Hungarian research group reported on the hypotensive activity of 3-(pyrazol-1-yl)pyridazines of type (72) in rats [253–256]. The activity of these compounds (in which both of the hydrazino nitrogen atoms are incorporated into a five-membered aromatic ring system) has been found to be mediated by inhibition of prostaglandin-catabolizing enzymes.

In Germany, a variety of 6-heteroaryl-3-hydrazinopyridazines as represented in formula (73) have been studied [257,258]. Imidazole-derived compounds have proved particularly active in this series.



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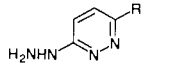
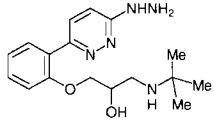


R= imidazol-1-yl, pyrazol-1-yl, 1,2,4-triazol-1-yl, indol-1-yl, carbazol-5-yl

(73)

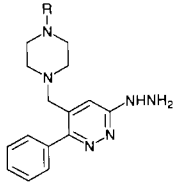
In the United Kingdom, the hydrazinopyridazine derivative prizidilol [SK&F 92657, CAS 59010-44-5, (74)] has been designed in search of a compound possessing both direct-acting vasodilator and β-adrenoceptor antagonistic properties [259,260]. The substituent attached to C-6 of the pyridazine system in this compound is a typical β-blocking moiety. There is also a patent on congeneric compounds in which C-3 of the phenol ether grouping is linked to the diazine moiety [261]. The pharmacology and toxicology of (74) have been studied extensively [262–285]. The therapeutic profile of this compound initially appeared to be close to ideal for an antihypertensive agent. However, adverse findings in long-term toxicological studies finally prevented the completion of development.



(74)

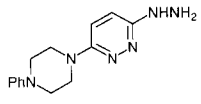
There are numerous patents claiming 3-hydrazinopyridazine derivatives with amino-substituted alkoxy side-chains at C-6 as β-blocking vasodilating agents [188,286–293]. Other 3-hydrazinopyridazine-derived antihypertensive agents are covered by patents [294–301], or described in the literature [302–306]. It should be noted that with structurally very simple hydrazinopyridazines like 6-hydrazino-3-pyridazinecarboxamide (hydracarbazine, 2105 TH; CAS 3164-47-9), antihypertensive activity has been observed.

In Spain, various 6-phenyl-5-aminomethyl-3-hydrazinopyridazines have been prepared and investigated [307]. The antihypertensive activity of compounds of type (75) has been found to exceed the activity of hydralazine. Similar activity has been observed with compound (76) [308]. The activity of the 4-piperazinopyridazine derivative (77) was found to equal that of hydralazine [309]. Also replacement of the phenylpiperazinomethyl substituent in compound (75) by a morpholinomethyl group was found to afford antihypertensive agents [310].



R=Ph,4-OCH_3-C_6H_4

(75)



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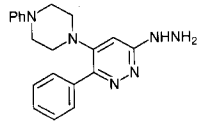
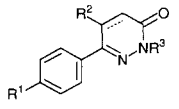


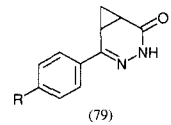
(77)

In addition to the hydrazinopyridazine derivatives discussed above, a wide variety of pyridazinones and 4,5-dihydropyridazinones exhibit interesting antihypertensive activity. Some selected examples of such compounds claimed in patents are given in formulae (78) [18–20,51,111,116,129,311–335], (79) [169,336], (80) [169,337], (81) [338–341], (82) [52–56,59,60, 127,342–357], (83) [358–362], and (84) [363–365]. For many of these compounds also cardiotonic and/or antithrombotic activity has been claimed (compare Part 1 of this review [1]).



R^1=NH-acyl,NH-CH_2-heteroaryl,NH_2,Br,CN,F, NH-subst.alkyl,subst.alkyl,imidazolyl,pyrrolyl acyl,cycloamino,subst.guanidino,alkoxy
R^2=H,alkyl,hydroxyalkyl
R^3=H,alkyl

(78)



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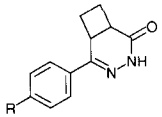

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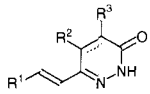
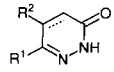


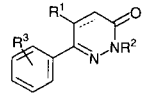
R^1=pyridyl,subst.pbenyl, subst.imidazolyl
R^2,R^3= H,alkyl

(81)



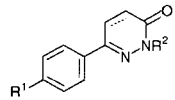
(82)

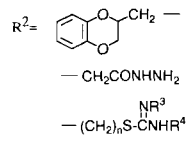
R^1=subst.thienyl,pyridyl,subst.piperazinyl, subst.imidazolyl,subst.benzimidazolyl, subst.benzoxazolyl,subst.benzotriazolyl, subst.quinolinyl,phenothiazinyl
R^2=H,alkyl



R^1,R^2=H,alkyl
R^3=subst.alkoxy

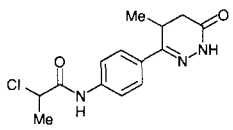
(83)





(84)

As representatives of antihypertensive 6-arylpyridazinones, amipizone [LU 23051; CAS 69635-63-8, (85)], motapizone [NAT 05239; CAS 90697-57-7, (86)] and the potent vasodilator (87) [366] may serve.



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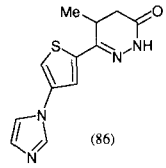
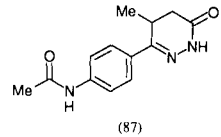


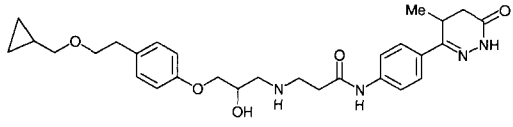
(86)



(87)

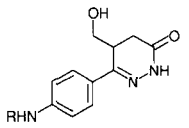
In order to design antihypertensive pyridazinones with vasodilator and β-adrenoceptor antagonistic activity, compound (87) has been combined

through a spacer link to an (aryloxy)propanolamine moiety [367]. From these and other pharmacological studies in the U.K., compound SK&F 95018 (88) emerged as a development candidate [368,369]. The four stereoisomers of (88) have been prepared and it was found that each of them has a different pharmacological profile. The isomer with *R*-configuration of pyridazine-C-5 and *S*-configuration of the alcoholic function in the side-chain appeared to be the most interesting one [369]. Recent toxicological studies however, indicated that SK&F 95018 induces red-cell haemolysis and thus further development of this compound was abandoned [370].

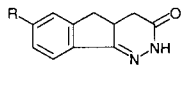


(88)

An Italian team reported that pyridazinones (89) hydroxymethylated at C-5 induced a high decrease in systolic blood pressure in rats [371]. In Italy, much effort has been devoted also to the preparation of conformationally restricted congeners of antihypertensive pyridazinones. In a structure–activity study, it has been found that indeno[1,2-*c*]pyridazinones, in particular compounds (90), are potent antihypertensive agents [372].



R=H,MeCO
(89)

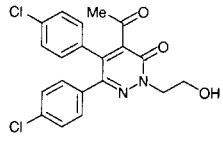


R=NH_2,NHCOMe
(90)

A variety of 6-aryl-4,5-dihydro-3(2*H*)-pyridazinones have been prepared in Germany in search of novel platelet aggregation-inhibiting drugs. Many of these compounds, in particular those bearing a chloroalkanoylamino substituent at C-4 of the phenyl group together with a methyl group at C-5 of the heterocyclic system have been found also to reduce blood pressure [373].

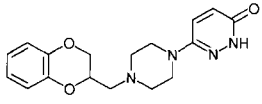
In the United States also, 5,6-diarylpyridazinone derivatives have been studied extensively as potential antihypertensive agents [374–377]. From experiments with spontaneously hypertensive rats and the deoxycorticosteroid model of hypertension, it turned out that substitution of the phenyl rings by halogen, introduction of an electron-attracting group (like acetyl or

cyano) at C-4 and attachment of a β-substituted alkyl group to N-2 of the pyridazine system leads to the most potent compounds. Structure–activity relationships in this series have been studied [378]. Esterification of the alcoholic function of a typical compound (91) leads to increased lipid and aqueous solubility (depending on the nature of the acyl group introduced) causing larger decrease of blood-pressure than the parent compound [379].

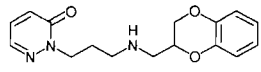


(91)

A recent report from Italy indicates that combination of a benzodioxane moiety with a C-6-substituted pyridazinone system as represented in (92) affords a compound causing strong and lasting decrease in arterial blood pressure at 0.3 mg/kg (anaesthetized cats) [380]. Antihypertensive compounds in which a benzodioxane group is linked to N-2 of 3-(2*H*)-pyridazinone have been prepared and investigated in Hungary [381,382]. The most interesting representative is GYKI-12743 (93).

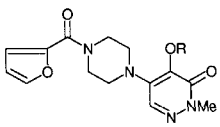


(92)



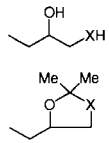
(93)

Marked hypotensive activities have been observed with 4-alkoxy-5-furoylpiperazinylpyridazin-3(2*H*)-ones (94) which have been prepared in Spain [356,383,384]. Hypotensive diarylpyridazinones of type (95) have been synthesized in India [385]. Regression analysis has been applied to the hypotensive activities of pyridazinones [386].

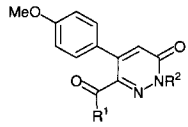


(94)

R=



X=O,N-C(CH$_3$)$_3$



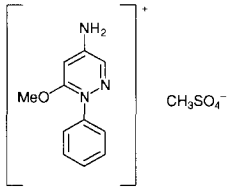
R^1=dialkylamino,cycloamino
R^2=Ph,o-tolyl

(95)

Finally, it should be mentioned that various C-4-substituted 1,4-dihydropyridazine derivatives (mainly 3,5-dicarboxylates) have been claimed as antihypertensive agents [171,387–389]. There are also some patents on antihypertensive hexahydropyridazine derivatives [390,391].

ANTIHYPOTENSIVE AGENTS

In Germany, an interesting novel antihypotensive agent, amezinium metilsulphate [Regulton, Lismont, Supratonin, LU 1631; CAS 30578-37-1, (96)], has been developed for the treatment of orthostatic dysregulation [392]. The indirect sympathomimetic effect of amezinium metilsulphate has been attributed to its uptake by adrenergic neurons [393]. Compound (96) inhibits noradrenaline re-uptake and enhances the pressor effect of exogenous noradrenaline [394]. In addition, (96) causes reversible specific inhibition of intraneuronal monoamine oxidase (MAO) type A [395–397]. Biochemical aspects of the mechanism of action have been published [398,399]. Some examples have been given of how amezinium metilsulphate can be used as a pharmacological tool [400].



(96)

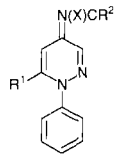
The effects of (96) on blood pressure and heart rate [401], on resistance vessels and capacitance vessels [402], on visceral, renal, metabolic, and sensory functions [403], and on central nervous functions [404] have been studied in various animal models (mice, rats, cats, dogs). Amezinium metilsulphate acts by stimulating mainly vascular α- and cardiac β_1-receptors; a comparatively long-lasting rise in arterial blood pressure has been observed. In an experiment using dogs, vasocontractile activity of (96) has been found only in those regions where circulation is affected by the local release of noradrenaline [405]. The effects of (96) on the sympathetic nervous system have been reviewed [400]. A study has been made of the pharmacokinetics in rats and dogs using ^{14}C-labelled amezinium metilsulphate [406] and of the toxicity of the drug [407].

In man, (96) proved to be an efficient antihypotensive agent, as it pro-

duces a significant dose-dependent increase in blood and pulse pressure, while the pulse-rate has been found to drop [408]. Besides raising arterial blood pressure, the activity pattern consists of a positive inotropic effect [392,408,409]. A study in man revealed the effects on the circulation after oral administration (10, 20, 40 mg) to be similar to those after i.v. administration (5, 10 mg) [408]. It has been stated that (96) permits a better adaptation to orthostatic dysregulation than norfenefrine and dihydroergotamine [410]. Studies of the utility of (96) for blood-pressure stabilization during epidural anaesthesia [411] and of its psychoactivity have appeared [412]. It has been shown that moderate doses of (96), when systemically injected, do not cause any effects on central MAO catabolism *in vivo*, whereas *in vitro* it is a potent inhibitor of MAO inside central noradrenergic neurons. Probably, this is due to exclusion of the amezinium ion from the brain by the blood-brain barrier [399].

The pharmacokinetics of amezinium metilsulphate in man have been reported [413–415]. The first step of metabolism consists in demethylation affording the pharmacologically inactive, uncharged 5-amino-2-phenyl-3(2*H*)-pyridazinone; hydroxylated pyridazinones and sulphuric acid conjugates thereof have been isolated [413].

Additional patents on the synthesis of (96) [416,417] and on antihypotensive pyridazinonimines of type (97) have been granted [418].

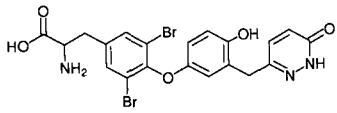


R^1=SR,CN,NCNH
X=O,S,NH
R^2=alkyl,alkenyl,Ph

(97)

HYPOCHOLESTEROLAEMIC AGENTS

Within a programme aimed at the development of thyroid hormone analogues as potentially useful plasma cholesterol-lowering agents, the pyridazinone derivative SK&F L-94901 (98) has been prepared and investigated in the U.K. [419–422]. Whereas naturally occurring thyroid hormones cannot be employed for this purpose because of their undesirable effect on heart rate, (98) has been found to represent a potent thyromimetic which retains hepatic activity but lacks cardiac activity. Structural modifications and QSAR studies have been carried out [422].

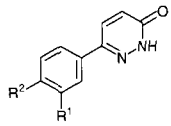


(98)

PYRIDAZINES AS BRONCHODILATORS

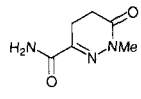
For a wide variety of 6-aryl-3(2*H*)-pyridazinones (including those discussed in the chapter on cardiotonic agents and antithrombotics [1]), bronchodilator activity has been claimed in patents [104,114–116,129,423,424]. Thus, for instance, the bronchospasmolytic effects (guinea-pig tracheal-chain preparations) of compounds of type (99) have been found to exceed those of xanthines [425]. The therapeutic index of these compounds (which inhibit phosphodiesterase at lower concentrations than xanthines and do not interact with adenosine receptors) is larger than that of xanthines.

Medazonamide [Medazoamide, L-1777, Catos; CAS 300-22-1 (100)] has been reported to exhibit a non-narcotic antitussive effect, which, however, is less than that of codeine [426]. Its toxic side-effects have been studied [427].



R^1=H,alkoxy,CF_3
R^2=H,alkoxy

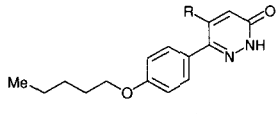
(99)



(100)

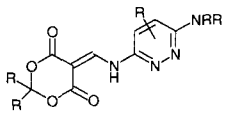
MISCELLANEOUS PHARMACOLOGICAL ACTIVITIES OF PYRIDAZINE DERIVATIVES

Various 6-arylpyridazinones of type (101) have been claimed as anthelmintics [428,429]. 3,6-Diaminopyridazine derivatives of type (102) have been patented as schistosomacides [430,431]. 4-Methyl-3-(morpholinoethylamino)-6-phenylpyridazine has been claimed as an agent for the treatment of secondary frigidity [432].



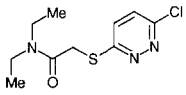
R=subst.amino

(101)



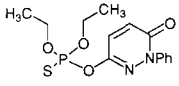
(102)

Azintamide (Azinthiamide, Biloral, Oragallin, Oragal, Rogalin, ST 9067, X-23; CAS 1830-32-6) (103) is a choleretic agent developed in Austria.

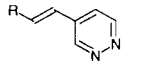


(103)

Anticholinesterase activity has been reported for pyridaphenthion [Pyridafenthion, Ofunack; CAS 119-12-0 (104)]. Some 4-styrylpyridazines (105) have been found to inhibit choline acetyltransferase *in vitro* [433].



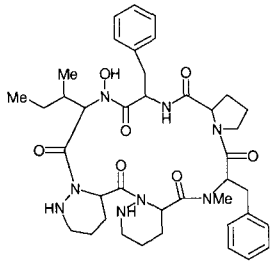
(104)



R= 3-Cl-phenyl,
3,4-diCl-phenyl,
3-OCH_3-phenyl,
1-naphthyl

(105)

Structurally unique, potent, and selective oxytocin antagonistic agents characterized by a cyclic hexapeptide structure have recently been isolated from the broth filtrate of a novel *Streptomyces silvensis* strain [434,435]. As shown in formula (106), these compounds contain two hexahydro-3-pyridazinecarboxylic acid moieties. A study has been made of their structural modifications (including oxidation of the reduced pyridazine nuclei) [436].



(106)

CONCLUSIONS

The increasing interest in the chemistry and in biological activities of pyridazines as observed within the past 15 years has resulted in the development of a wide variety of interesting novel drugs and pharmacological tools. It is also clearly reflected by an international symposium on this subject held in Strasbourg, France, in 1988 and by a second conference in Vienna, Austria, in 1990, where 70 scientists from 17 countries gathered to discuss recent advances in this field (a compilation of abstracts of this meeting was published [437]).

It is not surprising to see that most of the bio-active compounds discussed in this review are 1,2-diazine derivatives bearing heteroatom substituents either at C-3 or at C-3 and C-6, since pyridazinones and pyridazinediones, utilized as intermediates in the synthesis of such derivatives, have been known for a long period and are generally conveniently accessible. On the other hand, there are so far only a few examples of pyridazine-derived pharmacological agents in which the parent system is linked to a functionalized carbon side-chain only. This may be attributed to the fact that many of the required synthetic building blocks had remained unexplored until very recently [15,173,438–441]).

Accordingly, very little is known so far about the metabolic fate of the parent ring system and of simple derivatives thereof. In experiments using rats, it has been found that pyridazine undergoes extensive oxidative biotransformation to afford monohydroxy derivatives along with 4,5-dihydroxy- and 4,5-dihydro-4,5-dihydroxypyridazine. 3-Methylpyridazine was found to be metabolized under these conditions in a similar manner yielding additionally 3-hydroxymethylpyridazine [442].

Also the bio-isosteric potential of the pyridazine system hitherto remained largely unexplored, despite the fact that several examples discussed in this review indicate that the replacement of aromatic moieties in bio-active compounds by the highly polar pyridazine nucleus may well improve the pharmacodynamic and/or the pharmacokinetic profile of a drug molecule.

It is hoped that this review will not only facilitate the access to the literature on pharmacologically interesting pyridazines but also stimulate further research in this field.

ACKNOWLEDGEMENTS

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REFERENCES

1 Heinisch, G. and Frank, H. (1990) Progr. Med. Chem. 27, 1–49. Elsevier, Amsterdam.
2 Tišler, M. and Stanovnik, B. (1990) Adv. Heterocycl. Chem. 49, 385–474.
3 Godfraind, T. and Ghysel-Burton, J. (1979) Eur. J. Pharmacol. 60, 337–44.
4 Siphar, S.A. (1976) Ger. Offen. 2, 534, 981; (1976) Chem. Abstr. 85, 33365.
5 Humber, D.C., Jones, P.S., Phillipps, G.H., Dodds, M.G. and Dolamore, P.G. (1983) Steroids 42, 171–188.
6 Humber, D.C., Phillipps, G.H., Dodds, M.G., Dolamore, P.G. and Machin, I. (1983) Steroids 42, 189–203.
7 Lesher, G.Y., Dickinson, W.B. and Singh, B. (1981) Fr. Demande FR 2, 481, 284; (1982) Chem. Abstr. 96, 85571.
8 Lesher, G.Y. and Dickinson, W.B. (1981) U.S. Patent US 4, 304, 777; (1982) Chem. Abstr. 96, 85570.
9 Lesher, G.Y. and Dickinson, W.B. (1981) Fr. Demande FR 2, 481, 282; (1982) Chem. Abstr. 96, 122813.
10 Lesher, G.Y. and Dickinson, W.B. (1982) U.S. Patent US 4, 337, 253; (1982) Chem. Abstr. 97, 182443.
11 Lesher, G.Y. and Dickinson, W.B. (1984) U.S. Patent US 4, 486, 431; (1985) Chem. Abstr. 102, 90141.
12 Lesher, G.Y. and Dickinson, W.B. (1981) U.S. Patent US 4, 304, 775; (1982) Chem. Abstr. 96, 122812.
13 Dickinson, W.B. and Lesher, G.Y. (1981) Belg. 886, 574; (1981) Chem. Abstr. 95, 203980.
14 Lesher, G.Y. and Dickinson, W.B. (1986) U.S. Patent US 4, 590, 194; (1986) Chem. Abstr. 105, 78949.
15 Haider, N., Heinisch, G. and Offenberger, S. (1989) Pharmazie 44, 598–601.
16 Bristol, J.A., Sircar, I., Moos, W.H., Evans, D.B. and Weishaar, R.E. (1984) J. Med. Chem. 27, 1099–1101.
17 Sircar, I. and Bristol, J.A. (1982) U.S. Patent US 4, 353, 905; (1983) Chem. Abstr. 98, 53928.
18 Sircar, I. and Bristol, J.A. (1983) Eur. Pat. Appl. EP 75, 436; (1983) Chem. Abstr. 99, 88218.
19 Sircar, I., Bristol, J.A. and Skeean, R.W. (1986) Eur. Pat. Appl. EP 186, 484; (1986) Chem. Abstr. 105, 133898.
20 Hilboll, G. and Biedermann, J. (1984) Ger. Offen. DE 3, 306, 195; (1985) Chem. Abstr. 102, 6517.
21 Mertens, A., Von der Saal, W., Mueller-Beckmann, B. and Sponer, G. (1988) Ger. Offen. DE 3, 626, 865; (1988) Chem. Abstr. 108, 167493.
22 Sircar, I. (1985) Eur. Pat. Appl. EP 145, 450; (1985) Chem. Abstr. 103, 141997.
23 Sircar, I., Steffen, R.P., Bobowski, G., Burke, S.E., Newton, R.S., Weishaar, R.E., Bristol, J.A. and Evans, D.B. (1989) J. Med. Chem. 32, 342–350.
24 Sircar, I., Moos, W.H., Morrison, G.C., Duell, B.L., Bobowski, G. and Potoczak, R. (1989) Eur. J. Med. Chem. 24, 349–355.

25 Sircar, I., Duell, B.L., Bobowski, G., Bristol, J.A. and Evans, D.B. (1985) J. Med. Chem. 28, 1405–1413.
26 Sircar, I., Weishaar, R.E., Kobylarz, D., Moos, W.H. and Bristol, J.A. (1987) J. Med. Chem. 30, 1955–1962.
27 Sircar, I., Bobowski, G., Bristol, J.A., Weishaar, R.E. and Evans, D.B. (1986) J. Med. Chem. 29, 261–267.
28 Sircar, I., Duell, B.L., Cain, M.H., Burke, S.E. and Bristol, J.A. (1986) J. Med. Chem. 29, 2142–2148.
29 Warrington, B.H. (1986) Eur. Pat. Appl. EP 181, 145; (1986) Chem. Abstr. 105, 115083.
30 Sircar, I. (1985) J. Heterocycl. Chem. 22, 1045–1048.
31 Anon. (1985) Drugs Future 10, 449–450.
32 Steffen, R.P., Eldon, C.M. and Evans, D.B. (1986) J. Cardiovasc. Pharmacol. 8, 520–526.
33 Decker, N., Grima, M. and Schwartz, J. (1985) J. Pharmacol. Exp. Ther. 16, 391–402.
34 Steffen, R.P., Weishaar, R.E., Evans, D.B. and Kaplan, H.R. (1986) New Cardiovasc. Drugs 81–105.
35 Weishaar, R.E., Cain, M.H. and Bristol, J.A. (1985) J. Med. Chem. 28, 537–545.
36 Weishaar, R.E., Burrows, S.D., Kobylarz, D.C., Quade, M.M. and Evans, D.B. (1986) Biochem. Pharmacol. 35, 787–800.
37 Weishaar, R.E., Quade, M., Schenden, J.A., Boyd, D.K. and Evans, D.B. (1985) Eur. J. Pharmacol. 119, 205–215.
38 Weishaar, R.E., Quade, M.M., Schenden, J.A. and Evans, D.B. (1985) J. Cyclic Nucleotide Protein Phosphorylation Res. 10, 551–564.
39 Weishaar, R.E., Kobylarz-Singer, D.C., Quade, M.M., Steffen, R.P. and Kaplan, H.R. (1987) J. Cyclic Nucleotide Protein Phosphorylation Res. 11, 513–527.
40 Weishaar, R.E., Kobylarz-Singer, D.C., Steffen, R.P. and Kaplan, H.R. (1987) Circ. Res. 61, 539–547.
41 Moos, W.H., Humblet, C.C., Sircar, I., Rithner, C., Weishaar, R.E., Bristol, J.A. and McPhail, A.T. (1987) J. Med. Chem. 30, 1963–1972.
42 Hayes, A. and Chang, T. (1983) Pharmacologist 25, Abst 766.
43 Olson, S. and Chang, T. (1983) Pharmacologist 25, Abst 765.
44 Jafri, S.M. (1986) Am. J. Cardiol. 57, 254.
45 Hayes, A.G., Mehta, S. and Chang, T. (1984) J. Chromatogr. 336, 446–51.
46 Hays, S.J., Hicks, J.L. and Huang, C.C. (1987) J. Labelled Compd. Radiopharm. 24, 1185–1193.
47 Sircar, I., Morrison, G.C., Burke, S.E., Skeean, R. and Weishaar, R.E. (1987) J. Med. Chem. 30, 1724–1728.
48 Steffen, R.P., Evans, D.B., Kaplan, H.R. and Weisbach, J.A. (1986) U.S. Patent US 4, 584, 299; (1986) Chem. Abstr. 105, 72686.
49 Steffen, R.P. (1987) U.S. Patent US 4, 693, 996; (1987) Chem. Abstr. 107, 242651.
50 Geiss, K.H., Raschack, M., Lehmann, H.D., Gries, J., Ruebsamen, H. and Schmied, B. (1986) Ger. Offen. DE 3, 425, 632; (1986) Chem. Abstr. 105, 42828.
51 Geiss, K.H., Schmied, B., Kropp, R., Baldinger, V., Gries, J., Lehmann, H.D., Ruebsamen, H. and Traut, M. (1987) Ger. Offen. DE 3, 600, 275; (1987) Chem. Abstr. 107, 115602.
52 Austel, V., Kutter, E., Heider, J., Eberlein, W. and Kadatz, R. (1976) Ger. Offen. DE 2, 427, 943; (1976) Chem. Abstr. 84, 121880.
53 Dr. Karl Thomae GmbH. (1976) Fr. Demande 2, 273, 545; (1976) Chem. Abstr. 85, 192752.
54 Austel, V., Heider, J., Eberlein, W. and Diederen, W. (1980) Ger. Offen. DE 2, 922, 336; (1981) Chem. Abstr. 94, 175143.

55 Austel, V., Heider, J., Eberlein, W., Diederen, W. and Haarmann, W. (1982) U.S. Patent US 4, 361, 563; (1983) Chem. Abstr. 98, 72123.
56 Pruecher, H., Rochus, J., Piulats, J. and Klockow, M. (1986) Ger. Offen. DE 3, 505, 609; (1987) Chem. Abstr. 106, 33085.
57 Mertens, A., Saal, W., Friebe, W.G., Mueller-Beckmann, B. and Sponer, G. (1987) Ger. Offen. DE 3, 531, 658; (1987) Chem. Abstr. 106, 196452.
58 Von der Saal, W., Mertens, A., Friebe, W.G. and Mueller-Beckmann, B. (1987) Ger. Offen. DE 3, 611, 343; (1988) Chem. Abstr. 108, 6026.
59 Hauel, N., Noll, K., Narr, B., Heider, J., Psiorz, M., Bomhard, A., Diederen, W. and Van Meel, J. (1987) Ger. Offen. DE 3, 606, 254; (1988) Chem. Abstr. 108, 21915.
60 Hauel, N., Heider, J., Stein, H., Austel, V., Riffen, M., Diederen, W. and Haarmann, W. (1981) Ger. Offen. DE 3, 006, 671; (1981) Chem. Abstr. 95, 203981.
61 Von Meel, J.C. (1985) Arzneim. -Forsch. 35, 284–288.
62 Honerjaeger, P., Heiss, A., Schaefer-Korting, M., Schoensteiner, G. and Reiter, M. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 325, 259–269.
63 Mannhold, R. (1985) Drugs Future 10, 570–571 and refs. cited therein.
64 Ruegg, J.C., Pfitzer, G., Eubler, D. and Zeugner, C. (1984) Arzneim. -Forsch. 34, 1736–1738.
65 Ruegg, J.C. (1986) Circulation, Suppl. 73, III78-III84.
66 Verdouw, P.D., Hartog, J.M., Duncker, D.J., Roth, W. and Saxena, P.R. (1986) Eur. J. Pharmacol. 126, 21–30.
67 Duncker, D.J., Van Dalen, F.J., Hartog, J.M., Lamers, J.M., Rensen, R.J., Saxena, P.R. and Verdouw, P.D. (1986) Arzneim. -Forsch. 36, 1740–1744.
68 Bitterman, H., Smith, B.A. and Lefer, A.M. (1988) Arzneim. -Forsch. 38, 1389–1393.
69 Scholz, H. and Meyer, W. (1986) Circulation, Suppl. 73, III99-III108.
70 Duncker, D.J., Hartog, J.M., Levinsky, L. and Verdouw, P.D. (1987) Br. J. Pharmacol. 91, 609–615.
71 Verdouw, P.D., Levinsky, L., Duncker, D.J., Rutteman, A.M. and Saxena, P.R. (1987) Eur. J. Pharmacol. 137, 219–226.
72 Berger, C., Meyer, W., Scholz, H. and Starbatty, J. (1985) Arzneim. -Forsch. 35, 1668–1673.
73 Anon. (1988) Drugs Future 13, 693–694.
74 Robertson, D.W. (1985) Eur. Pat. Appl. EP 155, 798; (1986) Chem. Abstr. 104, 142264.
75 Robertson, D.W. (1986) Eur. Pat. Appl. EP 178, 876; (1986) Chem. Abstr. 105, 66459.
76 Robertson, D.W., Krushinski, J.H., Beedle, E.E., Wyss, V., Pollock, G.D., Wilson, H., Kauffman, R.F. and Hayes, J.S. (1986) J. Med. Chem. 29, 1832–1840.
77 Robertson, D.W., Jones, N.D., Krushinski, J.H., Pollock, G.D., Swartzendruber, J.K. and Hayes, J.S. (1987) J. Med. Chem. 30, 623–627.
78 Hayes, J.S., Pollock, G.D., Wilson, H., Bowling, N. and Robertson, D.W. (1987) J. Cardiovasc. Pharmacol. 9, 425–434.
79 Sandusky, G.E. and Means, J.R. (1987) Toxicol. Lett. 38, 177–186.
80 Kauffman, R.F., Crowe, V.G., Utterback, B.G. and Robertson, D.W. (1986) Mol. Pharmacol. 30, 609–616.
81 Robertson, D.W., Krushinski, J.H., Utterback, B.G. and Kauffman, R.F. (1989) J. Med. Chem. 32, 1476–1480.
82 Robertson, D.W. and Hayes, J.S. (1986) Drugs Future 11, 377–379.
83 Robertson, D.W., Krushinski, J.H. and Kau, D. (1986) J. Labelled Compd. Radiopharm. 23, 343–354.
84 Robertson, D.W., Krushinski, J.H., Pollock, G.D., Wilson, H., Kauffman, R.F. and Hayes, J.S. (1987) J. Med. Chem. 30, 824–829.

85 Robertson, D.W. (1986) Eur. Pat. Appl. EP 178, 875; (1986) Chem. Abstr. 105, 139619.
86 Robertson, D.W., Krushinski, J.H., Pollock, G.D. and Hayes, J.S. (1988) J. Med. Chem. 31, 461–465.
87 Ludmer, P.L., Baim, D.S., Antman, E.M., Gauthier, D.F., Rocco, M.B., Friedman, P.L. and Colucci, W.S. (1987) Am. J. Cardiol. 59, 1351.
88 Packer, M. and Leier, C.V. (1987) Circulation 75, IV-55.
89 Okushima, H., Narimatsu, A., Kobayashi, M., Shimooda, I., Kitada, Y. and Furuya, R. (1985) Eur. Pat. Appl. EP 145, 019; (1985) Chem. Abstr. 103, 141995.
90 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R. and Kitada, Y. (1986) Jpn. Kokai Tokkyo Koho JP 61, 183, 282; (1987) Chem. Abstr. 106, 33087.
91 Okujima, H., Narimatsu, A., Kobayashi, M., Shimooda, R. and Kitada, Y. (1986) Jpn. Kokai Tokkyo Koho JP 61, 289, 032; (1987) Chem. Abstr. 106, 149477.
92 Okushima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1987) J. Med. Chem. 30, 1157–1161.
93 Mitsubishi Chem. Ind. Co., Ltd. (1984) Jpn. Kokai Tokkyo Koho JP 59, 084, 883; (1984) Chem. Abstr. 101, 171274.
94 Mitsubishi Chem. Ind. Co., Ltd. (1985) Jpn. Kokai Tokkyo Koho JP 60, 087, 283; (1985) Chem. Abstr. 103, 141996.
95 Anon. (1987) Drugs Future 12, 856–859.
96 Wynsen, J.C., Shimshak, T.M., Preuss, K.C., Gross, G.J., Brooks, H.L. and Warltier, D.C. (1987) Fundam. Clin. Pharmacol. 1, 335–345.
97 Narimatsu, A., Kitada, Y., Satoh, N., Suzuki, R. and Okushima, H. (1987) Arzneim. -Forsch. 37, 398–406.
98 Hosono, M. and Taira, N. (1987) J. Cardiovasc. Pharmacol. 10, 692–698.
99 Kitada, Y., Narimatsu, A., Matsumura, N. and Endo, M. (1987) Eur. J. Pharmacol. 134, 229–231.
100 Katayama, S., Narimatsu, A., Suzuki, R., Iijima, T. and Taira, N. (1987) Jpn. J. Pharmacol. 44, 481–488.
101 Sellin, L.C., Alajoutsijärvi, A., Törnquist, K., Fraser, M., Pippuri, A. and Ojala, I. (1988) Arzneim. -Forsch. 38, 1787–1789.
102 Sircar, I. (1983) U.S. Patent US 4, 397, 854; (1983) Chem. Abstr. 99, 169531.
103 Sircar, I. (1983) U.S. Patent US 4, 404, 203; (1983) Chem. Abstr. 99, 194988.
104 Amschler, H., Eistetter, K., Eltze, M., Flockerzi, D., Kilian, U., Kolassa, N., Sanders, K. and Ulrich, W.R. (1985) Eur. Pat. Appl. EP 163, 965; (1986) Chem. Abstr. 104, 186435.
105 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 008, 015; (1983) Chem. Abstr. 98, 137630.
106 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 008, 016; (1983) Chem. Abstr. 98, 173193.
107 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 148, 865; (1984) Chem. Abstr. 100, 34555.
108 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 183, 618; (1984) Chem. Abstr. 100, 61786.
109 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 203, 978; (1984) Chem. Abstr. 100, 174845.
110 Okujima, H., Narimatsu, A., Kobayashi, M., Tsuda, K. and Kitada, Y. (1985) Jpn. Kokai Tokkyo Koho JP 60, 197, 660; (1986) Chem. Abstr. 104, 168480.
111 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R. and Kitada, Y. (1986) Jpn. Kokai Tokkyo Koho JP 61, 044, 873; (1986) Chem. Abstr. 105, 42832.
112 Okushima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Kitada, Y. and Tsuda, K. (1988) Eur. Pat. Appl. EP 252, 422; (1988) Chem. Abstr. 108, 150501.

113 Smith, Kline and Beckman Corp. (1986) Jpn. Kokai Tokkyo Koho JP 61, 212, 582; (1987) Chem. Abstr. 106, 102311.
114 Coates, W.J. (1987) Eur. Pat. Appl. EP 220, 044; (1988) Chem. Abstr. 108, 112503.
115 Coates, W.J. (1987) Eur. Pat. Appl. EP 233, 745; (1988) Chem. Abstr. 108, 21914.
116 Slater, R.A. (1985) PCT Int. Appl. WO 85, 003, 076; (1986) Chem. Abstr. 104, 34098.
117 Okushima, H., Narimatsu, A. and Shimooda, I. (1983) Eur. Pat. Appl. EP 85, 985; (1983) Chem. Abstr. 99, 194986.
118 Smith, Kline and French Lab., Ltd. (1986) Jpn. Kokai Tokkyo Koho JP 61, 221, 177; (1987) Chem. Abstr. 106, 84625.
119 Coates, W.J., Emmet, J.C. and Slater, R.A. (1987) Eur. Pat. Appl. EP 208, 518; (1987) Chem. Abstr. 106, 138461.
120 Smith, Kline and Beckman Corp. (1986) Jpn. Kokai Tokkyo Koho JP 61, 212, 583; (1987) Chem. Abstr. 106, 33108.
121 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 188, 815; (1984) Chem. Abstr. 100, 45303.
122 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 203, 977; (1984) Chem. Abstr. 100, 156629.
123 Morisawa, Y., Kataoka, M. and Koike, H. (1987) Jpn. Kokai Tokkyo Koho JP 62, 053, 972; (1987) Chem. Abstr. 107, 23352.
124 Meyer, H., Franckowiak, G., Rosentreter, U., Gross, R., Thomas, G., Schramm, M., Kayser, M., Seuter, F., Perzborn, E. and Bechem, M. (1986) Ger. Offen. DE 3, 445, 852; (1986) Chem. Abstr. 105, 152945.
125 Prain, H.D. and Warrington, B.H. (1987) Eur. Pat. Appl. EP 208, 517; (1987) Chem. Abstr. 106, 156489.
126 Mitsubishi Chem. Ind. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 183, 687; (1984) Chem. Abstr. 100, 85711.
127 Mitsui Toatsu Chem. Inc. (1984) Jpn. Kokai Tokkyo Koho JP 59, 053, 479; (1984) Chem. Abstr. 101, 90957.
128 Zoller, G., Beyerle, R., Just, M., Bohn, H., Martorana, P. and Nitz, R.E. (1986) Ger. Offen. DE 3, 434, 680; (1986) Chem. Abstr. 105, 97487.
129 Zoller, G., Beyerle, R., Just, M., Bohn, H., Martorana, P. and Nitz, R.E. (1986) Eur. Pat. Appl. EP 175, 363; (1986) Chem. Abstr. 105, 153074.
130 Kuhla, D.E., Campbell, H.F., Studt, W.L., Faith, W.C. and Molino, B.F. (1987) PCT Int. Appl. WO 87, 003, 201; (1988) Chem. Abstr. 108, 6037.
131 Combs, D.W. (1988) U.S. Patent US 4, 721, 784; (1988) Chem. Abstr. 108, 221711.
132 Okujima, H., Narimatsu, A. and Furuya, R. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 670; (1988) Chem. Abstr. 109, 190437.
133 Combs, D.W. (1988) Eur. Pat. Appl. EP 272, 914; (1988) Chem. Abstr. 109, 190436.
134 Von der Saal, W., Friebe, W.G., Mertens, A., Mueller-Beckmann, B. and Kling, L. (1988) Ger. Offen. DE 3, 631, 085; (1988) Chem. Abstr. 109, 22975.
135 Ogata, H., Matsumoto, H., Kimoto, S. and Ueda, M. (1987) Jpn. Kokai Tokkyo Koho JP 62, 273, 975; (1988) Chem. Abstr. 109, 73461.
136 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 671; (1988) Chem. Abstr. 109, 190450.
137 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 672; (1988) Chem. Abstr. 109, 190451.
138 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 673; (1988) Chem. Abstr. 109, 190452.
139 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 675; (1988) Chem. Abstr. 109, 190453.

140 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., Tsuda, K. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 674; (1988) Chem. Abstr. 109, 211090.
141 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 683; (1988) Chem. Abstr. 109, 231045.
142 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 154, 676; (1988) Chem. Abstr. 109, 231075.
143 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R. and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 215, 672; (1989) Chem. Abstr. 110, 75541.
144 Franckowiak, G., Kayser, M., Schramm, M., Thomas, G., Gross, R., Perzborn, E. and Seuter, F. (1988) Ger. Offen. DE 3, 712, 369; (1989) Chem. Abstr. 110, 95257.
145 Franckowiak, G., Rosentreter, U., Perzborn, E., Seuter, F., Kayser, M. and Thomas, G. (1988) Ger. Offen. DE 3, 712, 371; (1989) Chem. Abstr. 110, 95258.
146 Okujima, H., Narimatsu, A., Kobayashi, M., Furuya, R., and Kitada, Y. (1988) Jpn. Kokai Tokkyo Koho JP 63, 218, 666; (1989) Chem. Abstr. 110, 95277.
147 Pruecher, H. (1988) Ger. Offen. DE 3, 704, 879; (1988) Chem. Abstr. 109, 211070.
148 Sircar, I. and Bristol, J.A. (1988) U.S. US 4, 717, 730; (1988) Chem. Abstr. 109, 149554.
149 Sircar, I. and Bristol, J.A. (1988) U.S. US 4, 734, 415; (1989) Chem. Abstr. 111, 97259.
150 Herter, R., Engler, H., Moersdorf, P., Schickaneder, H., Pfahlert, V. and Ahrens, K.H. (1989) Eur. Pat. Appl. EP 327, 800; (1990) Chem. Abstr. 112, 55899.
151 Nomoto, Y., Takai, H., Ohno, T. and Kubo, K. (1989) Eur. Pat. Appl. EP 326, 307; (1990) Chem. Abstr. 112, 21004.
152 Moersdorf, P., Herter, R., Engler, H., Pfahlert, V., Weidner, R. and Ahrens, K.H. (1989) Eur. Pat. Appl. EP 339, 208; (1990) Chem. Abstr. 112, 198403.
153 Baader, E., Jendralla, H., Kerekjarto, B. and Beck, G. (1989) Eur. Pat. Appl. EP 324, 347; (1990) Chem. Abstr. 112, 21003.
154 Spada, A.P., Studt, W.L., Campbell, H.F. and Kuhla, D.E. (1988) PCT Int. Appl. WO 88 08, 706; (1989) Chem. Abstr. 111, 194783.
155 Schickaneder, H., Moersdorf, P., Pfahlert, V., Engler, H. and Ahrens, K.H. (1988) S. African ZA 88 00, 950; (1989) Chem. Abstr. 111, 97261.
156 Moersdorf, P., Schickaneder, H., Herter, R., Pfahlert, V., Engler, H. *et al.* (1988) S. African ZA 88 00, 951; (1989) Chem. Abstr. 111, 78017.
157 Austel, V., Noll, K., Eberlein, W., Heider, J., Van Meel, J., Diederen, W. and Haarmann, W. (1989) Ger. Offen. DE 3, 728, 244; (1989) Chem. Abstr. 111, 97263.
158 Burpitt, B.E., Crawford, L.P., Davies, B.J., Mistry, J., Mitchell, M.B., Pancholi, K.D. and Coates, W.J. (1988) J. Heterocycl. Chem. 25, 1689–1695; and literature cited therein.
159 Coates, W.J., Prain, H.D., Reeves, M.L. and Warrington, B.H. (1990) J. Med. Chem. 33, 1735–1741.
160 Davies, A., Warrington, B.H. and Vinter, J.G. (1987) J. Comput. -Aided Mol. Design 1, 97–120.
161 Anon. (1989) Drugs Future 14, 132–134.
162 Sastry, C.V., Rao, K.S., Krishnan, V.S., Rastogi, K., Jain, M.L., Verma, R.K., Tripathi, R.M., Singh, P.P., Rao, C.S. and Kaushal, R. (1989) Indian J. Chem., Sect. B 28B, 882–884.
163 Combs, D.W., Rampulla, M.S., Bell, S.C., Klaubert, D.H., Tobia, A.J., Falotico, R., Haertlein, B., Lakas-Weiss, C. and Moore, J.B. (1990) J. Med. Chem. 33, 380–386.
164 Jonas, R. and Piulats, J. (1987) Ger. Offen. DE 3, 527, 036; (1987) Chem. Abstr. 106, 176411.
165 Sircar, I. (1987) U.S. Patent US 4, 656, 170; (1987) Chem. Abstr. 106, 213965.
166 Kuhla, D.E., Campbell, H.F., Studt, W.L. and Faith, W.C. (1987) PCT Int. Appl. WO 87 02, 587; (1987) Chem. Abstr. 107, 217642.

167 Takasugi, H., Kuno, A., Sakai, H., Sugiyama, Y. and Takaya, T. (1987) Eur. Pat. Appl. EP 228, 845; (1988) Chem. Abstr. 108, 112475.
168 Tam, L., Yen, M.H. and Chow, S.Y. (1975) T'ai-wan I Hsueh Hui Tsa Chih 74, 480–488; (1976) Chem. Abstr. 84, 99156.
169 Franckowiak, G., Bossert, F., Heise, A. and Towart, R. (1980) Ger. Offen. DE 2, 834, 624; (1980) Chem. Abstr. 93, 46704.
170 Franckowiak, G., Wehinger, E., Thomas, G., Schramm, M. and Gross, R. (1986) Ger. Offen. DE 3, 431, 700; (1986) Chem. Abstr. 105, 60623.
171 Vogel, A. (1985) Patentschrift (Switz.) CH 650, 260; (1986) Chem. Abstr. 104, 68877.
172 Bayer A.G. (1978) Ger. Offen. DE 2, 658, 804.
173 Dostal, W., Heinisch, G., Holzer, W., Perhauc, I. and Zheng, C. (1990) J. Heterocycl. Chem. 27, 1313–1321.
174 Stout, D.M. and Matier, W.L. (1984) PCT Int. Appl. WO 84, 000, 489; (1984) Chem. Abstr. 101, 6802.
175 Cross, P.E., Arrowsmith, J.E. and Thomas, G.N. (1987) Eur. Pat. Appl. EP 242, 173; (1988) Chem. Abstr. 108, 167108.
176 Raabe, T., Bohn, H., Martorana, P.A. and Nitz, R.E. (1982) Eur. Pat. Appl. EP 54, 946; (1982) Chem. Abstr. 97, 182444.
177 Jablonka, B., Reibert, R., Schmidt, A. and Schraven, E. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 228.
178 Bohn, H., Martorana, P.A. and Raabe, T. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 227.
179 Meyer, B.H., Muller, F.O., Luus, H.G., Badian, M., Reifenberger, D. and Irmisch, R. (1986) IIIrd World Conf. Clin. Pharmacol. Ther., Abst. 810.
180 Howson, W., Kitteringham, J., Mistry, J., Mitchell, M.B., Novelli, R., Slater, R.A. and Swayne, G.T. (1988) J. Med. Chem. 31, 352–356.
181 Dorn, H. and Hilgetag, K.P. (1976) Ger. (East) 118, 641; (1977) Chem. Abstr. 86, 171482.
182 Pifferi, G. and Gaviraghi, G. (1980) Cron. Farm. 23, 186–187.
183 Pifferi, G., Parravicini, F., Carpi, C. and Dorigotti, L. (1975) J. Med. Chem. 18, 741–746.
184 Parravicini, F., Scarpitta, G., Dorigotti, L. and Pifferi, G. (1979) Farmaco, Ed. Sci. 34, 299–310.
185 Parravicini, F., Scarpitta, G., Dorigotti, L. and Pifferi, G. (1978) Farmaco, Ed. Sci. 33, 99–105.
186 Di Mola, N. (1982) Fr. Demande FR 2, 495, 147; (1982) Chem. Abstr. 97, 182445.
187 Farina, C., Pifferi, G. and Pinza, M. (1984) Eur. Pat. Appl. EP 124, 058; (1985) Chem. Abstr. 102, 113519.
188 Dorigotti, L., Gaviraghi, G., Pinza, M., Pifferi, G. and Semeraro, C. (1980) Eur. Pat. Appl. EP 18, 016; (1981) Chem. Abstr. 94, 121580.
189 Carpi, C., Dorigotti, L. and Pifferi, G. (1974) Ger. Offen. 2, 410, 201; (1974) Chem. Abstr. 82, 4280.
190 Pifferi, G. (1978) Swiss Patent 596, 188; (1978) Chem. Abstr. 89, 75375.
191 Dorigotti, L. and Parravicini, F. (1977) Ger. Offen. 2, 726, 191; (1978) Chem. Abstr. 88, 121225.
192 Carpi, C., Dorigotti, L. and Pifferi, G. (1977) Brit. Patent 1, 470, 747; (1977) Chem. Abstr. 87, 135374.
193 Pinza, M., Monguzzi, R. and Colombo, R. (1986) Eur. Pat. Appl. EP 202, 095; (1987) Chem. Abstr. 106, 50239.
194 Buffoni, F., Carpi, C., Banchelli Soldaini, G. and Raimondi, L. (1980) Farmaco, Ed. Sci. 35, 848–855.

195 Buffoni, F., Banchelli Soldaini, G., Toccafondi Ferroni, A. and Raimondi, L. (1980) Ital. J. Biochem. 29, 61–63.
196 Buffoni, F., Ignesti, G. and Pirisino, R. (1977) Farmaco, Ed. Sci. 32, 404–413.
197 Dorigotti, L., Rolandi, R. and Carpi, C. (1976) Pharmacol. Res. Commun. 8, 295–308.
198 Worcel, M. (1978) Br. J. Pharmacol. 62, 375.
199 Worcel, M. (1978) Vasc. Neuroeff. Mech. 36–37.
200 Worcel, M. and Da Ponte, F. (1978) J. Pharmacol. Exp. Ther. 207, 320–330.
201 Chevillard, C., Saiag, B. and Worcel, M. (1981) Br. J. Pharmacol. 73, 811–817.
202 Dorigotti, L. and Semeraro, C. (1980) Eur. J. Pharmacol. 67, 275–282.
203 Dorigotti, L., Carpi, C., Bertoli, G. and Mariani, P.L. (1984) Arzneim. -Forsch. 34, 876–879.
204 Bacher, S., Kraupp, O., Beck, A., Seitelberger, R. and Raberger, G. (1984) Basic. Res. Cardiol. 79, 588–597.
205 Bacher, S., Kraupp, O., Beck, A., Skoda, H. and Raberger, G. (1985) Arzneim. -Forsch. 35, 288–291.
206 Simonotti, L., Colombo, R. and Pifferi, G. (1979) Recent Dev. Mass Spectrom. Biochem. Med. 2, 27–41.
207 Ventura, P., Parravicini, F., Simonotti, L., Colombo, R. and Pifferi, G. (1981) J. Pharm. Sci. 70, 334–336.
208 Della Porta, G. and Dragani, T.A. (1983) J. Cancer Res. Clin. Oncol. 106, 97–101.
209 Glatt, H. and Oesch, F. (1985) Teratog. Carcinog. Mutagen. 5, 339–345.
210 Semeraro, C., Dorigotti, L., Banfi, S. and Carpi, C. (1981) J. Cardiovasc. Pharmacol. 3, 455–467.
211 Dorigotti, L., Ferni, G., Lombroso, M. and Semeraro, C. (1984) Arzneim. -Forsch. 34, 984–987.
212 Dorigotti, L. and Ferni, G. (1987) in New Cardiovascular Drugs (Scriabine, A., ed.) pp 155–171, Raven Press, New York.
213 Takahashi, S., Nagae, Y., Takahashi, H., Shimazu, H., Nishikawa, M., Anabuki, K. and Tamura, K. (1987) Yakuri to Chiryo 15, 3875–3899; (1988) Chem. Abstr. 108, 87786.
214 Bonardi, G., Rossi, E. and Pellegatti, M. (1983) Eur. J. Drug Metab. Pharmacokinet. 8, 25–33.
215 Schuetz, H., Faigle, J.W., Kueng, W. and Theobald, W. (1985) Eur. J. Drug Metab. Pharmacokinet. 10, 147–153.
216 Crolla, T., Santini, F., Visconti, M. and Pifferi, G. (1984) J. Chromatogr. 310, 139–149.
217 Schuetz, H. and Faigle, J.W. (1983) J. Chromatogr. 281, 273–280.
218 Terauchi, Y., Watari, S., Ishikawa, S., Takeyama, K., Sekine, Y., Hashimoto, M. and Hayashi, T. (1988) Arzneim. -Forsch. 38, 237–239.
219 Hauffe, S.A., Dubois, J.P. and Imhof, P.R. (1985) Eur. J. Drug Metab. Pharmacokinet. 10, 217–223.
220 Citerio, L., Visconti, M., Borsa, M. and Pifferi, G. (1981) Boll. Chim. Farm. 120, 222–233.
221 Simonotti, L., Zanol, M., Parravicini, F. and Pifferi, G. (1982) Eur. J. Drug Metab. Pharmacokinet. 7, 39–45.
222 Terauchi, Y., Watari, S., Ishikawa, S., Nakao, M., Negoro, T., Kagemoto, A., Sekine, Y. and Hashimoto, M. (1987) J. Pharmacobio-Dyn. 10, 758–766.
223 Watanabe, T.X., Shiono, K., Saito, K. and Sokabe, H. (1978) Jpn. J. Pharmacol. 28, 413–422.
224 Kawashima, K., Watanabe, T.X. and Sokabe, H. (1980) Jpn. J. Pharmacol. 30, 116–118.

225 Maxwell, G.M. (1976) Clin. Exp. Pharmacol. Physiol. 3, 343–348.
226 De Ponti, C., Bardi, U. and Marchetti, M. (1976) Arzneim. -Forsch. 26, 2089–2092.
227 Pasotti, C., Nicrosini, F., Marchetti, M., Manzini, A. and Nicolis, F.B. (1976) Farmaco, Ed. Prat. 31, 453–462.
228 Maseri, A., Pesola, A., L'Abbate, A., Contini, C. and Magini, G. (1976) J. Int. Med. Res. 4, 402–409.
229 Beretta, E., Cristina, T., Ferrari, P., Tuan, G., Zerilli, L.F. and Martinelli, E. (1979) Eur. J. Drug Metab. Pharmacokinet. 4, 29–33.
230 Assandri, A., Perazzi, A., Buniva, G. and Pagani, V. (1979) Eur. J. Drug Metab. Pharmacokinet. 4, 75–82.
231 Bellasio, E. and Campi, A. (1978) Farmaco, Ed. Sci. 33, 565–572.
232 Ferni, G., Farina, C., Lombroso, M. and Pinza, M. (1984) IUPHAR 9th Int. Congr. Pharmacol., Abstr. 1707P.
233 Dorigotti, L., Ferni, G. and Semeraro, C. (1984) IUPHAR 9th Int. Congr. Pharmacol., Abstr. 1706P.
234 Kosa, E., Borvendeg, J., Huszti, Z., Kosary, J., Szilagyi, G., Tardos, L., Kasztreiner, E., Nagy, L., Szucs, E. and Kiss, G. (1980) Ger. Offen. 2, 928, 395; (1980) Chem. Abstr. 92, 185940.
235 Szilagy, G., Kasztreiner, E., Kosary, J., Matyus, P., Huszty, Z., Cseh, G., Kenessey, A., Tardos, L., Kosa, E. et al. (1979) Ger. Offen. 2, 825, 861; (1979) Chem. Abstr. 90, 186985.
236 Szilagy, G., Kasztreiner, E., Matyus, P., Kosary, J., Czako, K., Cseh, G., Huszty, Z., Tardos, L., Kosa, E. and Jaszlits, L. (1984) Eur. J. Med. Chem. -Chim. Ther. 19, 111–117.
237 Tardos, L., Kosa, E., Jaszlits, L., Berzetei, I. and Ronai, A. (1980) Adv. Pharmacol. Res. Pract., Proc. Congr. Hung. Pharmacol. Soc., 3rd 1, 239–243; (1981) Chem. Abstr. 94, 185543.
238 Kosa, E., Jaszlits, L. and Tardos, L. (1980) in Ref. 237, 233–237. (1981) Chem. Abstr. 94, 185542.
239 Sutka, K. and Cseh, G. (1981) Proc. Hung. Annu. Meet. Biochem. 21st., 171–172; (1982) Chem. Abstr. 96, 193107.
240 Huszti, Z., Szilagy, G., Matyus, P. and Kasztreiner, E. (1981) J. Neurochem. 37, 1272–1281.
241 Lazar, A., Csanyi, E. and Elekes, I. (1981) Drugs, Biochem. Metab., Sci. Mater. Pap. Colloq., 223–227; (1982) Chem. Abstr. 96, 28203.
242 Bellasio, E., Di Mola, N., Campi, A. and Baldoli, E. (1981) U.S. 4, 247, 551; (1981) Chem. Abstr. 94, 192362.
243 Bellasio, E., Di Mola, N., Campi, A. and Baldoli, E. (1980) Eur. Pat. Appl. 9, 655; (1981) Chem. Abstr. 94, 4033.
244 Bellasio, E., Campi, A., Di Mola, N. and Baldoli, E. (1984) J. Med. Chem. 27, 1077–1083.
245 Baldoli, E., Bianchi, G., Corsico, N. and Di Francesco, G.F. (1985) Arzneim. -Forsch. 35, 818–826.
246 Di Francesco, G.F., Baldoli, E., Marchetti, G. and Glaesser, A. (1986) Arzneim. -Forsch. 36, 84–88.
247 Howden, C.W., Elliott, H.L., Lawrie, C.B. and Reid, J.L. (1983) J. Cardiovasc. Pharmacol. 5, 552–556.
248 Assandri, A., Bellasio, E., Bernareggi, A., Cristina, T., Perazzi, A. and Odasso, G. (1985) Arzneim. -Forsch. 35, 508–513.
249 Assandri, A., Perazzi, A., Baldoli, E., Ferrari, P., Ripamonti, A., Bellasio, E., Tuan, G., Zerilli, L.F. and Tarzia, G. (1985) Xenobiotica 15, 1069–1087.

250 Assandri, A., Tarzia, G., Bellasio, E., Ciabatti, R., Tuan, G., Ferrari, P., Zerilli, L., Lanfranchi, M. and Pelizzi, G. (1987) Xenobiotica 17, 559–573.
251 Assandri, A., Perazzi, A., Bellasio, E., Ciabatti, R., Tarzia, G., Ferrari, P., Ripamonti, A., Tuan, G. and Zerilli, L.F. (1985) Xenobiotica 15, 1089–1102.
252 Di Mola, N. and Bellasio, E. (1985) Farmaco, Ed. Sci. 40, 517–533.
253 Szilagy, G., Kasztreiner, E., Tardos, L., Kosa, E., Jaszlits, L., Cseh, G., Divald, A., Tolnay, P., Elek, S., Elekes, I. and Polgari, I. (1979) Ger. Offen. 2, 825, 906; (1979) Chem. Abstr. 90, 152224.
254 Szilagy, G., Kasztreiner, E., Tardos, L., Jaszlits, L., Kosa, E., Cseh, G., Tolnay, P. and Kovacs-Szabo, I. (1979) Eur. J. Med. Chem. -Chim. Ther. 14, 439–445.
255 Szilagy, G., Kasztreiner, E., Tardos, L., Kosa, E., Jaszlits, L., Cseh, G., Kovacs, I., Tolnay, P., Elek, S., Elekes, I. and Polgari, I. (1979) Belg. 868, 987; (1979) Chem. Abstr. 91, 20532.
256 Szilagy, G., Kasztreiner, E., Tardos, L., Kosa, E., Jaszlits, L., Cseh, G., Kovacs-Szabo, I., Tolnay, P., Elek, S., Elekes, I. and Polgari, I. (1979) Ger. Offen. 2, 831, 072; (1979) Chem. Abstr. 91, 107990.
257 Steiner, G., Gries, J. and Lenke, D. (1981) J. Med. Chem. 24, 59–63.
258 Steiner, G., Gries, J. and Lenke, D. (1981) Ger. Offen. 2, 935, 359; (1981) Chem. Abstr. 95, 43151.
259 Hopkins, S.J. (1980) Drugs Future 5, 554–556.
260 Coates, W.J., Roe, A.M., Slater, R.A. and Taylor, E.M. (1976) Ger. Offen. 2, 527, 066; (1976) Chem. Abstr. 84, 164819.
261 Coates, W.J., Slater, R.A., Roe, A.M. and Taylor, E.M. (1976) Belg. 836, 633; (1977) Chem. Abstr. 87, 6003.
262 Taylor, E.M., Roe, A.M. and Slater, R.A. (1979) Clin. Sci. 57, Suppl., 433s-436s.
263 Al-Falahi, H., May, P.M., Roe, A.M., Slater, R.A., Trott, W.J. and Williams, D.R. (1984) Agents Actions 14, 113–120.
264 Sybertz, E.J., Baum, T., Pula, K.K., Nelson, S., Eynon, E. and Sabin, C. (1982) J. Cardiovasc. Pharmacol. 4, 749–758.
265 Baum, T. and Sybertz, E.J. (1983) Fed. Proc., Fed. Am. Soc. Exp. Biol. 42, 176–181.
266 Pitcher, D.W., Curry, P.V. and Trounce, J.R. (1982) Br. J. Clin. Pharmacol. 13, 711–716.
267 Edmondstone, W.M., Waller, J.F., Manghani, K.K., Hill, P.L., Bell, A.J. and Denison, D.M. (1983) Br. J. Clin. Pharmacol. 16, 591–598.
268 Kawashima, K., Watanabe, T.X. and Sokabe, H. (1984) Jpn. J. Pharmacol. 36, 519–526.
269 Fariello, R., Alicandri, C.L., Agabiti-Rosei, E., Romanelli, G., Castellano, M., Beschi, M., Platto, L., Leto di Priolo, S. and Muiesan, G. (1981) Clin. Sci. 61, Suppl., 465s-468s
270 Boucher, M., Dubray, C. and Duchene-Marullaz, P. (1983) J. Cardiovasc. Pharmacol. 5, 712–716.
271 Bianchetti, M.G., Boehringer, K., Weidmann, P., Link, L., Schiffl, H. and Ziegler, W.H. (1982) Eur. J. Clin. Pharmacol. 23, 289–296.
272 Boehringer, K., Weidmann, P., Link, L., Bianchetti, M.G., Schiffl, H. and Reubi, F.C. (1983) Br. J. Clin. Pharmacol. 15, 181–188.
273 Fariello, R., Alicandri, C., Agabiti-Rosei, E., Montini, E., Boni, E., Zaninelli, A., Muiesan, M.L., Romanelli, G., Beschi, M., Castellano, M., Cinquegrana, A., Guarienti, P. and Muiesan, G. (1984) Clin. Trials J. 21, 67–79.
274 Collier, J.G. and Pitcher, D.W. (1980) Br. J. Clin. Pharmacol. 10, 567–571.
275 Taylor, E.M., Eden, R.J., Fielden, R. and Owen, D.A. (1981) J. Cardiovasc. Pharmacol. 3, 355–368.

276 Taylor, E.M., Cameron, D., Eden, R.J., Fielden, R. and Owen, D.A. (1981) J. Cardiovasc. Pharmacol. 3, 337–354.
277 Leonetti, G., Terzoli, L., Sala, C., Bianchini, C. and Zanchetti, A. (1980) Clin. Sci. 59, Suppl., 461s-463s.
278 Larsson, R., Karlberg, B.E., Norlander, B. and Wirsen, A. (1981) Clin. Pharmacol. Ther. 29, 588–593.
279 Edmondstone, W.M., Manghani, K.K., Bell, A.J., McLeod, M., Milton-Thompson, G.J. and Burland, W.L. (1981) Br. J. Clin. Pharmacol. 12, 567–572.
280 Steiner, J.A., Clancy, A., Manghani, K.K. and James, I.M. (1981) Br. J. Clin. Pharmacol. 12, 573–578.
281 Karlberg, B.E., Larsson, R. and Oehman, K.P. (1981) Clin. Sci. 61, Suppl., 461s-464s.
282 Watanabe, T.X., Sokabe, H. and Kawashima, K. (1985) Jpn. J. Pharmacol. 38, 273–279.
283 Owen, D.A., Eden, R.J., Lamb, M.S. and Taylor, E.M. (1986) J. Cardiovasc. Pharmacol. 8, 743–748.
284 Barnett, K.C., Leslie, G.B. and Walker, T.F. (1985) Hum. Toxicol. 4, 417–424.
285 Sutton, T.J., Darby, A.J., Johnson, P., Leslie, G.B. and Walker, T.F. (1986) Hum. Toxicol. 5, 183–187.
286 Teikoku Hormone Mfg. Co., Ltd. (1981) Jpn. Kokai Tokkyo Koho JP 81, 142, 272; (1982) Chem. Abstr. 96, 142874.
287 Teikoku Hormone Mfg. Co., Ltd. (1981) Jpn. Kokai Tokkyo Koho JP 81, 169, 675; (1982) Chem. Abstr. 96, 162722.
288 Teikoku Hormone Mfg. Co., Ltd. (1982) Jpn. Kokai Tokkyo Koho JP 82, 108, 075; (1982) Chem. Abstr. 97, 216202.
289 Briggs, M.T., Dowell, R.I. and Thornber, C.W. (1983) Eur. Pat. Appl. EP 92, 945; (1984) Chem. Abstr. 100, 121097.
290 Yasuda, K., Takezaki, T., Ohuchi, R., Ohuyabu, H., Tanimoto, Y., Seki, T., Yamaguchi, T., Izumi, A., Himori, N. and Ishimori, T. (1984) Eur. Pat. Appl. EP 97, 202; (1984) Chem. Abstr. 100, 156627.
291 Teikoku Hormone Mfg. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 225, 069; (1984) Chem. Abstr. 100, 209855.
292 Teikoku Hormone Mfg. Co., Ltd. (1984) Jpn. Kokai Tokkyo Koho JP 59, 122, 477; (1985) Chem. Abstr. 102, 62254.
293 Farina, C., Pinza, M., Cerri, A. and Parravicini, F. (1988) Eur. Pat. Appl. EP 274, 409; (1989) Chem. Abstr. 111, 153820.
294 Leclerc, G., Wermuth, C.G., Miesch, F. and Schwartz, J. (1976) Eur. J. Med. Chem. -Chim. Ther. 11, 107–113.
295 Naka, T. and Furukawa, S. (1979) Jpn. Kokai Tokkyo Koho 79, 138, 584; (1980) Chem. Abstr. 92, 163988.
296 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Jpn. Kokai Tokkyo Koho 80, 139, 376; (1981) Chem. Abstr. 94, 121579.
297 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Jpn. Kokai Tokkyo Koho 80, 160, 773; (1981) Chem. Abstr. 95, 62244.
298 Anderson, P.L., Houlihan, W.J. and Manning, R.E. (1976) U.S. 3, 978, 057; (1977) Chem. Abstr. 86, 16691.
299 Houlihan, W.J. and Manning, R.E. (1974) Can. 959, 845; (1975) Chem. Abstr. 83, 10124.
300 ISF SpA. (1984) Jpn. Kokai Tokkyo Koho JP 59, 206, 363; (1985) Chem. Abstr. 102, 185094.
301 Fuhrer, W. and Hofbauer, K.G. (1985) Ger. Offen. DE 3, 507, 608; (1986) Chem. Abstr. 104, 69162.

302 Baloniak, S., Drozdzynska, E., Linkowska, E., Filczewski, M., Mroczkiewicz, A., Oledzka, K. and Zyczynska-Baloniak, I. (1979) Acta Pol. Pharm. 36, 295–300.
303 Kasztreiner, E., Szilagy, G., Huszti, Z., Matyus, P., Kosary, J., Tardos, L., Kosa, E., Jaszlits, L. and Cseh, G. (1980) Adv. Pharmacol. Res. Pract., Proc. Congr. Hung. Pharmacol. Soc. 3rd, 1, 227–232; (1981) Chem. Abstr. 94, 185394.
304 Bluth, R. (1982) Pharmazie 37, 51–53.
305 Gafiteanu, E., Bobulescu, V. and Petrovanu, M. (1974) Rev. Med. -Chir. 78, 137–141.
306 Petrovanu, M., Gafiteanu, E., Caprosu, M. and Danet, R. (1977) Rev. Med. -Chir. 81, 659–664.
307 Raviña, E., Garcia Mera, G., Santana, L., Orallo, F. and Calleja, J.M. (1985) Eur. J. Med. Chem. -Chim. Ther. 20, 475–479.
308 Garcia Mera, G., Raviña, E., Santana, L., Orallo, F., Fontenla J.A. and Calleja, J.M. (1985) An. Quim., Ser. C 81, 110–112.
309 Garcia, G., Raviña, E., Santana, L., Orallo, F. and Calleja, J.M. (1985) An. R. Acad. Farm. 51, 23–31.
310 Orallo, F., Calleja, J.M., Garcia-Mera, G. and Raviña, E. (1985) Arch. Farmacol. Toxicol. 11, 171–182.
311 Takaya, M., Yamada, T., Fukuda, T. and Kishikawa, T. (1978) Jpn. Kokai 78, 012, 881; (1978) Chem. Abstr. 88, 190875.
312 Katakami, T., Fukazawa, N., Iizuka, H., Nishina, T., Kamiya, J., Tanaka, Y. and Nakano, T. (1983) PCT Int. Appl. WO 83, 001, 447; (1983) Chem. Abstr. 99, 88219.
313 Mitsui Toatsu Chemicals, Inc. (1983) Jpn. Kokai Tokkyo Koho JP 58, 069, 869; (1983) Chem. Abstr. 99, 88221.
314 Mitsui Toatsu Chemicals, Inc. (1982) Jpn. Kokai Tokkyo Koho JP 82, 046, 966; (1982) Chem. Abstr. 97, 55827.
315 Thyes, M., Franke, A., Koenig, H., Lenke, D., Lehmann, H.D. and Gries, J. (1982) Ger. Offen. DE 3, 022, 177; (1982) Chem. Abstr. 96, 142875.
316 Thyes, M., Koenig, H., Lenke, D., Lehmann, H.D. and Gries, J. (1982) Ger. Offen. DE 3, 022, 176; (1982) Chem. Abstr. 96, 104272.
317 Thyes, M., Gries, J., Lehmann, H.D., Lenke, D. and Kunze, J. (1980) Ger. Offen. 2, 854, 191; (1980) Chem. Abstr. 93, 239443.
318 Lebkuecher, R., Thyes, M., Koenig, H., Lehmann, H.D., Gries, J., Lenke, D. and Kunze, J. (1979) Ger. Offen. 2, 727, 481; (1979) Chem. Abstr. 90, 137849.
319 Allen, G.R. and McEvoy, F.J. (1975) U.S. 3, 888, 901; (1975) Chem. Abstr. 83, 193359.
320 McEvoy, F.J. and Allen, G.R. (1975) U.S. 3, 876, 786; (1975) Chem. Abstr. 83, 58793.
321 McEvoy, F.J. and Allen, G.R. (1975) U.S. 3, 876, 787; (1975) Chem. Abstr. 83, 48225.
322 Thyes, M., Rossy, P.A., Koenig, H., Lehmann, H.D., Gries, J. and Lenke, D. (1984) Ger. Offen. DE 3, 302, 442; (1985) Chem. Abstr. 102, 24641.
323 Rossy, P.A., Thyes, M., Franke, A., Koenig, H., Lehmann, H.D., Gries, J., Friedrich, L. and Lenke, D. (1984) Ger. Offen. DE 3, 302, 021; (1984) Chem. Abstr. 101, 230558.
324 Mitsui Toatsu Chemicals, Inc. (1984) Jpn. Kokai Tokkyo Koho JP 59, 053, 472; (1984) Chem. Abstr. 101, 90956.
325 Rossy, P.A., Thyes, M., Franke, A., Koenig, H., Gries, J., Lehmann, H.D. and Lenke, D. (1983) Ger. Offen. DE 3, 209, 159; (1984) Chem. Abstr. 100, 6536.
326 Rossy, P.A., Thyes, M., Franke, A., Koenig, H., Gries, J., Lehmann, H.D. and Lenke, D. (1983) Ger. Offen. DE 3, 209, 158; (1983) Chem. Abstr. 99, 175786.
327 Thyes, M., Franke, A., Koenig, H., Lenke, D., Lehmann, H.D. and Gries, J. (1981) Eur. Pat. Appl. EP 42, 513; (1982) Chem. Abstr. 96, 162721.
328 SmithKline Beckmann Corp. (1985) Jpn. Kokai Tokkyo Koho JP 60, 255, 776; (1986) Chem. Abstr. 104, 148894.

329 Teikoku Hormone Mfg. Co., Ltd. (1989) Austrian AT 389, 108; (1990) Chem. Abstr. 112, 198402.
330 Yasuda, K., Shibata, K., Honma, S., Seki, T., Hasumi, K., Masuda, T., Izumi, A., Ishimori, T., Gotanda, K. and Uno, M. (1988) Eur. Pat. Appl. EP 259, 835; (1988) Chem. Abstr. 109, 6533.
331 Katakami, T., Fukazawa, N., Iizuka, H., Nishina, T., Kamiya, J., Tanaka, Y. and Nakano, T. (1983) Eur. Pat. Appl. EP 84, 250; (1984) Chem. Abstr. 100, 6534.
332 Goeschke, R. (1986) Eur. Pat. Appl. EP 172, 141; (1986) Chem. Abstr. 105, 6517.
333 Teikoku Hormone Mfg. Co., Ltd. (1983) Jpn. Kokai Tokkyo Koho JP 58, 146, 570; (1984) Chem. Abstr. 100, 51594.
334 Hilboll, G., Doppelfeld, I.S. and Prop, G. (1983) Ger. Offen. DE 3, 212, 304; (1984) Chem. Abstr. 100, 51595.
335 Kasztreiner, E., Rabloczky, G., Makk, N., Jaszlits, L., Matyus, P., Cseh, G., Pribusz, I., Czako, K., Diesler, E. et al. (1987) Eur. Pat. Appl. EP 220, 735; (1987) Chem. Abstr. 107, 176053.
336 Thyes, M., Gries, J., Lehmann, H.D., Lenke, D. and Kunze, J. (1980) Ger. Offen. 2, 854, 475; (1981) Chem. Abstr. 94, 15760.
337 Rossy, P.A., Thyes, M., Franke, A., Koenig, H., Gries, J., Lehmann, H.D. and Lenke, D. (1983) Ger. Offen. DE 3, 135, 617; (1983) Chem. Abstr. 98, 198262.
338 Imperial Chemical Industries PLC (1983) Jpn. Kokai Tokkyo Koho JP 58, 090, 581; (1983) Chem. Abstr. 99, 139958.
339 Jonas, R., Piulats, J., Lues, I. and Schliep, H.J. (1987) Eur. Pat. Appl. EP 210, 530; (1987) Chem. Abstr. 106, 176412.
340 Hilboll, G., Prop, G., Borbe, H.O. and Uhlendorf, J. (1985) Ger. Offen. DE 3, 401, 911; (1986) Chem. Abstr. 104, 34096.
341 Biedermann, J., Borbe, H., Graf, E., Hilboll, G. and Prop, G. (1985) Ger. Offen. DE 3, 407, 508; (1986) Chem. Abstr. 104, 62076.
342 Nakao, T., Setoguchi, N. and Yaoka, O. (1979) Jpn. Kokai Tokkyo Koho 79, 019, 987; (1979) Chem. Abstr. 91, 39511.
343 Austel, V., Heider, J., Eberlein, W., Diederen, W. and Haarmann, W. (1980) Ger. Offen. 2, 837, 161; (1980) Chem. Abstr. 93, 114525.
344 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Jpn. Kokai Tokkyo Koho 80, 053, 284; (1980) Chem. Abstr. 93, 220765.
345 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Neth. Appl. 78, 010, 396; (1980) Chem. Abstr. 93, 239442.
346 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Jpn. Kokai Tokkyo Koho 80, 083, 781; (1981) Chem. Abstr. 94, 47349.
347 Nakao, T., Setoguchi, S. and Yaoka, O. (1980) Fr. Demande 2, 439, 196; (1981) Chem. Abstr. 94, 65712.
348 Nakao, T., Setoguchi, S. and Yaoka, O. (1980) Brit. UK Pat. Appl. 2, 031, 404; (1981) Chem. Abstr. 94, 103407.
349 Yoshitomi Pharmaceutical Industries, Ltd. (1980) Jpn. Kokai Tokkyo Koho 80, 141, 483; (1981) Chem. Abstr. 94, 121578.
350 Lesher, G.Y. and Dickinson, W.B. (1981) U.S. US 4, 298, 609; (1982) Chem. Abstr. 96, 69013.
351 Lautenschlaeger, H.H., Hilboll, G., Friehe, H. and Loehr, J.P. (1983) Ger. Offen. DE 3, 130, 252; (1983) Chem. Abstr. 98, 198225.
352 Hauel, N., Austel, V., Heider, J., Reiffen, M., Diederen, W. and Haarmann, W. (1983) Ger. Offen. DE 3, 129, 447; (1983) Chem. Abstr. 98, 198261.
353 Mitsui Toatsu Chemicals, Inc. (1984) Jpn. Kokai Tokkyo Koho JP 59, 053, 482; (1984) Chem. Abstr. 101, 90955.

354 Warner-Lambert Co. (1984) Jpn. Kokai Tokkyo Koho JP 59, 176, 279; (1985) Chem. Abstr. 102, 132059.
355 Hauel, N., Narr, B., Noll, K., Bomhard, A., Heider, J., Psiorz, M., Diederen, W. and Van Meel, J. (1986) Ger. Offen. DE 3, 511, 110; (1987) Chem. Abstr. 106, 18589.
356 Gomez Gil, M.C., Gomez Parra, V., Sanchez Alonso, F. and Torres Cebada, T. (1985) Span. ES 540, 813; (1987) Chem. Abstr. 106, 18587.
357 Gomez Gil, M.C., Gomez Parra, V., Sanchez Alonso, F. and Torres Cebada, T. (1985) Span. ES 525, 030; (1987) Chem. Abstr. 106, 5064.
358 Coates, W.J., Roe, A.M. and Slater, R.A. (1975) Ger. Offen. 2, 459, 468; (1975) Chem. Abstr. 83, 164219.
359 Coates, W.J., Roe, A.M. and Slater, R.A. (1977) U.S. US 4, 011, 321; (1977) Chem. Abstr. 87, 23316.
360 Coates, W.J., Roe, A.M. and Slater, R.A. (1977) Brit. 1, 488, 330; (1978) Chem. Abstr. 88, 152654.
361 Roe, A.M., Coates, W.J., Slater, R.A., Breukelman, S.P. and Meakins, G.D. (1985) Eur. Pat. Appl. EP 138, 344; (1985) Chem. Abstr. 103, 141993.
362 Morisawa, Y., Kataoka, M., Koike, H., Kobayashi, S. and Kumakura, S. (1987) Jpn. Kokai Tokkyo Koho JP 62, 142, 165; (1988) Chem. Abstr. 108, 37852.
363 Yamada, T., Yamaguchi, A., Shimamura, H. and Takatani, M. (1980) Jpn. Kokai Tokkyo Koho 80, 015, 455; (1980) Chem. Abstr. 93, 114554.
364 Shimamura, H., Kosegi, K., Yaginuma, H. and Oki, M. (1987) Jpn. Kokai Tokkyo Koho JP 62, 255, 486; (1988) Chem. Abstr. 109, 22974.
365 Stefanescu, E., Caprosu, M., Gafitanu, E., Danet, R. and Petrovanu, M. (1979) Rev. Med. -Chir. 83, 311–315.
366 Curran, W.V. and Ross, A. (1974) J. Med. Chem. 17, 273.
367 Slater, R.A., Howson, W., Swayne, G.T., Taylor, E.M. and Reavill, D.R. (1988) J. Med. Chem. 31, 345–351.
368 Swayne, G.T., Owen, D.A., Taylor, E.M., Eden, R.J., Slater, R.A. and Howson, W. (1987) Arch. Int. Pharmacodyn. Ther. 289, 251–266.
369 Howson, W., Kitteringham, J., Mistry, J., Mitchell, M.B., Novelli, R., Slater, R.A. and Swayne, G.T. (1988) J. Med. Chem. 31, 352–356.
370 Sutton, T.J., Luke, J.S. and Jones, H.B. (1988) Hum. Toxicol. 7, 243–248.
371 Barlocco, D., Martini, A., Pinna, G.A., Curzu, M.M., Sala, F. and Germini, M. (1987) Farmaco, Ed. Sci. 42, 585–594.
372 Cignarella, G., Barlocco, D., Pinna, G.A., Loriga, M., Tofanetti, O., Germini, M. and Sala, F. (1986) J. Med. Chem. 29, 2191–2194.
373 Thyes, M., Lehmann, H.D., Gries, J., Koenig, H., Kretzschmar, R., Kunze, J., Lebkuecher, R. and Lenke, D. (1983) J. Med. Chem. 26, 800–807.
374 Diamond Shamrock Corp. (1980) Jpn. Kokai Tokkyo Koho 80, 111, 473; (1981) Chem. Abstr. 94, 103406.
375 Depompei, M.F. and Hlynsky, A. (1981) U.S. 4, 281, 125; (1981) Chem. Abstr. 95, 187288.
376 Bimber, R.M., Buchman, R., Depompei, M.F. and Powers, L.J. (1981) U.S. US 4, 286, 091; (1982) Chem. Abstr. 96, 6747.
377 Powers, L.J., Ariyan, Z.S., Buchman, R., Scozzie, J.A., Moser, R.E. and Pyne, W.J. (1980) U.S. US 4, 238, 490.
378 Buchman, R., Scozzie, J.A., Ariyan, Z.S., Heilman, R.D., Rippin, D.J., Pyne, W.J., Powers, L.J. and Matthews, R.J. (1980) J. Med. Chem. 23, 1398–1405.
379 Fogt, S.W., Scozzie, J.A., Heilman, R.D. and Powers, L.J. (1980) J. Med. Chem. 23, 1445–1448.

380 Corsano, S., Strappaghetti, G. and Codagnone, A. (1989) Arch. Pharm. (Weinheim) 322, 833–835.
381 Matyus, P., Makk, N., Kasztreiner, E. and Jerkovich, G. (1989) Heterocycles 29, 67–77.
382 Birkas-Faigl, E., Zolyomi, G., Makk, N. and Kasztreiner, E. (1987) J. Labelled Compd. Radiopharm. 24, 1317–1324.
383 Gomez Gil, M.C., Gomez Parra, V., Sanchez, F. and Torres, T. (1985) Liebigs Ann. Chem., 1465–1473.
384 Gomez Gil, M.C., Gomez Parra, V., Sanchez, F. and Torres, T. (1986) Arch. Pharm. (Weinheim) 319, 60–64.
385 Deodhar, K.D., Kanekar, D.S. and Pabrekar, P.N. (1983) Indian J. Chem., Sect. B 22B, 512–514.
386 Kulkarni, V.M. (1977) Curr. Sci. 46, 801–803.
387 Vogel, A. (1983) Ger. Offen. DE 3, 239, 789; (1983) Chem. Abstr. 99, 105266.
388 Loev, B., Jones, H. and Shroff, J.R. (1984) U.S. US 4, 435, 395; (1984) Chem. Abstr. 101, 7179.
389 Bayer A.G. (1987) Jpn. Kokai Tokkyo Koho JP 62, 025, 662; (1988) Chem. Abstr. 108, 37853.
390 Huang, F.C., Jones, H. and Chan, W.K. (1984) U.S. US 4, 465, 679; (1984) Chem. Abstr. 101, 211193.
391 Huang, F.C., Jones, H. and Chan, W.K. (1987) U.S. US 4, 659, 711; (1987) Chem. Abstr. 107, 115600.
392 Belz, G.G., Aust, P.E., Belz, G., Appel, E. and Palm, D. (1982) Eur. J. Clin. Pharmacol. 23, 15–20.
393 Steppeler, A. and Starke, K. (1982) Biochem. Pharmacol. 31, 1075–1080.
394 Steppeler, A., Pfaendler, R., Hedler, L. and Starke, K. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 314, 1–11.
395 Lenke, D., Gries, J. and Kretzschmar, R. (1981) Arzneim. -Forsch. 31, 1558–1565.
396 Araujo, D., Caramona, M.M. and Osswald, W. (1983) Eur. J. Pharmacol. 90, 203–214.
397 Steppeler, A. and Starke, K. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 314, 13–16.
398 Traut, M., Brode, E. and Hoffmann, H.D. (1981) Arzneim. -Forsch. 31, 1566–1574.
399 Steppeler, A., Doering, C., Hedler, L. and Starke, K. (1982) Biochem. Pharmacol. 31, 2395–2402.
400 Starke, K. (1983) Trends Pharmacol. Sci. 4, 269–272.
401 Gries, J., Schuster, J., Giertz, H., Lehmann, H.D., Lenke, D. and Worstmann, W. (1981) Arzneim. -Forsch. 31, 1533–1543.
402 Lehmann, H.D., Giertz, H., Kretzschmar, R., Lenke, D., v. Philipsborn, G., Raschack, M. and Schuster, J. (1981) Arzneim. -Forsch. 31, 1544–1557.
403 Mueller, C.D., Gries, J., Lenke, D., Teschendorf, H.J. and Weifenbach, H. (1981) Arzneim.-Forsch. 31, 1574–1579.
404 Teschendorf, H.J., Kretzschmar, R., Kreiskott, H. and Weifenbach, H. (1981) Arzneim. -Forsch. 31, 1580–1588.
405 Kretzschmar, R., Lehmann, H.D., Lenke, D. and Raschack, M. (1983) Gen. Pharmacol. 14, 163–165.
406 Traut, M., Brode, E., Neumann, B. and Kummer, H. (1981) Arzneim. -Forsch. 31, 1594–1604.
407 Miyazaki, Y., Misawa, H., Nakagawa, H., Iida, M. and Ohnishi, K. (1988) Yakuri to Chiryo 16, 1471–1480; (1988) Chem. Abstr. 109, 85976.
408 Wilsmann, K., Neugebauer, G., Kessel, R. and Lang, E. (1981) Arzneim. -Forsch. 31, 1638–1646.

409 Neugebauer, G., Kaumeier, H.S., Kehrhahn, O.H. and Morgenthaler, H. (1983) Eur. J. Clin. Pharmacol. 24, 185–190.
410 Wilsmann, K., Neugebauer, G., Kessel, R. and Lang, E. (1981) Arzneim. -Forsch. 31, 1647–1652.
411 Mueller, H., Braehler, A., Boerner, U., Boldt, J., Stoyanov, M. and Hempelmann, G. (1985) Reg. -Anaesth. (Berlin) 8, 43–49; Pub. in Anaesthesist. 34, 7; (1985) Chem. Abstr. 103, 205997.
412 Saletu, B., Gruenberger, J. and Linzmayer, L. (1980) Curr. Ther. Res. 28, 800–826.
413 Traut, M., Kummer, H. and Brode, E. (1981) Arzneim. - Forsch. 31, 1616–1622.
414 Kaumeier, S., Kehrhahn, O.H., Morgenthaler, H. and Neugebauer, G. (1981) Arzneim. -Forsch. 31, 1653–1657.
415 Traut, M. and Brode, E. (1981) Arzneim. -Forsch. 31, 1605–1615.
416 Goday Baylina, E. and Puigdellivol Llobet, P. (1986) Span. ES 549, 271; (1987) Chem. Abstr. 106, 67337.
417 Goday Baylina, E. and Puigdellivol Llobet, P. (1986) Span. ES 549, 028; (1987) Chem. Abstr. 106, 84626.
418 Schlecker, R., Kropp, R., Reicheneder, F., Gries, J., Hofmann, H.P., Teschendorf, H.J. and Traut, M. (1985) Ger. Offen. DE 3, 436, 550; (1985) Chem. Abstr. 103, 215309.
419 Hickey, D.M., Leeson, P.D., Carter, S.D., Goodyear, M.D., Jones, S.J., Lewis, N.J., Morgan, I.T., Mullane, M.V. and Tricker, J.Y. (1988) J. Chem. Soc., Perkin Trans. I, 3097–3102.
420 Lewis, N. (1988) Chem. Ind. (London), 109–113.
421 Underwood, A.H., Emmett, J.C., Ellis, D., Flynn, S.B., Leeson, P.D., Benson, G.M., Novelli, R., Pearce, N.J. and Shah, V.P. (1986) Nature (London) 324, 425.
422 Leeson, P.D., Emmett, J.C., Shah, V.P., Showell, G.A., Novelli, R., Prain, H.D., Benson, M.G., Ellis, D., Pearce, N.J. and Underwood, A.H. (1989) J. Med. Chem. 32, 320–336.
423 Amschler, H., Ulrich, W.R. and Eltze, M. (1984) Ger. Offen. DE 3, 417, 368; (1985) Chem. Abstr. 102, 113520.
424 Amschler, H., Ulrich, W.R., Eltze, M., Kilian, U. and Sanders, K. (1984) Ger. Offen. DE 3, 417, 391; (1985) Chem. Abstr. 102, 113035.
425 Kilian, U., Amschler, H., Beume, R., Eltze, M., Kolassa, N. and Schudt, C. (1985) Curr. Clin. Pract. Ser. 19, 209–213.
426 Nosalova, G., Strapkova, A. and Korpas, J. (1986) Farmakol. Toksikol. (Moscow) 49, 81–84; (1986) Chem. Abstr. 104, 81831.
427 Nosalova, G., Strapkova, A. and Korpas, J. (1979) Prog. Pathophysiol., Proc. Int. Congr., 2nd 1975, 608–609; (1981) Chem. Abstr. 94, 206.
428 Zikan, V., Vosatka, V. and Danek, J. (1988) Czech. CS 255, 242; (1989) Chem. Abstr. 111, 153821.
429 Zikan, V. and Vosatka, V. (1988) Czech. CS 255, 244; (1989) Chem. Abstr. 111, 97260.
430 Lesher, G.Y. and Opalka, C.J. (1978) Ger. Offen. 2, 737, 542; (1978) Chem. Abstr. 88, 170179.
431 Lesher, G.Y. and Chester, J. (1978) U.S. US 4, 104, 385; (1979) Chem. Abstr. 90, 121625.
432 Lombard, J.P. (1980) U.S. 4, 238, 489; (1981) Chem. Abstr. 94, 114723.
433 Heinisch, G. and Mayrhofer, A. (1980) Arch. Pharm. (Weinheim) 313, 53–60.
434 Goetz, M.A., Schwartz, C.D., Koupal, L.R., Liesch, J.M., Hensens, O.D., Freidinger, R., Anderson, P.S., Pettibone, D.J. and Woodruff, B.H. (1988) Eur. Pat. Appl. EP 256, 847; (1988) Chem. Abstr. 109, 72049.
435 Goetz, M.A., Schwartz, C.D., Koupal, L.R., Liesch, J.M., Hensens, O.D., Freidinger,

R., Anderson, P.S., Pettibone, D.J. and Woodruff, B.H. (1989) Eur. Pat. Appl. EP 301, 199; (1990) Chem. Abstr. 112, 215199.

436 Pettibone, D.J., Clineschmidt, B.V., Anderson, P.S., Freidinger, R.M., Lundell, G.F., Koupal, L.R., Schwartz, C.D., Williamson, J.M., Goetz, M.A., Hensens, O.D., Liesch, J.M. and Springer, J.P. (1989) Endocrinology (Baltimore) 125, 217–222.

437 Proc. 2nd Int. Symp. Pyridazines (1990) Sci. Pharm. 58, 167–229.

438 Heinisch, G. (1987) Heterocycles 26, 481–496.

439 Easmon, J., Heinisch, G., Holzer, W. and Rosenwirth, B. (1989) Arzneim. -Forsch. 39, 1196–1201.

440 Heinisch, G. and Huber, T. (1989) J. Heterocycl. Chem. 26, 1787–1791.

441 Haider, N., Heinisch, G. and Kemetmueller, G. (1990) J. Heterocycl. Chem. 27, 1645–1647.

442 Borchert, H.H., Pfeifer, S., Helbig, B., Franke, P. and Heinisch, G. (1989) Pharmazie 44, 625–630.

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5 Centrally Acting Dopamine D2 Receptor Ligands: Agonists

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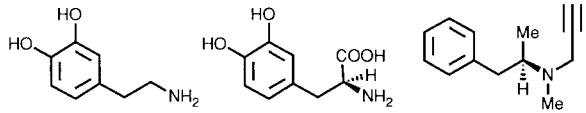
INTRODUCTION

Research on central dopamine (DA) (1) receptors and their functions has evolved during the past 30 years and is now more active than ever [1]. There is a great potential to develop new and efficient drugs, both DA agonists and DA antagonists, for treating diseases related to disturbances in DA functions in the central nervous system (CNS). So far, only a few clinically useful DA agonists have been developed. The indirectly acting aromatic amino acid L-dopa (2) is used for treating Parkinson's disease, and is still the dominating therapeutic agent [2–4]. There is an interesting synergistic interrelationship between the dopamine D1 and D2 receptors, with a possible anti-parkinsonian implication [5]. It has also been speculated that selective D1 receptor activation would be the most beneficial therapy against Parkinson's disease, since D2 agonists are connected with side-effects like nausea, vomiting and inhibition of prolactin secretion [6]. In recent years, L-dopa has found an increasing use in alleviating the disturbing, and sometimes painful symptoms of restless legs [7–10].



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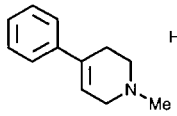
Many DA receptor antagonists (neuroleptics) for treating psychoses (for example, schizophrenia) have become efficient medicines. However, most of them induce severe extrapyramidal side-effects (EPS) akin to parkinsonian symptoms and also, more seriously, they induce tardive dyskinesias (TD). There is a need for improvements in the neuroleptics in the clinic. The substituted benzamides are D2 antagonists, some of which display a high degree of limbic selectivity. Such a regional selectivity has been suggested to be beneficial from the side-effects point of view [11,12].

An alternative to neuroleptics would be to develop partial DA receptor agonists. Such agonists will preferentially stimulate the sensitive D2 autoreceptors and block the less sensitive postsynaptic D2 receptors. Still, such compounds should have a some degree of intrinsic efficacy for the postsynaptic DA receptors in order not to be cataleptogenic [13]. Neuroleptic-induced catalepsy in rats is considered to be predictive for the precipitation of EPS in the clinic. There are now quite a few partial D2 receptor agonists under clinical evaluation (see below), and the near future will show whether this approach offers some advantage over the classical neuroleptics, which all potently block postsynaptic DA receptors.

The mechanisms for presynaptic regulation of dopaminergic neurotransmission by the striatum, the regulation by corticostriatal glutamatergic neurons and the involvement of a thalamo-cortico-striatal neuronal loop in the presynaptic control of dopamine release has been reviewed [14]. A closely related hypothesis on the involvement of different neurotransmitter functions in the regulation of the cortico-striato-thalamic feedback loop was presented by Carlsson [15]. In this model, thalamus is considered to be the system which filters sensory inputs in order to limit the amount of signals reaching cortex. Increased cortical input will increase the glutamatergic, excitatory input to the striatum, which via GABA neurons makes the filter tighter, letting less signal through to cortex. The model is relevant for regulation of both mental and motor functions [15]. Related theories on the etiology of schizopherenia have been presented [16,17]. In addition, the antipsychotic effect of neuroleptics has been suggested to be caused by a nonspecific, postjunctional subsensitivity at glutamatergic synapses, which in turn develops concomitantly with the dopaminergic supersensitivity [17].

Another potential clinical application of potent, peripherally acting DA agonists is the lowering of intraocular pressure in, for example, glaucoma [18]. There is *in vivo* and *in vitro* evidence that dopamine receptors might modulate the intraocular pressure. The influence of both agonists and antagonists for D1 and D2 receptors has been studied. Some human data are also available [19].

The Parkinson Disease Group has initiated a large clinical study in the US on the use of the MAO-inhibitor deprenyl (selegiline®) (3) for slowing down the progression of Parkinson's disease. The name of the study is DATATOP, and some of the positive results have been presented [20]. The background to the study was a published, retrospective study from Europe that indicated positive effects of deprenyl in Parkinsonians. Another important factor for selecting deprenyl was that it is a well-established drug. In addition, the discovery of the neurotoxic agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (4), and the finding that inhibitors of monoamine oxidase-B (MAO-B) protect against the development of its neurotoxicity, led to the speculation that oxidative processes could be a contributing factor to the development of Parkinson's disease [21]. Animal models have also been used to study the protective effects of MAO-B inhibition [22,23]. In addition, a primate model of Parkinson's disease has been developed using MPTP to induce the diseased state [24]. In the attempts to deduce the toxic effects of MPTP, several analogues of MPTP and methylphenylpyridinium (MPP^+), the oxidation product, have been investigated [25]. In particular, polar and charged compounds have successfully been administered by direct infusion using the microdialysis technique [26].



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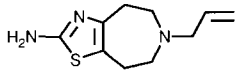

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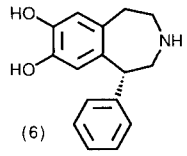


(6)

The clear synergistic relationship between D1 and D2 receptors, at least from the behavioural point of view, is well documented [27–29]. As an example, if reserpinized rats are given a small dose of the DA autoreceptor agonist BHT-920 (talipexole®) (5), the animals display a behaviour consisting of occasional jerks. If such a treatment is combined with a small dose of the D1 agonist SKF 38393 (6), which in itself does not stimulate the behaviour, a very powerful synergism between the D1 and the D2 effects evolves, as evidenced by an intense locomotor activity [30–32]. The D1/D2 interaction can also be seen *in vitro* in receptor binding experiments [33].

STATUS OF THE MOLECULAR BIOLOGY AND PHARMACOLOGY OF THE D2 RECEPTOR

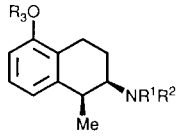
Work on the purification, isolation, cloning and expression of the different DA receptors has had a great success recently, and its development has been described [34,35].

Several molecular biology studies on the D2 receptor(s) have been reported [36–39]. An extra 29-amino-acid sequence in the third cytoplasmic loop was found in these studies. This region of the receptor is known to be involved in G-protein coupling, and its amino acid sequence varies dramatically between different receptor subtypes. The two D2 receptor isoforms (D2L (long) consisting of 444 amino acid units and D2S (short) consisting of 415 amino acid units) were cloned and expressed in transfected COS-7 cells. These two isoforms were shown to have different pharmacological characteristics in binding studies using [^{125}I]iodosulpiride. The mRNA of the D2S receptor seems to be the least abundant in all regions tested, representing 2% in pituitary, 12% in the olfactory bulb, 20–25% in striatum, olfactory tubercle, substantia nigra, cortex and hippocampus, 40–45% in hypothalamus and pons-medulla. It is worth noting that neither of the two receptor subtypes displays the high affinity for substituted benzamide derivatives (for example, raclopride), which is otherwise characteristic for the putative D2 receptor subtype.

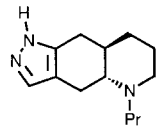
Neurons are long-lived cells, and as such they have lost their ability to replicate DNA. In those cases, alternative splicing is a reversible post-

transscriptional mechanism that enables proteins to be rapidly generated, for example in response to environmental stimuli. This has been speculated to be the mechanism behind the adaptivity exhibited by D2 receptors: for example, the increase in the number of D2 receptors found in the brains of schizophrenic patients and/or following long-term treatment with antipsychotics [38].

In addition to the two D2 receptor isoforms discussed above, still another DA receptor subtype has been cloned and expressed. This DA receptor has been named the D3 receptor and has a 446-amino-acid sequence [40]. This sequence is 52% homologous to that of the D2 receptor subtype. The homology is even higher (75–80%) when considering only the seven transmembral regions, and there are other similarities too. Some interesting binding studies have emerged and there are speculations that atypical neuroleptics like clozapine would have a different D2/D3 binding affinity ratio from the classical neuroleptics like haloperidol. The putative DA autoreceptor antagonists *cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(n-propylamino)tetralin ((+)-AJ 76; *cis*-(+)-7) and its di-n-propylamino analogue ((+)-UH 232; *cis*-(+)-8) have 3–4-times higher affinity for D3 than for D2 receptors [40].



compd	R^1	R^2	R^3
(cis-(+)-7)	H	Pr	Me
(cis-(+)-8)	Pr	Pr	Me
(cis-(+)-9)	Pr	Pr	H



(trans-(-)-4aR-10)

However, it should be emphasized that extensive (that is, many data points) D2 binding data can be mathematically analyzed to give information about both the high ($D2^{high}$) and the low ($D2^{low}$) affinity state of the D2 receptor [41]. It has been stated [40] that the DA agonist quinpirole ((−)-LY171555; 4a*R-trans*-10) exhibits the greatest D3 selectivity. However, taking the $D2^{high}$ binding affinity into account, the D3/D2 binding affinity ratio is close to unity [42].

Just recently, Civelli and co-workers described the cloning of the D4 receptor [43]. This gene has introns, which align with those found in the D2 and D3 genes. The D4 receptor is found more frequently in mesocortical and mesolimbic structures than in the basal ganglia.

PHARMACOLOGICAL METHODS USED IN D2 RESEARCH

Several of the most used pharmacological methods in modern DA research have recently been surveyed by Horn [1].

IN VITRO ASSAYS

(a) In vitro *binding*

Several radioactively labelled ligands for *in vitro* binding studies in D2 research have been developed. These represent both agonist and antagonist binding ligands. The D2 binding studies have revealed, in some cases, two different states of the D2 receptor, namely $D2^{high}$ and $D2^{low}$ [44] (see also above).

Beart and co-workers studied a series of N-substituted 2-aminoteralins from the $D2^{high}/D2^{low}$ point of view. A detailed analysis of [^{3}H]spiperone binding data for potent *N*,*N*-dialkylated 5- and 7-hydroxy-2-aminotetralins show that these compounds bind to two sites, $D2^{high}$ and $D2^{low}$, respectively. $D2^{high}$ accounts for approximately 80% of the total binding. 2-Aminotetralins with one of the two N-substituents being *N*-n-propyl and the other one a lipophilic group like phenylethyl or (2-thienyl)ethyl display very high D2 receptor affinities [45]. It has been suggested that [^{3}H]spiperone binding is indicative of low-affinity and high-capacity binding [46].

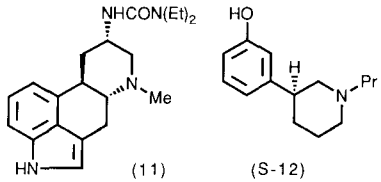
(b) Adenylate cyclase

Dopamine-sensitive adenylate cyclase activity was early demonstrated in both the retina and the cervical ganglion of the cow [47] and later in homogenates of the caudate-putamen of the rat brain [48]. Kebabian has recently reviewed the biochemical components of dopamine-sensitive adenylate cyclase and the physiological role of the D1 receptor [49]. D1 and D2 agonists stimulate and inhibit adenylate cyclase activity, respectively.

(c) Prolactin release

The DA receptor regulating prolactin release in the pituitary is of the $D2^{high}$ type [50]. This view is substantiated by the studies of Carlsson and coworkers, who reported that prolactin release response is different in male and female rats given partial DA agonists of different intrinsic efficacy [13]. In these studies, endogenous DA was depleated and prolactin levels were

raised through the administration of γ-butyrolactone (GBL). The partial DA agonists *trans*-dihydrolisuride (TDHL) (11) and (−)-(*S*)-3-PPP (12) gave dose-dependent reductions in serum prolactin levels in male rats, but had only slight effects in females. This was interpreted to be due to the fact that the DA levels in pituitary portal circulation are higher in the female than in the male rat, which leads to a reduced responsiveness of hypophyseal DA receptors in females.



(11) (S-12)

IN VIVO ASSAYS

(a) Behaviour

Locomotor activity is measured in motility boxes equipped with photocells. Both horizontal and vertical movements can be registered in the modern boxes. Pretreatment with reserpine (18 h) renders the animals virtually without movement, an akinetic Parkinson-like state. It also makes postsynaptic D2 receptors supersensitive, since the receptors have been exposed to low concentrations of DA for a long period of time. The model is thus useful to reveal D2 agonists with a low degree of intrinsic efficacy and, the intrinsic efficacy of a series of partial agonists can be rated by this model.

Unilateral lesions can be obtained through different means: hemisection, 6-OH-DA and MPTP injections. Supersensitivity is developed at the postsynaptic receptors on the lesioned side, giving rise to an asymmetry in the animals treated. Administration of a DA agonist to a lesioned animal will give rise to a rotating behaviour [51].

(b) Biochemistry

The level of DOPA accumulation (decreased by agonists and increased by antagonists) in different brain parts after the administration of dopaminergic test compounds can be taken as an indirect measure of the DA synthesis rate. Such a biochemical test model can be used in normal animals and in reserpine or GBL pretreated animals (see below).

(c) Electrophysiology

Single unit recording is one of the most direct techniques for monitoring neuronal events. In particular, iontophoretic application of drugs avoids problems connected with the passage of the blood-brain barrier and also metabolic problems. In addition, even though most experiments are run under anaesthesia, today these experiments can be performed in non-anaesthetized animals giving the opportunity to correlate the electrophysiological events with behavioural observations. A comprehensive review of the electrophysiology of dopamine-containing neurons in the CNS has been given by Chiodo [52].

(d) Intracranial self-stimulation, i.v. self-administration, drug discrimination and conditioned place preference

The intracranial self-stimulation technique is used to characterize a compound as being a CNS stimulant or depressant. The test animal can, by pressing a lever, introduce a small electric pulse in a pleasure-inducing brain-area. Self-administration can reveal the abuse potential of a stimulant. The animal is able to inject itself i.v. with the test drug by pressing a lever. In the drug discrimination paradigm, an animal is trained to discriminate a standard compound from saline. In the test situation, the compound under investigation is given instead of the standard. If the animal recognizes the test compound as the standard, it is considered to be likely that the two compounds induce similar effects. Conditioned place preference has a similar background philosophy. These techniques have recently been reviewed [53].

(e) Positron emission tomography (PET)

The dopaminergic system is one of the best studied CNS receptor systems with the PET imaging technique [54,55]. ^{11}C-labelled spiperone and raclopride, a pure D2 antagonist, have been used for PET studies in schizophrenic patients. Treatment with ten chemically different classical neuroleptics resulted in a 65–89% occupancy of D2 receptors. This finding strongly supports the dopaminergic hypothesis of schizophrenia. The two patients treated with clozapine showed a D2 occupancy of 65% and 40%, respectively. A D1 receptor occupancy of 42% was found in one of the patients treated with clozapine, and it was speculated that the neuroleptic effects of clozapine may be related to a combined effect on both D1 and D2 receptors [55–57]. Performing kinetic and Scatchard analyses of PET data greatly improves the value of these investigations [58,59].

A SUITABLE SET OF PHARMACOLOGICAL METHODS FOR SCREENING NEW COMPOUNDS WITH POTENTIAL ACTION ON CENTRAL DA RECEPTORS

(a) RESERPINE PRETREATED RATS

This model identifies compounds that are agonists (ranging from classical agonists to partial agonists with very low intrinsic efficacy). 18 h after reserpine administration the behaviour of the rats is studied in photocell motility meters for 30 min (the behaviour can also be recorded with a video camera). Classical DA receptor agonists (acting both at pre- and postsynaptic receptors) reverse the reserpine-induced akinesia and induce locomotor activity. At higher doses, stereotypes such as sniffing, rearing and licking can be seen. A partial DA receptor agonist induces behavioural effects according to its degree of intrinsic efficacy. Compounds with a rather high level of intrinsic efficacy will induce occasional locomotion consisting of jerks, whereas compounds with a low intrinsic efficacy fail to induce detectable behavioural effects in the reserpinized rats. The degree of intrinsic efficacy can be detected by the use of the brain DOPA/5-HTP accumulation assay in the animals of the same experiment.

A 5-HT1A receptor agonist will be identified in this model as a compound which induces the '5-HT behavioural syndrome' (flat body posture, abducted hind and forelegs and forepaw treading). In addition, an α-adrenergic agonist induces piloerection in the reserpinized rat.

After the motility measurements, the rats are injected with the L-aromatic-amino acid decarboxylase inhibitor NSD1015 (3-hydroxybenzylhydrazine) 30 min before killing the animals. The amounts of DOPA and 5-HTP accumulated is an indirect measure of the synthesis rates of the respective monoamines DA and 5-HT. These precursors are measured by means of HPLC with electrochemical detection. A DA receptor agonist will decrease the DOPA accumulation in the DA-rich limbic and striatal brain areas. This effect is considered to be the result of an activation of the inhibitory autoreceptors. Classical DA receptor agonists will produce a maximal decrease in DOPA accumulation (to 20% of controls in the striatum). By the same reasoning, 5-HT1A receptor agonists (and partial agonists) decrease the formation of 5-HTP in the three brain regions studied, while α-adrenergic agonists decrease the DOPA accumulation in the NA-rich remaining cortical region.

(b) NON-PRETREATED RATS

This model detects both agonists and antagonists at monoamine receptors.

Compounds that are inactive in the reserpine model should always be tested in non-pretreated rats before they are classified as inactive. In the motor activity experiments, classical DA receptor agonists induce a biphasic response with hypomotility and hypermotility noted after low and high doses, respectively. These effects are considered to reflect a stimulation of autoreceptors and postsynaptic receptors after low and high doses, respectively. Partial DA receptor agonists induce a monophasic decrease in locomotion. The degree of hypomotility noted depends upon the mixed agonist/antagonist properties of the partial agonist. Classical DA receptor antagonists produce strong hypomotility with catalepsy noted at high doses. The preferential DA autoreceptor antagonists induce a weak locomotor stimulation, which is more pronounced in habituated animals. 5-HT1A receptor agonists induce the 5-HT behavioural syndrome also in non-pretreated animals, however, the behaviour is less pronounced when compared with the effects in reserpinized rats. α-Adrenergic drugs induce piloerection also in non-pretreated animals.

After a 30 min activity session in the motility boxes, the rats are injected with NSD1015 (see above). The DOPA accumulation in striatum and in the limbic brain region will be decreased and increased by DA receptor agonists and antagonists, respectively. Partial DA receptor agonists induce an increased or decreased DOPA formation depending upon their intrinsic efficacy. DA receptor antagonists (the classical ones or those acting preferentially at the autoreceptors) induce a dose-dependent increase in DOPA accumulation. 5-HT1A receptor agonists decrease the 5-HTP formation, whereas α-adrenergic ligands affect the DOPA formation in the cortical region.

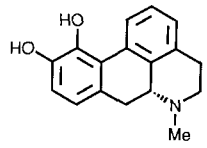
In summary, these two basic screening models offer the possibility of revealing both monoaminergic agonists and antagonists. The models will produce information about the potency and selectivity of the drugs tested, and also whether the compounds have classical agonist/antagonist profiles, or are partial agonists, or antagonists with preference for the DA autoreceptors.

STRUCTURAL CLASSES OF D2 AGONISTS

The dopamine D2 agonist SAR area has been reviewed by Hacksell and coworkers from the perspective of stereochemistry and pharmacological profiles of the enantiomers of the compounds synthesized by that research group during a period of 10 years. The structural classes surveyed were 3-phenylpiperidines, 2-aminotetralins and their ring-methylated analogues and octahydrobenzo[*f*]quinolines (OHB[f]Qs) [60].

(a) APORPHINE DERIVATIVES

One of the early, classical methods of SAR studies, the dissection of a potent molecule into smaller fragments carrying important pharmacophoric groups, has been applied to the potent DA agonist apomorphine (6a*R*-13). Conformational analysis of such fragment molecules by molecular mechanics were undertaken. There was a strong correlation between the energy penalty that has to be paid to achieve a conformation that fits well and the observed loss of potency in biological activity [61].

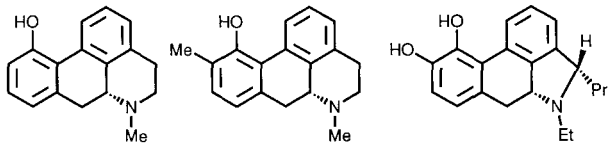


(6aR-13)

The aporphine SAR from the *N*-alkyl substituent point of view has been studied and, taken together, the potency order was: *N*-cyclopropylmethyl > *N*-allyl > *N*-nPr = *N*-Et. The branched analogues (*N*-iPr and *N*-iBu) were inactive [62]. This result is compatible with the results from studies on phenylpiperidines and OHB[f]Qs [63,64] (see under Modelling below). The *N*-allyl derivatives of apomorphine and 11-hydroxyapomorphine were prepared in order to achieve precursors to the corresponding ^{3}H-labelled *N*-nPr derivatives. These *N*-allyl derivatives were evaluated for their dopamine D1 and D2 receptor affinity. In addition, [^{3}H]ADTN binding, which is considered to represent high affinity DA agonist binding, was performed on aporphine analogues. The most interesting result of this study was that (6a*R*)-(−)-11-hydroxy-*N*-allylnoraporphine has a very high affinity (IC_{50} = 0.034 nM) in [^{3}H]ADTN binding. Such a high-affinity [^{3}H]ADTN binding usually is consistent with a high potency functionally [65]. In another aporphine study from the same laboratory, the (6a*R*)-(−)-enantiomer of 2-fluoro-*N*-n-propylnorapomorphine was shown to display an extremely high D2 affinity (IC_{50} = 0.071 nM) in [^{3}H]spiperone binding [66]. The D1/D2 affinity ratio is extremely high, and such high affinity and selective D2 ligands, when radioactively labelled, might prove very useful in future binding studies and in PET imaging.

The mono-phenolic aporphine derivative (6a*R*)-(−)-11-hydroxyaporphine (6a*R*-14) is a DA agonist. Very interestingly, its 10-methyl analogue (6a*R*-15) is devoid of DA effects but instead it potently binds to and stimulates central 5-HT1A receptors [67]. A ring-contracted aporphine analogue

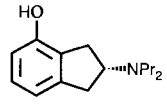
(*R*-16), an abeorphine derivative, was shown to have potent dopaminergic effects in several of the classical test models [68]. It is orally active and long-lasting, but no true estimation of the oral bioavailability can be made from the data presented in the paper, since the only route of administration of compound (*R*-16) was orally.



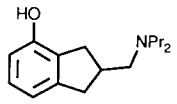
(b) 2-AMINOINDAN AND 2-AMINOTETRALIN DERIVATIVES

The 2-aminoindans have not been studied as extensively as the 2-aminotetralins (see below). Both 4- and 5-hydroxy-2-(dialkylamino)indans display dopaminergic properties [69]. Conformational analyses by the molecular mechanics method (MM2) on differently substituted 2-aminoindans has been reported [70]. It can be anticipated that the active enantiomer (*R*)-4-hydroxy-2-(*N*,*N*-di-n-propylamino)indan (*R*-17) should have its N atom in the equatorial position, placing it close to the aromatic ring plane. This is one of several prerequisites for potent DA receptor agonism. The di-Me model compound of the moderately potent dopaminergic phenylpropylamine analogue 4-hydroxy-2-(*N*,*N*-di-n-propylaminomethyl)indan (18) has also been studied with conformational calculations. Two stable conformations, which fit current DA agonist SAR, were found [70]. This emphasizes that the phenylethylamine moiety in the DA agonists is not an absolute requirement for activity. A compound which has the necessary functional groups at the right places in space and does not protrude sterically outside the receptor volume will show agonist activity.

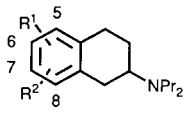
Two *o*-fluorinated tetralins, 6-fluoro-5-hydroxy- and 7-fluoro-8-hydroxy-2-(*N*,*N*-di-n-propylamino)tetralin (6-F-5-OH- and 7-F-8-OH-DPAT, respectively) (19 and 20) retain the dopaminergic and serotonergic receptor stimulating properties of their non-fluorinated analogues 5-OH- and 8-OH-DPAT (21 and 22), respectively [71]. The most interesting implication of this result is that the strong H-bond between the two aromatic substituents is likely to prevail also at the drug–receptor interaction, giving rise to a refinement of the SAR of the phenolic OH groups in these two compounds. It is thus likely that the optimal O–H bond direction in both the DA and the 5-HT1A receptor at the drug-receptor interaction is the one where the



(R-17)



(18)



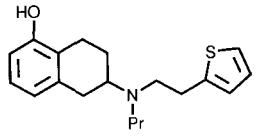
compd	R^1	R^2
(19)	5-OH	6-F
(20)	8-OH	7-F
(21)	5-OH	H
(22)	8-OH	H
(23)	7-OH	H

phenolic H is on the side of the fluorine atom, which is small enough not to impose any steric bulk (the size of a fluorine atom is close to that of a hydrogen atom).

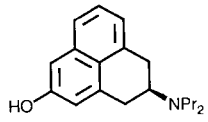
A new and detailed tetralin study deals with the resolution and the determination of the optical purity of the enantiomers of 5-OH-DPAT (21). Of particular interest is that an analytical HPLC method that measures very small amounts of optical impurity was used [72]. It could be shown that the *R*-enantiomer really possesses antagonistic properties, as measured biochemically, in non-pretreated animals [73]. These findings support the modelling results of Froimowitz and co-workers [74,75]. Other studies have not revealed such properties of (*R*-21) [64,76]. These new and interesting findings have implications for the atypical D2 antagonsits with preferential action on D2 autoreceptors, developed from D2 agonists, as discussed below.

The selective D2 receptor agonist (±)-N-0437 (24), and its enantiomers, have been speculated to have potential use in the treatment of Parkinson's disease and schizophrenia [77]. In addition, the enantiomers have been extensively tested for their pharmacological actions on D2 autoreceptors both *in vivo* (DA release in microdialysis after local application) and *in vitro* (effects on the electrically stimulated release of [^{3}H]DA from striatal slices). It is now documented that (*S*)-(−)-N-0437 is an agonist at receptors controlling DA release. (*R*)-(+)-N-0437 is an antagonist at postsynaptic D2 receptors both *in vivo* and *in vitro*. However, since it is known that (*R*)-(+)-N-0437 is an agonist at D2 autoreceptors, it is clear that this enantiomer has the properties of a partial agonist. The intrinsic efficacy of (*S*)-(−)-N-0437 is thus higher than that of its (*R*)-(+)-enantiomer [78].

The more active enantiomer of the tetrahydrophenalenamine (25) was shown to have the *R* absolute configuration. It should be compared to (*R*-23), according to the McDermed concept. The extra benzene ring in (25)



(24)



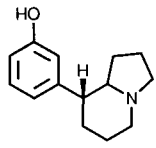
(R-25)

is likely to impose some degree of steric hindrance at the drug–receptor interaction, rendering the molecule an autoreceptor selective profile, as emphasized in an electrophysiological study of (25) and its enantiomers [79].

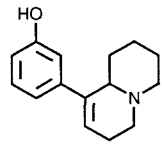
C1- and C3-methyl-substituted 5- and 7-OH-DPAT have been investigated both conformationally and pharmacologically for activity at central DA receptors [80–84]. All compounds were less potent or inactive, as compared with their non-methylated analogues. However, very interesting SAR results have come out of this series of methylated 2-aminotetralins, and *cis*-(+)-(1*S*,2*R*)-5-OH-1-Me-DPAT ((+)-UH242; *cis*-(+)-1*S*,2*R*-9) was the first compound revealed to have the profile of a DA receptor antagonist with a preferential action on DA autoreceptors (see below) [80].

(c) PHENYLPIPERIDINES AND DERIVATIVES

The phenylpiperidine 3-(3-hydroxyphenyl)-*N*-n-propylpiperidine (3-PPP) (12) and its enantiomers have been thoroughly studied for 10 years now [85, 86]. It is well documented that (*S*)-(−)-3-PPP (S-12) is a partial DA agonist. It displays agonistic properties at the supersensitive D2 autoreceptors, while it is antagonistic at the normosensitive postsynaptic D2 receptors. These two effects should work in the same direction to lower the dopaminergic activity. Such a pharmacological profile has been speculated to be beneficial in psychoses. The idea behind the concept is that a partial DA agonist would block the postsynaptic D2 receptors while it would not be strong enough to precipitate extrapyramidal side-effects (EPS and TD) [87]. Beside the phenylpiperidines, the potential dopaminergic effects of phenylindolizidines and phenylquinolizidines have been studied [88]. Only compound (8*R*-26) showed DA receptor agonist properties, while others, for example (27),



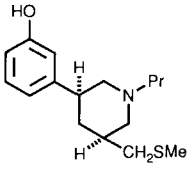
(8R-26)



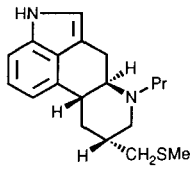
(27)

showed DA receptor antagonistic properties. These close analogues of 3-PPP were further studied by conformational analysis with molecular mechanics (see below) and the results made it possible to refine a previously proposed DA receptor model [89,90].

A 5-(methylthio)methyl-substituted derivative (*cis*-28) of (±)-3-PPP has been reported [91]. The background to the study was the structural similarity between pergolide (29) and (*cis*-28). However, the biological testing of (*cis*-28) showed that it is inactive *in vivo* (GBL model), while an *in vitro* assay (inhibition of tyrosine hydroxylation) showed (*cis*-28) to be equipotent to racemic 3-PPP itself. These results indicate that the steric bulk in (*cis*-28) is not compatible with potent DA receptor interaction. However, since compound (*cis*-28) was not resolved, there is a possibility that one or both of the enantiomers of (*cis*-28) might have antagonistic properties [89].



(cis-28)



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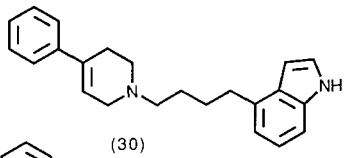

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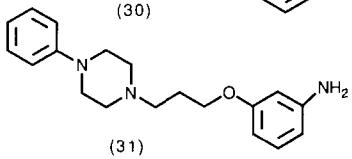
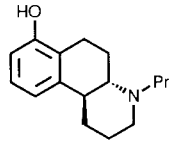


(31)

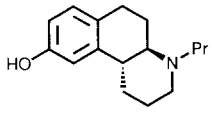
A compound possessing DA autoreceptor agonist properties is EMD23448 (30) [92,93]. The (aminoalkoxy)anilines represent a new class of compounds with dopaminergic properties. The analogue 3-[3-(4-phenyl-1-piperazinyl)propoxy]benzenamine (31) was classified as a selective DA autoreceptor agonist based on the proper pharmacological models for revealing such a profile. In addition, compound (31) showed a good oral availability in a conditioned avoidance response (CAR) test in squirrel monkeys and it was shown to have no propensity for inducing EPS in a primate model. Compound (31) is claimed to be a more selective DA autoreceptor agonist than both 3-PPP and EMD23448 [94].

(d) OHB[f]Qs AND OHB[g]Qs

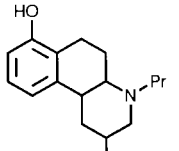
Trans-fused 7- and 9-hydroxyoctahydrobenz[*f*]quinolines (OHB[f]Qs) (for example (32) and (33), respectively) have been found to possess potent dopaminergic properties, provided that the nitrogen atom is properly substituted ($\leqslant$nPr for *trans*-9-OH in the 'downwards' direction and any size of an alkyl group for *trans*-7-OH in the 'upwards' direction; see below) [95]. These results are further substantiated by the fact that the 2-substituted *trans*-7-OH-OHB[f]Qs (for example (34)) display low dopaminergic activity (too bulky 'downwards'), while their 9-OH analogues (for example (35)) are very potent dopaminergics [96]. Similarly, new phenolic (*cis*)-and (*trans*)-1,2,3,4,4a,5,10,10a-octahydrobenz[*g*]quinolines (OHB[g]Qs) have been



(trans-4aS,10bS-32)



(trans-4aR,10bR-33)



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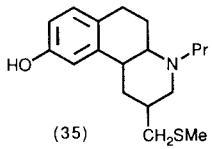
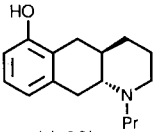
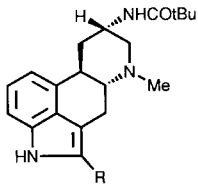


(35)

studied in pharmacological models monitoring both D1 and D2 effects. In contrast to their the *cis* analogues, the *trans*-6-hydroxy compounds were active. The agonist effects increased with increasing *N*-alkyl size in the "downwards" direction up to and including nPr (*trans*-(−)-36). The corresponding *N*-nBu derivative was inactive, supporting the previously presented interrelationship between chirality, phenolic position and *N*-alkyl size [64,97]. For further SAR details, see under Modelling, below.



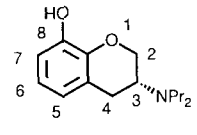
(trans-(-)-36)



compd	R
(37)	Me
(38)	Cl

(e) ERGOLINES

The ergolines have served as lead structures for the development of several different classes of compounds, for example the partial DA agonists transdihydrolisuride (TDHL) (11), SDZ 208–911 (37) and SDZ 208–912 (38). These compounds were compared with haloperidol, a classical DA antagonist (neuroleptic), in several pharmacological test models, emphasizing differences in the intrinsic efficacy between these partial agonists. SDZ 208–912 has the lowest intrinsic efficacy of the three, showing antagonistic properties in most test models. However, it lowers prolactin secretion and has a greatly reduced propensity to induce catalepsy. Both SDZ 208–911 and TDHL have higher intrinsic efficacies and display similar pharmacological properties [98].



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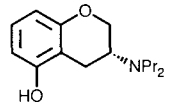

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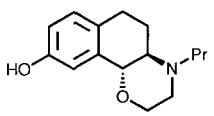
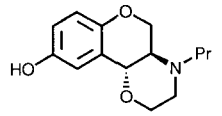


(trans-(+)-4aR,10bR-41)



(trans-(+)-4aR,10bR-42)

(f) 3-AMINOCHROMAN DERIVATIVES

The chromanamines 8- (39) and 5-OH-DPCA (40) have been shown to be potent, centrally acting agonists at D2 and 5-HT1A receptors, respectively [99]. 8-OH-DPCA is a much more potent dopaminergic agonist than its 6-OH analogue [100,101]. A different relationship exists between their tetralin analogues 5-(21) and 7-OH-DPAT (23), respectively, which both are potent DA agonists [64,102]. There is a good peroral (p.o.) to intraperitoneal (i.p.) ratio for 8-OH-DPCA, which is an indication of a good absorption from the gastrointestinal tract and a limited metabolism during the absorption process. One of the few DA agonists that has been tested clinically is reduced (+)-4-propyl-9-hydroxynaphthoxazine (41). This compound is active against Parkinson's disease, both alone and in the combination with L-dopa. Since the oral bioavailability of (41) is low, a continuous delivery system

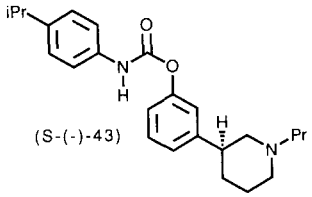
was used [24]. The pharmacological testing of the dioxygenated tricyclic compound *trans*-3,4,4a,10b-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol (42) showed that it is a potent D2 agonist with preference for D2 autoreceptors [103]. In contrast to this result, another group has claimed that this compound displays very weak D2 agonist effects [104]. These authors argue that it is the low pK_a value of the compound, giving a low degree of protonation (2%) under physiological conditions, which makes it weak. The discrepancy between the two papers might depend on the different pharmacological methods used [103].

METABOLIC PROBLEMS AND BIOISOSTERIC REPLACEMENTS IN D2 AGONISTS

METABOLISM OF DA AGONISTS

Due to the fact that they contain aromatic hydroxy functionalities, most of the potent DA receptor agonists known (for example, apomorphine, the aminoindans and the aminotetralins) have poor pharmacokinetic properties. They are likely to be conjugated (as glucuronides and/or as sulphates) on their first passage through the liver after p.o. or i.p. administration. In addition, an electronically activated aromatic nucleus such as a phenol or an anisol is susceptible to aromatic hydroxylation by the cytochrome *P*-450 enzyme system. This pathway is more pronounced in the rat than in man. If present in the compound administered, or if formed *in vivo*, catechols are also degraded by the enzyme catechol-*O*-methyltransferase (COMT). For example, the metabolic degradation of the very potent D2 agonist N-0437 (24) has been studied in the rat and in the conscious monkey after both i.v. and p.o. administration using radioactively labelled (ditritiated) N-0437 [105]. The metabolic inactivation of N-0437 proceeds mainly through conjugation, and the biliary metabolic profile showed mainly glucuronidation in both species [106,107]. The urinary profile in the rat showed almost exclusively sulphation, while monkey urine contained three main metabolites, including the glucuronide and the suphate [105]. In an attempt to improve the bioavailability of this compound, several esters were prepared as potential prodrugs. The ester hydrolysis rate was assayed *in vitro*, and the *in vivo* activity of these compounds was monitored with the Ungerstedt rotation model [51]. Large differences were found *in vitro*, but *in vivo* these differences were much smaller and, in fact, did not correlate with the *in vitro* data [108]. Similar disappointing results have previously been found for pivaloyl ester prodrugs of *N*,*N*-di-*n*-propylated *m*-tyramine and *N*,*N*-di-*n*-propylated DA [109].

Using HPLC with electrochemical detection to investigate the potential *o*-hydroxylation in the phenylpiperidine 3-PPP, it was shown that a catecholic metabolite is formed. However, such a metabolite was suggested not to play a significant role in the *in vivo* pharmacological profile exhibited by 3-PPP itself [110–112]. Unfortunately, no urine samples were collected in these studies. An extensive series of carbamate esters of (−)-3-PPP was investigated as potential prodrugs [113]. The 4-isopropylphenylcarbamate (43) was the best prodrug, and it did not induce convulsions, which some of the carbamates in that series did. The plasma levels of (−)-3-PPP 4 h after an oral dose of compound (43) of 100 μmol/kg was 90 times higher than those after the parent drug itself after the same time and dose.



(S-(-)-43)

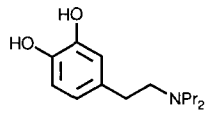
Some of the new DA agonists do not suffer from these pharmacokinetic problems. Most of the new compounds have bioisosteric heterocyclic aromatic systems instead of the phenolic ring systems in the older analogues. Some examples of these new agonists will be given here.

HETEROCYCLIC BIOISOSTERES OF DA AGONISTS

In a series of 3,4-disubstituted anaolgues of *N*,*N*-di-n-propyldopamine (DPDA; 44), the 3-OH function was replaced by different bioisosteric groups. These analogues were evaluated for D1- and D2-receptor affinity. The 3-methanesulphonamide analogue (45) displayed a somewhat higher affinity for the D2 receptor than DPDA and was also more selective for this receptor. In addition, the 3-formamide analogue (46) had a high D2 affinity. These compounds were also active *in vivo* in a model (peripheral) monitoring cardiovascular and renal effects in the anaesthetized rat [114]. Both (45) and (46) are electronically different from the catechol DPDA. They have a lesser electronic density in the aromatic ring system and are thus likely to be less prone to oxidation by the liver oxidizing enzymes.

Another series of heterocyclic bioisosters of DA and *m*-tyramine were evaluated for their affinity and agonist activity at D1 and D2 receptors [46]. Compound (47), in which the *m*-hydroxyl in DPDA has been replaced with a thiazol-2-one ring system, has a higher D2 receptor affinity and a more

potent activity *in vitro*, as compared to DPDA itself. It is also more potent than the oxindole (48) previously reported [115].



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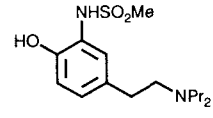

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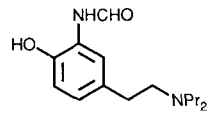

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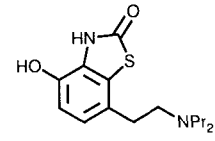

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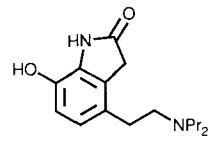

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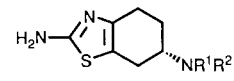
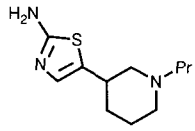


compd	R^1	R^2
(S-49)	H	Pr
(S-50)	Pr	Pr

The heterocyclic aminothiazoloazepine derivative BHT920 (5) is a DA autoreceptor agonist [116]. Attempts to utilize the aminothiazole functionality in other structural classes known to be dopaminergic have been made. Compound (S-49), which is homochiral to the potent DA agonist (*S*)-5-OH-DPAT (S-21), is a D2 agonist and has been suggested to have DA autoreceptor selectivity [117]. It is interesting to note that compound (*S*-50), the *N*,*N*-di-nPr analogue of (*S*-49), was not included in the study. It can be predicted, with high probability, that (*S*-50) would be more potent than (*S*-49). In addition, it is likely that (*S*-50) is less selective for the DA autoreceptors than (*S*-49). Interestingly, compound (*S*-50) has been published in several recent patent applications [118,119].

Similar reasoning formed the background to a study on heterocyclic analogues of 3-PPP [120]. In particular, the low oral bioavailability of 3-PPP [121,122] was emphasized as a possible obstacle for clinical studies with 3-PPP. Modelling studies of the ring-to-ring dihedral angle of the 2-amino-4-thiazolyl piperidine (51) and its tetrahydropyridine analogue (52) showed a

preference, due to the resonance effect, of the latter for a coplanar ring-to-ring relationship. Such a conformation is believed to be the preferred D2 agonist conformation of 3-PPP itself [89,90]. While compound (51) was shown to be inactive in the biological test systems, the best tetrahydropyridine analogues (compounds (52) and (53)) were active. These compounds exhibit some selectivity for DA autoreceptors at lower doses, but at higher doses they clearly stimulate postsynaptic DA receptors. The biological models used in that study are quite relevant to the conclusions drawn. Of particular interest is the fact that these compounds show oral activity. They are thus less prone to metabolic inactivation than 3-PPP itself.



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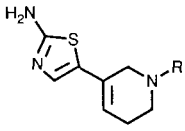
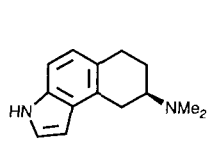
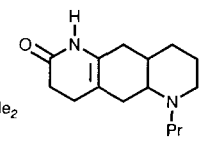


compd	R
(52)	Pr
(53)	allyl

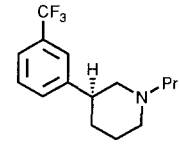
The enantiomers of the dopaminergic, and orally active 6,7,8,9-tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine (54) were studied for their actions on central dopamine and serotonin (5-HT) receptors [123]. The dopaminergic effects were shown to reside in the (+)-(*R*)-enantiomer. Very interestingly, it was shown that racemic (54) and its (+)-(*R*)-enantiomer possess potent central 5-HT1A receptor stimulating properties, which was not reported originally [124]. Still another indication of the trend in DA agonist SAR towards heterocyclic bioisosteres is given by compound (55) [125].



(R-54)



(trans-55)



(S-56)

THE QUESTION AS TO WHETHER THE BASIC NITROGEN ATOM OF A DA LIGAND IS CHARGED OR NOT AT THE DRUG–RECEPTOR INTERACTION

This problem has been thoroughly addressed by Miller and co-workers in

a series of publications regarding both DA agonists and DA antagonists [126–132] and also by Ross and Burkman [133]. These studies conclude that permanently charged structural analogues of dopamine have affinity for, and can activate, the D2 receptor. These analogues contain an N, S or Se atom in the side-chain. However, they are less potent than DA itself, indicating that, whereas the ability of a compound to exist in an uncharged form is not a requirement, both charged and uncharged forms of the agonist molecule appear to play a role in D2 agonist activity [126].

MOLECULAR MODELLING OF D2 LIGANDS

In two recent articles, the use of computers in modern SAR modelling studies has been reviewed [134,135].

Modelling of macromolecules

A computer generated receptor model from the primary sequence of the cloned D2 receptor [36] was presented by Dahl [136]. The Kollman program AMBER (Assisted Model Building and Energy Refinement) [137–139] was used in the constuction of the model. The seven putative transmembrane helices were put together to form a cylinder, inside which the receptor was suggested to be located. Docking experiments in this model with agonists and antagonists indicated that these different types of compound bind at different sites in the receptor model [140]. Despite the advanced approach, this model is still very tentative and crude, and a much more detailed study of the inside of the proposed cylinder must be undertaken before it can be used in a predictive fashion for the design of new agonists and antagonists for the D2 receptor(s). The power of this receptor model is that it is conceptually very appealing and natural. It is interesting to note this development in the macromolecular modelling of a receptor and, in a not too distant future, the receptor concepts that are built from the small molecules (molecular modelling of agonists and antagonists, see below) and from amino acid sequences will merge into one single concept. However, the increasing number of subreceptors (for example the D3 receptor) identified will make this an even more complicated area of research.

Modelling of small molecules

Molecular modelling studies using small molecules, which have been conformationally investigated before they are compared and fitted, can be performed by using a potent and rigid analogue as a template molecule. Alter-

natively, a theoretical receptor model with defined pharmacophoric points in space could be used as the template. Quantification of the quality of a fit is achieved with root-mean-squares (RMS) calculations.

Theoretical calculations can also provide physicochemical data, which can be used as an input for 3D-QSAR correlation studies [134,141]. There is a current trend in the area of lead-finding in the pharmaceutical industry to use calculations to set up computer search profiles to be used in the screening of large structural databases, such as the chemical suppliers' databases and the internal company databases.

Using electrostaic maps and point charges can be an alternative to using the midpoint of rings and individual atoms in the RMS fits [142,143]. An interesting approach when fitting molecules with a basic nitrogen, which is likely to be protonated at the drug-receptor interaction (see above), is to utilize the N–H ammonium hydrogen vector and a hydrogen bonding distance (2.8 Å) in an attempt to mimic the situation when a drug binds to the receptor [144]. The dummy atom at the H-bonding distance will thus be a point in space to be included in the RMS calculation together with the other pharmacophoric groups. Different molecules could have their N-atoms at different sites and still satisfy a good RMS fit, when superimposed on another molecule or a template. Recent molecular modelling studies on D2 SAR have emerged [75,89,90,145,146].

DA AGONIST RECEPTOR MODELS

Several authors have tried to rationalize their SAR results in more or less complicated models. The following are examples: Shepphard and Burghardt [147], Grol and Rollema [148,149], Goldberg [150], Dandiya [151], McDermed [102], Seiler and Markstein [97,152], Nichols [153], Erhardt [154,155], Humber [156,157,158, 159], Seeman [160,161], Neumeyer [162], Wikström [64], Kaiser and Jain [163], Liljefors and Wikström [90]. A few of these conceptual models formed the basis of a more advanced study with computers (see below) [145].

In hindsight, the single most intelligent interpretation of DA SAR was made by McDermed and co-workers [102], and it is worthwhile to recapitulate the background of their concept. The two potent DA receptor agonists 5-OH-DPAT (21) and 7-OH-DPAT (23) were resolved, and it was shown that the active enantiomers of these two isomers were (*S*-21) and (*R*-23), respectively. Since the 2-aminotetralins are almost planar structures, a simple upside-down turn of one of the isomers, together with a fit of the nitrogens and the hydroxyl groups gave a fit as displayed in *Figure 5.1.* Here (*S*-21)

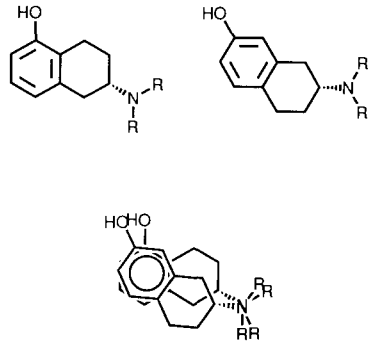


Figure 5.1.

and the (*R*-23) are homochiral, that is, the C2-N bond direction is downwards in both molecules. This has been the basic concept underlying many of the DA receptor models mentioned above. In a study based on the McDermed concept, and applied to the dopaminergic monohydroxylated 3-phenylpiperidines and octahydrobenzo[*f*]quinolines (OHB[f]Qs), it was found that there is a relationship between the absolute configuration, the ring-position of the hydroxyl group and the size of the *N*-alkyl substituent. These parameters taken together define two different directions of the *N*-alkyl substituents in these molecules (*Figure 5.2*) [64]. The combination of the stereochemical concept and the *N*-substituent direction concept has proved useful for explaining the activities and inactivities of several of the chiral compounds tested for their effects on DA receptors. Naturally, a strong concept like this can also be utilized in the design of new dopaminergic compounds, and for setting limits to synthetic programmes in the DA research field. See also note on p. 216.

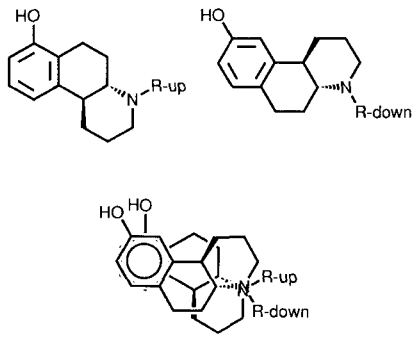
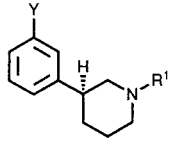


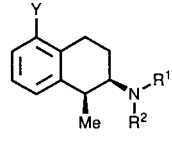
Figure 5.2.

D2 AUTORECEPTOR ANTAGONISTS, FUNCTIONAL D2 RECEPTOR AGONISTS

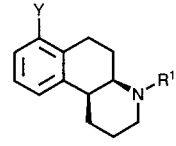
In the course of developing new DA receptor agonists with autoreceptor selectivity (partial agonists), that is, functional analogues of 3-PPP, the enantiomers of *cis*-1-methyl-5-hydroxy-2-(di-n-propylamino)tetralin (UH242; *cis*-9) were synthesized and tested [81]. Racemic (*cis*-9) has a selective DA autoreceptor-selective profile much like that of racemic 3-PPP. However, as was the case with 3-PPP, the pharmacological testing of the enantiomers of (*cis*-9) showed that (*cis*-(−)-1*R*,2*S*-9) is a classical DA agonist stimulating both pre- and postsynaptic DA receptors, while (*cis*-(+)-1*S*,2*R*-9) has DA receptor antagonist properties. Thus, it stimulates the DA synthesis rate like the classical DA antagonists but it does not induce any locomotor activity depression. Instead, a tendency of a weak stimulation of locomotor activity can be seen. The *O*-methylated analogue, compound ((+)-UH232; *cis*-(+)-1*S*,2*R*-8) was then tested in the same pharmacological paradigms. This compound was shown to induce a strong biochemical effect and a clear locomotor stimulation, especially in habituated rats [80,81,164]. It is now clear that the stereochemistry at the 2-position in the 5-substituted 2-aminotetralins is determining which effect, agonistic or antagonistic (2-*S* and 2-*R* absolute configuration in the 5-substituted 2-aminotetralins, respectively), a compound will have at the D2 receptor [60]. This has also been pointed out by others [75]. In the case of an agonist, a more potent effect will be seen with the 5-OH substitution pattern, while for antagonistic effects a 5-OMe group is more efficient than a 5-OH group, implying that it should be quite relevant to study the H-bonding aspects (strength and directionality) in such compounds. The relevance of this reasoning can be seen with *S*-3-(3-trifluoromethyl-*N*-n-propyl)piperidine (*S*-56), an analogue of (*S*)-3-PPP, which displays a pharmacological profile similar to that of *cis*-(+)-UH232 (*cis*-(+)-(1*S*,2*R*)-8) [165]. Thus, it should be possible, by selecting the proper aromatic substituents, to design new 3-substituted (*S*)-3-phenylpiperidines (*S*-57), *cis*-(1*S*,2*R*-5)-substituted 1-methylated-2-aminotetralins (*cis*-1*S*,2*R*-58) and 7-substituted *cis*-(4a*R*,10b*S*)-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines (*cis*-(4a*R*,10b*S*)-59) [166] with preference for the DA autoreceptor.



(S-57)



(cis-1S,2R-58)



(cis-4aR,10bS-59)

Such D2 receptor antagonists are functionally D2 receptor agonists, and may thus be useful in several clinical conditions like geriatric conditions, Parkinson's disease, minimal brain dysfunction (MBD), depression and in obesity, as an anorectic drug [165]. It will be crucially important for the development of any stimulant compound of this class to establish its tendency for inducing abuse. Pharmacological methods that can be used for such investigations are for example intracranial self-stimulation, self-administration, conditioned place-preference and drug discrimination tests.

SUMMARY

The collected amount of research on D2 agonists is immense. The D2 research field has been, and still is, very dynamic. Despite this fact, only a few compounds have reached the clinic so far. At present there are clinical trials ongoing with partial D2 agonists possessing a range of intrinsic efficacies. Some of these agonists are tested for their potential effects in Parkinson's disease (high intrinsic efficacy), while others are tested for potential anti-psychotic effects (low intrinsic efficacy). An interesting possibility has arisen through the research on the synergism between D1 and D2 receptors. This could possibly be utilized in the alleviation of Parkinsonian symptomatology. The near future will show whether the compounds under evaluation hold promise for being new, valuable medicines for treating major diseases like Parkinson's disease and schizophrenia.

REFERENCES

1 Horn, A.S. (1990) in Comprehensive Medicinal Chemistry (Emmett, J.C., ed.), 3, pp 229–291, Pergamon Press, Oxford.
2 Quinn, N.P. (1984) Drugs 28, 236–262.
3 Carlsson, A., Lindqvist, M.L. and Magnusson, T. (1957) Nature (London) 180, 1200.
4 Hornykiewicz, O. (1960) Pharmacol. Rev. 18, 925.
5 Clark, D. and White, F.J. (1987) Synapse 1, 347–388.
6 Markstein, R., Seiler, M.P., Vigouret, J.M., Urwyler, S., Enz, A. and Dixon, K. (1988) Neurol. Neurobiol., 42B (Progr. Catecholamine Res., Pt. B) 59–64.
7 Akpinar, S. (1987) Clin. Neuropharmacol. 10, 69–77.
8 Brodeur, C., Montplaisir, J., Godbout, R. and Marinier, R. (1988) Neurology 38, 1845–1848.
9 Montplaisir, J., Godbout, R., Poirier, G. and Bedard, M.A. (1986) Clin. Neuropharmacol. 9, 456–463.
10 Sandyk, R., Bernick, C., Lee, S.M., Stern, L.Z., Iacono, R.P. and Bamford, C.R. (1987) Int. J. Neurosci. 35, 233–235.

11 Ögren, S.-O. and Högberg, T. (1988) Isi Atlas Sci.: Pharmacol. 2, 141–147.
12 Högberg, T., Rämsby, S., Ögren, S.-O. and Norinder, U. (1987) Acta Pharm. Suec. 24, 289–328.
13 Carlsson, M., Carlsson, A. and Eriksson, E. (1987) Eur. J. Pharmacol. 142, 39–43.
14 Glowinski, J., Cheramy, A., Romo, R. and Barbeito, L. (1988) Cell. Mol. Neurobiol. 8, 7–17.
15 Carlsson, A. (1989) Fidia Res. Found. Symp. Ser., 2 (Neurochem. Pharmacol.-Tribute B.B. Brodie) 13–22.
16 Deutsch, S.I., Mastropaolo, J., Schwartz, B.L., Rosse, R.B. and Morihisa, J.M. (1989) Clin. Neuropharmacol. 12, 1–13.
17 Freed, W.J. (1989) Pharmacol. Biochem. Behav. 32, 337–345.
18 Turner, P. and Mekki, Q.A. (1985) Trends Pharmacol. Sci. 6, 348–349.
19 Karnezis, T.A. and Murphy, M.B. (1988) Trends Pharmacol. Sci. 9, 389–390.
20 The Parkinson disease study group (DATATOP) (1989) N. Engl. J. Med. 321, 1364–1371.
21 Steventon, G., Humfrey, C., Sturman, S., Warling, R.H. and Williams, A.C. (1990) Lancet i, 180.
22 Knoll, J. (1988) Mech. Ageing Dev. 46, 237–262.
23 Knoll, J. (1988) Mt. Sinai J. Med. 55, 67–74.
24 Coleman, R.J., Jenner, P., Marsden, C.D. and Stahl, S.M. (1988) Neurol. Neurobiol., 42C (Progr. Catecholamine Res., Pt. C) 19–23.
25 Johnson, E.A., Wu, E.Y., Rollema, H., Booth, R.G., Trevor, A.J. and Castagnoli, N.J. (1989) Eur. J. Pharmacol. 166, 65–74.
26 Rollema, H., De Vries, J.B., Damsma, G., Westerink, B.H.C., Kranenborg, G.L., Kuhr, W.G. and Horn, A.S. (1988) Toxicology 49, 503–511.
27 Hyttel, J. and Arnt, J. (1988) Neurol. Neurobiol., 42B (Progr. Catecholamine Res., Pt. B) 53–57.
28 Walters, J.R., Bergstrom, D.A., Carlson, J.H., Chase, T.N. and Braun, A.R. (1987) Science (Washington, DC) 236, 719–722.
29 Walters, J.R., Carlson, J.H., Weick, B.G. and Bergstrom, D.A. (1988) Neurol. Neurobiol., 42B (Progr. Catecholamine Res., Pt. B) 65–71.
30 Grabowska Andén, M. and Andén, N.E. (1987) J. Pharm. Pharmacol. 39, 660–661.
31 Andén, N.E. and Grabowska Andén, M. (1988) Acta Physiol. Scand. 134, 285–290.
32 Andén, N.E. and Grabowska Andén, M. (1989) J. Pharm. Pharmacol. 41, 490–492.
33 Seeman, P. and Niznik, H.B. (1988) Isi Atlas Sci.: Pharmacol. 2, 161–170.
34 Beart, P.M. (1989) Clin. Exp. Pharmacol. Physiol. 16, 511–515.
35 Sibley, D.R. (1991) Trends Pharmacol. Sci. 12, 7–9.
36 Bunzow, J.R., van Tol, H., Grandy, D., Albert, P., Salom, J., Christie, M., Machida, C., Neve, K. and Civelli, O. (1988) Nature (London) 336, 783–787.
37 Eidne, K.A., Taylor, P.L., Zabavnik, J., Saunders, P.T.K. and Inglis, J.D. (1989) Nature (London) 342, 865.
38 Giros, B., Sokoloff, P., Martes, M.-P., Riou, J.-F., Emorine, L.J. and Schwarz, J.-C. (1989) Nature (London) 342, 923–926.
39 Monsma, F.J.J., McWittie, L.D., Gerfen, C.R., Mahan, L.C. and Sibley, D.R. (1989) Nature (London) 342, 926–929.
40 Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.L. and Schwartz, J.C. (1990) Nature (London) 347, 146–151.
41 Seeman, P., Grigoriadis, D., George, S.R., Watanabe, M. and Ulpian, C. (1984) Dopaminergic Syst. Their Regul., [Proc. Symp.], Toronto, Canada, 97–109.
42 Seeman, P. (1991) personal communication.

43 Van Tol, H., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B. and Civelli, O. (1991) Nature (London) 350, 610–614.
44 Wreggett, K.A. and Seeman, P. (1984) Mol. Pharmacol. 25, 10–17.
45 Beart, P.M., Cook, C.J., Cincotta, M., De Vries, D.J., Tepper, P., Dijkstra, D. and Horn, A.S. (1987) Naunyn Schmiedeberg's Arch. Pharmacol. 336, 487–493.
46 Weinstock, J., Gaitanopoulos, D.E., Stringer, O.D., Franz, R.G., Hieble, J.P., Kinter, L.B., Mann, W.A., Flaim, K.E. and Gessner, G. (1987) J. Med. Chem. 30, 1166–1176.
47 Kebabian, J.W. and Greengard, P. (1971) Science 174, 1346–1349.
48 Kebabian, J.W., Petzold, G.L. and Greengard, P. (1972) Proc. Natl. Acad. Sci. USA 69, 2145–2149.
49 Kebabian, J.W. (1988) Adv. Exp. Med. Biol. 235, 19–31.
50 George, S.R., Watanabe, M., Di Paolo, T., Falardeau, P., Labrie, F. and Seeman, P. (1985) Endocrinology (Baltimore) 117, 690–697.
51 Ungerstedt, U. (1976) Pharmacol. Ther., Part B 2, 37–40.
52 Chiodo, L.A. (1988) Neurosci. Biobehav. Rev. 12, 49–91.
53 Fibiger, H.C. and Phillips, A.G. (1988) Ann. N.Y. Acad. Sci. 537, 206–215.
54 Barrio, J.R., Huang, S.C. and Phelps, M.E. (1988) Annu. Rev. Pharmacol. Toxicol. 28, 213–230.
55 Sedvall, G., Farde, L., Nybäck, H., Pauli, S., Persson, A., Savic, I. and Wiesel, F.A. (1989) Fidia Res. Found. Symp. Ser., 2 (Neurochem. Pharmacol.-Tribute B.B. Brodie) 271–278.
56 Farde, L., Hall, H., Ehrin, E. and Sedvall, G. (1986) Science 231, 258–261.
57 Farde, L., Wiesel, F.A., Nordström, A.L. and Sedvall, G. (1989) Psychopharmacol. (Berlin) Suppl. 28–31.
58 Farde, L., Eriksson, L., Blomquist, G. and Halldin, C. (1989) J. Cereb. Blood Flow Metab. 9, 696–708.
59 Blomqvist, G., Pauli, S., Farde, L., Eriksson, L., Persson, A. and Halldin, C. (1989) Nuklearmedizin, Suppl. (Stuttgart) 25, 324–327.
60 Hacksell, U., Johansson, A.M., Karlén, A., Svensson, K. and Grol, C.J. (1990) in Chirality and biological activity (Holmstedt, B., Frank, H. and Testa, B., eds.), 23, pp 247–266, Alan R. Liss, New York.
61 Pettersson, I. and Liljefors, T. (1987) J. Comput. Aided Mol. Des. 1, 143–152.
62 Gao, Y.G., Ram, V.J., Campbell, A., Kula, N.S., Baldessarini, R.J. and Neumeyer, J.L. (1989) J. Med. Chem. 33, 39–44.
63 Wikström, H., Sanchez, D., Lindberg, P., Hacksell, U., Arvidsson, L.E., Johnsson, A.M., Thorberg, S.O., Nilsson, J.L.G. and Svensson, K. (1984) J. Med. Chem. 27, 1030–1037.
64 Wikström, H., Andersson, B., Sanchez, D., Lindberg, P., Arvidsson, L.E., Johansson, A.M., Nilsson, J.L.G., Svensson, K., Hjorth, S. and Carlsson, A. (1985) J. Med. Chem. 28, 215–225.
65 Neumeyer, J.L., Gao, Y., Kula, N.S. and Baldessarini, R.J. (1991) J. Med. Chem. 34, 24–28.
66 Neumeyer, J.L., Gao, Y., Kula, N.S. and Baldessarini, R.J. (1990) J. Med. Chem. 33, 3122–3124.
67 Cannon, J.G., Mohan, P., Bojarski, J., Long, J.P., Bhatnagar, R.K., Leonard, P.A., Flynn, J.R. and Chatterjee, T.K. (1988) J. Med. Chem. 31, 313–318.
68 Jaton, A.L., Giger, R.K.A., Vigouret, J.M., Enz, A., Frick, W., Closse, A. and Markstein, R. (1986) Life Sci. 38, 155–163.
69 Hacksell, U., Arvidsson, L.-E., Svensson, U., Nilsson, J.L.G., Wikström, H., Lindberg, P., Sanchez, D., Hjorth, S., Carlsson, A. and Paalzow, L. (1981) J. Med. Chem. 24, 429–434.

70 Wikström, H., Lii, J.H. and Allinger, N.L. (1987) J. Med. Chem. 30, 1115–1120.
71 Wikström, H. (1988) Neurol. Neurobiol., 42A (Progr. Catecholamine Res., Pt. A) 409–414.
72 Karlsson, A., Pettersson, C., Sundell, S., Arvidsson, L.-E. and Hacksell, U. (1988) Acta Chem. Scand., Ser. B, B42 231–236.
73 Karlsson, A., Björk, L., Pettersson, C., Andén, N.-E. and Hacksell, U. (1991) Chirality 2, 90–95.
74 Neumeyer, J.L., Froimowitz, M., Baldessarini, R.J., Campbell, A. and Gao, Y.G. (1988) J. Recept. Res. 8, 83–96.
75 Froimowitz, M. and Baldessarini, R., J. (1987) J. Pharm. Sci. 76, 557–564.
76 McDermed, J.D., McKenzie, G.M. and Freeman, H.S. (1976) J. Med. Chem. 19, 547–549.
77 Horn, A.S., Van der Weide, J., Tepper, P.G. and De Vries, J.B. (1988) Neurol. Neurobiol., 42B (Progr. Catecholamine Res., Pt. B) 13–17.
78 Timmerman, W., Dubocovich, M.L., Westerink, B.H.C., De Vries, J.B., Tepper, P.G. and Horn, A.S. (1989) Eur. J. Pharmacol. 166, 1–11.
79 Piercy, M.F., Broderick, P.A., Hoffmann, W.E. and Vogelsang, G.D. (1990) J. Pharm. Exp. Ther. 254, 369–374.
80 Hacksell, U., Johansson, A.M., Arvidsson, L.-E., Nilsson, J.L.G., Hjorth, S., Carlsson, A., Wikström, H., Sanchez, D. and Lindberg, P. (1984) J. Med. Chem. 27, 1003–1007.
81 Johansson, A.M., Arvidsson, L.-E., Hacksell, U., Nilsson, J.L.G., Svensson, K., Hjorth, S., Clark, D., Carlsson, A., Sanchez, D., Andersson, B. and Wikström, H. (1985) J. Med. Chem. 28, 1049–1053.
82 Johansson, A.M., Nilsson, J.L.G., Karlén, A., Hacksell, U., Sanchez, D., Svensson, K., Hjorth, S., Carlsson, A., Sundell, S. and Kenne, L. (1987) J. Med. Chem. 30, 1827–1837.
83 Johansson, A.M., Nilsson, J.L.G., Karlén, A., Hacksell, U., Svensson, K., Carlsson, A., Kenne, L. and Sundell, S. (1987) J. Med. Chem. 30, 1135–1144.
84 Johansson, A.M., Arvidsson, L.E., Hacksell, U., Nilsson, J.L.G., Svensson, K. and Carlsson, A. (1987) J. Med. Chem. 30, 602–611.
85 Clark, D., Hjorth, S. and Carlsson, A. (1985) J. Neural Transm. 62, 1–52.
86 Clark, D., Hjorth, S. and Carlsson, A. (1985) J. Neural Transm. 62, 171–207.
87 Kikuchi, T., Ohshiro, Y. and Hiyama, T. (1989) Kagaku, Zokan (Kyoto) 116, 191–200.
88 Bøgesø, K.P., Arnt, J., Lundmark, M. and Sundell, S. (1987) J. Med. Chem. 30, 142–150.
89 Liljefors, T., Bøgesø, K.P., Hyttel, J., Wikström, H., Svensson, K. and Carlsson, A. (1990) J. Med. Chem. 33, 1015–1022.
90 Liljefors, T. and Wikström, H. (1986) J. Med. Chem. 29, 1896–1904.
91 Kelly, T.R., Howard, H.R., Koe, B.K. and Sarges, R. (1985) J. Med. Chem. 28, 1368–1371.
92 Seyfrid, C.A. and Fuxe, K. (1982) Arzneim.-Forsch. 32, 892.
93 Martin, G.E. and Pettibone, D.J. (1985) J. Neural Transm. 61, 115–123.
94 Jaen, J.C., Wise, L.D., Heffner, T.G., Pugsley, T.A. and Meltzer, L.T. (1988) J. Med. Chem. 31, 1621–1625.
95 Wikström, H., Sanchez, D., Lindberg, P., Arvidsson, L.E., Hacksell, U., Johansson, A., Nilsson, J.L.G., Hjorth, S. and Carlsson, A. (1982) J. Med. Chem. 25, 925–931.
96 Craig, C.J., Torkelson, S.M., Findell, P.L. and Weiner, R.I. (1989) J. Med. Chem. 32, 961–968.
97 Seiler, M.P., Markstein, R., Walkinshaw, M.D. and Boelsterli, J.J. (1989) Mol. Pharmacol. 35, 643–651.

98 Karobath, M., Coward, D., Enz, A., Haefliger, W., Jaton, A., Markstein, R., Palacios, J.M., Shearman, G., Supavilai, P., Wiederhold, K.H. and Vigouret, J.M. (1988) Neurol. Neurobiol., 42B (Progr. Catecholamine Res., Pt. B) 19–26.
99 Thorberg, S.-O., Hall, H., Åkesson, C., Svensson, K. and Nilsson, J.L.G. (1987) Acta Pharm. Suec. 24, 169–182.
100 Vermue, N.A., Dijkstra, D. and Horn, A.S. (1988) J. Pharm. Pharmacol. 40, 574–577.
101 Vermue, N.A., Kaptein, B., Tepper, P.G., deVries, J.B. and Horn, A.S. (1988) Arch. Int. Pharmacodyn. Ther. 293, 37–56.
102 McDermed, J.D., Freeman, H.S. and Ferris, R.M. (1979) in Catecholamines, Basic and Clinical Frontiers (Usdin, E., Kopin, I.J. and Barchas, J., eds.), 1, pp 568–570, Pergamon Press, New York.
103 DeWald, H.A., Heffner, T.G., Jaen, J.C., Lustgarten, D.M., McPhail, A.T., Meltzer, L.T., Pugsley, T.A. and Wise, L.D. (1990) J. Med. Chem. 33, 445–450.
104 Dijkstra, D., Mulder, T.B.A., Rollema, H., Tepper, P.G., Van der Weide, J. and Horn, A.S. (1988) J. Med. Chem. 31, 2178–2182.
105 Gerding, T.K., Drenth, B.F.H., De Zeeuw, R.A. and Horn, A.S. (1988) Eur. Soc. Biochem. Pharmacol., Eleventh European Workshop on Drug Metabolism, University of Konstanz F.R.G., poster 3.37.
106 Gerding, T.K., Drenth, B.F.H., Roosenstein, H.J., De Zeeuw, R.A., Tepper, P.G. and Horn, A.S. (1990) Xenobiotica 20, 515–524.
107 Gerding, T.K., Drenth, B.F.H., Roosenstein, H.J., De Zeeuw, R.A., Tepper, P.G. and Horn, A.S. (1990) Xenobiotica 20, 525–536.
108 den Daas, I., Tepper, P., G. and Horn, A., S. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 186–191.
109 Wikström, H., Lindberg, P., Martinson, P., Hjorth, S. and Carlsson, A. (1978) J. Med. Chem. 21, 864–867.
110 Rollema, H., Mastebroek, D., Wikström, H. and Horn, A.S. (1985) J. Med. Chem. 37, 314–319.
111 Rollema, H., Mastebroek, D., Wikström, H., Svensson, K., Carlsson, A. and Sundell, S. (1986) J. Med. Chem. 29, 1889–1895.
112 Thorberg, S.-O., Ahlénius, S., Johansson, L., Lundstöm, J., Säfholm, C., Teiling, A. and Lennmark, M. (1987) Acta Pharm. Suec. 24, 183–192.
113 Thorberg, S.-O., Berg, S., Lundström, J., Pettersson, B., Wikström, A., Sanchez, D., Lindberg, P. and Nilsson, J.L.G. (1987) J. Med. Chem. 30, 2008–2012.
114 Clark, R.D., Caroon, J.M., Isaac, N.E., McClelland, D.L., Michel, A.D., Petty, T.A., Rosenkranz, R.P. and Waterbury, L.D. (1987) J. Pharm. Sci. 76, 411–415.
115 Huffman, W.F., Hall, R.F., Grant, J.A., Wilson, J.W., Hieble, J.P. and Hahn, R.A. (1983) J. Med. Chem. 26, 933–935.
116 Andén, N.-E., Nilsson, H., Roos, B.-E. and Thornström, U. (1983) Acta Pharmacol. Toxicol. 52, 51–56.
117 Schneider, C.S. and Mierau, J. (1987) J. Med. Chem. 30, 494–498.
118 Laguzza, B.C. and Turner Jr., W.W. (1985) US. Patent 85–747748; (1987) Chem. Abstr. 106(17): 131726w.
119 Griss, G., Schneider, C., Hurnaus, R., Kobinger, W., Pichler, L., Bauer, R., Mireau, J., Hinzen, D. and Schingnitz, G. (1984) Ger. Patent 84–3447075; (1986) Chem. Abstr. 106(1): 5015a.
120 Jaen, J.C., Wise, L.D., Caprathe, B.W., Tecle, H., Bergmeier, S., Humblet, C.C., Heffner, T.G., Meltzer, L.T. and Pugsley, T.A. (1990) J. Med. Chem. 33, 311–317.
121 Bhaird, N.N.A., Fowler, C.J., Thorberg, S.-O. and Tipton, K.F. (1985) Biochem. Pharmacol. 34, 3599–3601.

122 Högberg, K. and Lundstöm, J. (1988) Eur. Soc. Biochem. Pharmacol., Eleventh European Workshop on Drug Metabolism, University of Konstanz F.R.G., poster 3.41.
123 Wikström, H., Andersson, B., Svensson, A., Humber, L.G., Asselin, A.A., Svensson, K., Ekman, A., Carlsson, A., Nilsson, I. and Chidester, C. (1989) J. Med. Chem. 32, 2273–2276.
124 Asselin, A., Humber, L., Voit, K. and Metcalf, G. (1986) J. Med. Chem. 29, 648–654.
125 Schaus, J.M. (1989) U.S. Patent 89–383673; (1990) Chem. Abstr. 113(21): 191185y.
126 Wallace, R.A., Farooqui, T., Wallace, L., Ares, J., Chang, Y.A., Miller, D.D. and Uretsky, N. (1987) Biochem. Pharmacol. 36, 3903–10.
127 Sabol, B., Boldry, R., Farooqui, T., Chang, Y.A., Miller, D.D. and Uretsky, N. (1987) Biochem. Pharmacol. 36, 1679–85.
128 Harrold, M.W., Chang, Y.A., Wallace, R.A., Farooqui, T., Wallace, L.J., Uretsky, N. and Miller, D.D. (1987) J. Med. Chem. 30, 1631–5.
129 Chang, Y.A., Ares, J., Anderson, K., Sabol, B., Wallace, R.A., Farooqui, T., Uretsky, N. and Miller, D.D. (1987) J. Med. Chem. 30, 214–18.
130 Wallace, R.A., Farooqui, T., Wallace, L., Ares, J., Chang, Y.A., Miller, D.D. and Uretsky, N. (1988) Biochem. Pharmacol. 37, 2077–84.
131 Miller, D.D., Harrold, M., Wallace, R.A., Wallace, L.J. and Uretsky, N.J. (1988) Trends Pharmacol. Sci. 9, 282–4.
132 Wallace, R.A., Wallace, L., Harrold, M., Miller, D.D. and Uretsky, N.J. (1989) Biochem. Pharmacol. 38, 2019–25.
133 Ross, P.C. and Burkman, A.M. (1988) Proc. Soc. Exp. Biol. Med. 188, 87–91.
134 Marshall, G.R. and Cramer, R.D.I. (1988) Trends Pharmacol. Sci. 9, 285–289.
135 Cohen, N.C., Blaney, J.M., Humblet, C., Gund, P. and Barry, D.C. (1990) J. Med. Chem. 33, 883–894.
136 Dahl, S. (1989) Psychiatry Today: Accomplishments and Promises. VIII World Congress of Psychiatry, Athens, Greece, 489, Poster 1889.
137 Weiner, P.K. and Kollman, P.A. (1981) J. Comput. Chem. 2, 287–303.
138 Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, G., Profeta Jr., S. and Weiner, P. (1984) J. Am. Chem. Soc. 106, 765784.
139 Weiner, S.J., Kollman, P.A., Nguyen, D.T. and Case, D.A. (1986) J. Comput. Chem. 7, 230–252.
140 Dahl, S. (1989) ECNP, Göteborg, Sweden.
141 Cartier, A. and Rivail, J.L. (1987) Chemom. Intell. Lab. Syst. 1, 335–347.
142 Kocjan, D., Hodiscek, M. and Hadzi, D. (1986) J. Med. Chem. 29, 1418–1423.
143 Van de Waterbeemd, H., Carrupt, P.A. and Testa, B. (1987) Pharmacochem. Libr. 10, 195–197.
144 Lloyd, E.J. and Andrews, P.R. (1986) J. Med. Chem. 29, 453–462.
145 Tonani, R., Dunbar, J.J., Edmonston, B. and Marshall, G.R. (1987) J. Comput. Aided Mol. Des. 1, 121–132.
146 Manallak, D.T. and Beart, P.M. (1988) J. Pharm. Pharmacol. 40, 422–428.
147 Sheppard, H. and Burghardt, C.R. (1974) Mol. Pharmacol. 10, 721–726.
148 Grol, C.J. and Rollema, H. (1977) J. Pharm. Pharmacol. 29, 153–156.
149 Grol, C.J., Jansen, L.J. and Rollema, H. (1985) J. Med. Chem. 28, 679–683.
150 Goldberg, L.I., Kohli, J.D., Kotake, A.N. and Volkman, P.H. (1978) Fed. Proc. Fed. Am. Soc.Exp. Biol. 37, 2396.
151 Dandiya, P.C., Sharma, H.L., Patni, S.K. and Gambhir, R.S. (1975) Experientia 31, 1441–1443.
152 Seiler, M.P. and Markstein, R. (1982) Mol. Pharmacol. 22, 281–289.
153 Nichols, D.E. (1983) ACS Symp. Ser. 224 (Dopamine Recept.) 201–218.

154 Erhardt, P.W. (1980) J. Pharm. Sci. 69, 1059–1061.
155 Erhardt, P.W. (1983) ACS Symp. Ser. 224, 275–280.
156 Humber, L.G., Philipp, A.H., Bruderlein, F.T., Goetz, M. and Voith, K. (1979) ACS Symp. Ser., (Comput.-Assisted Drug Des.), Montreal, PQ H3C 3J1, Can., 112, 227–241.
157 Philipp, A.H., Humber, L.G. and Voith, K. (1979) J. Med. Chem. 22, 768–73.
158 Humber, L.G., Bruderlein, F.T., Philipp, A.H., Goetz, M. and Voith, K. (1979) J. Med. Chem. 22, 761–767.
159 Humber, L.G. (1980) Med. Chem. Adv., Proc. Int. Symp., Montreal, PQ, Can., 179–190.
160 Seeman, P. (1980) Pharmacol. Rev. 32, 229–313.
161 Seeman, P., Watanabe, M., Grigoriadis, D., Tedesco, J.L., George, S.R., Svensson, U., Nilsson, J.L.G. and Neumeyer, J.L. (1985) Mol. Pharmacol. 28, 391–399.
162 Neumeyer, J.L., Arana, G.W., Ram, V.J. and Baldessarini, R.J. (1983) Acta Pharm. Suec., (Suppl. 2, Dopamine Recept. Agonists 2) 11–24.
163 Kaiser, C. and Jain, T. (1985) Spec. Publ. - R. Soc. Chem. Med. Chem. Symp. 55, 133–68.
164 Svensson, K. (1986) Thesis: Dopamine autoreceptor antagonists: A new class of central stimulants, University of Göteborg, Göteborg, Sweden, ISBN 91–7900–078–9.
165 Nedelec, L., Guillaume, J. and Dumont, C. (1976) Fr. Patent 76–3933; (1977) Chem. Abstr. 87, 152038.
166 Wikström, H., Andersson, B., Elebring, T., Svensson, K., Carlsson, A. and Largent, B. (1987) J. Med. Chem. 30, 2169–2174.

NOTE ADDED IN PROOF

Very recently, new reports of modelling studies of the D2 receptor have appeared:

Hibbert, M.F., Trumpp-Kallmeyer, S., Bruinrels, A. and Hoflack, J. (1991) Mol. Pharmacol. 40, 8–15.

Dahl, S.G., Edvardsen, Ø. and Sylte, J. (1991) Proc. Natl. Acad. Sci. USA 88, 8111–8115.

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6 Progress in the Medicinal Chemistry of the Herb Feverfew

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INTRODUCTION

During the past 10 to 15 years the herb feverfew has received much attention from both the general public and the scientific community. In the late

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1970's, claims from members of the public about its efficacy in prevention of migraine were widely publicized in the British press. This attention brought feverfew back into the limelight from obscurity since the Middle Ages. Producers of herbal and homeopathic remedies were quick to act in bringing their feverfew preparations to the (U.K.) market, thereby increasing the availability of the herb to the general public. Thus, the situation arose that some estimated hundreds of thousands of people were consuming feverfew daily, yet the medical profession had little or no information to substantiate the claimed benefits, or worse, to inform the users about possible side-effects associated with this herb.

It was against this background that the research into feverfew in the early 1980's was performed. A number of investigations have now been carried out into the plant's *in vitro* biological actions, the chemical components responsible for these actions and suggestions have been put forward as to its mode of action. Furthermore, a limited amount of data are available about the clinical effects of feverfew. The aim of this chapter is to give an overview to date of the facts about the herb feverfew with respect to its chemical, biological and clinical properties.

BACKGROUND ASPECTS

HISTORY OF THE MEDICINAL USE OF FEVERFEW

The use of feverfew as a medicinal plant can be traced back to the earliest written record of the Herbals, the Greek herbal 'Materia Medica' by Dioscorides. Later herbal writers are thought to have used the 'Materia Medica' as a source of information and feverfew was described by Dodoens in 1619, by Gerard in 1636 and Culpeper in 1650. Their descriptions of the properties of the plant became more extensive and the conditions for which feverfew was reputed to be beneficial can be divided into three main areas: (1) fever, headache and migraine; (2) women's conditions such as difficulties in labour, threatened miscarriages and the regulation of menstruation; (3) the relief of stomach-ache, toothache and insect bites [1].

Feverfew is a corruption of the Old English name 'febrifuge' from the Latin 'febrifugia', pointing to one of its acclaimed benefits in reducing fever. It is for this reason that feverfew has sometimes been referred as a 'mediaeval aspirin'. In some other European countries, this herb is referred to as 'motherherb', for example, 'Mutterkraut' in Germany, indicating its acclaimed beneficial properties in various women's conditions. Other names for feverfew include featherfoil, flirtwort and bachelor's buttons.

Feverfew was administered in a variety of ways. It was recommended that the herb be consumed as a dried powder taken with honey (presumably because of its bitter taste), to bath in a decoction, sometimes made in wine (for the women's conditions) or to make a syrup to be taken through the winter months. In most cases it was recommended that the leaves be used, but for some conditions it was suggested that the flowers could also be beneficial.

TODAY'S CLAIMS AND USES

Today's widespread use of feverfew essentially started in 1978 when the British press reported that a group of migraine sufferers from Wales had found relief from attacks after taking the leaves of the plant for some time. The initiative of self-medication was lead by a lady migraine sufferer with frequent attacks, who found that after 10 months of taking the leaves she was completely free of migraine attacks [2, p. 19]. Following this newspaper report the interest in feverfew grew quickly. Many personal accounts from other migraine sufferers followed, describing how feverfew had helped to relieve or had abolished their condition, usually after they had tried many other remedies unsuccessfully.

Following the success of feverfew in migraine, the herb was also tried in many other conditions and it has now been claimed (but not scientifically proven) to be effective in arthritis, psoriasis and stress, amongst others [3, p. 36]. However, although many people were willing to try this herb, little hard information was available to the general public on such basic matters as how much of the herb to take. Just how great the interest in feverfew was became clear when a source of this kind of information was revealed in a daily newspaper and 25,000 requests were received [4].

ADMINISTRATION OF THE HERB FEVERFEW

Today, as in the Middle Ages, there are different ways in which feverfew is employed with respect to the part of the plant consumed, the frequency and the dose of administration. The vast majority of users appear to consume the leaves [2, p. 46], which are said to taste quite bitter. Commercial feverfew preparations are widely available in the U.K. and many other countries. They contain either dry powdered leaves or whole plant in tablet or capsule form.

Feverfew appears to be used predominantly as a prophylactic agent in both migraine [2, p. 41] and arthritis [3, p. 48], although there are incidental

reports of the use of single high doses for migraine attacks [2, p.39]. The dose which is taken for prophylaxis has been estimated at 2 to 3 fresh leaves per day [2, p.39]. The period of time that people need to gain benefit from the herb as a prophylactic agent varies, although some generalized estimates can be made. In arthritis, it is claimed that on average 7 days are needed to gain relief from most of the pain. To reduce the inflammation and improve mobility, for example, an average of 14 to 28 days are needed. The time needed to achieve relief for migraine is more difficult to judge, as attacks may occur sporadically and reported beneficial effects vary from instant relief to relief over a period of months [2, p. 54].

Complaints of side-effects by feverfew users have been limited. Mouth ulcers have been suggested to be the most common side-effect which seems to be a systemic effect [2, pp. 79,80]. A survey carried out among 300 feverfew users revealed that 18% experienced side-effects and 11.3% of users reported mouth ulcers [2, pp. 77–80]. In a different analysis of 164 patients who had stopped taking feverfew, 21% did so because of side-effects [2, pp. 82,83]. A prospective clinical trial [5] carried out to assess the efficacy of feverfew in a controlled way reported that mouth ulcers were more common during the placebo treatment.

SCIENTIFIC ASPECTS

BOTANICAL CLASSIFICATION

Tanacetum parthenium (L.) Schultz Bip. is the official Latin name for the member of the Compositae family which is more commonly referred to as feverfew [6]. The plant had previously been classified as *Chrysanthemum parthenium* (L.) Bernh., so the latter name is strictly speaking no longer correct. Other old classifications for feverfew are *Leucanthemum parthenium* (L.) Gren. & Godron or *Pyrethrum parthenium* (L.) Sm.

Feverfew has been described as "a perennial plant growing to a height of 14–45 cm with strong-smelling greenish-yellow bipinnate (feather-like) leaves" [7]. The flowerheads "are arranged in a loose terminal corymb, the central disc florets being yellow and a single layer of outer ray florets being white" [7]. "It is a mountain scrub and grows in rocky places. It has been long cultivated for ornament and as a medicinal plant and naturalized in hedges and waste places throughout a large part of Europe" [6].

There are at least two varieties of feverfew, the wild variety and a cultivated variety, also known as the 'golden' feverfew or *Tanacetum parthenium aureus* [7]. The leaves of the golden variety are much yellower than those

of the wild kind, making it a more attractive looking plant. It must be stressed that most of the chemical and biological investigations have been carried out on the wild variety and it is not known whether the results would also apply to the 'golden variety'. A widely available double variety (double row of outer ray florets) has also been cultivated.

The confusion over the correct variety of feverfew is further complicated by the existence of another genus of plants, *Matricaria*, the leaves of which are very similar to those of feverfew but the flowers of which are different [7] (for example, Snow Dwarf, with all white flowers, Golden Ball with all yellow flowers, and Tom Thumb, which looks like a cross between the two). These attractive looking plants are sometimes sold as 'feverfew'. Again, there has been no reported scientific work on these related species *per se* and the work quoted here for the true wild feverfew may not necessarily apply to these feverfew-like plants.

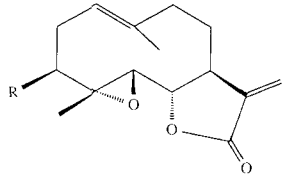
CHEMICAL CONSTITUENTS OF FEVERFEW

Sesquiterpene lactones are secondary metabolites that are widespread throughout the plant kingdom. The common feature in this class of compounds is an α-methylenebutyrolactone (formerly α-methylene-γ-lactone) moiety. These compounds can be classified according to their basic structure into different types such as germacranolides (10-membered ring), eudesmanolides (two fused 6-membered rings) and guaianolides (two fused 5- and 7-membered rings).

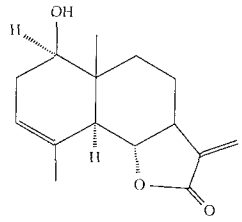
In 1959–61, a Czech group [8,9] reported the isolation of a new sesquiterpene lactone from *Chrysanthemum* (*Tanacetum*) *parthenium* as part of a study of the sesquiterpene lactones of the Compositae family. They named it parthenolide. The initial structure for parthenolide was later revised [10,11] and the accepted structure for parthenolide today is represented by structure (1). Parthenolide is a germacranolide-type sesquiterpene lactone. The crystal structure for parthenolide has also been reported [12]. Extractions of *C.* (*T.*) *parthenium* grown in Mexico, known locally as santamaria, did not yield parthenolide [13], but a closely related compound was isolated and named santamarine (3). This suggests that regional variations in the chemical constituents may occur.

Parthenolide was only the first of many sesquiterpene lactones to be isolated from feverfew. Two further compounds were isolated and named chrysartemin A (4) and B (5) [14]. The structure of chrysartemin B was later revised [15]. Recently, several studies have shown that chrysartemin A and B are not present in feverfew as the structures shown in (4) and (5) but are in fact the isomeric canin (19) and artecanin (20), respectively (see

below). In another study [16], santamarine (3) was again isolated from *T. parthenium* along with reynosin (6), 8β-hydroxyreynosin (7), 1β-hydroxyarbusculin (8) and magnolialide (9). Santamarine (3) is an example of a eudesmanolide-type sesquiterpene lactone.

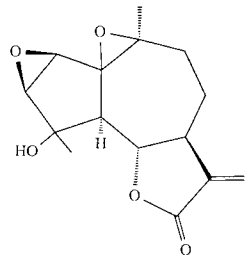


(1) R = H
(2) R = OH



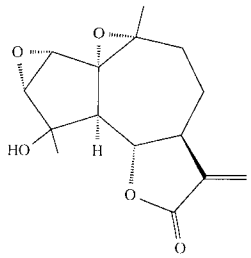
(3)

A detailed investigation of the chemical constituents of *T. parthenium* was carried out by Bohlmann and Zdero [17]. They did not find any sesquiterpene lactones in the roots. However, the aerial parts contained a complex mixture of such compounds. The less polar fractions contained only one sesquiterpene lactone, costunolide (10). The more polar fractions gave a complex mixture of sesquiterpene lactones which were difficult to separate and were mostly present in minute amounts. The main component was, however, parthenolide (1). Further, two esters (12,13), 3β-hydroxycostunolide (11), reynosin (6), 3β-hydroxyparthenolide (2), artemorin (15), the hydroxyketone (16), the epoxide (17) together with the ketone (18) corresponding to artemorin (15), and traces of 8α-hydroxyestafiatin (14) were identified. A second series of closely related compounds were identified as canin (19), its stereoisomer artecanin (20), another diepoxide (21), two endoperoxides, tanaparthin α- (22) and β-peroxide (23) and two secoguaianolides secotanapartholide A (24) and B (25). The stereochemical structure given in [17] for canin was different from that reported subsequently [18]. As a result, the stereochemistry of the isomers canin and artecanin was recently reinvestigated [19]. It was concluded that the original stereochemistry as given in [18] was correct and the structures for canin and artecanin are correctly represented by (19) and (20), respectively. Further, it was shown [18] that canin is identical with chrysartemin A and that artecanin is the same compound as chrysartemin B. Therefore, it is concluded that the chrysarthemin



(4)

A (4) and B (5) structures originally presented [15] were wrong and that these compounds do not occur in feverfew. Canin (19) is an example of a guainolide-type sesquiterpene lactone.

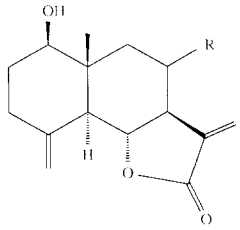


(5)

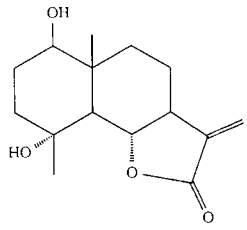
An extract of *T. parthenium* is described in a patent [20] which, besides the known sesquiterpene lactones parthenolide (1) and chrysartemin A (4, see above), also contained previously unreported partholide (26) and chrysanthemolide (27), both of unknown stereochemistry. Further, dimers and trimers of the sesquiterpene nucleus such as chrysanthemonin (28) were said to be present in this extract which had not been reported before.

There is little information on the stability of the sesquiterpene lactones. However, one report [21] states that a chloroform solution of parthenolide stored at room temperature was found to contain costunolide diepoxide (29) after a few days, probably formed by aerial oxidation. The literature also suggests that parthenolide can polymerize on prolonged storage [22].

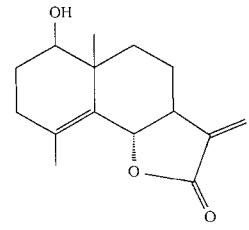
(6) R = H
(7) R = OH



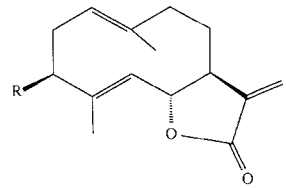
(8)



(9)

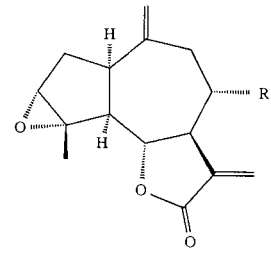


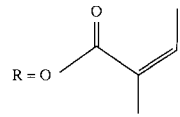
(10) R = H
(11) R = OH



There have been no reports on the chemical synthesis of parthenolide or indeed on the modification of the biological activity (see below) of parthenolide or the sesquiterpene lactones in general. However, routes for the synthesis of constunolide have been reported [23–25].

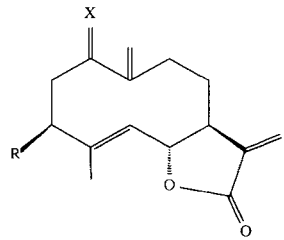
(12) R = O*i*Bu
(13) R = OAng
(14) R = OH



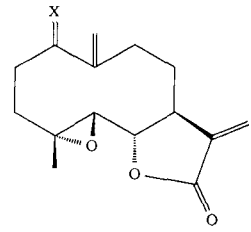
OAng: 

Sesquiterpene lactones are not exclusive to feverfew and most of the compounds extracted from the plant, including parthenolide [26], have also been

(15) R=H,X= *β*-OH,H
(16) R=OH,X=0



(17) X = OH, H
(18) X = O



isolated from other plant species. A study of the sesquiterpene lactone content of 14 different Chrysanthemum species, including feverfew, and bitter

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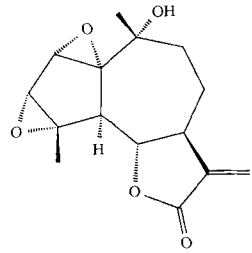

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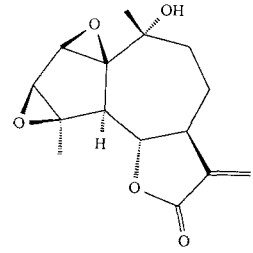

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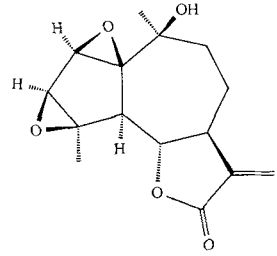

principle indexes revealed a high correlation between these two parameters [27].

In summary, feverfew appears to be a source of a range of sesquiterpene lactones of three different types: germacranolides, eudesmanolides and guaianolides.

BIOLOGICAL ACTIVITY

Effects in in vitro *systems and evidence for possible modes of action*

One of the first biological activities for which sesquiterpene lactones were screened was their possible anti-tumour activity. Studies have shown that

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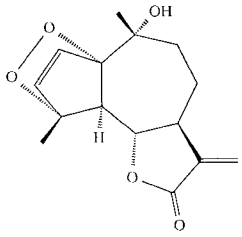

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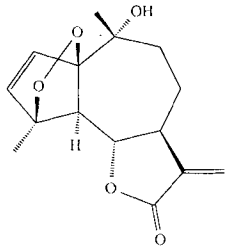
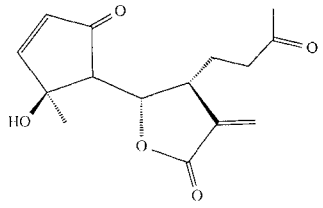


(24) α-OH
(25) β-OH



a number of sesquiterpene lactones displayed cytotoxic activity towards four different human cell lines [28,29]. It was postulated that the α-methylenebutyrolactone moiety in the sesquiterpene structure was crucial for its activity, as compounds without this feature did not possess any cytotoxic

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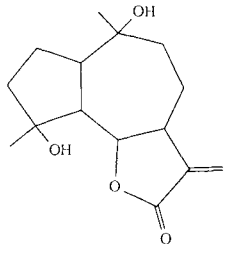

activity. In the case of feverfew all the sesquiterpenes isolated to date from the plant contain an α-methylenebutyrolactone unit. Parthenolide in partic-

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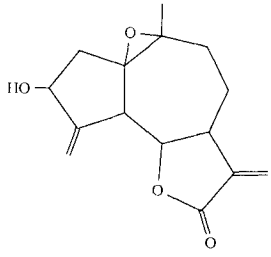
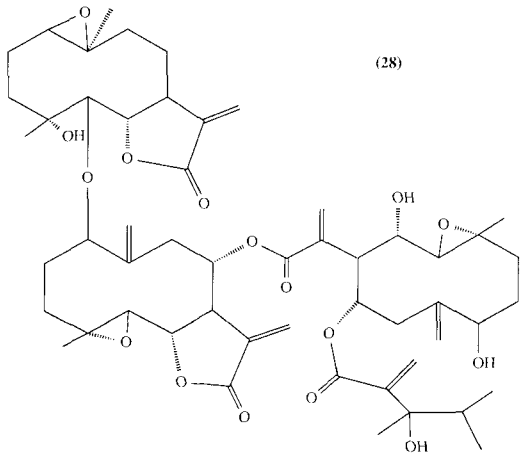


ular was shown to have cytotoxic activity towards cell lines derived from normal human fibroblast, human laryngeal carcinoma, human cells transformed with simian virus 40 [28] and the human epidermoid carcinoma of the nasopharynx test system [29]. Further, parthenolide was shown to inhib-



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it incorporation of radioactive thymidine into DNA of HeLa cells and it was suggested that the anti-tumour activity occurs at the DNA replication

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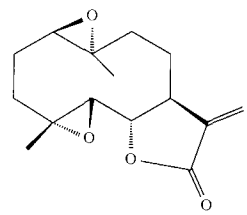

level [30], probably by interfering with the DNA-template [31]. The ability to inhibit DNA synthesis correlated well with the cytotoxicity of parthenolide and other sesquiterpene lactones under study. Further, sesquiterpene lactones containing an α-methylenebutyrolactone moiety were shown to inhibit DNA polymerase activity [32]. In the same study, *in vitro* aerobic basal respiration and oxidative phosphorylation processes in Ehrlich ascites were inhibited by the same group of compounds.

Parthenolide was found to be located in the glands on the surface of the leaves and seeds and was shown to have antimicrobial properties [33]. It was able to inhibit the growth of Gram-positive bacteria, yeast and filamentous

fungi, but not of Gram-negative bacteria. It was suggested that parthenolide may protect the plant against pathogens.

In the early 1980's, a number of studies investigating the effects of crude feverfew extract on prostaglandin biosynthesis were reported coinciding with the renewed interest in the plant as a herbal remedy. Prostaglandins, the mediators of inflammation were an obvious candidate for study, since feverfew had been reputed to be beneficial in inflammatory conditions such as arthritis and because the herb had been claimed to have fever lowering properties. Sesquiterpene lactones with an α-methylenebutyrolactone unit themselves had previously been shown to have anti-inflammatory activity [34]. Collier *et al.* [35] did show inhibition of prostaglandin biosynthesis by an extract of feverfew in bull seminal vesicles but their results did not suggest inhibition of cyclo-oxygenase enzyme, the site of action for the well known anti-inflammatory agent acetylsalicylic acid (aspirin). In a similar study, inhibition of prostaglandin synthesis was confirmed [36] and 3 active compounds were identified as parthenolide (1), chrysanthenyl acetate (30) and michefuscalide (31). It was noted, however, that in the case of chrysanthenyl acetate (30) inhibition may have been due to an acetylation reaction of the cyclooxygenase part of the prostaglandin synthetase complex similar to aspirin. It was further shown [37] that crude feverfew extract was a potent inhibitor of aortic smooth muscle phospholipase A_2 and inhibited the formation of both cyclooxygenase and lipoxygenase products in rat peritoneal leucocytes [38].

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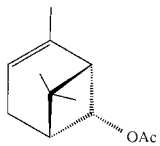

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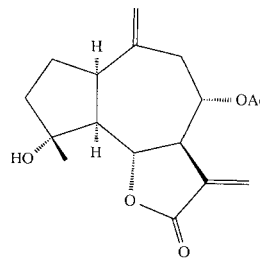

The pharmacological effects of crude feverfew extract on agonist-induced responses in two human blood cells, platelets and neutrophils have been studied. Both types of cells are able to secrete substances from intracellular granules in response to cellular agonists. The secreted substances are often able to augment any response elicited by the original agonist. Cellular responses to agonist can be measured by assaying for the secreted substances. A change in the secretion of such substances, for example, in the presence of inhibitors, indicates a change in the cell's response to the agonist. Fur-

ther, in response to an agonist, platelets can form aggregates *in vitro* and this response can be measured by light densitometry.

Makheja and Bailey [39,40] were the first to demonstrate inhibition of platelet aggregation by feverfew extract when aggregation was induced by adenosine diphosphate (ADP), collagen or thrombin but not when it was induced by arachidonic acid. They also found inhibition of prostaglandin synthesis in platelets and suggested that inhibition of the enzyme phospholipase A_2 was involved. (For a review of arachidonic acid metabolism see [41] and *Figure 6.1.*) However, inhibition of phospholipase A_2 did not provide the full explanation for the results of Heptinstall *et al.* [42]. In their experiments, feverfew extract inhibited platelet aggregation induced by adrenaline, collagen and U46619 (a stable thromboxane A_2 mimetic), while the irreversible aggregation induced by ADP and arachidonic acid was modified to a reversible response. The latter result contradicts that of Makheja and Bailey [39,40]. Thromboxane synthesis was not inhibited by feverfew extract when arachidonic acid, ADP or thrombin were used to stimulate the platelets but in response to adrenaline, no thromboxane B_2 synthesis could be detected in the presence of feverfew extract. Further, feverfew extract inhibited platelet 5HT (5-hydroxytryptamine) secretion induced by ADP, adrenaline or arachidonic acid in a dose-dependent fashion, while secretion induced by collagen and by U46619 was also inhibited. In contrast, neither aggregation nor secretion induced by the calcium ionophore A23187 appeared to be inhibited by feverfew extract. However, it was later shown that A23187-induced responses in platelets can be inhibited by feverfew extract when lower concentrations of the calcium ionophore are used [43]. In the same study, feverfew extract also inhibited the 5HT-secretion induced by phorbol esters, agents that activate protein kinase C.

The ability of feverfew extract to inhibit 5HT-secretion in platelets was used to identify compounds responsible for the inhibitory effects on platelets [44]. A crude feverfew extract was separated by chromatography and fractions were screened for antisecretory activity induced by adrenaline. In five active fractions compounds were identified as the sesquiterpene lactones parthenolide (1), canin (19), artecanin (20), secotanapartholide A (24) and 3β-hydroxyparthenolide (2). The ability of parthenolide to affect platelet activity induced by other agents was confirmed in a study of direct comparison of crude feverfew extract with parthenolide [43]. Parthenolide in the micromolar concentration range inhibited platelet 5HT-secretion.

In polymorphonuclear leucocytes (PMNL, neutrophils), secretory responses measured as vitamin B_{12}-binding protein release were inhibited by feverfew extract when the response was induced by the chemotactic peptide FMLP or arachidonic acid but not when the calcium ionophore A23187 was

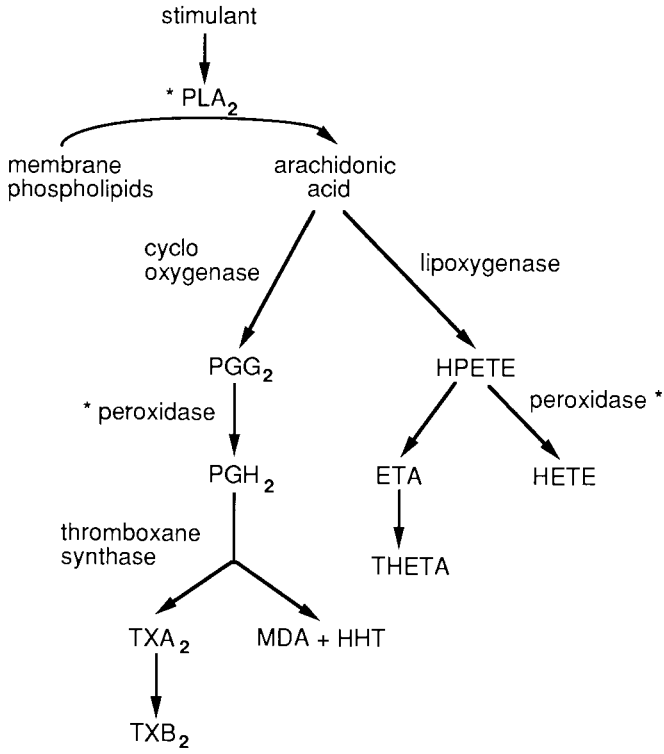

*Figure 6.1. Arachidonic acid metabolism in platelets. Activation of platelets by stimulating agents leads to activation of membrane phospholipase A_2 (PLA_2). This results in liberation of arachidonic acid which can be acted upon by two enzymes: cyclooxygenase or lipoxygenase. Cyclo-oxygenase action results in the formation of prostaglandin G_2 (PGG_2), which is converted rapidly into prostaglandin H_2 (PGH_2) by a peroxidase associated with the cyclooxygenase enzyme. PGH_2 is a substrate for the enzyme thromboxane synthase. Thromboxane A_2 (TXA_2) is produced which is a very potent platelet-stimulating agent. TXA_2 is very unstable and rapidly broken down to thromboxane B_2 (TXB_2) which is stable. As well as TXA_2 and TXB_2, malondialdehyde (MDA) and a 17-hydroxyfatty acid HHT are formed. Lipoxygenase action on arachidonic acid results in 12-hydroperoxyeicosatetraenoic acid (HPETE) formation from which 12-hydroxyeicosatetraenoic acid (HETE) is formed by a peroxidase. Lipoxygenase action further results in the formations of hydroxylated compounds eicosatrienoic acid (ETA) and trihydroxyeicosatrienoic acid (THETA). Possible points of feverfew interaction are indicated by *. For a review on the above pathways, see [41].*

used [42]. However, later data showed that aggregation of PMNL's induced by A23187 as well as FMLP was dose-dependently inhibited by feverfew extract [45]. Neutrophil phagocytotic function assayed by a number of different methods can also be inhibited by feverfew extract [45,46] along with the chemiluminescence response that occurs during phagocytosis. Neutrophil killing activity in the presence of feverfew extract was shown to be inhibited, but extracellular killing was not affected [46]. In one study [45] LDH release as a measure of cell lysis was the same in the presence and absence of feverfew indicating that the inhibitory effects were not merely a reflection of cell death. However, both feverfew extract and parthenolide have been found to inhibit mitogen-induced human mononuclear cell proliferation and this response was indistinguishable from a cytotoxic effect in the system studied [47]. It was noted that the time course in these experiments was longer than those employed in the platelet and neutrophil studies quoted above.

In mast cells, histamine release induced by IgE or A23187 was also inhibited by feverfew extract [48] which also reduced spasmolytic activity of smooth muscle induced by acetylcholine, 5HT, histamine, prostaglandin E_2 and bradykinin [20].

It has been suggested that the α-methylenebutyrolactone unit, common to the biologically active sesquiterpene lactones identified thus far, can interact with biological nucleophiles such as sulphydryl (SH) groups [49–51]. A 'Michael-type' reaction between the exocyclic methylene group on the sesquiterpene lactone and the SH-group would be irreversible and leads to a stable product (*Figure 6.2*). A number of studies have been carried out, particularly in platelets, to determine whether the effects seen on platelet behaviour may be related to interaction with SH-groups.

Agents that contain SH-groups such as cysteine or 2-mercaptopropionyl glycine were able to neutralize feverfew inhibitory activity on platelets [52]. Further, the same study demonstrated a dramatic reduction in the number of acid-soluble SH-groups, both by feverfew and parthenolide. These effects

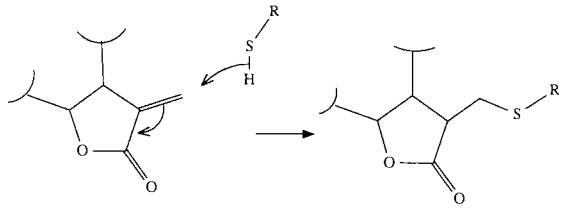

Figure 6.2. Michael-type reaction between an α-methylenebutyrolactone group and a sulphydryl-group-containing compound.

were dose and time-dependent and correlated well with the inhibition of 5HT secretion from the platelets induced by the phorbol ester PMA or arachidonic acid. Cysteine was able to prevent a reduction in the number of acid-soluble groups by feverfew extract. In each of the above cases, amino acids with other functional groups (hydroxy or amino groups) were unable to interfere with the effects of feverfew.

The acid-soluble SH-groups in platelets are mainly those of glutathione (GSH). GSH is a cofactor for enzymes such as peroxidase. If feverfew is able to interfere with this cofactor, enzyme function may be impaired. One pathway that may be affected in this way is the metabolism of arachidonic acid (*Figure 6.1*). In the presence of feverfew extract an increase was found in lipoxygenase product formation and impaired conversion of HPETE to HETE, for which GSH is a cofactor [52]. Inhibition of the liberation of [^{14}C]arachidonic acid from phospholipids was also found [53], which implies impairment of phospholipase A_2 activity and for which SH-groups are thought to be important.

Only a very small reduction in the number of acid-insoluble SH-groups in platelets was seen in the presence of feverfew extract [52]. A change in the high molecular weight protein pattern was only seen after the platelets had been activated. Such changes are indicative of polymerization of the proteins and the formation of S–S bridges and this can lead to disturbances in membrane-cytoskeletal interactions. Uptake of [^{14}C]arachidonic acid into phospholipids was inhibited by feverfew [53], which may be a result of altered cytoskeletal–membrane interaction.

Feverfew extract was shown to inhibit deposition, aggregate formation and spreading of platelets on collagen fibres [54]. Changes in membrane–cytoskeletal interaction leading to a change in expression of membrane receptors involved in these processes may explain this result. Feverfew extract was further able to protect the endothelial monolayer in rabbit aortas from perfusion injury and some reversible increase in cAMP levels in the aorta segments was found [55].

Evidence of a reduction in the number of acid-soluble SH-groups by feverfew extract in neutrophils at concentrations similar to those inhibiting neutrophil behaviour has been described [45]. It was suggested that interference with SH-groups alters assembly of microtubules which are known to be involved in phagocytosis and degranulation of neutrophils.

Although it seems that interaction of feverfew components with SH-groups brings about changes in aspects of platelet and neutrophil behaviour, no single event seems to be able to explain all the effects seen by feverfew on these cells *in vitro*.

Effects in in vivo *models*

There are only a few reports on the efficacy of feverfew in an *in vivo* situation. Inhibition of collagen-induced bronchoconstriction in an *in vivo* guinea-pig model was demonstrated [56] and it was concluded that this was consistent with *in vivo* phospholipase A_2 inhibition. In a rat model of experimentally induced nephrocalcinosis, parthenolide was shown to protect the rats against this condition. Inhibition of prostaglandin biosynthesis may have been the mechanism of action of parthenolide in this case, as prostaglandins are thought to be involved in nephrocalcinosis [57].

Effects in ex vivo *studies*

Johnson and co-workers [58] were unable to show any inhibition of platelet aggregation induced by ADP or thrombin after prolonged usage of feverfew in 10 patients compared to control subjects. However threshold levels for the response to U46619 and 5HT appeared increased. It was concluded that the results seen *in vitro* may not be directly applicable to the clinical situation. As part of a large clinical trial of 59 migraine patients (see below) of the effects of feverfew in migraine, no *ex vivo* reduction in 5HT-secretion from platelets could be demonstrated after ingestion of feverfew for up to 4 months (Groenewegen, W.A., unpublished data).

CLINICAL TRIALS CONDUCTED WITH FEVERFEW

In a clinical study of 17 migraine sufferers who had been taking feverfew for some years, patients were randomized either to continue to receive feverfew (2×25 mg/day) or placebo for 6 months. This rather unconventional approach was the best accepted from an ethical point of view at the time of the study (pre-1985). A statistically significant increase in the frequency and severity of attacks was found in the placebo group, with no change of symptoms of migraine in the feverfew group [59]. It was concluded that feverfew taken prophylactically did have beneficial effects in patients suffering from migraine.

A larger randomized double-blind placebo-controlled trial [5] of 59 migraine sufferers (who had not taken feverfew before) found that feverfew treatment was associated with a reduction in the mean number and severity of attacks and the degree of vomiting, but the duration of each individual attack was unaltered. The study period was 9 months in total consisting of 1 month placebo run-in followed by 2×2 months of either feverfew (~ 82 mg/day) or placebo after which patients were switched to the other leg of

treatment. Thus, each patient served as his/her own control. Both of the studies of the effects of feverfew in the prevention of migraine therefore indicated beneficial effects from the herb and neither study reported any serious side-effects as a consequence of feverfew administration (see below).

A study of the efficacy of feverfew ($\sim$76 mg/day) in rheumatoid arthritis in 40 patients (double-blind, placebo-controlled) showed no apparent benefit from oral feverfew over a 6-week study-period [60].

No serious side-effects were reported during the two clinical studies of the effects of feverfew in migraine. The incidence of mouth ulcers in these studies were not found to be increased on taking feverfew. A study [61] of possible genetoxic effect of feverfew in 30 migraine patients after taking feverfew (mean dose 73 mg/day, range 12.5–250 mg) for a mean of 2.9 years (range 0.9–11.5 years) revealed no difference in any of the parameters measured compared to a matched control group who had never taken feverfew. The parameters studied were chromosomal abberations, sister chromatid exchanges and mutagenicity of urine samples (Ames test).

As early as 1950, feverfew was reported to cause contact dermatitis in at least one patient [62] and parthenolide was found to cause an allergic reaction in patients with the same condition [63]. Since then there have been many reports [64–68] of feverfew-induced allergic reactions, the condition being worst in the summer months coinciding with the flowering season of the plant. The α-methylene butyrolactone group was found to be a partial requirement for activity in contact dermatitis [69].

COMMERCIALLY AVAILABLE PREPARATIONS OF FEVERFEW

Many preparations of feverfew are available to the general public from health food shops and similar outlets in the U.K. They take the form of herbal or homeopathic preparations. The herbal preparations may contain just dried leaf in tablet or capsule form or indeed the dried whole plant. There are many different dosages on the market ranging from 25 to 390 mg per tablet or capsule. When the platelet antisecretory activity that could be extracted from some of these preparations was compared with that extracted from known amounts of feverfew, the apparent feverfew content of most preparations was found to be lower that that stated on the preparation [70]. In the case of the homeopathic preparation, no antisecretory activity was detected.

The amount of parthenolide in capsules of the dried leaves given in two studies [5,60] measured as anti-secretory activity in platelets was estimated at 2–3 μmol by comparison with a known concentration of parthenolide.

The capsules contained a mean of 82 mg feverfew and thus the level of activity calculated as parthenolide can be estimated as 0.67%. Other studies quote 0.87% sesquiterpene lactones calculated as parthenolide [27] and 0.25–0.30% of active (antimicrobial) material [33]. Bohlmann's extraction of individual components from the dried plant yielded ~116 mg sesquiterpene lactones/kg dried feverfew which amounts to less than 0.01% [17]. Another extraction yielded 330 mg endoperoxides/kg and 56 mg canin/kg alone [19] which would amount to a level of sesquiterpene lactones of at least 0.04%. Thus, the level of sesquiterpene lactones appears to vary with different sources of the plant and this could have been due to a number of factors including the conditions in which feverfew was grown, the season in which it was picked and the way in which it was stored. Another important determinant of the parthenolide content of feverfew appears to be the geographical location. A recent survey of commercial preparations found that all the North American commercial products tested contained less that 0.1% parthenolide, wheras much higher values were obtained for British products. A minimum level of 0.2% parthenolide in commercial products has been proposed by the Health Protection Branch of Health and Welfare Canada [71].

Quantitative analysis of feverfew with regards to sesquiterpene lactone content has been carried out by TLC [72,73] and HPLC [71,74,75] and ^{1}H-NMR spectroscopy [75]. Measurements of parthenolide by HPLC correlated well with measurements by bioassays based on 5HT-secretion from platelets [75]. The availability of several techniques for quantitation of parthenolide levels in feverfew, makes some standardization of commercial preparations possible.

CONCLUSIONS

In the past 10 years, more information has become available about the medicinal herb feverfew. The active components are sesquiterpene lactones which contain an α-methylene butyrolactone moiety. This functional group can undergo a 'Michael-type' reaction with biological nucleophiles such as sulphydryl groups. This interaction may go some way towards explaining the effects of extracts of feverfew on *in vitro* cell systems such as platelets and neutrophils. However, Woynarowski *et al.* [30,31] suggested that interaction of the sesquiterpene lactones with DNA directly, rather than with SH-goups on enzymes involved in DNA synthesis, was the sole mechanism of action for these compounds. The evidence provided by other workers suggests, however, that the mechanism of action of the sesquiterpene lactones is more general. Platelets for instance do not possess any nuclear

DNA and the effects of feverfew in these cells correlate well with an interaction with SH-groups, although the precise mechanism is still unclear.

Two clinical trials of the effects of feverfew in migraine have shown beneficial effects by feverfew but no clinical benefit could be demonstrated for arthritis. There have been suggestions that platelets and neutrophils may play a role in migraine and arthritis respectively, although no study has clearly established a link between any *in vitro* effects of feverfew and possible clinical benefits. Few side-effects have been noted and although the components of feverfew have been shown to have cytotoxic properties under some experimental conditions, the relevance of this to the clinical situation is not clear.

REFERENCES

1 Berry, M.I. (1984) Pharm. J. 232, 611–614.
2 Johnson, S. (1984) Feverfew. A Traditional Herbal Remedy for Migraine and Arthritis, Sheldon Press, London.
3 Hancock, K. (1986) Feverfew. Your Headache may be Over. Keats Publishing, New Canaan, CT.
4 Editorial (1985) Lancet i, 1084.
5 Murphy. J.J., Heptinstall, S. and Mitchell, J.R.A. (1988) Lancet ii, 189–192.
6 Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. and Webb, D.A. (1976) Flora Europaea, 4, pp.171, Cambridge University Press, Cambridge.
7 Hylands, D.M. (1984) Migraine Matters 2, 25–27.
8 Herout, V., Souček, M. and Šorm, F. (1959) Chem. Ind. (Lond.) 1069–1070.
9 Souček, M., Herout, V. and Šorm, F. (1961) Collect. Czech. Chem. Commun. 26, 803–810.
10 Govindachari, T.R., Joshi, B.S. and Kamat, V.N. (1964) Tetrahedron Lett. 3927–3933.
11 Govindachari, T.R., Joshi, B.S. and Kamat, V.N. (1965) Tetrahedron, 21, 1509–1519.
12 Quick, A. and Rogers, D. (1976) J. Chem. Soc. Perkin Trans. 2, 465– 469
13 Romo de Vivar, A. and Jiménez, H. (1965) Tetrahedron 21, 1741–1745.
14 Romo, J., Romo de Vivar, A., Treviño, R., Joseph-Nathan, P. and Díaz, E. (1970) Phytochemistry 9, 1615–1621.
15 Osawa, T., Taylor, D., Suzuki, A. and Tamura, S. (1977) Tetrahedron Lett. 1169–1172.
16 Stefanović, M., Mladenović, S., Dermanović, M. and Ristić, N. (1985) J. Serb. Chem. Soc. 50, 435–441.
17 Bohlmann, F. and Zdero, C. (1982) Phytochemistry 21, 2543–2549.
18 Kelsy, R.G., Shafizadeh, F., Campbell, J.A., Craig, A.C. Campana, C.F. and Craig, R.E.R. (1983) J. Org. Chem. 48, 125–127.
19 Begley, M.J. Hewlett, M.J. and Knight, D.W. (1989) Phytochemistry 28, 940–943.
20 Johnson, E.S. Hylands, P.J. and Hylands, D.M. (1982) GB Patent 82/14572; (1984) Chem. Abstr. 100, 19778.
21 El-Feraly, F.S. and Chan, Y.M. (1978) J. Pharm. Sci. 67, 347–350.
22 Joshi, B.S. (1976) J. Sci. Ind. Res. 35, 239–243.
23 Grieco, P.A. and Nishizawa, M. (1977) J. Org. Chem. 42, 1717–1720.

24 Shibuya, H., Ohashi, K., Kawashima, K., Hori, K., Murakami, N. and Kitagawa, I. (1986) Chem. Lett. 85–86.
25 Takahashi, T., Nemoto, H., Kanda, Y., Tsuji, J. and Fujise, Y. (1986) J. Org. Chem. 51, 4315–4316
26 Fischer, N.H. Olivier, E.J. and Fischer, H.D. (1979) Prog. Chem. Org. Nat. Prod. 38, 47–390.
27 Bloszyk, E. and Drozdz, B. (1978) Acta Soc. Bot. Pol. 47, 3–13.
28 Lee, K.H., Huang, E.S., Piantadosi, C., Pagano, J.S. and Geissman, T.A. (1971) Cancer Res. 31, 1649–1654.
29 Hoffmann, J.J., Torrance, S.J., Wiedhopf, R.M. and Cole, J.R. (1977) J. Pharm. Sci. 66, 883–884.
30 Woynarowski, J.M. and Konopa, J. (1981) Mol. Pharmacol. 19, 97–102.
31 Woynarowski, J.W., Beerman, T.A. and Konopa, J.(1981) Biochem. Pharmacol. 30, 3005–3007.
32 Hall, I.H., Lee, K.H., Starnes, C.O., Eigebaly, S.A., Ibuka, T., Wu, Y.S., Kimura, T. and Haruna, M. (1978) J. Pharm. Sci. 67, 1235–1239.
33 Blakeman, J.P. and Atkinson, P. (1979) Physiol. Plant Pathol. 15, 183– 192.
34 Hall, I.H., Lee, K.H., Starnes, C.O., Sumida, Y., Wu, R.Y., Waddell, T.G., Cochran, J.W. and Gerhart, K.G. (1979) J. Pharm. Sci. 68, 537–542.
35 Collier, H.O.J., Butt, N.M., McDonald-Gibson, W.J. and Saeed, S.A. (1980) Lancet ii, 922.
36 Pugh, W.J. and Sambo, K. (1988) J. Pharm. Pharmacol. 40, 743–745.
37 Thakkar, J.K., Sperelakis, N., Pang, D. and Franson, R.C. (1983) Biochim. Biophys. Acta 750, 134–140.
38 Capasso, F. (1986) J. Pharm. Pharmacol. 38, 71–72.
39 Makheja, A.N. and Bailey, J.M. (1981) Lancet ii, 1054.
40 Makheja, A.N. and Bailey, J.M. (1982) Prostaglandins Leukotrienes Med. 8, 653–660.
41 Smith, J.B. (1987) in Heamostasis and Thrombosis: Prostaglandins in platelet aggregation and haemostasis (Bloom, A.L. and Thomas, D.P., eds.), pp.78–89, Churchill Livingstone, Edinburgh.
42 Heptinstall, S., White, A., Williamson, L. and Mitchell, J.R.A. (1985) Lancet i, 1071–1074.
43 Groenewegen, W.A. and Heptinstall, S. (1990) J. Pharm. Pharmacol. 42, 553–557.
44 Groenewegen, W.A., Knight, D.W. and Heptinstall, S. (1986) J. Pharm. Pharmacol. 38, 709–712.
45 Lösche, W., Michel, E., Heptinstall, S., Krause, S., Groenewegen, W.A., Pescarmona, G.P. and Thielmann, K. (1988) Planta Med. 54, 381–384.
46 Williamson, L.M., Harvey, D.M., Sheppard, K.J. and Fletcher, J. (1988) Inflammation 12, 11–16.
47 O'Neill, L.A.J., Barrett, M.L. and Lewis, G.P. (1987) Br. J. Clin. Pharmacol. 23, 81–83.
48 Hayes, N.A., and Foreman, J.C. (1987) J. Pharm. Pharmacol. 39, 466–470.
49 Kupchan, S.M., Fessler, D.C., Eakin, M.A. and Giacobbe, T.J. (1970) Science (Washington, DC) 168, 376–377.
50 Lee, K.H., Hall, I.H., Mar, E.C., Starnes, C.O., Elgebaly, S.A., Waddell, T.G., Hadgraft, R.I., Ruffner, C.G. and Weidner, I. (1977) Science (Washington, DC) 196, 533–536.
51 Talaga, P., Schaeffer, M., Mattes, H., Benezra, C. and Stampf, J.L. (1989) Tetrahedron 45, 5029–5038.
52 Heptinstall, S., Groenewegen, W.A., Spangenberg, P. and Loesche, W. (1987) J. Pharm. Pharmacol. 39, 459–465.

53 Loesche, W., Groenewegen, W.A., Krause, S., Spangenberg, P. and Heptinstall, S. (1988) Biomed. Biochim. Acta 47, S241–243.
54 Lösche, W., Mazurov, A.V., Heptinstall, S., Groenewegen, W.A., Repin, V.S. and Till, U. (1987) Thromb. Res. 48, 511–518.
55 Voyno-Yasenetskaya, T.A., Loesche, W., Groenewegen, W.A., Heptinstall, S., Repin, V.S. and Till, U. (1988) J. Pharm. Pharmacol. 40, 501–502.
56 Keery, R.J. and Lumley, P. (1986) Br. J. Pharmacol. 89, 834P.
57 Buck., A.C., Pugh, J., Davies, R., Moffat, D.B. and Lote, C.J. (1986) Abstract from Congress sur les Prostaglandines, pp.53
58 Biggs, M.J., Johnson, E.S., Persaud, N.P. and Ratcliffe, D.M. (1982) Lancet ii, 776.
59 Johnson, E.S., Kadam. N.P., Hylands, D.M. and Hylands, P.J. (1985) Br. Med. J. 291, 569–573.
60 Pattrick, M., Heptinstall, S. and Doherty, M. (1989) Ann. Rheum. Dis. 48, 547–549.
61 Johnson, E.S., Kadam, N.P., Anderson, D., Jenkinson, P.C., Dewdney, R.S. and Blowers, S.D. (1987) Hum. Toxicol. 6, 533–534.
62 Vickers, H.R. (1950) Practitioner 164, 226–233.
63 Schultz, K.H. (1975) Arch. Dermatol. Res. 25, 235–244.
64 Hausen, B.M. and Osmundsen, P.E. (1983) Acta Derm. Venereol. 63, 308–314.
65 Corres, L.F. (1984) Contact Dermatitis 11, 74–79.
66 Schmidt, R.J. and Kingston, T. (1985) Contact Dermatitis 13, 120–127.
67 Wedner, H.J., Zenger, V.E. and Lewis, W.H. (1987) Int. Arch. Allergy Appl. Immunol. 84, 116–122.
68 Guin, J.D. and Skidmore, G. (1987) Arch. Dermatol. 123, 500–502.
69 Evans, F.J. and Schmidt, R.J. (1980) Planta Med. 38, 289–316.
70 Groenewegen, W.A. and Heptinstall, S. (1986) Lancet i, 44–45.
71 Awang, D.V.C., Dawson, B.A., Kindack, D.G., Crompton, C.W. and Heptinstall, S. (1991) J. Nat. Prod., in press.
72 Droździ, B. and Bloszyk, E. (1978) Planta Med. 33, 379–384.
73 Picman, A.K., Ranieri, R.L., Towers, G.H.N. and Lam, J. (1980) J. Chromatogr. 189, 187–198.
74 Strack, D., Proksch, P. and Gülz, P.G. (1980) Z. Naturforsch. 35c, 915–918.
75 Heptinstall, S., Awang, D.V.C., Dawson, B.A., Kindack, D.G., Knight, D.W. and May, J. (1992) J. Pharm. Pharmacol., in press.

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7 Ondansetron and Related 5-HT_3 Antagonists: Recent Advances

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INTRODUCTION

5-Hydroxytryptamine has many physiological functions, most of which are poorly understood. Several of these actions are apparently opposing in nature and have stimulated the interest of scientists for many years. In the last decade there has been an explosion of interest toward characterizing these responses with respect to the interaction of 5-HT with receptor subtypes. One area which has probably attracted most interest is the physiological roles of the 5-HT$_3$ receptor. These studies have already led to the use of the 5-HT$_3$ receptor antagonist, ondansetron (Zofran^R), in the treatment of nausea and vomiting caused by cancer chemotherapy and radiotherapy. In addition, there is now abundant evidence from animal studies which suggests that 5-HT$_3$ receptor antagonists could be useful in treating several psychiatric disorders. This review describes the medicinal chemistry which led to the identification of ondansetron and related structures having selective 5-HT$_3$ receptor antagonist activity.

5-HT RECEPTOR CLASSIFICATION

At least seven types of 5-HT receptor have been identified on the basis of distinct pharmacological profiles [1]. The 5-HT$_3$ receptor (previously known as the 5-HT 'M' receptor) [2] is identified as being stimulated by selective agonists, such as 2-methyl-5-HT [3] and 1-(*m*-chlorophenyl)biguanide (mCPBG) [4, 5], and antagonized by nanomolar concentrations of antagonists such as MDL 72222 [6], ICS 205–930 [3] and ondansetron [7]. Antagonists of other 5-HT receptors (for example, methiothepin and ketanserin) are not active except at very high concentrations.

All of the 5-HT receptors, except the 5-HT$_3$ receptor, belong to the single-subunit, seven-helical-domain, G-protein-linked family of receptors [8]. The 5-HT$_3$ receptor is unique in belonging to the multisubunit ligand-gated ion-channel family of receptors [9]. These receptors appear to be located exclusively on neurones in both the autonomic and central nervous systems [10], where they mediate some of the excitatory effects of 5-HT. Electrophysiological analysis of 5-HT$_3$ receptor function reveals that agonist interaction causes the opening of an ion channel permeable to monovalent cations. This results in a rapid depolarization which equally rapidly desensitizes [11]. There is increasing evidence that 5-HT$_3$ receptors have a modulatory role on the release of other neurotransmitters [12–17].

BIOLOGICAL TEST SYSTEMS FOR 5-HT_3 RECEPTORS

Many *in vitro* assay systems have been developed for studying substances which interact with the 5-HT_3 receptor. Outlined in the next section are those most commonly used to assess 5-HT_3 receptor antagonist activity. This is followed by a section on the *in vivo* tests to assess 5-HT_3 receptor activity.

IN VITRO ASSAYS

The guinea-pig isolated ileum contracts in response to the application of selective 5-HT_3 receptor agonists. This effect is blocked by the muscarinic antagonist, atropine, and by selective 5-HT_3 receptor antagonists. Studies have revealed that 5-HT acts on 5-HT_3 receptors on myenteric neurones to cause the release of acetylcholine (and possibly substance P) which stimulates muscarinic receptors on the smooth muscle to cause a contraction [2, 13, 18]. This is a reliable and reproducible preparation for the assessment of agonist and antagonist activity at 5-HT_3 receptors in the guinea-pig. Ondansetron (1×10^{-7}–1×10^{-6} M) causes a concentration-related rightward shift in the concentration–response curve to 2-methyl-5-HT (*Figure 7.1*).

However, recent evidence indicates that the guinea-pig ileum may not be

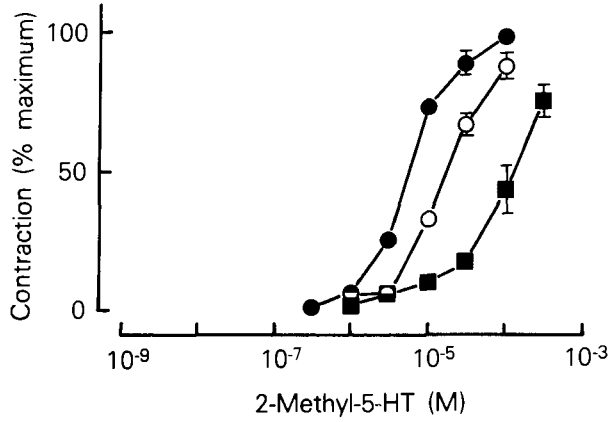
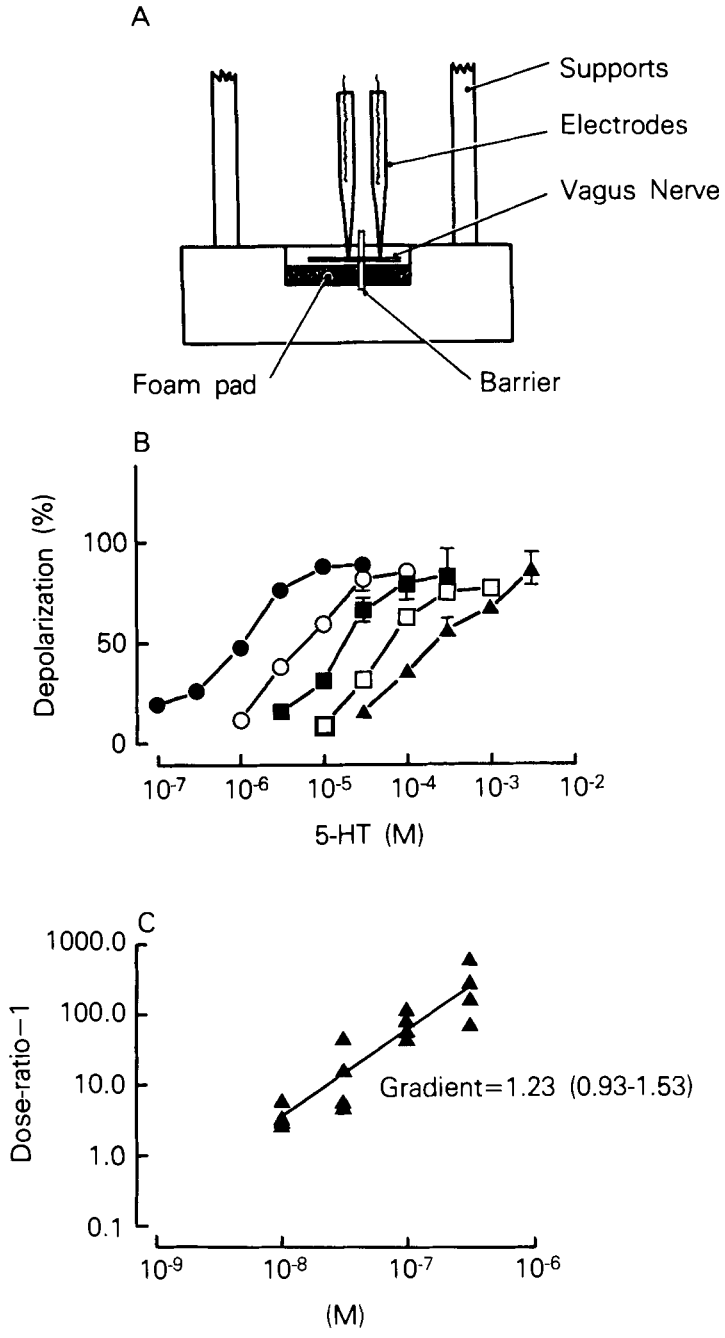


Figure 7.1. The effect of ondansetron on contractile responses to 2-methyl-5-HT in the guinea-pig isolated longitudinal muscle myenteric plexus preparation. Symbols indicate control responses (●) or in the presence of ondansetron at 1 × 10^{-7} M (○) or 1 × 10^{-6} M (■). Results ± S.E.M. of at least four separate observations. Experiments were performed as described by Butler et al. [7].


A
Supports
Electrodes
Vagus Nerve
Foam pad
Barrier
B
Depolarization (%)
100
50
0
10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2}
5-HT (M)
C
Dose-ratio−1
1000.0
100.0
10.0
1.0
0.1
Gradient=1.23 (0.93-1.53)
10^{-9} 10^{-8} 10^{-7} 10^{-6}
(M)

the most appropriate preparation on which to predict the activity of 5-HT_3 receptor antagonists in man. It is now clear that the guinea-pig 5-HT_3 receptor exhibits a pharmacological profile different from that of the rat [19]. This is manifest in lower apparent affinities for most 5-HT_3 receptor antagonists. The human 5-HT_3 receptor appears to resemble more closely that present in the rat [20].

A second assay which has been used extensively in the characterization of 5-HT_3 receptors is the rabbit isolated heart. Both the sympathetic and parasympathetic inputs to the rabbit heart can be influenced by stimulation of 5-HT_3 receptors; this preparation is usually performed in the presence of atropine to block parasympathetic effects [12]. Under these conditions, 5-HT and other 5-HT_3 receptor agonists exert positive effects on rate and contractile force. Selective 5-HT_3 receptor antagonists such as MDL 72222 [6], ICS 205–930 [3] and ondansetron [7] block the agonist effects in a concentration-related manner.

Both of the preparations described above provide indirect measures of the 5-HT_3 receptor. That is, the end tissue response is mediated by another neurotransmitter. This has a disadvantage in that compounds may interfere with the secondary neurotransmitter and give a false affinity estimate. A preparation that provides a direct measure of 5-HT_3 receptor activity and which was used at Glaxo Group Research as a primary screen for 5-HT_3 receptor antagonists is the rat isolated vagus nerve [21] (*Figure 7.2A*). 5-HT_3 receptor agonists cause a rapid depolarization of the vagus nerve. Ondansetron causes parallel, rightwards displacements of the agonist concentration response curve (*Figure 7.2B*); indeed, ondansetron was first identified as a 5-HT_3 receptor antagonist using this preparation.

A more recently identified assay for 5-HT_3 receptor affinity is radioligand binding. Various ligands have now been used to label 5-HT_3 receptors (see [23]), but the first used in brain tissue – which also provided the first direct evidence for the existence of 5-HT_3 receptors in the brain – was [^{3}H]GR65630 (see *Figure 7.3*) [10]. Radioligand binding is a very useful screening technique because of the rapidity and accuracy of the assay. How-

Figure 7.2. (A) The greased-gap technique for recording depolarizations of the rat isolated vagus nerve. (B) The effect of ondansetron on depolarizations of the rat isolated vagus nerve induced by 5-HT. Symbols indicate controls (●) or in the presence of ondansetron at 1×10^{-8} M (○), 3×10^{-8} M (■), 1×10^{-7} M (□) or 3×10^{-7} M (▲). Results are the mean ± S.E.M. of at least four determinations. Experiments were performed as described by Ireland and Tyers [21]. (C) Data from the experiments illustrated in B plotted according to Arunlakshana and Schild [22]. Each point is the result from a separate tissue. The gradient of straight line (95% confidence limits) was determined using linear regression analysis.

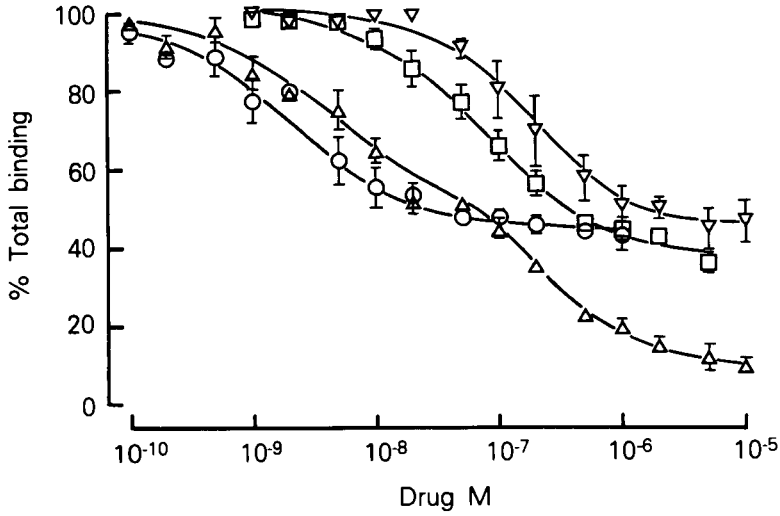


Figure 7.3. The inhibition of [^{3}H]GR65630 binding to homogenates of rat entorhinal cortex by ICS 205-930 (○), ondansetron (△), MDL 72222 (□) and 5-HT (▽). Results are the mean ± S.E.M. of at least three separate determinations. Experiments were performed as described by Kilpatrick et al. [10].

ever, such assays must be carefully characterized to ensure that the specific binding site is the same as the functional receptor under study. It should also be remembered that in binding assays it is difficult to determine whether compounds with apparent affinity also have intrinsic agonist activity.

Initial studies with [^{3}H]GR65630 revealed that it labelled a site in rat brain with all the characteristics of the 5-HT$_3$ receptor as characterized in functional models. Of most importance was the observation that 5-HT$_3$ receptor antagonists inhibited binding with affinities very close to those observed in functional assays [10]. Ondansetron and other 5-HT$_3$ receptor antagonists potently inhibited approximately 60 % of total binding. However, ondansetron and other compounds in the same chemical series also inhibited a further portion of binding, albeit with lower affinity (*Figure 7.3*). This means that it is necessary to apply complex two-site analysis using the computer program LIGAND [24] in order to obtain exact 5-HT$_3$ receptor affinities. Other 5-HT$_3$ receptor antagonists do not inhibit binding to this low-affinity site, indicating that it is not related to the 5-HT$_3$ receptor.

These are the assays most frequently used to detect 5-HT$_3$ receptor activity. However, there are other *in vitro* assays which could be used. Amongst these are neurotransmitter release assays using brain tissue; for example, 5-

HT_3 receptor agonists have been shown to enhance the release of endogenous dopamine from striatal slices [14] and to reduce acetylcholine release from sections of rat entorhinal cortex [15]. Furthermore, there are several electrophysiological techniques using a variety of neurones, including those in the gut [25] and primary cultures of brain tissue [26] which respond to 5-HT_3 agonist stimulation.

IN VIVO TESTS

In vivo tests for drug activity provide information for two purposes: firstly, to obtain data on the potency and duration of action of novel compounds and, secondly, animal models of disease states may be used in order to predict potential therapeutic uses. Models for both of these purposes have been established for agents which interact with the 5-HT_3 receptor.

The most frequently used preparation for assessing the *in vivo* activity of 5-HT_3 receptor agents is the von Bezold Jarisch reflex (the BJ test) in anaesthetized animals [27, 28]. This preparation is relatively simple to perform and can provide rapid comparative data on a series of compounds, although it gives no indication of potential therapeutic uses.

We have found the anaesthetized cat to be the most reliable preparation, although ondansetron was first tested in the anaesthetized rat. Bolus i.v. injections of 5-HT or a 5-HT_3 receptor agonist cause transient, dose-dependent reflex falls in heart rate and blood pressure which are believed to result from the depolarization of afferent nerve endings in the wall of the right ventricle [3]. This can be blocked by the prior administration of a 5-HT_3 receptor antagonist such as ondansetron (*Figure 7.4*). We quantified the duration of antagonistic effects by measuring the time that a DR_5 was maintained. The DR_5 represents a 5-fold shift in the concentration-response curve to an agonist, that is, if after the administration of an antagonist, the dose of agonist must be increased 5 times to exert the same effect, then the DR_5 level has been reached. For ondansetron, a dose of 30 μg/kg i.v. maintained a DR_5 for over 100 min in the anaesthetized cat.

Richardson and colleagues [3] identified a simple *in vivo* assay for the 5-HT_3 receptor in man. They showed that the application of 5-HT to the exposed base of a blister evokes a pain and a wheal and flare response. These can be blocked by 5-HT_3 receptor antagonists. Obviously one needs willing volunteers for such studies and suitable safety data must be available, but this is a useful model for assessing 5-HT_3 receptor antagonist activity in man.

Indications for several therapeutic uses of 5-HT_3 receptor antagonists have come from behavioural studies. These compounds have little or no ef-

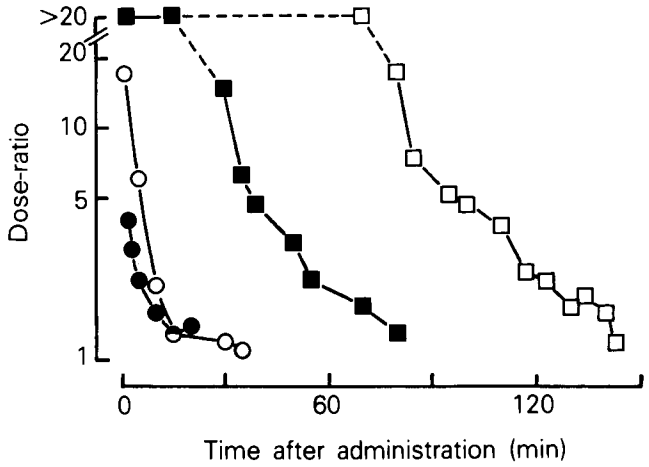


Figure 7.4. Antagonism by ondansetron of the von Bezold Jarisch reflex in the anaesthetized cat induced by the administration of 2-methyl-5-HT. Symbols indicate the time course of antagonism following intravenous administration of ondansetron at 1 (●), 3 (○), 10 (■) or 30 μg/kg (□). Each set of data points represents data from a single animal. Experiments were performed as described by Butler et al. [7].

fect on normal behaviour but exert profound effects in some animal models of psychiatric disease states. These can be divided into three broad sections, disinhibitory effects, controlling effects on disturbed mesolimbic dopamine systems and enhancing effects on cognitive function.

The disinhibitory effects of 5-HT$_3$ receptor antagonists are now well documented [29, 30]. These compounds act to restore normal behaviour to animals in conditions which are mildly aversive, such as a novel brightly lit test area. Such effects may be predictive of anxiolytic activity. An example of such disinhibition is the effect of ondansetron in the rat social interaction test in which the level of interaction between two rats is measured under certain defined conditions [29]. In non-aversive conditions this type of behaviour is quite marked, but it is suppressed in novel highly illuminated conditions. Ondansetron overcomes this suppression, as do known anxiolytics such as diazepam.

Mesolimbic dopamine pathways are thought to be involved in the rewarding effects of drugs of abuse and an imbalance of this pathway is thought to be causal in psychoses. Several studies have revealed that 5-HT$_3$ receptor antagonists can correct such imbalances. Thus, ondansetron inhibits the behavioural hyperactivity resulting from direct stimulation of this

system with discrete injections of dopamine or amphetamine into the nucleus accumbens [31] or stimulation of dopamine cell bodies in the ventral tegmental area with a stable analogue of substance P [32]. Ondansetron and other 5-HT_3 receptor antagonists have also been shown to block some of the rewarding effects of drugs of abuse such as nicotine, morphine and amphetamine [33, 34] and the consumption of ethanol in dependent marmosets [35] and rats [36]. This effect may be mediated in mesolimbic systems, since the direct measurement of dopamine in these areas of the brain reveals increases after the administration of substances such as morphine. These increases in dopamine release are inhibited by 5-HT_3 receptor antagonists [37]. Further studies indicate that 5-HT_3 receptor antagonists inhibit the behavioural consequences of withdrawing from chronic treatment with drugs of abuse [38–40].

Cognitive enhancing effects of 5-HT_3 receptor antagonists have been observed in several animal models, using both normal animals and animals in which a cognitive deficit has been induced by the administration of the muscarinic antagonist scopolamine [41]. An example is the Wisconsin test, in which marmosets learn to discriminate which of two objects conceal a food reward and, after learning this, the task is reversed such that the food is hidden beneath the other object. Treatment with ondansetron has been shown to enhance the ability of marmosets to learn the reversal task.

Thus, it is clear that 5-HT_3 receptor antagonists such as ondansetron have effects in animal models of human psychiatric disease states such as anxiety, psychoses, drug dependence and cognitive dysfunction.

PHARMACOLOGICAL AND ANTIEMETIC PROPERTIES OF ONDANSETRON

As described above, ondansetron has potent and highly selective antagonist properties at the 5-HT_3-receptor [7]. The selectivity of action of ondansetron for the 5-HT_3 receptor has been demonstrated using a number of *in vitro* preparations which respond to activation of a number of different neurotransmitter receptors. The selectivity ratio for ondansetron on 5-HT_3-receptors compared with other receptor types is greater than 1000. In animals and in man, ondansetron has no overt actions on cardiovascular parameters and there are no effects on normal behaviour [7].

The severe nausea and vomiting induced by cytotoxic drugs and radiation in man can be reduced by metoclopramide given either alone or in combination with other drugs, such as dexamethasone. However, the extrapyramidal side-effects induced by metoclopramide, due to antagonism of dopamine re-

ceptors, limit the dose and hence its effectiveness. Metoclopramide has both dopamine (D_2) and 5-HT$_3$ receptor-blocking properties, but until more recently it was not known whether antagonism of 5-HT$_3$ receptors contributed to the anti-emetic effect of metoclopramide. We therefore decided to evaluate the effects of ondansetron as an anti-emetic in the ferret. The ferret had previously been shown to be sensitive to the antiemetic effect of metoclopramide against emesis induced by the cytotoxic drug cisplatin [42].

Cisplatin (review [42a]) is a highly emetogenic cytotoxic drug in man. In ferrets, a dose of 9 mg/kg i.v. causes retching and vomiting after a delay of about 60–90 min. These effects are preceded by a number of behavioural changes such as salivation, irregular breathing and restlessness, which have been described as 'nausea'. Cyclophosphamide, 200 mg/kg i.p., also causes marked nausea and vomiting after a short onset latency (30–60 min). Whole body X-radiation (800 rads 250 kV) also causes nausea and vomiting after a delay of about 30 min and a prolonged response. Ondansetron, 0.01–0.1 mg/kg i.v. or 0.1–0.5 mg/kg p.o. compared with metoclopramide 1–4 mg/kg i.v. caused dose-dependent inhibitions of the nausea and vomiting induced by each of these procedures [43]. In contrast to ondansetron, metoclopramide also caused moderate to marked behavioural depression. Since metoclopramide is about 50-times more potent as a dopamine D_2-receptor antagonist than as a 5-HT$_3$-receptor antagonist, the behavioural depression is almost certainly due to blockade of dopamine receptors.

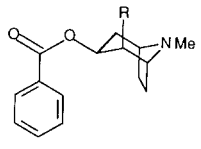
Clinical studies in cancer patients have also shown ondansetron to be a highly effective antiemetic drug and to be significantly more effective than metoclopramide [44–46]. As expected, there are no reports of extrapyramidal side-effects in patients receiving ondansetron.

5-HT$_3$ receptors are densely located in the hind brain area known as the vomiting system (that is, the area postrema, dorsovagal nucleus, nuclear solitary tract) (see Pratt et al. [47]). Discrete injections of ondansetron into this brain area in ferrets inhibit emesis induced by cisplatin given intraperitoneally, suggesting that the vomiting system is an important site for the antiemetic actions of 5-HT$_3$-antagonists [48]. Further studies have also shown that cisplatin increases the turnover of 5-HT in the duodenum [49]. Since 5-HT$_3$-receptors are known to be present on vagal afferent fibres, it could be that there are two sites of action. The initiation of the vomiting response may be due to a release of 5-HT from 5-HT-rich enterochromaffin cells in the intestinal mucosa. The released 5-HT excites vagal afferents which project to the vomiting system. 5-HT released in the vomiting system propagates the response to initiate the efferent arm of the vomiting reflex. The antiemetic effects of 5-HT$_3$-antagonists could, therefore, be mediated at both the peripheral vagal site and at the brain stem vomiting system.

Ondansetron is the first 5-HT_3-receptor antagonist to be used for the treatment of nausea and vomiting induced by cancer therapy. Its high receptor selectivity is reflected in clinical studies which show ondansetron to be a very effective antiemetic drug with few side-effects.

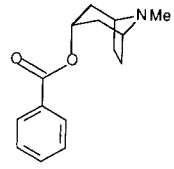
AROMATIC ESTERS AND AMIDES AS 5-HT_3 ANTAGONISTS

The search for selective antagonists at the 5-HT_3 receptor was first initiated by J.R. Fozard and his colleagues at the University of Manchester, screening compounds using the rabbit heart preparation. This followed the report by Rocha e Silva et al. (1953) [50] that (−)-cocaine (1) was an antagonist of 5-HT in the guinea-pig isolated ileum. Fozard et al. [51] showed that cocaine was also an antagonist (pA_2 6.24) in the rabbit heart against 5-HT-induced tachycardias. Of related compounds that were tested on the rabbit heart, benzoylpseudotropine (2), with similar stereochemistry to (−)-cocaine, was one of the most potent (pA_2 6.96), but the *endo* isomer, benzoyltropine (3), was marginally even more active (pA_2 7.19).



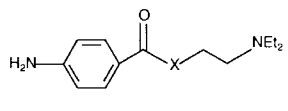
(1) R = CO_2Me

(2) R = H



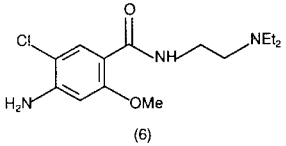
(3)

Other local anaesthetics such as procaine (4) and procainamide (5) were also shown to be antagonists of the sympathetic neuronal 5-HT_3 receptor at concentrations well below those causing anaesthesia and led to the testing of metoclopramide (6), a D_2 receptor antagonist and stimulant of gastrointestinal motility. This compound too was found to be a potent antagonist (pA_2 7.2) at 5-HT_3 receptors [52].



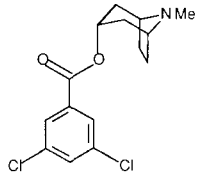
(4) X = O

(5) X = NH

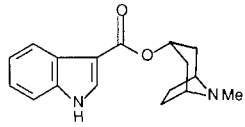


(6)

Continuing this work in the laboratories of the Merrell International Research Centre at Strasbourg, Fozard and Gittos [53] investigated a series of tropine benzoate esters from which emerged the first selective and highly potent 5-HT$_3$ antagonist to be published, MDL 72222 (7) (pA_2 9.3). Their therapeutic target was the treatment of migraine based on a hypothesis [54] that 5-HT released locally in blood vessels of the head would contribute to the headache by activating sensory 5-HT$_3$ receptors innervating the cranial vasculature.

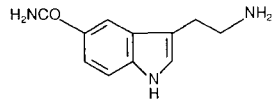


(7)



(8)

About the same time, and independently of this study, a group at Sandoz reported [3] the synthesis of 3-indolyltropine esters such as ICS 205-930 (8) with a pharmacological profile similar to that of MDL 72222. In this case the strategy [55] had been to select 5-HT, metoclopramide and 5-carboxamidotryptamine (9) [56] as the starting points and carry out a systematic modification of the carboxamido group and the side-chain at C-3 on the indole nucleus.

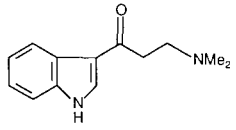


(9)

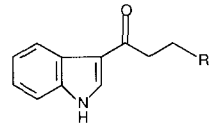
N-LINKED IMIDAZOLYL INDOLYLPROPANONES AS 5-HT$_3$ ANTAGONISTS

Glaxo had also started a programme to identify selective agonists and antagonists for the 5-HT$_3$ receptor. Although aromatic tropine esters and metoclopramide were established prototype molecules for designing a selective 5-HT$_3$ antagonist, there were no published data defining the essential structural features required for activity at the time we began our study. We concluded a new approach was needed and investigated the possibility that an indole derivative to which a basic side-chain was attached might provide

a novel lead. Selective screening soon revealed that the indolylpropanone (10) was a weak antagonist of 5-HT-induced depolarization of rat vagus nerve (pA_2 6.5), but of sufficient potency to initiate a chemical programme. This work, which led to the discovery of ondansetron, is the basis of this review.

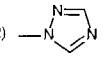


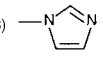
(10)



R

(11) 

(12) 

(13) 

Systematically increasing the size and lipophilic character of the tertiary amino group by replacing the dimethylamino substituent with acyclic and cyclic amines failed to produce much increase in activity, and the weakly basic pyrazole (11) and triazole (12) were both inactive. However, the *N*-imidazolyl compound (13) was unexpectedly found to be at least 10-times more potent (RVN pA_2 7.61) than the parent dimethylamino derivative (10) and became a template for further elaboration.

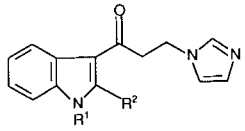
Initially, it was found that introducing a methyl group at the 1-position and a methyl or phenyl substituent at the 2-position of the indole ring in this series produced only small changes in activity, despite a marked increase in lipophilicity of the phenyl-substituted compounds (*Table 7.1*).

The quaternary ammonium salts required for preparing the above derivatives were synthesized by standard reactions from the appropriate indole (*Scheme 7.1*).

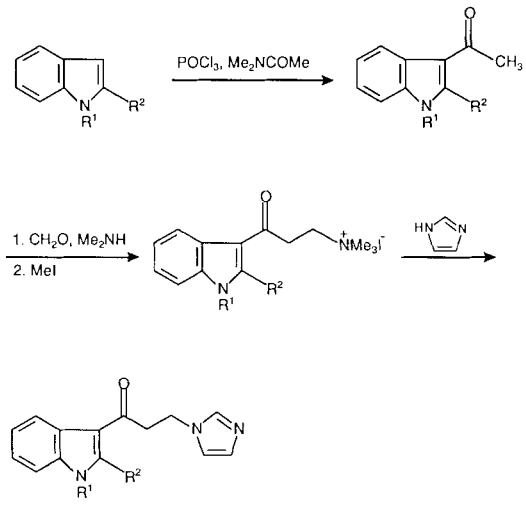
EFFECT OF SUBSTITUTING THE IMIDAZOLE RING

The 1-methylindole derivative (14) (see *Table 7.1*), which was marginally more potent than (13) *in vitro*, showed good oral activity in the BJ test in the rat (ED_{50} 0.11 mg/kg). However, it potentiated the pentobarbitone sleeping time in the mouse, suggesting it had the capacity to bind to and inhibit the hepatic cytochrome *P*-450-linked oxidase enzyme system. Optical

Table 7.1. N-LINKED IMIDAZOLYPROPANONES



	R^1	R^2	pA_2 (RVN)
(13)	H	H	7.61
(14)	Me	H	7.67
	H	Me	7.48
	H	Ph	7.47
	Me	Ph	7.37



Scheme 7.1.

difference spectra confirmed type II binding to *P*-450 in which the imidazole ring was probably co-ordinated at the nitrogen atom at the 3-position to the ferric ion of the enzyme haemoprotein. It was predicted from a study [57] of the steric factors that determine the inhibitory interaction of imidazoles with microsomal enzymes that introducing a methyl group at the 2- or 4-position of the imidazole ring in (14) would provide sufficient steric hindrance to prevent this undesirable interaction. Fortuitously, we found that this had the desired effect without compromising 5-HT$_3$ antagonist activity. The 2-methyl derivative (15) was the more potent of the two compounds

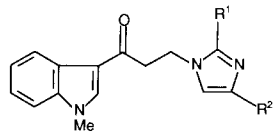
in vitro, but in the BJ test in the rat it was weakly active by the oral route (ED_{50} 2.5 mg/kg), though very potent by intravenous administration (ED_{50} 3.8 μg/kg), indicating the probability of rapid metabolism. The demethyl compound (14), by comparison, was potent by either route of administration, presumably because it inhibited its own metabolism. A range of compounds was prepared in which the 2-methyl group on the imidazole ring was replaced by bulkier and more lipophilic substituents, but these had adverse effects on potency (*Table 7.2*). The methyl group was preferred.

Further modification of this series failed to give a compound with significant advantages over the 2-methylimidazole (15).

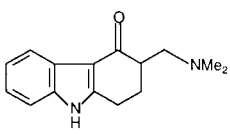
N-LINKED IMIDAZOLYLMETHYL TETRAHYDROCARBAZOLONES

Incorporating the side-chain into the tetrahydrocarbazolone system furnished the conformationally restrained compound (16) (RVN pA_2 6.9), which was only marginally more potent than the indolylpropanone (10). The imidazolyl derivative (17) (ondansetron) (pA_2 8.6) was similarly more potent than the acyclic ketone (15) on rat vagus nerve and it had greatly enhanced oral activity (ED_{50} 7 μg/kg p.o.) in the rat and a long duration of action (> 3 h at 30 μg/kg i.v.) against the BJ response in the anaesthetized cat [7]. Ondansetron had no effect on the cytochrome *P*-450 oxidase system but, in common with other drugs of this class, for example [3], showed high speci-

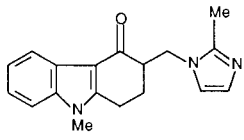
Table 7.2. EFFECT OF SUBSTITUENTS ON THE IMIDAZOLE RING



	R^1	R^2	pA_2 (RVN)
(14)	H	H	7.67
(15)	Me	H	8.0
	H	Me	7.4
	Et	H	7.6
	iPr	H	7.8
	tBu	H	6.6
	Ph	H	6.3
	CH_2Ph	H	7.6



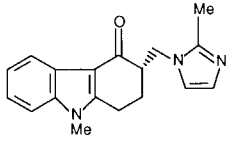
(16)



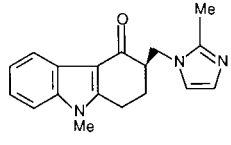
(17)

ficity and was found to be more than 1000-fold selective for the 5-HT$_3$ receptor compared to any other receptor type examined, including 5-HT$_2$, 5-HT$_1$-like, GABA, histamine, acetylcholine, and most importantly, dopamine receptors.

Ondansetron (17) is a racemic compound not easy to resolve by chemical means because the carbonyl function is poorly reactive so it is difficult to form chiral derivatives. However, a resolution was achieved by the classical method of forming diastereomeric salts with an optically active acid and then separating the salts by recrystallisation. A number of acids were tried, but only the salts prepared from (+)- and (−)-di-*p*-toluoyltartaric acid could be separated in this way. Each isomer was obtained in greater than 95 %ee. The absolute stereochemistry of the isomer from the (+)-acid was determined by X-ray crystallography (Williams, D., personal communication) and shown to possess the *S*-configuration (18).

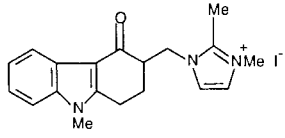


(18) (S)-ISOMER
pA_2 8.55 (RVN)



(19) (R)-ISOMER
pA_2 8.95 (RVN)

It was unusual to find that both enantiomers had similar activity *in vitro*, but these are relatively planar molecules in which the asymmetric centre is well separated from the basic end group, so there is close coincidence of the key recognition sites of the indole nucleus, carbonyl function and basic imidazole nitrogen atom. The quaternary derivative (20) of ondansetron retained activity (RVN pA_2 8.4), suggesting that the imidazole ring is protonated in the binding interaction with the receptor.

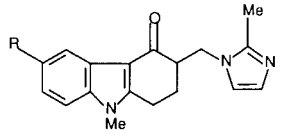


(20)

MODIFICATION OF THE INDOLE NUCLEUS

Many tetrahydrocarbazolone derivatives were prepared but none had advantages over ondansetron. Substituting the indole nucleus at C-6, a potential site of metabolism, to increase the duration of action produced disappointing results. Most analogues, with the exception of the 6-fluoro derivative, were significantly less potent than ondansetron (*Table 7.3*). There appears to be a lack of steric tolerance at this position and a similar phenomenon has been reported for indazole amides [58] and indolyl esters [55] with an azabicyclic end-group. None of the compounds was longer acting than ondansetron.

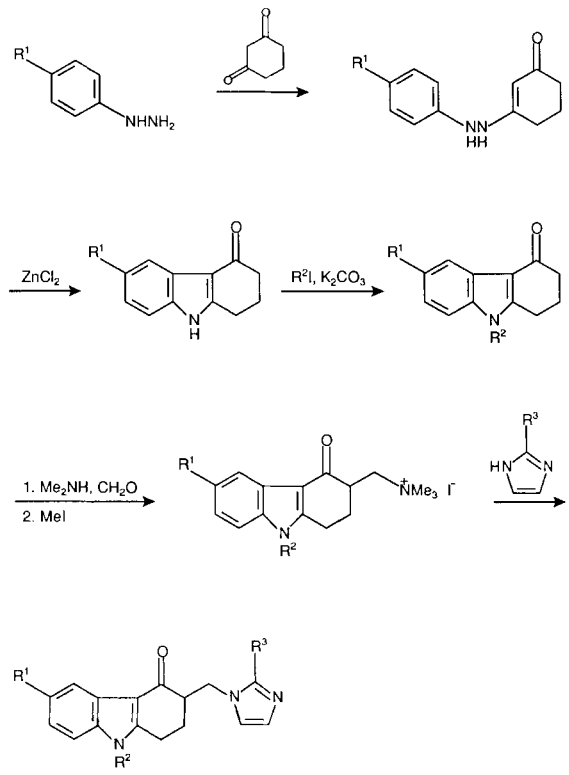
Table 7.3. RING-SUBSTITUTED TETRAHYDROCARBAZOLONES



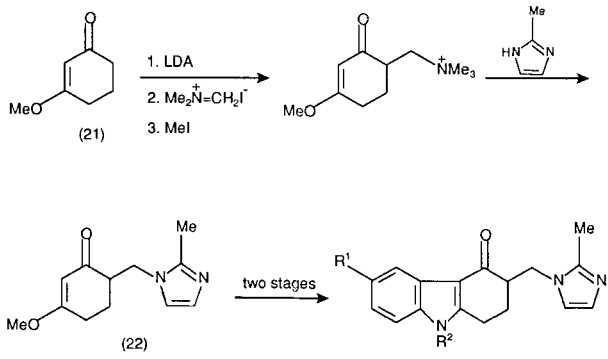
R	pA_2 (*RVN*)
H	8.6
F	8.2
Br	7.5
MeO	6.1
HO	8.2
Me_2N	7.9

A method of synthesis which has been used generally in this series employed a Fischer reaction at an early stage to form the tetrahydrocarbazolone nucleus [59] (*Scheme 7.2*). We devised a second route employing the Fischer method in which the key intermediate was the cyclohexenone (22) (*Scheme 7.3*). This was readily prepared by treating the enolate of the methyl enol ether (21) with dimethyl(methylene)ammonium iodide [60] to form the Mannich base which was then condensed with 2-methylimidazole to give (22).

Substitution at the 9-position was well tolerated, but some long-chain lipophilic substituents or acyl groups reduced activity. Alkylation or acylation of the unsubstituted compound in the presence of sodium hydride afforded derivatives substituted on the indole nitrogen atom (*Table 7.4*).

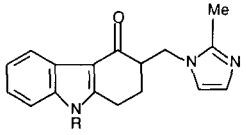


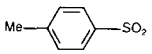
Scheme 7.2.



Scheme 7.3.

Table 7.4. 9-SUBSTITUTED TETRAHYDROCARBAZOLONES



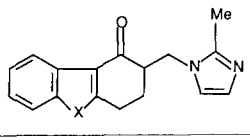
R	*pA₂ (RVN)*
Et	8.7
	8.4
$PhCH_2$	8.2
$Me(CH_2)_5$	7.8
$Me(CH_2)_4C{\equiv}CCH_2$	7.7
$Me(CH_2)_9$	7.2
$Ph(CH_2)_3$	6.8
Me_2NCO	8.4
$MeSO_2$	7.9
	7.4

We studied the effect of replacing the indole nucleus by other non-basic ring systems and prepared the benzofuran and benzothiophen derivatives by the standard route from the appropriate ketone. It may be seen from *Table 7.5* that both derivatives were less active than ondansetron.

MODIFICATION OF THE CARBONYL FUNCTION

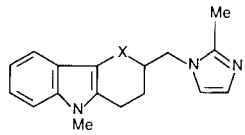
The carbonyl function in this series was found to be an essential feature for potent 5-HT_3 antagonist activity. The racemic *cis*-alcohols (23) and corre-

Table 7.5. EFFECT OF CHANGING THE INDOLE RING



X	*pA₂ (RVN)*
NMe	8.6
O	7.5
S	7.3

Table 7.6. VARIATION OF THE CARBONYL FUNCTION



Ring X	*pA$_2$ (RVN)*
	8.6
(23) β-OH (*cis*)	6.9
(24) α-OH (*trans*)	7.1
(25) CH_2	6.4

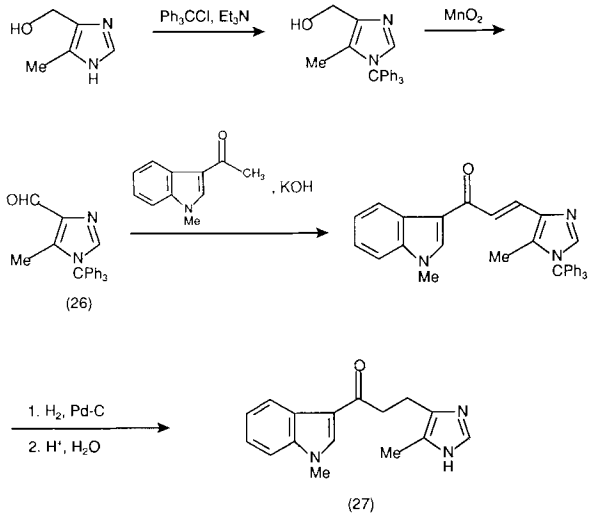
sponding racemic *trans*-compounds (24) were about 30-times less active than ondansetron in the vagus preparation, whereas the tetrahydrocarbazole (25) was at least 100-times less potent than the tetrahydrocarbazolone (*Table 7.6*).

C-LINKED IMIDAZOLYL KETONES

We investigated the possibility that the N-linked 2-methylimidazole ring might not be the optimum one for 5-HT$_3$ antagonist activity and considered the possibility that C-linked imidazole derivatives might have advantages. One of the first compounds prepared was the indolyl ketone (27), which was chosen on grounds of accessibility and synthesized by standard reactions from the protected aldehyde (26) (*Scheme 7.4*) [61].

We were greatly surprised to find that (27) was approximately 100-times more potent (RVN pA_2 9.9) than the N-linked imidazole (15). A radiolabelled form of this high-affinity ligand, [^{3}H]GR 65630 (28), has been used to demonstrate the existence and distribution of 5-HT$_3$ receptors in brain tissue from several species, including man [62].

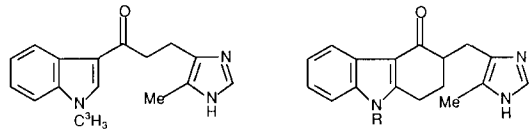
A similar route again employing the protected aldehyde (26) in an aldol condensation afforded the C-linked tetrahydrocarbazolone (29) [63]. This, too, was a highly potent 5-HT$_3$ antagonist (RVN pA_2 10.2) and has also been employed as the radioligand [^{3}H]GR 67330 (30) in binding experiments [64]. However, the 2-imidazolylmethyl tetrahydrocarbazolone (31), prepared by the aldol route from trityl protected imidazole-2-carboxaldehyde, was less active than ondansetron, though nonetheless a potent antagonist (RVN pA_2 7.7).



(26)

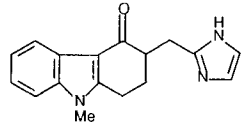
(27)

Scheme 7.4.



(28)

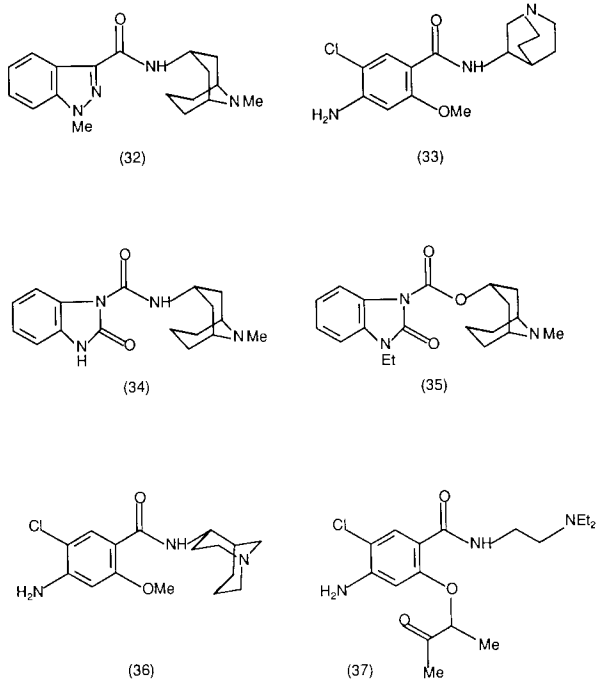
(29) R = Me
(30) R = C^3H_3



(31)

OTHER 5-HT_3 RECEPTOR ANTAGONISTS

Several other highly potent 5-HT_3 receptor antagonists have been described and representative examples of the main structural types (*Scheme 7.5*) include granisetron ((32), BRL 43694) [65], zacopride (33) [66], DAU 6287 (34), DAU 6236 (35) [67, 68], renzapride ((36), BRL 24924) [69] and BMY 25801 (37) [70]. Few of them have been studied extensively in the clinic, but



Scheme 7.5.

chemotherapy-induced emesis was controlled in open trials by MDL 72222 [71], BRL 43694 [72–74], ICS 205-930 [75] and BMY 25801 [76].

Recently, attempts have been made to rationalize the activity of several 5-HT$_3$ antagonists of diverse structural type employing computer-modelling techniques. Hibert *et al.* [77] computed low-energy conformations to define a pharmacophore and receptor map that may account for the activity of ondansetron, metoclopramide and some tropane-based esters and amides. The basic pharmacophore consists essentially of a carbonyl group coplanar to an aromatic ring and a basic centre with the relative positions and dimensions illustrated in *Figure 7.5*. The predictive value of this model was demonstrated by the synthesis and observed activity of the ketoindole (38), which was found to have potent 5-HT$_3$ antagonist properties. Interestingly, quipazine (39), a non-selective ligand with high affinity for 5-HT$_3$ receptors, does not have the required carbonyl oxygen defined by the pharmacophore but will fit the model if the lone pair of electrons on the quinoline nitrogen is allowed to substitute for the carbonyl oxygen atom. Hence, the quinoline nitrogen acts as a bioisostere for the carbonyl group. Rizzi *et al.* [78] also identified a similar pharmacophore working with a series of thiazole-con-

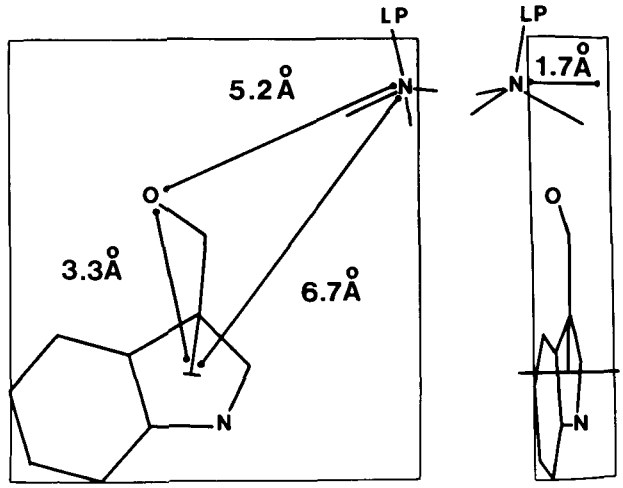
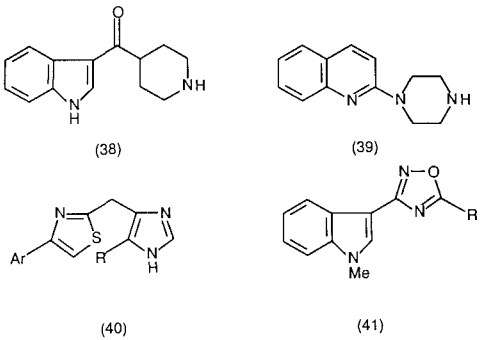


Figure 7.5. Basic pharmacophore for 5-HT$_3$ receptor antagonists. (Reproduced with permission from J. Med. Chem., 33 (1990) 1594; copyright 1990, American Chemical Society.)

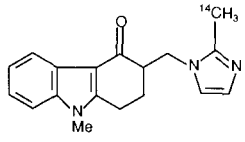
taining ligands (40) [79] and suggested that in the binding process the thiazole nitrogen atom acted as a weak hydrogen bond acceptor analogous to a carbonyl group. A group from MSD [80] introduced the 1,2,4-oxadiazole ring as a bioisostere for the ester function in a series of novel indoles (41) with potent 5-HT$_3$ antagonist activity. The group R was a basic amine, preferably an azabicyclic system. It was shown from electrostatic potential maps that both the oxadiazole nitrogen atoms were capable of accepting a hydrogen bond, but only the interaction at N-2 was essential for high activity.



Scheme 7.6.

THE METABOLISM OF ONDANSETRON

The fate of ondansetron has been investigated in laboratory animals and man during studies undertaken as part of its development [81]. Radiolabelled ondansetron (42) has been used to determine the disposition of total drug-related material and sensitive chromatographic methods [82, 83] have been developed for the specific determination of unchanged ondansetron in biological fluids.



(42)

The pharmacokinetics and metabolism of ondansetron will first be considered in rat and dog, the major species used in safety evaluation of the compound (*Table 7.7*). Comparison will then be made with the available information on the pharmacokinetics of ondansetron in man.

After either oral or intravenous administration of ondansetron to laboratory animals the elimination of the drug is rapid. The short elimination half-lives ($t_{1/2}$) (*Table 7.7*) reflect the high plasma clearance (CL_P) in these species. Renal clearance (CL_R) is below glomerular filtration rate, indicating that the major component of systemic clearance is metabolism. Ondansetron is rapidly absorbed after oral administration, peak concentrations in plasma being achieved within 40 min of dosing. However, the oral bioavailability is low. The similarity between concentrations of total drug-related material in plasma after oral and intravenous doses indicates that the low

Table 7.7. A SUMMARY OF THE PHARMACOKINETIC PARAMETERS OF ONDANSETRON IN ANIMALS

	Rat (1 mg/kg)	*Dog (1 mg/kg)*
C_{max} (p.o., ng/ml)	15	8
$t_{1/2}$(h)	0.2	0.5
T_{max} (p.o., min)	30	40
CL_P (ml/min per kg, i.v.)	117	71
CL_R (ml/min per kg, i.v.)	<6	<4
V_d (area) (l/kg)	2	3.5
Bioavailability (%)	<10	<10

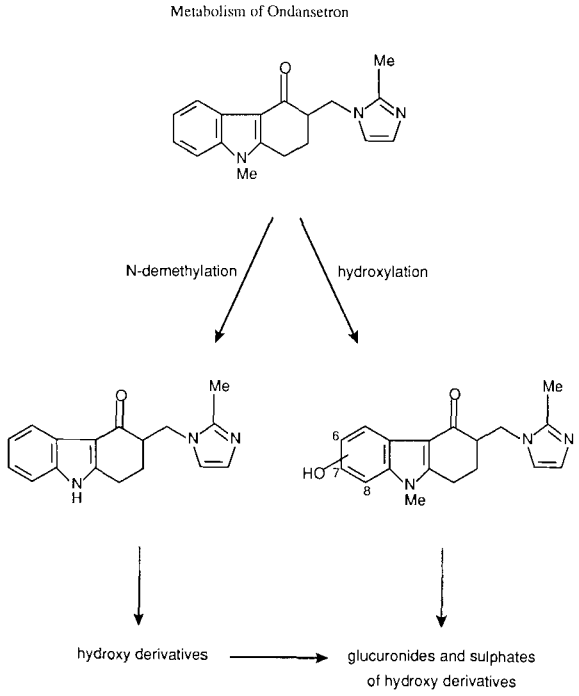
bioavailability is caused by extensive first-pass metabolism rather than ineffective transfer of ondansetron across the gastrointestinal tract.

The apparent volume of distribution (V_d) exceeds total body water, indicating that ondansetron distributes to tissues and is consistent with the physicochemical properties of the compound. When [^{14}C]ondansetron is given to laboratory animals, radioactive drug-related material is rapidly and widely distributed throughout the body. Elimination of drug-related material is also rapid, so that by 6 h after dosing radioactivity is mainly restricted to the kidney and liver, the major organs of excretion. By 24 h, only low concentrations of ondansetron and metabolites remain in the body, although in pigmented animals low quantities persist in the melanin-containing tissues such as skin and uveal tract. This pattern of tissue distribution is independent of the route of administration.

After either oral or intravenous administration of [^{14}C]ondansetron to rats the majority (about 80 %) of the radioactive dose is voided in the faeces, the remainder of the dose being excreted in the urine. In the dog, faecal elimination accounts for about half of the dose and is independent of the route of administration. Evidence from animals with cannulated bile-ducts indicates that the major route of excretion is via the bile. In both species, less than 5 % of the dose is excreted unchanged in urine, suggesting that extensive metabolism of ondansetron occurs.

The metabolites of ondansetron have been examined in urine and bile from rat and dog. The major pathways for metabolism of ondansetron are *N*-demethylation and hydroxylation (*Scheme 7.7*). However, whereas *N*-demethylation predominates in dog, this is only a minor metabolic route in rat. Hydroxylation may occur at the 6, 7 or 8 position in the carbazolone ring. Hydroxy metabolites of ondansetron are excreted predominantly as glucuronide or sulphate conjugates. Studies with immobilised glucuronyltransferase (Heath, S.E., personal communication) have demonstrated that *O*- and *N*-glucuronidation of ondansetron metabolites may occur.

The pharmacokinetics of ondansetron in man have been determined in healthy volunteers after single and repeat doses [84]. The clinical pharmacokinetics (*Table 7.8*) showed many similarities with the kinetics in animals, but also some important differences. Elimination is rapid, but less so than in animals. The volume of distribution is similar in animals and man. As in animals, the clearance of ondansetron in man is predominantly by metabolism. However, metabolic clearance in man is considerably lower than in animals, resulting in a lower first-pass metabolism and a significantly greater oral bioavailability of 60 %. Steady-state concentrations of ondansetron are consistent with the single-dose kinetics of the compound and show no evidence of significant accumulation.



Scheme 7.7.

In elderly volunteers, the pharmacokinetic profile is similar to that in younger subjects. However, clearance is reduced from 541 ml/min to 421 ml/min and the half-life is increased from 3.2 to 5.0 h.

Table 7.8. A SUMMARY OF THE PHARMACOKINETIC PARAMETERS OF ONDANSETRON IN MAN

	Single 8 mg i.v. dose	*8 mg oral dose*	
		single	*steady-state*
C_{max} (ng/ml)	80 ±33	33 ±11	42 ±16
T_{max} (h)	0.12±0.05	1.2±0.4	1.1±0.3
$t_{1/2}$ (h)	2.8 ±0.6	3.2±0.7[a]	3.3±0.8[b]
CL_p (ml/min)	702 ±167		
CL_R (ml/min)	–	19.2±9.9[a]	18.3±10.5[a]
V_d (l)	163 ±25		

[a]$n=14$; [b]$n=9$.

The metabolic fate of ondansetron has been investigated in healthy volunteers given intravenous doses of ^{14}C-labelled drug. About 60 % of the radioactive drug-related material appears in urine [84]. Examination of urine showed that unchanged ondansetron was a minor component. The routes of metabolism in man are qualitatively similar to those seen in animals. As in rat, *N*-demethylation is a minor metabolic route. Hydroxylation, the major metabolic route is regioselective, occurring in the rank order $8 > 7 > 6$ position. The hydroxy metabolites are excreted in urine as glucuronide and sulphate conjugates. Hydroxy metabolites of ondansetron have some $5HT_3$-antagonist activity; however, the systemic levels are significantly lower than those of ondansetron. It is therefore improbable that metabolites of ondansetron contribute significantly to therapeutic effect.

CONCLUSION

A direct comparison between different 5-HT_3 antagonists awaits the results of clinical trials, but there is no doubt that 5-HT_3 antagonists represent a major advance in antiemetic therapy. The rank order of potency of such compounds as antiemetic agents in animal models compares favourably with their 5-HT_3 affinities in a binding assay (*Table 7.9*). Ondansetron and granisetron are the most selective compounds in the group and have no detectable affinity for dopamine receptors. The benzamide derivatives, renzapride and BMY 25801, in contrast to metoclopramide, exhibit little dopamine antagonist activity, but renzapride and zacopride also possess significant agonist activity at the recently characterised 5-HT_4 receptor [92]. ICS 205-930 is a selective 5-HT_3 antagonist, but it too has other actions at high doses, including antiarrhythmic activity and weak antagonist properties against 5-HT_4 receptors [93].

The early suggestion by Fozard [54] that 5-HT_3 receptor antagonists may be useful in the treatment of migraine has yet to be established, although MDL 72222 appeared to be effective in limited clinical trials [94, 95]. There are exciting prospects for other clinical applications of 5-HT_3 antagonists in psychiatric disorders which have been predicted from behavioural studies in rodents and primates, and several clinical trials are underway in psychiatric patients.

Table 7.9. ANTIEMETIC ACTIVITY OF 5-HT$_3$ ANTAGONISTS

Compound	*Species*	*Emetic challenge*	*Antiemetic doses (mg/kg)*	*[^{3}H]ICS 205-930 NIE 115 cells (pK_i)*
Ondansetron	ferret	cisplatin	0.01, 0.1 i.v. [43]	7.87 [91]
		cyclophosphamide	0.1, 0.5 s.c. [43]	
		radiation	0.1, 0.5 s.c. [43]	
		cisplatin	0.01–1 μg into area postrema [48]	
Granisetron	ferret	cisplatin	0.05, 0.5 i.v. [85]	8.85 [91]
		cyclophosphamide plus doxorubicin	2 × 0.5 i.v. [85]	
		radiation	0.5 i.v. or p.o. [85]	
Metoclopramide	ferret	cisplatin	4 i.v. [86]	6.65 [91]
		cyclophosphamide plus doxorubicin	2 × 2.5 i.v. [87]	
		radiation	ID_{50} = 0.6 i.v. [70]	
	dog	cisplatin	ID_{50} = 0.2 s.c. [70]	
BRL 24924	ferret	cisplatin	2 × 0.65, 1.25 i.v. [70]	8.50 [91]
		cyclophosphamide	5 s.c. [88]	
		cyclophosphamide plus doxorubicin	2 × 0.65, 1.25 i.v. [87]	
		radiation	0.25, 1.25 i.v., 1.5 s.c. [87]	
Zacopride	ferret	cisplatin	0.01–0.1 i.v. [89]	9.49 [91]
	cat	cisplatin	0.02 i.c.v. [90]	
	dog	cisplatin	0.001–0.01 i.v. [90]	
		doxorubicin	1 p.o.	
BMY 25801	ferret	cisplatin	ID_{50} = 0.5 i.v. [70]	–
		cyclophosphamide	ID_{50} = 0.4 i.d. [70]	
		radiation	ID_{50} = 0.2 i.v. [70]	
ICS 205-930	ferret	cisplatin	0.01, 0.1 i.v. [89, 86]	9.09 [91]
MDL 72222	ferret	cisplatin	2 × 0.05, 0.5 i.v. [48]	8.2 [91]

REFERENCES

1 Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986) Neuropharmacology 25, 563–576.
2 Gaddum, J.H.R. and Picarelli, Z.P. (1957) Br. J. Pharmacol. Chemother. 12, 323–328.
3 Richardson, B.P., Engel, G., Donatsch, P. and Stadler, P.A. (1985) Nature (London) 316, 126–131.
4 Fastier, F.N. (1962) Pharmacol. Rev. 14, 37–90.
5 Kilpatrick, G.J., Butler, A., Burridge, J. and Oxford, A.W. (1990) Eur. J. Pharmacol. 182, 193–197.
6 Fozard, J.R. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 326, 36–44.
7 Butler, A., Hill, J.M., Ireland, S.J., Jordan, C.C. and Tyers, M.B. (1988) Br. J. Pharmacol. 94, 397–412.
8 Peroutka, S.J. (1990) Pharmacol. Toxicol. 67, 373–383.
9 Derkach, V., Surprenant, A. and North, R.A. (1989) Nature (London) 339, 706–709.
10 Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Nature (London) 330, 746–748.
11 Peters, J.A. and Lambert, J.J. (1989) Trends Pharmacol. Sci. 10, 172–175.
12 Fozard, J.R. and Mwaluko, G.M.P. (1976) Br. J. Pharmacol. 57, 115–125.
13 Buchheit, K.-H., Engel, G., Mutschler, E. and Richardson, B. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 36–41.
14 Blandina, P. Goldfarb, J. and Green, J.P. (1988) Eur. J. Pharmacol. 155, 349–350.
15 Barnes, J.M., Barnes, N.M., Costall, B., Naylor, R.J. and Tyers, M.B. (1989) Nature (London) 338, 762–763.
16 Galzin, A.M., Poncet, V. and Langer, S.Z. (1990) Br. J. Pharmacol. 100, 307P.
17 Raiteri, M. and Paudice, P. (1991) Br. J. Pharmacol. 102, 48P.
18 Fox, A.J. and Morton, I.K.M. (1990) Br. J. Pharmacol. 101, 553–556.
19 Butler, A., Elswood, C.J., Burridge, J., Ireland, S.J, Bunce, K.T., Kilpatrick, G.J. and Tyers, M.B. (1990) Br. J. Pharmacol. 101, 591–598.
20 Barnes, J.M., Barnes, N.M., Costall, B., Ironside, J.W. and Naylor, R.J. (1989) J. Neurochem. 53, 1787–1793.
21 Ireland, S.J. and Tyers, M.B. (1987) Br. J. Pharmacol. 90, 229–238.
22 Arunlakshana, O. and Schild, H.O. (1959) Br. J. Pharmacol. Chemother. 14, 48–58.
23 Kilpatrick, G.J., Bunce, K.T. and Tyers, M.B. (1990) Med. Res. Rev. 10, 441–475.
24 Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–230.
25 Surprenant, A. and Crist, J. (1988) Neuroscience 24, 283–295.
26 Yakel, J.L., Trussell, L.O. and Jackson, M.B. (1988) J. Neurosci. 8, 1273–1285.
27 Fastier, F.N., McDowall, M.A. and Waal, H. (1959) Br. J. Pharmacol. Chemother. 14, 527–535.
28 Collins, D.P. and Fortune, D.H. (1983) Br. J. Pharmacol. 80, 570P.
29 Jones, B.J., Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J., Oakley, N.R. and Tyers, M.B. (1988) Br. J. Pharmacol. 93, 985–993.
30 Tyers, M.B. (1989) in The Behavioural Pharmacology of 5-HT (Bevan, P., Cools, A.R. and Archer, T., eds.), pp. 353–360, Laurence-Erlbaum, New Jersey.
31 Costall, B., Domeney, A.M., Naylor, R.J. and Tyers, M.B. (1987) Br. J. Pharmacol. 92, 881–894.
32 Hagan, R.M., Jones, B.J., Jordan, C.C. and Tyers, M.B. (1990) Br. J. Pharmacol. 99, 227–232.
33 Carboni, E., Acquas, E., Leone, P., Perezzani, L. and Di Chiara, G. (1988) Eur. J. Pharmacol. 151, 159–160.

34 Van der Hoek, G.A., Cooper, S.J., Jones, B.J. and Tyers, M.B. (1989) Proc. Int. Symp. on Serotonin, Florence, p. 55.
35 Oakley, N.R., Jones, B.J., Tyers, M.B., Costall, B. and Domeney, A.M. (1988) Br. J. Pharmacol. 95, 870P.
36 Sellers, E.M., Kaplin, H.L., Lawrin, M.O., Somer, G., Naranio, C.A. and Frecker, R.C. (1988) 18th Annu. Meet. Soc. Neurosci., Toronto, Abstr. No. 21.4.
37 Imperato, A. and Angelucci, L. (1989) Neurosci. Lett. 101, 214–217.
38 Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S. and Tyers, M.B. (1990) Pharmacol. Biochem. Behav. 36, 339–344
39 Oakley, N.R., Jones, B.J. and Tyers, M.B. (1988) Br. J. Pharmacol. 95, 764P.
40 Goudie, A.J. and Leathley, M.J. (1990) Eur. J. Pharmacol. 185, 179–186.
41 Barnes, J.M., Costall, B., Coughlan, J., Domeney, A.M., Gerrard, P.A., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tomkins, D.M. and Tyers, M.B. (1990) Pharmacol. Biochem. Behav. 35, 955–962.
42 Florczyk, A.P., Schurig, J.E. and Bradner, W.T. (1982) Cancer Treat. Rep. 66, 187–189.
42a Dabrowiak, J.C. and Bradner, W.T. (1987) Prog. Med. Chem. 24, 129.
43 Stables, R., Andrews, P.L.R., Bailey, H.E., Costall, B., Gunning, S.J., Hawthorn, J., Naylor, R.J. and Tyers, M.B. (1987) Cancer Treat. Rev. 14, 333–336.
44 Priestman, T.J. (1989) Eur. J. Cancer Clin. Oncol. 25, Suppl. 1, S29–S33.
45 Schmoll, H.-J. (1989) Eur. J. Cancer Clin. Oncol. 25, Suppl. 1, S35–S39.
46 Marty, M. (1989) Eur. J. Cancer Clin. Oncol. 25, Suppl. 1, S41–S45.
47 Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., Leslie, R.A., Barnes, N.M., Naylor, R.J., Jones, B.J., Nelson, D.R., Palacios, J.M., Slater, P., Reynolds, J.M. (1990) Trends Pharmacol. Sci. 11, 135–136.
48 Higgins, G.A., Kilpatrick, G.J., Bunce, K.T., Jones, B.J. and Tyers, M.B. (1989) Br. J. Pharmacol. 97, 247–255.
49 Gunning, S.J., Hagan, R.M. and Tyers, M.B. (1987) Br. J. Pharmacol. 90, 135P.
50 Rocha e Silva, M., Valle, J.R. and Picarelli, Z.P. (1953) Br. J. Pharmacol. 8, 378–388.
51 Fozard, J.R., Mobarok Ali, A.T.M. and Newgrosh, G. (1979) Eur. J. Pharmacol. 59, 195–210.
52 Fozard, J.R. and Mobarok Ali, A.T.M. (1978) Eur. J. Pharmacol. 49, 109–112.
53 Fozard, J.R. and Gittos, M.W. (1983) Br. J. Pharmacol. 80, 511P.
54 Fozard, J.R. (1982) in Headache: Physiopathological and Clinical Concepts. Advances in Neurology, Vol. 33 (Critchley, M., Friedman, A., Gorini, S. and Sicuteri, F., eds.), pp. 295–307, Raven Press, New York.
55 Giger, R.K.A., Donatsch, P., Engel, G., Richardson, B. and Stadler, P.A. (1984) in Proc. VIII Int. Symp. Med. Chem. Symp. pp. 133–135.
56 Feniuk, W., Humphrey, P.P.A. and Watts, A.D. (1981) Br. J. Pharmacol. 73, 191P.
57 Rogerson, T.D., Wilkinson, C.F. and Hetarski, K. (1977) Biochem. Pharmacol. 26, 1039–1042.
58 Bermudez, J., Fake, C.S., Joiner, G.F., Joiner, K.A., King, F.D., Miner, W.D. and Sanger, G.J. (1990) J. Med. Chem. 33, 1924–1929.
59 Bell, J.A., Coates, I.H., Ewan, G.B. and Humber, D.C. (1984) G.B. Patent 2153821A; (1986) Chem. Abstr. 104, 19589.
60 Danishefsky, S., Kitahara, T., McKee, R. and Schuda, P.F (1976) J. Am. Chem. Soc. 98, 6715–6717.
61 Cavalla, D.J. and Oxford, A.W. (1986) European Patent A-242973; (1988) Chem. Abstr. 108, 112457.
62 Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1989) Eur. J. Pharmacol. 159, 157–164.
63 Bell, J.A., Bradshaw, J., Coates, I.H., Ewan, G.B., Humber, D.C. and Mitchell, W. (1986) G.B. Patent 2202530A; (1988) Chem. Abstr. 109, 231018.

64 Kilpatrick, G.J., Butler, A., Hagan, R.M., Jones, B.J. and Tyers, M.B. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 22–30.
65 Sanger, G.J. and Nelson, D.R. (1989) Eur. J. Pharmacol. 159, 113–124.
66 Smith, W.W., Sancilio, L.F., Owera-Atepo, J.B., Naylor, R.J. and Lambert, L. (1988) J. Pharm. Pharmacol. 40, 301–302.
67 Giraldo, E., Monti, L., Turconi, M., Nicola, M., Donetti, A. and Ladinsky, H. (1989) Proc. Int. Symp. on Serotonin, Florence p. 69.
68 Turconi, M., Nicola, M., Quitero, M.G., Maiocchi, L., Micheletti, R., Giraldo, E. and Donetti, A. (1990) J. Med. Chem. 33, 2101–2108.
69 Cooper, S.M., McLelland, C.M., McRitchie, B. and Turner, D.H. (1986) Br. J. Pharmacol. 88, 383P.
70 Gylys, J.A., Wright, R.N., Nicolosi, W.D., Buyniski, J.P. and Crenshaw, R.R. (1988) J. Pharmacol. Exp. Ther. 244, 830–837.
71 Servais, J., Robin, M. and Orwin, J. (1989) Proc. Int. Symp. on Serotonin, Florence p. 161.
72 Carmichael, J., Cantwell, B.M.J., Edwards, C.M., Rapeport. W.G. and Harris, A.L. (1988) Br. Med. J. 297, 110–111.
73 Cassidy, J., Raina, V., Lewis, C., Adams, L., Soukop, M., Rapeport, W.G., Zussman, B.D., Runkin, E.M. and Kaye, S.B. (1988) Br. J. Cancer 58, 651–653.
74 Joss, R.A., Richner, J., Brunner, K.W., Rohrbach, D., Pirovino, M., Terrey, J.P. and Upward, J. (1988) J. Natl. Cancer Inst. 80, 1340–1341.
75 Leibundgut, U. and Lancranjan, I. (1987) Lancet i, 1198.
76 Monkovic, I. (1989) Drugs Future 14, 41–49.
77 Hibert, M.F., Hoffman, R., Miller, R.C. and Carr, A.A. (1990) J. Med. Chem. 33, 1594–1600.
78 Rizzi, J.P., Nagel, A.A., Rosen, T., McLean, S. and Seeger, T. (1990) J. Med. Chem. 33, 2721–2725.
79 Rosen, T., Nagel, A.A., Rizzi, J.P., Ives, J.L., Daffeh, J.B., Ganong, A.H., Guarino, K., Heym, J., McLean, S., Nowakowski, J.T., Schmidt, A.W., Seeger, T.F., Siok, C.J. and Vincent, L.A. (1990) J. Med. Chem. 33, 2715–2720.
80 Swain, C.J., Baker, R., Kneen, C., Moseley, J., Saunders, J., Seward, E.M., Stevenson, G., Beer, M., Stanton, J. and Watling, K. (1991) J. Med. Chem 34, 140–151.
81 Saynor, D.A. and Dixon, C.M. (1989) Eur. J. Cancer Clin. Oncol. 25 (Suppl. 1), S75–S79.
82 Colthup, P.V. and Palmer, J.L. in Ref. 81, S71–S74.
83 Colthup, P.V., Felgate, C.C., Palmer, J.L. and Scully, N.M. J. Pharm. Sci. (in press).
84 Blackwell, C.P. and Harding, S.M. (1989) Eur. J. Cancer Clin. Oncol. 25 (Suppl. 1), S21-S24.
85 Bermudez, J., Boyle, E.A., Miner, W.D. and Sanger, G.J. (1988) Br. J. Cancer 58, 644–650.
86 Costall, B., Domeney, A.M., Naylor, R.J. and Tattersall, F.D. (1986) Neuropharmacology 25, 959–961.
87 Miner, W.D., Sanger, G.J. and Turner, D.H. (1987) Br. J. Cancer 56, 159–162.
88 Hawthorn, J., Ostler, K.J. and Andrews, P.L.R. (1988) Q. J. Exp. Physiol. 73, 7–21.
89 Costall, B., Domeney, A.M., Gunning, S.J., Kelly, M.E., Nohria, V., Owera-Atepo, J.B., Simpson, K.M., Tan, C.C.W. and Tattersall, D. (1987) Neuropharmacology 26, 669–677.
90 Smith, W.L., Callaham, E.M. and Alphin, R.S. (1988) J. Pharm. Pharmacol. 40, 142–143.
91 Hoyer, D. and Neijt, H.C. (1988) Mol. Pharmacol. 33, 303–309.

92 Eglen, R.M., Swank, S.R., Walsh, L.K.M. and Whiting, R.L. (1990) Br. J. Pharmacol. 101, 513–520.
93 Dumuis, A., Sebben, M. and Bockaert, J. (1989) Naunyn-Schmiedeberg's Arch Pharmacol. 340, 403–410.
94 Fozard, J.R., Loisy, C. and Tell, G.P. (1985) in Proceedings 5th Migraine Symposium (Clifford-Rose, ed.), p. 264, Karger, Basel.
95 Loisy, C., Beorchia, S., Centonze, V., Fozard, J.R., Schechter, P. J. and Tell, G.P. (1985) Cephalalgia 5, 79–82.

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8 Synthetic Inhibitors of Bacterial and Mammalian Interstitial Collagenases

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INTRODUCTION

Enzymes have continued to be attractive targets for rational drug design. Nowhere is this more evident than in the design and synthesis of specific, potent interstitial collagenase inhibitors. Because of the involvement of interstitial collagenases in the pathological breakdown of collagenous components of the extracellular matrix, these enzymes and homologous members of the collagenase gene family have become attractive targets for drug development. The goal of this chapter is to review recent progress in the development of both bacterial and mammalian collagenase inhibitors. To facilitate this goal, we provide relevant biochemical background information on these enzymes and their assay methods. In addition, we review briefly the many roles that these collagenases are believed to play in both normal and pathological processes. Last, we summarize systematically the inhibitors that have been developed and conclude by discussing results on the use of a few collagenase inhibitors in physiological assays. The reader is referred to an earlier review [1] for other discussion of this subject.

BACKGROUND

STRUCTURE AND FUNCTION OF COLLAGENS

The extracellular matrix in humans is composed of a variety of macromolecules that serve both structural and organizational roles [2,3]. These include collagens, elastin, proteoglycans, fibronectin, laminin, and other poorly characterized minor substituents. The collagens account for about one-third of human protein and are the primary components of most connective tissues including skin, bone, teeth, tendon, cartilage, basement membrane, blood vessels, cornea and the vitreous humor. They are ubiquitous in higher organisms, in which they occur as aggregates and function as structural proteins. The term collagen actually refers to a class of molecules derived from

one or several multigene families [4,5]. At least eleven different types of collagen (designated type I–XI, or by other symbols) have been described to date, all of which are thought to consist of three polypeptide chains referred to as α chains. The present discussion focuses primarily on the classical interstitial collagen types I, II and III, which consist of homologous α chains with a molecular weight of approximately 95,000, each of which contains an uninterrupted Gly-X-Y repeating sequence throughout 90% or more of its length.

The basic functional collagen monomer, often called tropocollagen, consists of three parallel α chains that associate to form a tightly coiled triple helix [6]. Each of the three chains is coiled into a poly-Pro II left-handed helix and the three chains wrap around one another to form a right-handed superhelix with the Gly residues in each chain occupying the helical core. Thus, from a macroscopic viewpoint, collagen exists as a long, stiff rod with a diameter of 15 Å and a length of 3000 Å. It is this rod-like structure that enables collagens to serve as the structural support network of tissue. On raising the temperature, the triple helix is dissociated into the constituent α chains to form gelatin, which is unordered and can no longer serve as a structural building block. The melting temperature is higher for collagens that contain greater amounts of Pro + Hyp [6]. The various collagen types differ with respect to the identity of the constituent α chains, where each α chain has a different sequence. Type I collagen is a heterotrimer that consists of two identical α1(I) chains and a nonidentical α2(I) chain and is designated $[\alpha 1(I)]_2\alpha 2(I)$. The central 1014 residues of each chain consist of the Gly-X-Y sequence repeated 338 times and form the triple helical core. There are short telopeptides at both the N- and C-terminal ends of the α chains that do not have this repeating sequence and, hence, do not adopt the triple helical conformation. Type II and III collagens are homotrimers composed of three identical chains, designated α1(II) and α1(III), respectively. The complete sequences of the α1(I), α2(I), and α1(III) chains from calf skin, and a partial sequence of the α1(II) chain from bovine cartilage are known [7,8].

In tissue, interstitial collagens exist as fibrils that consist of precisely oriented collagen monomers stacked head-to-tail with a one-quarter stagger between adjacent rows. Crosslinking of collagen chains both within the same molecule and between adjacent molecules occurs to an extent dependent upon the collagen type, tissue type, and age of the organism. This crosslinking is essential for proper structural integrity. The letters β and γ designate covalently crosslinked dimers and trimers of α chains, respectively. Collagen fibrils are more resistant to thermal denaturation than soluble collagens and can retain their structure up to 55°C. The distribution of the interstitial collagens is tissue specific and reflects different tissue function.

Type I collagen is by far the most abundant collagen and comprises a large percentage of skin, bone and tendon. Type III collagen is found together with type I in tissues requiring elasticity such as skin, arteries and uterus. In contrast, type II collagen is found complexed with proteoglycans in tissues that require viscoelastic properties such as cartilage, intervertebral disc and vitreous humor.

This review is restricted to the collagenases that catalyze the degradation of types I, II and III collagens. These collagens were the first to be discovered and are often referred to as the classical fibril-forming interstitial collagens. Hence, the collagenases that act on these have come to be known as the interstitial collagenases. It is appropriate, however, to consider also type IV and V collagens, since they are hydrolyzed by separate collagenases distinct from the interstitial collagenases. Type V collagen is another fibril-forming collagen that has a structure very similar to types I, II and III collagens. It is composed of α1(V), α2(V), and α3(V) chains in the combinations $[\alpha 1(V)]_3$, $[\alpha 1(V)\alpha 2(V)\alpha 3(V)]$, and $[\alpha 1(V)]_2\alpha 2(V)$. Type IV collagen is one of a different group of collagens which consists of α chains with a molecular weight of greater than 95,000, and which have helical domains that are separated by nonhelical regions that do not contain a repeating Gly-X-Y triplet sequence. The chain composition of type IV collagen may also be variable. There is considerable evidence for the heterotrimer $[\alpha 1(IV)]_2\alpha 2(IV)$, but the homotrimers $[\alpha 1(IV)]_3$ and $[\alpha 2(IV)]_3$ may also exist *in vivo* [9]. The type IV collagen molecule is composed of an N-terminal triple-helical region called the 7S domain, a short adjacent non-triple helical region called the NC2 domain, a long helical domain and a C-terminal globular segment called the NC1 domain. The long helical domain, however, contains numerous relatively short loci at which the $(\text{Gly-X-Y})_n$ sequence is interrupted in at least one α chain [9]. Because of its domain structure and flexibility, type IV collagen does not form fibrils *in vivo*. Instead, it forms sheet-like networks and is found almost exclusively in basement membranes. The N- and C-terminal regions of type IV collagen appear to function as crosslinking sites during assembly of these sheets, with the intervening sections of each molecule acting as flexible spacers.

BACTERIAL AND MAMMALIAN COLLAGENASES

The interstitial collagens are highly resistant to proteolytic attack under physiological conditions due to their tightly coiled triple-helical structure. Indeed, the normal and pathological breakdown of these collagens is catalyzed by a highly specific class of enzymes called *interstitial collagenases*. By definition, interstitial collagenases are enzymes that catalyze the hydrolytic

cleavage of the undenatured, triple-helical portions of types I, II or III collagens at physiological pH and temperature. This definition excludes telopeptidases that are capable of hydrolyzing the non-triple helical telopeptides under physiological conditions, and also enzymes (for example, certain cathepsins) that can degrade the triple helical regions of collagen at acid pH. It also excludes a variety of proteinases that can hydrolyze type III collagen in solution, but not type III fibrils [10], since interstitial collagens are only found in fibrillar form *in vivo*. However, the definition is broad in that it permits inclusion of enzymes that make either few or multiple scissions, thus allowing two categories of collagenases to be distinguished. The first are collagenases produced by bacteria and other micro-organisms that attack collagens at multiple sites along the triple helix, while the second are mammalian or tissue collagenases that make a single scission in these collagens (*Figure 8.1*). The important properties of these two categories of collagenases are considered briefly below.

Bacterial collagenases

A number of bacteria have been shown to produce true collagenases [11] that most likely serve either a nutritional role or are used as a means of invasion of the host. In fact, the first collagenases (EC 3.4.24.3) to receive any detailed study were those produced by the pathogenic anaerobe *Clostridium histolyticum* that is partially responsible for gas gangrene. *Achromobacter iophagus* collagenase (AIC) has also been studied in reasonable detail [12,13]. Bacterial collagenases are characterized by their ability to make multiple scissions within the triple helical region of collagens and to attack almost all collagen types. The scissions in collagens are made on the N-terminal

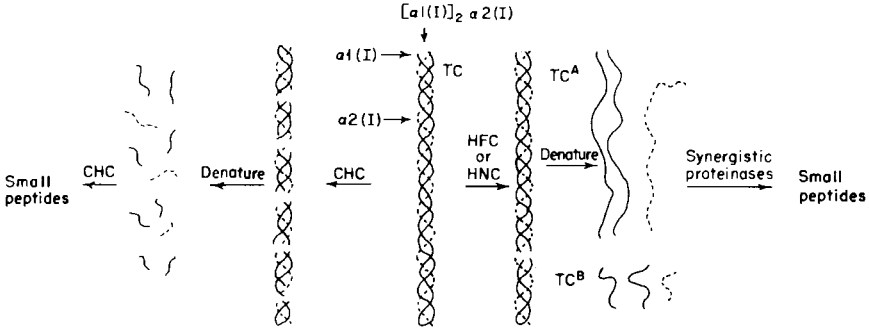


Figure 8.1. Schematic showing the hydrolysis of the type I tropocollagen (TC) heterotrimer [α1(I)]2α2(I), by tissue (right) and bacterial (left) collagenases.

side of Gly residues. Thus, in the repeating Gly-X-Y sequence, these collagenases hydrolyze Y-Gly bonds, where Y may be a variety of different residues (see below). For simplicity, discussion is limited to the *Clostridium histolyticum* collagenases (CHC). The CHC are known to ultimately degrade native collagens to a mixture of small, dialyzable peptides [11]. Because of their effectiveness in dissolving connective tissue, a crude preparation of the CHC is widely used as a reagent in cell dissociation experiments, and is one of the largest selling research enzymes.

Seven collagenases, denoted by the letters α, β, γ, δ, ε, ζ and η, have been purified to homogeneity from the culture filtrate of *Clostridium histolyticum* [14,15]. Some of their properties [14–16] including recently determined k_{cat}/K_M values for type I collagen (Mallya, S.K., Mookhtiar, K.A. and Van Wart, H.E., unpublished data) are listed in *Table 8.1.* All of these CHC are single polypeptide chains with molecular weights that range from 68,000 to 130,000. Their isoelectric points are very similar and lie between 5.35 and 6.20. The metal content of these enzymes has been measured by atomic absorption spectroscopy and all contain approximately one atom of Zn(II) per protein chain and variable amounts (between 1.9 and 6.8 atoms per chain) of Ca(II). Both types of metal ion are essential for activity where Zn(II) is at the active site and performs a catalytic role and Ca(II) serves to stabilize the tertiary structure of the protein chain. No carbohydrate has been detected in any of the CHC. These enzymes can be divided into two classes (designated class I and II) on the basis of the sequence homologies in their polypeptide chains, as revealed from a comparison of their tryptic digests

Table 8.1. PROPERTIES OF *CLOSTRIDIUM HISTOLYTICUM* COLLAGENASES

Collagenase assays were carried out in 50 mM Tricine, 0.2 M NaCl, 10 mM $CaCl_2$ (pH 7.5) at 30°C using rat type I collagen as substrate.

Enzyme	*Molecular weight*	*pI*	*Collagenase activity* ($k_{cat}/K_M \times 10^{-6}$, $M^{-1}\,min^{-1}$)	*Metal content (mol/mol protein)*	
				Zn(II)	*Ca(II)*
α	68,000	5.85, 5.90	690	0.80	1.9
β	115,000	5.55, 5.60, 5.75	530	1.03	6.8
γ	79,000	6.10, 6.20	290	0.85	2.4
η	130,000	–	430	–	–
δ	100,000	5.80	320	1.10	2.6
ε	110,000	5.90, 5.95	290	0.91	3.5
ζ	125,000	5.35	200	0.88	5.1

[17]. This division into classes is also supported by a comparison of their specificities toward peptide substrates [15,18,19], their interaction with substrate-analogue inhibitors [20], their response to active site metal substitutions [21,22], and their mode of attack of triple helical collagens [23] (French, M.F., Bhown, A. and Van Wart, H.E., unpublished data).

The specificity of the CHC toward synthetic peptide substrates containing a variety of specific amino-acid residues in subsites P_3 through P'_3 (nomenclature of Schechter and Berger [24]) has been examined in some detail [15,18,19]. Because of the relevance of these studies to the rational design of inhibitors, these results will be summarized briefly. A variety of furanacryloyl-blocked tetrapeptides, or unblocked penta- and hexapeptides, are good substrates for the CHC. Both classes rapidly hydrolyze peptides with many residue types in subsite P'_3, but with a much greater variation for the class I enzymes. Subsite P'_3 corresponds to the Y position of the repeating Gly-X-Y sequence of collagen. Interestingly, peptides with P'_3 residues such as Hyp, Ala and Arg, which occur most frequently in the Y-position in collagen, are hydrolyzed rapidly. The class I CHC prefer Hyp in subsite P'_3. In subsite P'_2, the class I CHC prefer Pro. Substitution by Ala lowers the rate over 20-fold and substitution by other residues decreases the rate to barely detectable levels. The class II enzymes also prefer Pro in this subsite, but substitution by Ala only lowers the rate by approximately 3-fold. Substitution by other amino acids results in lower, but still appreciable, rates. The requirement for Gly in subsite P'_1 is strict, since even substitution by Ala lowers the rates to below detectable levels for all of the CHC.

Both classes of CHC exhibit a marked preference in subsite P_1 for large hydrophobic residues that are not branched at the β-carbon, such as Phe, Tyr or Leu. Smaller residues such as Ser and Ala are also hydrolyzed, but at a lower rate, while Glu is very unfavourable in this subsite. Interestingly, the class I enzymes are better able to accommodate Hyp and Pro in this subsite than the class II enzymes. In subsite P_2, both classes of CHC prefer large hydrophobic groups (for example, furanacryloyl, cinnamoyl). With respect to amino acids, the class II CHC prefer Pro, while the class I enzymes are less restrictive and can accommodate residues as large as Leu. Peptides with Glu in subsite P_2 are poor substrates for both classes of CHC, even though Glu occurs frequently in this position, which corresponds to the X-position, in collagen. Both classes of enzymes prefer Gly in subsite P_3, although Ala can substitute better in this subsite than in subsite P'_1. The complementary specificities of the two classes of CHC undoubtedly account for the extensive digestion of collagens by mixtures of these enzymes and may be the underlying basis for their synergistic action on collagens [25]. The initial proteolytic events in the attack of the CHC on triple helical types I, II and III

collagens have been elucidated and are very similar for the enzymes within each class, but different for the two classes (23; French, M.F., Bhown, A. and Van Wart, H.E., unpublished data). The class I enzymes first hydrolyze loci near the ends of the triple helical domains of these collagen molecules, while the class II enzymes make their initial cleavages in the interior. The basis for the hyper-reactivity of these sites is not yet understood. Subsequent to these initial cleavages, the triple helical fragments thermally denature and are degraded further by the CHC, which are also good gelatinases (*Figure 8.1.*). The kinetic parameters for the hydrolysis of types I, II and III collagens have been measured (Mallya, S.K., Mookhtiar, K.A. and Van Wart, H.E., unpublished data) and are similar in magnitude to those for the tissue collagenases [26]. Unlike human collagenases, however, the CHC exhibit relatively little preference for one of these three collagen types over the others.

Mammalian collagenases and the matrix metalloproteinases

A body of evidence accumulated over the past two decades suggests that the metabolic degradation of the major extracellular matrix macromolecules in vertebrates and mammals is catalyzed by a family of unique and homologous metalloproteinases that are produced by resident and inflammatory cells [3]. These enzymes have come to be known as the matrix metalloproteinases (MMP). The MMP are zinc proteinases that are synthesized as zymogens and activated extracellularly by mechanisms that are poorly understood. They also share the property that they are inhibited by tissue inhibitors of metalloproteinase (TIMP-1 or TIMP-2), ubiquitous natural inhibitors that form 1:1 inactive complexes with the MMP. In humans, the MMP family currently consists of two distinct interstitial collagenases (EC 3.4.24.7) (fibroblast-type collagenase, HFC [27], and neutrophil-type collagenase, HNC [26,28-30]), a 72 kDa gelatinase isolated from fibroblasts [31], a 92 kDa gelatinase found in human neutrophils and also produced by transformed fibroblasts [32], fibroblasts stromelysin-1 [33–35], tumour stromelysin-2 [36] and -3 [37], and putative metalloproteinase-1 [36]. Members of this family that have been sequenced from other mammalian species include rat [38], pig [39], and rabbit [40] interstitial collagenases, rabbit stromelysin [41] and the rat analogues of the stromelysins referred to as transin [42] and transin-2 [43]. The MMP have also been designated by a numerical code, where HFC is designated MMP-1 and HNC is MMP-8 [44,45].

Collectively, the MMP are capable of degrading all of the major protein constituents of the extracellular matrix. The interstitial collagenases that are the focus of this review dissolve collagen fibrils by making a single scission across all three α chains at a specific, sensitive locus of exposed type I, II

and III tropocollagen (TC) monomers approximately 3/4 from the NH_2-terminus to produce the so called TC^A and TC^B fragments. This unique mode of attack was first elucidated by Gross and co-workers with tadpole collagenase [46,47]. These fragments retain their triple helical structure and are resistant to further degradation at 25°C. However, at the physiological temperature of 37°C, they denature and become susceptible to nonspecific proteolysis by synergistic proteinases. This suggests that the physiological committed step in collagen degradation is the initial cleavage of these collagens by interstitial collagenases (*Figure 8.1*). HFC and HNC are the only known human enzymes capable of catabolizing the interstitital collagens at an appreciable rate and the evidence for their *in vivo* role in the degradation of the extracellular matrix is the strongest. HFC also degrade types VII [48] and X [49–51] collagens, but at different loci.

The biologic function of the other MMP is less clearly understood. However, it is clear that the gelatinases are capable of cleaving a number of proteins, including the native form of types IV and V collagens [31,52–54], neither of which is hydrolyzed by the interstitial collagenases. For this reason, they have been referred to as type IV collagenases. Several matrix macromolecules are substrates for the stromelysins, including proteoglycan core protein, laminin, types IV and V collagens, and fibronectin [33,44,55,56]. In the remainder of this review, the only MMP that will be considered further are the interstitial collagenases HFC and HNC or their analogues from other species, and the term collagenase is used interchangeably with interstitial collagenase.

The early studies on collagenases were characterized by reports attributing a wide variety of molecular weights, isoelectric points, etc., to collagenases isolated from different tissues and cell types [57,58]. However, it now appears that the collagenases produced by synovial fibroblasts [59], macrophages [60], keratinocytes [61], transformed osteoblasts [62], and probably most other mammalian cell types are very similar to the well-characterized HFC produced by skin fibroblasts [27]. In contrast, HNC is a homologous, but distinct collagenase [26,28] that has only been found so far in neutrophils. Some properties of these two enzymes are summarized in *Table 8.2* [26–30,34,48–51,60,63–83].

Both HFC and HNC are endopeptidases that contain one Zn(II) atom located at the active site (Springman, E., Birkedal-Hansen, H. and Van Wart, H.E., unpublished observations) and require Ca(II) for stability [84]. The protein chains of both are very similar in their sequence and construction. The HFC and HNC preproenzymes consist of 468 and 467 amino acids, of which the first 18 and 20, respectively, represent the signal peptides [27,29,30]. Thus, the zymogens pro-HFC and pro-HNC consist of 450 and

Table 8.2. PROPERTIES OF HUMAN INTERSTITIAL COLLAGENASES

Collagenase assays were carried out in 50 mM Tricine, 0.2 M NaCl, 10 mM $CaCl_2$ (pH 7.5) containing 0.05% Brij-35 and 50 μM $ZnSO_4$ at 30°C on *p*-chloromercuribenzoate-activated HNC and trypsin-activated HFC using rat type I collagen as substrate. α-Ms denote α-macroglobulins; α_1-PI, α_1-proteinase inhibitor.

Enzyme	*Zymogen molecular weight*	*Collagenase activity* ($k_{cat}/K_M \times 10^{-6}$, M^{-1} min^{-1})	*Protein substrates*	*Human cells producing enzyme*	*Reference to sequence*
Human fibroblast Collagenase (HFC)	57,000 (glycosylated) 52,000	0.32	Type I, II, III, VII and X collagens; gelatins; α-Ms; casein	fibroblasts keratinocytes macrophages U937 cells endothelial cells	[27,34]
Human neutrophil Collagenase (HNC)	75,000 (glycosylated) 52,000	12	Type I, II and III collagens, gelatins; α_1-PI	neutrophils	[29,30]

447 residues, respectively, and both have a molecular mass of approximately 52 kDa. The two collagenases exhibit 58% homology and, although they are single polypeptide chains, both can be viewed as consisting of three domains. Both collagenases have a C-terminal domain that bears a weak sequence homology with hemopexin, a haem-binding serum protein [85,86]. Both HFC [87] and HNC [29] undergo an autolytic cleavage at a site very close to the boundary between these two domains that is referred to as the autolytic degradation site. This event, which causes these enzymes to lose their ability to hydrolyze collagens, is a major source of instability and has greatly hampered their study. The N-terminal portion of these two collagenases is called the propeptide domain. After activation by organomercurials, pro-HFC [88] and pro-HNC [26] undergo an autolytic cleavage that releases this domain, lowering the molecular mass to approximately 43 kDa. This cleavage occurs at a site referred to as the autolytic activation site because this event accompanies activation. However, the appearance of activity always precedes this autolytic step and chain cleavage is not necessary for activity. The remaining central portion of these collagenases is called the catalytic domain, which is an active proteinase that contains the Zn(II)-binding site. Thus, pro-HFC and pro-HNC share a three-domain structure, if possible. There are a number of key differences between HFC and HNC. With regard to carbohydrate processing, some of the HFC molecules contain complex N-linked carbohydrate due to glycosylation at one of two possible Asn residues [89]. This raises the apparent molecular mass as determined by SDS-PAGE by 5 kDa, but does not alter the functional properties of the enzyme in any way that is currently appreciated. In contrast, HNC *only* occurs as a glycoprotein and is thought to be glycosylated at three Asn residues to add a total of approximately 17 kDa to the apparent molecular mass [26]. Another difference between these two collagenases is that HFC is produced by cells by *de novo* synthesis in response to specific stimuli. It is not stored intracellularly after synthesis and is immediately secreted as a zymogen. In contrast, HNC is synthesized by neutrophils only during their maturation in bone marrow and is subsequently stored intracellularly in secondary granules. No resynthesis of HNC has been observed to take place in mature neutrophils. HNC is released by neutrophils on various stimuli and can be activated oxidatively by HOCl produced by myeloperoxidase from Cl^- and H_2O_2 [90].

HFC and HNC also differ with respect to their peptide and collagen specificities. Both HFC and HNC cleave all three α chains of types I, II and III collagens following the Gly residue of the partial sequences Gly-[Ile or Leu]-[Ala or Leu] in these collagens [91,92] at a single site located approximately three-fourths from the NH_2-terminus. This single scission of these

collagen chains is remarkably specific, since similar sequences occurring at other sites in these collagens are not hydrolyzed. The basis for the hyperreactivity of the interstitial collagen cleavage sites toward tissue collagenases is not yet understood [93]. HFC and HNC exhibit markedly different specificities toward soluble types I, II and III collagens. HNC preferentially hydrolyzes type I [26,74,75], while HFC prefers type III collagen [26,66].

The peptide specificities of HFC and HNC have been systematically investigated by measuring the rate of hydrolysis of 60 synthetic oligopeptides [94]. The amino acids in subsites P_4 through P'_4 all influence the hydrolysis rates for both collagenases and the effects of many substitutions at these sites are distinctive for the two enzymes. This provides a rational basis for the design of selective inhibitors of these two collagenases (see below). For peptides with unblocked N- and C-termini, occupancy of subsites P_3 through P'_3 is necessary for rapid hydrolysis. Compared to the α1(I) cleavage sequence, none of the substitutions investigated at subsites P_3, P_2 and P'_4 produces markedly improved substrates. In contrast, many substitutions at subsites P_1, P'_1 and P'_2 improve specificity. In subsite P_1, the overall preference for HFC is Ala > Pro > Met > His > Tyr > Gly > Phe > Gln > Glu > Leu > Val > Arg, while for HNC the order is Glu = Ala > Pro > Tyr > Phe > Gln > Met > Gly > Leu > His > Arg > Val. Thus, a wide variety of substitutions are allowable at this position, with Ala the most favourable and Arg and Val the most unfavourable. It is interesting to note that Glu is favoured by HNC, but not HFC.

In subsite P'_1, the residue preference of HFC is Leu > Ile > Met > Tyr > Gln > Val > Ser > Trp, Pro, Glu, Phe, Nph, Arg, while for HNC the order is Tyr > Leu > Ile = Met > Trp = Phe > Val = Gln > Pro, Glu, Ser, Nph, Arg. Thus, most substitutions at subsite P'_1 are detrimental relative to Ile. Interestingly, HNC generally tolerates aromatic amino acids better in this subsite than HFC. For subsite P'_2, substitution of Ala by Phe, Trp, Leu, or Arg increases the rates of hydrolysis markedly for both enzymes. The substitution by Trp is most dramatic and causes increases in rate of 7.3- and 8.3-fold for HFC and HNC, respectively. This clearly demonstrates the importance of the interaction of the substrate side-chain in this subsite with these collagenases. Substitution by Glu lowers the rates for both enzymes, while Hyp abolishes measurable hydrolysis.

An aspect of these collagenases that bears directly on inhibition studies is that of their activation mechanism(s). *In vitro* studies have shown that pro-HFC and pro-HNC, as well as the other pro-MMP, can be activated by a variety of seemingly disparate means, including treatment with proteinases, heavy metals, oxidants, disulphide compounds, alkylating agents, and conformational perturbants. The molecular basis for the latency of these zy-

mogens and for their activation has been traced to the disposition of a single Cys residue in their propeptide domains. In the pro-MMP, the sulphydryl group of the side chain of this Cys residue is believed to be complexed to the active site Zn(II) [95,96]. This complex blocks the active site and renders the zymogens inactive. All modes of activation of these zymogens are believed to involve the dissociation of this Cys residue from the active site Zn(II) and its replacement by water, with the concomitant exposure of the active site. This is thought to be the primary event that precedes the well-known autolytic release of the propeptide domain that is observed following the appearance of collagenase activity. The dissociation of this Cys residue from the Zn(II) in the zymogen switches the role of the Zn(II) from a non-catalytic one to a catalytic one and switches the catalytic activity on. Accordingly, we have termed this the 'cysteine switch' mechanism of activation. This mechanism is unique in enzymology and offers the opportunity for multiple modes of physiological activation of these important enzymes. At this time, the physiological routes of activation of pro-HFC and pro-HNC have not yet been fully delineated, although an oxidative pathway of pro-HNC activations seems to have been convincingly established [90].

One important consequence of the existence of multiple modes of activation of pro-HFC and pro-HNC for inhibition measurements is that there is not a single active form of these enzymes and it is unclear which active form best represents the physiological one that should be used for inhibition studies. There is the possibility of considerable diversity in the properties of the activated enzymes. First, each activation method can produce a full-size active species or a lower molecular weight active species due to loss of the propeptide. Second, since each activator (proteinase, oxidant, etc.) produces a chemically distinct (that is, the cysteine-switch residue is modified differently) full size species, each may have different kinetic properties. A well-known example of the profound effect of slight differences in activation on the functional properties of collagenases is the superactivation of pro-HFC by stromelysin [97–99]. In this case, the superactivation is thought to be due to hydrolysis of the propeptide by stromelysin at the Gln-Phe bond that immediately precedes the Phe-Leu bond that is autolytically cleaved subsequent to organomercurial activation [99]. This slight difference in activation leads to 10-fold higher activity. Such diversity as the result of different activation routes may mean that different values of K_I will be obtained for collagenases activated by different procedures and this must be considered in comparing data from different studies.

COLLAGENASE ASSAYS

In order to assess the potency of any putative collagenase inhibitor, one

must obviously have reliable assay methods. Such methods can be divided into two types – those based on the hydrolysis of natural protein substrates (that is, intact, triple helical collagens) vs. those that utilize peptide substrates. There are advantages and disadvantages to both types of assay. Since peptide-based assays cannot be considered specific for collagenases, only collagen-based assays can be used to follow the enzyme through purification and isolation. However, peptide-based assays are preferred in experiments with purified collagenases. We have devoted a substantial effort to developing assays of both types, which are described below.

Collagen-based assays

Numerous means have been developed to measure the hydrolysis of collagens, as evidenced by the wide variety of assays that have been reported. Of central importance are the choice of the state of the collagen used as the substrate and the method used to detect the hydrolysis products. Native, triple helical collagens can exist in many forms, including insoluble irreversibly crosslinked fibrils, fibrils or gels reconstituted from acid- or salt-soluble collagens, or soluble collagens. Assays have been developed that use all three of these forms as the substrate. For sensitive collagenase assays that obey the accepted principles of enzyme kinetics, soluble radiolabelled collagens are the substrates of choice.

The use of radiolabelled collagens in such assays is not straightforward and is complicated by numerous subtleties. The state that a collagen will adopt is markedly dependent on the environmental conditions (temperature, pH, ionic strength) as well as on the collagen concentration, the collagen source and type, and whether the collagen has intact telopeptides or has been chemically modified. All of these parameters influence whether the collagen will exist as soluble collagen, as fibrils or gels, or as gelatin. To aid in the development of sensitive assays, the preparation and properties of radiolabelled type I, II, and III collagens have been systematically investigated [100]. The influence of multiple parameters on the state of these collagens has also been determined to provide an information base with which to choose conditions for collagenase assays that utilize collagens in different states as the substrate. Based on these data, we have developed rapid, sensitive, and convenient assays for the hydrolysis of soluble [^{3}H]acetylated type I, II, and III collagens by both bacterial and tissue collagenases [101].

The assays are carried out at any temperature in the 1–30°C range on pre-activated enzymes in a single reaction tube and the progress of the reaction is monitored by withdrawing aliquots as a function of time, quenching with 1,10-phenanthroline, and quantitating the concentration of hydrolysis

fragments by scintillation counting. The latter is achieved by selective thermal denaturation of these fragments by incubation under conditions that are slightly different for each collagen [101]. The assays give percentages of hydrolysis for all three collagen types by tissue collagenases that agree well with the results of gel electrophoresis experiments. The initial rates of hydrolysis of all three collagens determined in this way are proportional to the concentration of both tissue or Clostridial collagenases over a wide range of enzyme concentrations. All three assays can be carried out at collagen concentrations that range from 0.06 to 2 mg/ml and give linear double-reciprocal plots for both tissue and bacterial collagenases that can be used to evaluate the kinetic parameters K_M, and k_{cat} or V_{max}. These assays can also be used to quantitate K_I values of inhibitors and to assess their mode of inhibition. The assay developed for the hydrolysis of rat type I collagen is more sensitive by at least one order of magnitude than comparable assays that use rat type I collagen fibrils or gels as substrate.

Peptide-based assays

We routinely employ two types of chromogenic peptide-based collagenase assay, each of which has its own unique properties. The first assay is that developed by Weingarten and associates and is based on the hydrolysis of the thio peptolide Ac-Pro-Leu-Gly-(S-Leu)-Leu-Gly-OEt [102,103]. The assay was originally developed for HFC, but we have shown that it works for HNC and the other MMP as well. Routine assays are carried out at a substrate concentration of 20 μM in the presence of a 10-fold excess of dithionitrobenzoate (DTNB). Upon hydrolysis of the scissile Gly-(S-Leu) bond, the free thiol HS-Leu is liberated and reacts with DTNB to produce a continuous increase in absorbance at 412 nm that is used to obtain an initial rate. This is the fastest, most sensitive assay available and is the method of choice. However, the substrate itself is rather unstable and is spontaneously hydrolyzed at a slow, but non-negligible rate. This rate is higher in the presence of nucleophilic buffers and at high pH, and this spontaneous rate must be subtracted from the assay rates. This assay cannot be used with mercaptan inhibitors or substances that absorb appreciably near 400 nm. The sensitivity of this assay could be improved by optimizing the sequence of the peptolide to match the specificities of HFC and HNC discussed in an earlier section. Since the K_M values for the hydrolysis of this substrate by both HFC and HNC greatly exceed its solubility, true K_I values cannot be obtained with this assay and we use it for measuring IC_{50} values only.

Stack and Gray have described a convenient, continuously recording, fluorescent assay for rabbit collagenase and gelatinase based on the hydroly-

sis of DNP-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2 [104]. The key features of this peptide are the presence of the fluorescent Trp residue in subsite P_2' and the 2,4-dinitrophenol (DNP) quenching group at the N-terminus. Hydrolysis of the Gly-Leu bond relieves the quenching of the Trp fluorescence in the intact substrate, and the resulting fluorescence increase serves as the basis for the assay. Based on the results of our substrate specificity studies, we have prepared four similar fluorogenic heptapeptide substrates with sequences that are optimized for five MMP [105]. DNP-Pro-Leu-Ala-Leu-Trp-Ala-Arg has been prepared as a substrate for HFC and DNP-Pro-Leu-Ala-Tyr-Trp-Ala-Arg for HNC. An initial rate measurement with submicromolar enzyme concentrations can be obtained in less than 10 min for both reactions. The kinetic parameters for the hydrolysis of these peptides by HFC and HNC have been determined from double-reciprocal plots. These plots are linear, indicating that there are no kinetic anomalies and that Michaelis-Menten kinetics are obeyed. The maximum substrate solubility exceeds K_M for all reactions, insuring accurate determination of kinetic parameters. The values of these parameters for the hydrolysis of DNP-Pro-Leu-Ala-Leu-Trp-Ala-Arg by HFC are $k_{cat} = 4400\ h^{-1}$ and $K_M = 130\ \mu M$, and of DNP-Pro-Leu-Ala-Tyr-Trp-Ala-Arg by HNC are $k_{cat} = 11{,}000\ h^{-1}$ and $K_M = 7.7\ \mu M$. These assays allow the accurate determination of K_I values for inhibitors, as well as for the elucidation of the mode (for example, competitive, mixed, etc.) of inhibition. However, these assays are not compatible with inhibitors that absorb near 280 nm or that fluoresce near 330 nm.

INVOLVEMENT OF COLLAGENASES IN HEALTH AND DISEASE

In this section, we review some of the physiological processes in which collagenases are believed to participate. We must emphasize from the outset that conclusions pertaining to the involvement of individual enzymes in the physiological degradation of collagens and other components of the extracellular matrix are largely circumstantial and are based on the observations that the enzyme can catabolize a specific macromolecule *in vitro*, coupled with the belief that the enzyme in question is present in an active form at the tissue site where such destruction takes place *in vivo*. Thus, the specific involvement of a collagenase in any physiological process is difficult to confirm. As noted earlier, however, the involvement of interstitial collagenases in the metabolic turnover of interstitial collagens is on stronger footing than analogous roles postulated for the other MMP due to the unique resistance of these collagens to all other known human proteinases. In the final analysis,

the confirmation of any putative physiological role for a collagenase can only be achieved when specific potent inhibitors are developed and are shown in physiological assays to selectively block the process in question. The field is just reaching this point and already some evidence of this type has been forthcoming (see last section).

NORMAL PROCESSES

While collagenases have attracted a great deal of attention because of their involvement in pathological processes, it must be understood that they are also believed to be intimately involved in many essential normal functions. In general, collagenases are believed to function during any process that involves the turnover or destruction of interstitial collagens. The first tissue collagenase to be identified was from the primary culture of tail tissue from tadpoles undergoing metamorphosis [46]. The action of this collagenase is presumably required for the tissue resorption that occurs as the tail disappears and legs form. This same type of requirement for collagenases is found in a number of related circumstances in mammals. This includes growth and development [57], wound healing [106,107], resorption of the uterus after parturition [108,109], blood vessel angiogenesis and neovascularization [110–112], thinning of the follicle wall during ovulation [113] and tooth eruption [114]. It is not yet known whether highly mobile normal cells such as macrophages or neutrophils use a collagenase to facilitate their migration through connective tissue.

DISEASE PROCESSES

There are a variety of pathological states in which the action of collagenases is strongly suspected. Some of these disease states are considered briefly below. Since current knowledge concerning the cellular source of the collagenases in these states is often conflicting, a discussion of this subject has been purposely avoided. Instead, possible sources are simply mentioned.

All forms of arthritis are characterized by the erosion of the articular cartilage of affected joints. Since cartilage consists primarily of proteoglycans and type II collagen, proteinases capable of attacking both macromolecules have been implicated in the progression of these diseases [115–118]. Thus, attention has focused on stromelysins and interstitial collagenases, respectively. The loss of proteoglycan generally precedes the loss of collagen, but collagen catabolism is necessary for the disease to progress. In osteoarthritis, there is little or no inflammatory component to the disease and the destructive proteinases are believed to arise from resident cells of the cartilage

which are capable of secreting collagenase and stromelysin, such as chondrocytes. In rheumatoid arthritis, there is a pronounced inflammatory response affecting the synovial tissue. This is associated with the infusion of large numbers of macrophages and neutrophils, making it impossible to pinpoint definitively the sources of degradative proteinases. Thus, the involvement of both the fibroblast-type and neutrophil-type collagenases in the destruction of joint collagen must be considered.

Periodontal disease is the principal cause of tooth loss over the age of 35. It is also an inflammatory disease that is triggered by bacteria that inhabit the gingival cavity. Periodontitis is characterized by the progressive loss of the attachment apparatus of the teeth. Since the major protein component of gingival tissue and bone is type I collagen, collagenases are believed to participate in the progression of this disease [119,120]. Possible sources of the collagenases include resident gingival fibroblasts, inflammatory cells including neutrophils and macrophages, and the oral bacteria themselves. Such bacteria have been shown convincingly, in at least one case, to produce a true collagenase [121].

A number of bone resorption diseases including Paget's disease, osteoporosis, hyperparathyroidism, and cholesteatoma are believed to involve the action of collagenases [122–127]. The resorption of bone involves the simultaneous removal of both the mineral and collagenous matrix. The solubilization of the mineral components is believed to be accomplished by a local acidification process, while the degradation of the type I collagen is accomplished with the aid of collagenases together with lysosomal cysteine proteinases. Osteoblasts are known to secrete collagenase on stimulation by parathyroid hormone [128,129] and may be the source of the collagenase.

A wide variety of other pathological processes may also involve collagenases. The metastasis of tumour cells is a process that is inhibited by the connective tissue barriers of the host. The association of both interstitial collagenases and proteinases capable of degrading the type IV collagen found in basement membranes has been well documented and it is certainly reasonable that such enzymes could facilitate metastasis [130–134]. Corneal ulceration can be brought about by chemical or thermal burns, infections (for example, *Pseudomonas*, *Staphylococcus*, herpes or fungal), Stevens-Johnson syndrome, Mooren's ulcer, vitamin A deficiency, and many other diseases [3,135,136]. The ulcers may lead to scarring and loss of vision, or perforation of the cornea and loss of the eye. The corneal stroma is composed predominantly of type I collagen that is progressively degraded during ulceration. The origin of the cells that produce the collagenase could be corneal epithelial cells, neutrophils, macrophages, or stromal fibroblasts. Another condition that may be aided by collagenase action is epidermolysis bullosa, in which there is a separation of the dermis and epidermis [137].

INHIBITOR DESIGN

Although some collagenase inhibitors have been reported that were not obtained by rational design (see below), most are substrate analogues that have been developed by using the substrate specificity of these enzymes as a starting point. In this section, several factors in the rational design of specific, potent collagenase inhibitors are considered.

OVERALL DESIGN PRINCIPLES

An approach to the design of collagenase inhibitors that has a proven track record involves the development of non-cleavable substrate analogues. The active sites of mammalian and bacterial metalloproteinases can be divided conceptually into two regions. The first is the region occupied by the scissile peptide bond of the substrate which includes the zinc atom and other residues (for example, Glu, etc.) that participate in the bond breaking step. The second region surrounds the first and consists of specificity pockets (S_n ... S'_n) on the surface of the enzyme that govern its substrate specificity (*Figure 8.2*). The C=O group of the scissile bond of the substrate is thought to interact electrostatically with the active site Zn(II) during binding and catalysis, while the amino-acid side-chains in the P_n through P'_n positions of the substrate are postulated to interact with the corresponding S_n through S'_n subsites on the surface of the enzyme [24]. Those substrates with optimal $S_n...P_n$ interactions are hydrolyzed with the highest k_{cat}/K_M values. In the transition state for peptide bond hydrolysis, the carbonyl carbon of the substrate is thought to become tetrahedral, which allows the substrate to bind more tightly to the enzyme.

To prepare an inhibitor for a particular metalloproteinase, the design principle is to replace either the C=O group, NH group, or both groups of the scissile peptide bond of a specific substrate by other moieties that the enzyme cannot hydrolyze, to give peptide bond surrogates or mimetics. More often than not, only the N- or C-terminal portion of the substrate is used and the resultant analogue terminates with a group that substitutes for the scissile amide moiety. This procedure produces what is called a substrate analogue. The underlying concept is that the similarity in the structure of a non-cleavable substrate analogue to that of a specific substrate will cause it to be bound tightly to the enzyme and, hence, to act as an inhibitor. Moreover, to the extent that the substrate that is mimicked is specific for that enzyme, the resultant substrate-analogue may reasonably be expected to be a specific inhibitor. If the scissile peptide bond mimetic has the same planar geometry as the original peptide bond, it is considered to be a ground-state

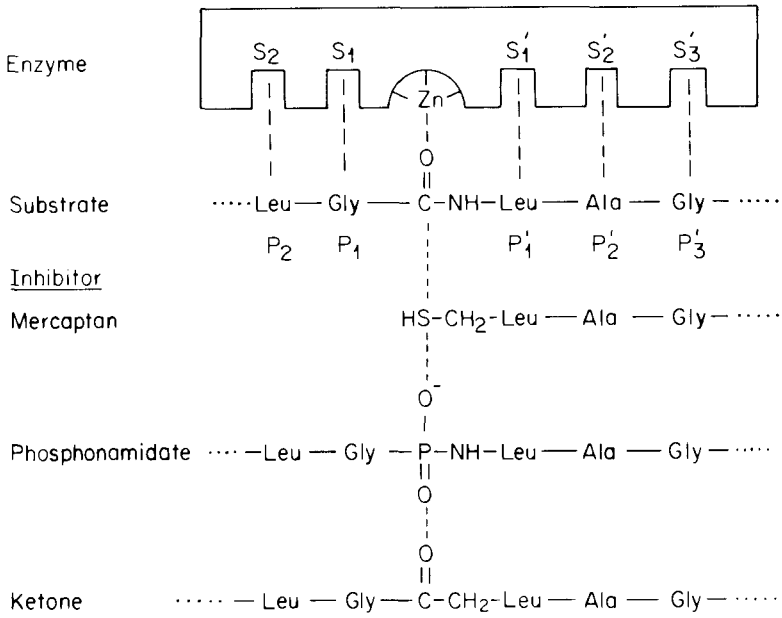


Figure 8.2. Schematic showing the interaction between the S_n subsites on the surface of a zinc proteinase and the P_n subsites of a substrate. Below the substrate are three examples of substrate-analogue inhibitors.

analogue inhibitor, while if it has a geometry that mimics the transition state (tetrahedral geometry about the carbonyl carbon in the case of peptide bond hydrolysis), it may act as a transition-state analogue inhibitor. The development of analogues with the transition state geometry is often preferred because they are believed to be able to take advantage of stronger binding interactions with the enzyme than analogous ground-state analogues. In either case, however, the K_I value obtained will reflect how favourable the interactions are between the enzyme and both the peptide bond mimetic and the amino-acid side-chains of the inhibitor.

Ondetti and associates have used this approach to develop inhibitors of angiotensin-converting enzyme (ACE) by choosing moieties for peptide bond replacement that were specifically capable of interacting favourably with the active site Zn(II) [138–140]. In reality, however, the interactions between the Zn(II) of the enzyme and the peptide bond surrogate that determine inhibitor potency are more complex and also involve hydrogen bonding to nearby catalytic residues. The existence of these hydrogen bonds has

been clearly documented from the crystallographic determination of the structures of more than twenty inhibitor complexes of thermolysin [14]. It may also be that substrate analogues can fortuitously take advantage of attractive interactions with the enzyme that were not possible for the substrate. A schematic of the active site of a Zn proteinase is pictured in *Figure 8.2* where the interactions with the substrate Leu-Gly-Leu-Ala-Gly are shown. Below the substrate are the structures of analogue inhibitors of this substrate in which the scissile (C=O)-NH bond has been replaced by the $HS\text{-}CH_2$, $P(=O)(OH)\text{-}NH$ and $(C=O)\text{-}CH_2$ groups to give mercaptan, phosphonamidate and ketone inhibitors, respectively.

DESIGN INFORMATION

The design principle outlined above is purely generic in that it allows for the design of inhibitors containing widely different scissile peptide bond mimetics and does not specify how the peptide portions of the inhibitor are chosen. The choice of peptide bond mimetics for collagenase inhibitors has largely followed those found to be successful for ACE and other metalloproteinases and, in our discussion of collagenase inhibitors given below, the inhibitors are grouped accordingly. By and large, this choice is made empirically from a small number of groups (mercaptan, hydroxamate, ketone, phosphinate, etc.) found to be effective for other metalloproteinases. The reason for the empiricism is because the potency of a hydroxamate- or mercaptan-type inhibitor for one metalloproteinase cannot necessarily be assumed to hold for another. This is unfortunate in the sense that it makes the development process more empirical, but it has the hidden benefit that differences in response of individual metalloproteinases to certain functional groups can be used as a means of developing selectivity (see below). In summary, no true 'design principles' have evolved with respect to the choice of scissile bond replacement, other than that one is best off screening from within those moieties with the ability to interact favourably with the Zn(II) and capable of forming hydrogen bonds to nearby catalytic residues.

The design of the peptide portion of substrate-analogue inhibitors, however, is less empirical and can be greatly facilitated by several types of information. Specifically, the structure of the substrate that is to be mimicked can be chosen from substrate specificity data, from examination of an X-ray structure of the enzyme, or from computer modelling of the enzyme active site. Unfortunately, no crystallographic structure is currently available for any collagenase or any member of the MMP gene family. Such studies have progressed slowly due to problems relating to the autolytic degradation of HFC and HNC at the concentrations needed for crystal growth. Only the

porcine analogue of HFC has been crystallized [142], but a structural determination has yet to be achieved. Thus, crystallographic input into collagenase inhibitor design is not yet possible and must await a successful structure determination.

The choice of the substrates that have been mimicked in the development of collagenase inhibitors has so far been guided primarily from substrate specificity considerations. Initial attempts were logically based upon the sequence of the collagenase cleavage site in interstitial collagens. This proved to be unfortunate, since the detailed specificity studies of HFC and HNC discussed in an earlier section have since shown that the optimal peptide substrates have markedly different sequences. It now seems clear that the collagenase cleavage site in collagens is determined largely by presently unappreciated local conformational features in the cleavage site region, rather than by the 'sequence specificity' of the collagenases [93]. Marked improvements in the potency of inhibitors initially prepared by reference to the collagenase cleavage site have been made empirically by changing the residues present at the various subsites (see below). In retrospect, it is now clear that the best inhibitors developed in this way generally mimic the structures of the most potent substrates identified in recent substrate specificity studies. Thus, the use of substrate specificity data can be quite helpful in rational inhibitor design.

Another method of designing inhibitors entails computer modelling. A computer modelling approach to collagenase inhibitor development has been described by Johnson and associates [1]. Two methods to model inhibitors have been suggested. The first is based on the concept that a good inhibitor should have a conformation like that found in the collagen triple helix and is called the 'collagen' model. The second approach assumes that there is structural homology between collagenases and other zinc peptidases whose structures are known, such as thermolysin, and is called the 'active site' model. The two models lead to inhibitor structure predictions that are incompatible and the 'active-site' model has been discarded. One must clearly be cautious in using thermolysin as a model for mammalian collagenases, since there is a very low homology between these enzymes. The 'collagen' model was subsequently used to refine the structure of several inhibitors based on the assumption that the side-chains of the residues in subsites P'_2 and P'_3 do not interact with the enzyme [143].

While this is in principle a valid approach to collagenase inhibitor design, there is some question as to whether or not the assumptions on which this model is based are valid. Specifically, there are several reasons for challenging the assumption that collagenases prefer substrate conformations that mimic those found in collagen. First, an examination of a model of collagen

shows that the Gly residues occupy the centre of the triple helix and are sterically inaccessible. Thus, hydrolysis of either the Gly-X bond by tissue collagenases or the Y-Gly bond by bacterial collagenases must be preceded by a local unwinding of the helix to gain accessibility to the scissile bond. Second, a comparison of the kinetic data for the hydrolysis of collagens vs. small synthetic substrates with identical cleavage sequences shows that the collagens are hydrolyzed with much lower k_{cat} values [68,93], reflecting the difficulty of their gaining access to the scissile bond. These values increase markedly as the thermal denaturation temperature is approached and this structure is loosened (Mallya, S.K. and Van Wart, H.E., unpublished data). Thus, there is currently no evidence to support the notion that the optimal conformation for a collagenase substrate or inhibitor resembles that of a collagen chain in the triple helix. Moreover, the assumption used in this modelling procedure that the side chains of residues in subsites P'_2 and P'_3 do not interact with the enzyme directly contradicts the substrate specificity data [94]. Thus, one must be cautious with respect to the assumptions that are used in such modelling studies.

ACHIEVING SELECTIVITY

One of the issues in the design of collagenase inhibitors that has received very little attention is that of selectivity. If a collagenase inhibitor is to be useful as a therapeutic agent, this may require that it does not simultaneously inhibit other essential proteinases. The same holds true if the inhibitor is to be useful in cell-mediated breakdown assays or cell migration systems, for the purposes of establishing the role of a collagenase in the catabolism of certain tissues or in cellular migration or invasion, respectively. This selectivity may need to be specific not only with respect to unrelated or distantly related proteinases such as ACE, but also against other members of the MMP family and, in certain circumstances, for one of the two known human collagenases.

The extent of need for selective collagenase inhibitors is not yet clear since, as emphasized above, the precise roles of HFC and HNC in normal physiological processes and individual diseases have not yet been convincingly delineated. It may be true that inhibitors which are effective against both HFC and HNC, or HFC and HFS, or even all of the MMP may be desired to treat a certain disease. However, in view of the essential roles that collagenases are believed to play in normal processes such as growth, development and wound healing, it seems likely that selectivity will be a critical issue. The very fact that the essential roles of collagenases are uncertain forces serious consideration of this question.

In our work, we have focused on the development of inhibitors that are specific for one or a small subset of the human MMP. In the context of the substrate-analogue design principle discussed above, the selectivity of an inhibitor can be viewed as arising from either the 'sequence' of the peptide portion of the analogue or the identity of the scissile peptide bond mimetic, since the interactions of both with the active sites of different metalloproteinases may differ widely. We have found that selectivity can be built in to even the initial design of collagenase inhibitors by optimizing the sequence of the substrate analogue to conform to that predicted from substrate specificity studies. In most cases, we find that this specificity is transferrable, at least qualitatively if not quantitatively, from substrate to inhibitor. In addition, we have found that added selectivity can be achieved, sometimes quite dramatically, by changing the peptide bond mimetic. This effect cannot be predicted in the same way as effects due to changes in sequence. Hence, the best mimetics for each enzyme must be discovered empirically. Lastly, it should be pointed out that the achievement of selectivity can often only be accomplished by sacrificing potency and these trade-offs must be judged in the context of the need for selectivity in efficacy.

INHIBITOR NOMENCLATURE AND ABBREVIATIONS

In order to facilitate reference to, and comparison of, the substrate analogue inhibitors considered below, a convention of abbreviations has been adopted to describe the amino-acid surrogates on either side of the scissile bond. A modification to the C=O group of residue P_1 is signified by the usual three-letter code for the residue followed by the formula of the group that has replaced the C=O, separated by a hyphen, all enclosed in parentheses. Modification of the NH group of residue P'_1 is analogously indicated by the three-letter code for the residue preceded by the formula of the group substituting for the NH, separated by a hyphen, all enclosed in parentheses. The stereochemistry at the α-carbon of the modified residue is indicated as being different from or analogous to that of the parent L-amino acid residue by inclusion of D- or L-, respectively, within the parentheses; DL-indicates either that the stereochemical mixture was used or that the diastereomers were separately assayed but not identified. If the diastereomers are separately assayed, this is indicated in the tables. Thus, a dipeptide analogue of Ala-Leu in which the amide bond is replaced by a phosphonamide bond (C=O replaced by P(=O)OH) is designated as (Ala-L-PO_2H)-Leu, and the corresponding phosphinic acid analogue (C=O replaced by P(=O))OH and NH replaced by CH_2) as (Ala-L-PO_2H)-(CH_2-L-Leu). Additional examples of

the application of this nomenclature are given in each of the tables, along with structural formulas for selected inhibitors. A bond designated by a wavy line in a structural formula designates either a stereochemical mixture at that site, or separated but not assigned diastereomers of the inhibitor. A variety of common abbreviations for organic groups is utilized, such as Me for methyl, Et for ethyl, and Ph for phenyl, without further definition; less common abbreviations are defined in the tables in which they appear.

The many inhibition studies on mammalian interstitial collagenases cited below have unfortunately used enzymes from numerous species (for example, human) and cell (for example, fibroblast) or tissue (for example, skin) types. In our discussion of the inhibitors from each study, the collagenase used by the investigators is specified. Examples include tadpole skin collagenase (TSC), human synovial collagenase (HSC), pig synovial collagenase (PSC), rabbit bone collagenase (RBC), and rabbit corneal collagenase (RCC). At first sight, this makes comparison of the results from different studies a bewildering task. However, several simplifying assumptions based on the discussion of HFC and HNC given in an earlier section can be invoked. First, it can reasonably be assumed based on current information that there are essentially two types of collagenase – the fibroblast-type and the neutrophil-type. Second, the same collagenase-type from different species seem, from the limited data available, to be very similar in their response to inhibitors. Third, any collagenase isolated from tissue or organ cultures is almost assuredly the fibroblast-type enzyme, since neutrophil-type collagenase has not yet been successfully grown in culture. Thus, to a first approximation, PSC, HSC, TSC, RBC and RCC can all be considered analogues of HFC. Conversely, collagenase extracted from neutrophils of any species can be assumed to be an analogue of HNC. Last, collagenases obtained from intact (uncultured) tissue can potentially be the fibroblast-type, the neutrophil-type, or a mixture of the two, depending upon the physiological circumstances. For the bacterial collagenases, only inhibition data for the CHC and AIC are presented. With respect to the CHC, some studies have employed the individual CHC (*Table 8.1*) for inhibition testing, while others have used a commercial preparation which consists of an undefined mixture of class I and II CHC. In tabulating the inhibitor data that follow, no attempt has been made to indicate the assay or activation method (if any) used. However, it should be noted that these factors can markedly affect comparisons of different studies.

INHIBITORS OF BACTERIAL COLLAGENASES

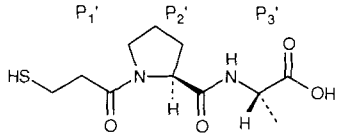
The development of potent inhibitors for the bacterial collagenases has centred on the use of mercaptan, ketone, and phosphonamidate moieties as scissile bond surrogates [20,144-154]. C-terminal analogue inhibitors (for example, those with residues on the P'_n of the substrate only) containing the mercaptan functionality have proven to be moderately effective against either AIC or a CHC mixture, with the best K_I values found to be in the 200–600 nM range (*Table 8.3.*). The most potent members of this group have a bulky side-chain in subsite P'_3, but it does not seem to matter whether or not that side-chain is charged (compare compounds (8)–(9) and (13)–(16) in *Table 8.3).*

Utilization of the ketone functionality as the scissile bond surrogate allows incorporation of both N-terminal and C-terminal (i.e., P_n and P'_n) residues into the inhibitor. The series of ketones (17)–(24) has been assayed as a diastereomeric mixture against β- and ε-CHC, with the most potent inhibitors exhibiting K_I values of 1–3 μM for the former and 20–30 nM for the latter (*Table 8.4*). The rigid cinnamoyl residue in subsite P_2 is far more effective than is its more flexible dihydro derivative (compare (21) and (22), *Table 8.4*), and the most favourable P'_3 residues are either Pro or those with an extended side-chain. These trends mimic the substrate specificity of these CHC discussed in an earlier section. That these compounds are acting as transition-state analogue inhibitors is strongly suggested by the linearity, with slopes near unity, of plots of log K_I for inhibitors (18)–(21) and (23)–(24) vs. log K_M/k_{cat} for their matched substrates for both β- and ε-CHC [20]; the ketone inhibitors are presumably binding to the enzymes in their *gem*-diol (ketone hydrate) form. Confirmation of this type of binding has been obtained from the ^{13}C-NMR spectrum of ^{13}C-enriched ketone (26), which shows a resonance at 102.6 ppm in the presence of a CHC mixture attributable to the bound *gem*-diol form of the inhibitor [149].

Phosphonamidate peptide analogues mimicking the $P_1...P'_3$ substrate residues have been prepared and evaluated against a CHC mixture (*Table 8.5*). Inhibitors with an aromatic side-chain in subsite P_1 are some 20-fold more effective than are those with an aliphatic one (compare (31) to (29) or (30), *Table 8.5*). An opposite, albeit less pronounced, preference at this site appears to exist for the ketone inhibitors for β-CHC ((17) vs. (25) and (21) vs. (26), *Table 8.4*). However, this difference could be attributable to a predominance of class II CHC in the mixture assayed in *Table 8.5*. Thus, comparisons of this sort must be made with caution. Introduction of a double bond into the P_1 side-chain decreases the potency of the phosphonamidate by more than a factor of 10 ((41) vs. (42), *Table 8.5*), perhaps because of

Table 8.3. MERCAPTAN INHIBITORS OF BACTERIAL COLLAGENASES

Example:



HS-(CH_2-Gly)-Pro-Ala-OH
(1)

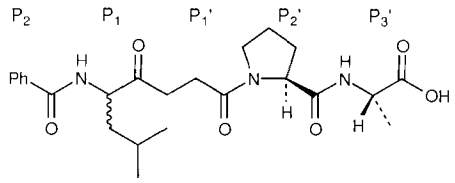
	Inhibitor [a]			$K_I\{IC_{50}\}$ (μM)			
No.	P_1'	P_2'	P_3'	*AIC*	*Ref.*	*CHC*[b]	*Ref.*
1	HS-(CH_2-Gly)	Pro	Ala-OH	{3}	144	2.5	146
1	HS-(CH_2-Gly)	Pro	Ala-OH	2.1	145		
2	HS-(CH_2-Gly)	Pro	Ala-OMe	8	145	200	146
3	HS-(CH_2-Gly)	Gly	Ala-OH	{77}	144		
4	HS-(CH_2-Gly)	Ala	Ala-OH	{55}	144		
5	HS-(CH_2-Gly)	Pro	Leu-OH	{21}	144	1.2	146
5	HS-(CH_2-Gly)	Pro	Leu-OH	2.7	145		
6	HS-(CH_2-Gly)	Pro	Phe-OH	2.2	145		
7	HS-(CH_2-Gly)	Pro	Pro-OH	0.8	145	0.6	146
8	HS-(CH_2-Gly)	Pro	Arg-OH	0.5	145	0.4	146
9	HS-(CH_2-Gly)	Pro	Arg-OMe	0.6	145		
10	HS-(CH_2-Gly)	Pro	NH_2			250	147
11	HS-(CH_2-Gly)	Pro	NHBn			170	147
12	HS-(CH_2-Gly)	Pro	NH*p*-NO_2Bn			20	147
13	HS-(CH_2-Gly)	Pro	Lys-OH			0.32	146
14	HS-(CH_2-Gly)	Pro	Lys(Boc)-OH			0.35	146
15	HS-(CH_2-Gly)	Pro	Har-OH			0.21	146
16	HS-(CH_2-Gly)	Pro	Ahx-OH			0.29	146

[a]Abbreviations: Ahx, (*S*)-2-aminohexanoyl; Bn, benzyl; Har, homoarginyl: *p*-NO_2Bn, *para*-nitrobenzyl. [b]An undefined mixture of CHC was used.

the conformational restraint imposed by the double bond. An extended side-chain in subsite P'_3 again enhances potency, and the best inhibitor of the CHCs reported to date (K_I = 5 nM) is the phosphonamidate (43), which has a polarized aromatic side-chain in subsite P_1 and an aminohexanoic acid group in subsite P'_3. The phosphonate moiety is approximately 300-fold less effective than the phosphonamidate as a scissile bond surrogate ((38) vs. (33)), and the phosphoramidate (27) is similarly ineffective.

Table 8.4. KETONE INHIBITORS OF BACTERIAL COLLAGENASES

Example:



Bz-(DL-Leu)-(CH_2-Gly)-Pro-Ala-OH
(17)

	Inhibitor[a]					$K_I\{IC_{50}\}$ (μM)		
No.	P_2	P_1	P_1'	P_2'	P_3'	*β-CHC*	*ε-CHC*	*Ref.*
17	Bz	DL-Leu	(CH_2-Gly)	Pro	Ala-OH	52	44	20
18	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Ala-OH	12	0.11	20
19	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Ala-OMe	60	1.2	20
20	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Leu-OH	2.8	0.030	20
21	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Pro-OH	2.7	0.018	20
22	$PhCH_2CH_2CO$	DL-Leu	(CH_2-Gly)	Pro	Pro-OH	220	2.9	20
23	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Arg-OH	1.0	0.030	20
24	PhCH=CHCO	DL-Leu	(CH_2-Gly)	Pro	Arg-OMe	1.1	0.023	20
25	Bz	DL-Phe	(CH_2-Gly)	Pro	Ala-OH	{120}[b]		148
26	PhCH=CHCO	DL-Phe	(CH_2-Gly)	Pro	Pro-OH	40[b]		149

[a]The diastereomeric mixture was assayed in each case. [b]Undefined mixture of CHC was used.

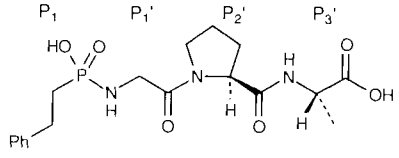
A number of other scissile bond replacements have been explored in an attempt to produce inhibitors of the bacterial collagenases, but all have exhibited relatively low potency (*Table 8.6*). These include a compound with an *N*-methylamide residue in subsite P'_1 (46), two carboxylates (47)–(48), the hydroxamate N-terminal (49) and C-terminal analogues (50)–(51), and one *N*-carboxyalkyl (52) derivative.

INHIBITORS OF MAMMALIAN COLLAGENASES

The selection of inhibitors considered in this section is complete in terms of the scissile bond surrogate functionalities that have been investigated, and representative in terms of residue substitutions, but it is not exhaustive

Table 8.5. PHOSPHONAMIDATE INHIBITORS OF BACTERIAL COLLAGENASES

Example:



$(Ph(CH_2)_2PO_2H)$-Gly-Pro-Ala-OH

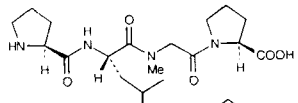
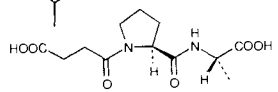
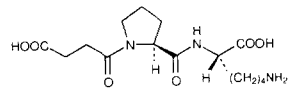
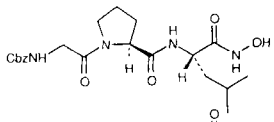
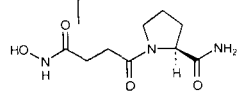
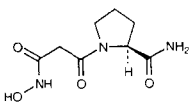
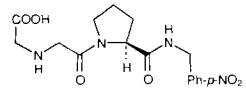
(31)

	Inhibitor[a]					
No.	P_1	P_1'	P_2'	P_3'	K_I {IC_{50}} (μM)[b]	*Ref.*
27	$(HOPO_2H)$	Gly	Pro	Ala-OH	{780}	150
28	$(EtPO_2H)$	Gly	Pro	Ala-OH	{46}	150
29	$(Me_2CH(CH_2)_2PO_2H)$	Gly	Pro	Ala-OH	{16}	150
30	$Me(CH_2)_9PO_2H)$	Gly	Pro	Ala-OH	{14}	150
31	$(Ph(CH_2)_2PO_2H)$	Gly	Pro	Ala-OH	0.8	151
32	$(Ph(CH_2)_2PO_2H)$	Gly	Pro	Tyr-OH	0.25	151
33	$(Ph(CH_2)_2PO_2H)$	Gly	Pro	Ahx-OH	0.06	151
34	$(Ph(CH_2)_2PO_2H)$	Gly	Ala	Ahx-OH	23	151
35	$(Ph(CH_2)_2PO_2H)$	Ala	Pro	Ahx-OH	2	151
36	$(Ph(CH_2)_2PO_2H)$	D-Ala	Pro	Ahx-OH	5	151
37	$(Ph(CH_2)_2PO_2H)$	(NMe-Gly)	Pro	Ahx-OH	50	151
38	$(Ph(CH_2)_2PO_2H)$	(O-Gly)	Pro	Ahx-OH	20	151
39	$(MePO_2H)$	Gly	Pro	Ahx-OH	2.6	151
40	$(PhCH_2PO_2H)$	Gly	Pro	Ahx-OH	0.36	151
41	$(Ph(CH_2)_3PO_2H)$	Gly	Pro	Ahx-OH	0.05	151
42	$(PhCH=CHCH_2PO_2H)$	Gly	Pro	Ahx-OH	0.7	151
43	$(p\text{-}NO_2Ph(CH_2)_2PO_2H)$	Gly	Pro	Ahx-OH	0.005	151
44	$(p\text{-}CF_3Ph(CH_2)_2PO_2H)$	Gly	Pro	Ahx-OH	0.024	151
45	$(Nap(CH_2)_2PO_2H)$	Gly	Pro	Ahx-OH	0.018	151

[a]Abbreviations: Ahx, (*S*)-2-aminohexanoyl; Nap, naphthyl (α- or β- not specified); $p\text{-}NO_2Ph$, *para*-nitrophenyl; $p\text{-}CF_3Ph$, *para*-(trifluoromethyl)phenyl. [b]Undefined mixture of CHC was used.

in terms of actual compounds synthesized and evaluated; numerous non-productive and/or non-instructive examples have been omitted. Inhibition results obtained with tadpole skin collagenase (TSC) are included in this section because it is a tissue collagenase that is very similar to the mammalian collagenases.

Table 8.6. MISCELLANEOUS INHIBITORS OF BACTERIAL COLLAGENASES

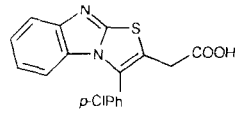
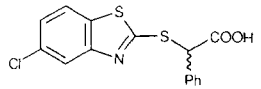
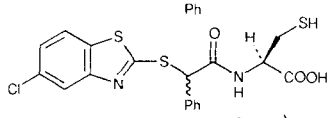
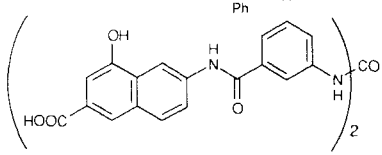
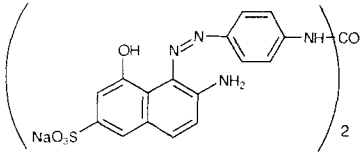
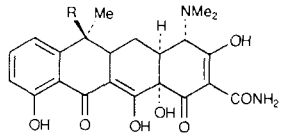
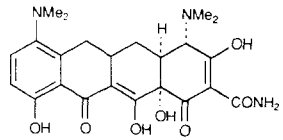
No.	Inhibitor P_3 P_2 P_1 P_1' P_2' P_3'	K_I (μM)	Enz.	Ref.
46		2200	AIC	152
47		400	AIC	153
48		20	AIC	153
49		240	CHC[a]	147
50		440	CHC[a]	147
51		4600	CHC[a]	147
52		1200	CHC[a]	147

[a]Undefined mixture of CHC was used.

NON-SUBSTRATE ANALOGUE INHIBITORS

Non-substrate analogue inhibitors of mammalian collagenases have been described, primarily in the patent literature (*Table 8.7*) [155–165]. The thiazolobenzimidazole and benzothiazolylthio derivatives (53) and (54), respectively, are weak inhibitors of HFC, but attachment of the latter moiety to a Cys residue as in (55) greatly enhances potency. Large aromatic ureas containing charged end-groups such as (56) and (57) are moderately effective inhibitors of HFC. The tetracycline antibiotics (58)–(60) have been shown to be inhibitors of rabbit corneal collagenase (RCC), with doxycycline (58) being 20-fold more potent than its hydroxylated congener tetracycline (59),

Table 8.7. NON-SUBSTRATE ANALOGUE INHIBITORS OF MAMMALIAN COLLAGENASES

No.	*Inhibitor*[a]	*IC_{50} {K_i} (μM) or % Inhibition*	*Enz.*	*Ref.*
53		350	HFC	155
54		>100	HFC	156
55		5.7	HFC	157
55		{3.5}	HFC	158
56		92% @ 45 μM	HFC	159
57		79% @ 38 μM	HFC	160
58		R = H 15	RCC	161
59		R = OH 350	RCC	161
60		190	RCC	161

[a]Abbreviations: *p*-ClPh, *para*-chlorophenyl.

while minocycline (60) is intermediate in potency. The nature of the binding interactions of any of these compounds with the enzyme is difficult to envision, with the exception of that of the Cys derivative (55), which is likely to interact with the Zn(II) via its sulphydryl group.

SUBSTRATE ANALOGUE INHIBITORS

As indicated earlier, the primary strategy for collagenase inhibitor design has been to incorporate scissile bond surrogate functionalities into specific peptide substrates. In some cases, the functionalities that have been utilized have allowed incorporation of substrate residues at both P_n and P_n' positions, while in others only one side of the scissile bond of the substrate has been mimicked. In the latter cases, the C-terminal analogues (containing P_n' residues) have generally been more potent inhibitors. The relatively large number of inhibitors that have been reported are categorized below according to type of peptide bond surrogate functionality.

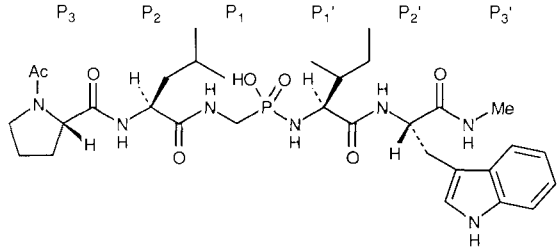
Phosphorus-based inhibitors

Both phosphonamidate and phosphinate functionalities have proven to be effective scissile bond replacements, while phosphoramidates and phosphonates have been less useful [143,166–171]. Representative data for the inhibition of HFC and HNC by phosphonamidates and phosphoramidates are summarized in *Table 8.8*. The best inhibitors in this series have the Gly-Ile or Gly-Leu sequences at subsites P_1-P_1' and have an aromatic residue in subsite P_2'. The phosphoramidate (61) and its isosteric phosphonamidate counterpart (62) are moderately good and approximately equipotent inhibitors of HFC. The incorporation of a phthaloyl group into subsite P_2 as in (65) improves potency, and this is in fact more effective than extending the inhibitor on the N-terminal side with substrate residues in subsites P_2 and P_3 (compare (65) with (66), *Table 8.8*). The value of K_I is almost the same whether there is a methyl, benzyl or α-methylbenzyl group in subsite P_3' ((65), (67) and (68), respectively), but an α-methylbenzyl group of the wrong stereochemistry clearly interferes with binding ((68) vs. (69)). A dependence of K_I on pH has been noted in this series, with phosphonamidate (67) being approximately 3-fold less potent at pH 7.5 than at pH 6.5, while the phosphoramidate (61) is somewhat more potent at the higher pH. The phosphonamidates (70)–(72) are only moderately effective inhibitors of HNC, and it is again noted that the incorporation of a substrate residue into subsite P_2 has little effect on inhibitor potency.

The inhibition of HFC by a series of phosphinate and phosphonate substrate analogues is summarized in *Table 8.9*. Since phosphinate inhibitors require synthesis of the P_1' residue, they are usually prepared as diastereomeric pairs. If the diastereomers were separated and assigned stereochemistry, this information is included in *Table 8.9*. A potentially significant finding in this series is that a very large aromatic group in subsite P_2 enhances

Table 8.8. PHOSPHONAMIDATE AND PHOSPHORAMIDATE INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



Ac-Pro-Leu-(Gly-PO_2H)-Ile-Trp-Me
(66)

	Inhibitor[a]						K_I (μM)		
No.	P_3	P_2	P_1	P_1'	P_2'	P_3'	*HFC*	*HNC*	*Ref.*
61			(EtO-PO_2H)	Ile	Trp	NHMe	1.5[b]		166
61			(EtO-PO_2H)	Ile	Trp	NHMe	0.9[c]		166
62			(Pr-PO_2H)	Ile	Trp	NHMe	2		167
63			(Hex-PO_2H)	Ile	Trp	NHMe	0.6		167
64			(Dec-PO_2H)	Ile	Trp	NHMe	0.2		167
65		Pht	(Gly-PO_2H)	Ile	Trp	NHMe	0.05		167
66	Ac-Pro	Leu	(Gly-PO_2H)	Ile	Trp	NHMe	0.3		167
67		Pht	(Gly-PO_2H)	Ile	Trp	NHBn	0.025[b]		168
67		Pht	(Gly-PO_2H)	Ile	Trp	NHBn	0.066[c]		168
68		Pht	(Gly-PO_2H)	Ile	Trp	NHαMeBn(*S*)	0.02		167
69		Pht	(Gly-PO_2H)	Ile	Trp	NHαMeBn(*R*)	2		167
70		Cbz	(Gly-PO_2H)	Leu	Ala	OH		78	169
71	Cbz	Phe	(Gly-PO_2H)	Leu	Ala	OH		71	169
72		Cbz	(Gly-PO_2H)	Leu	Ala	Gly-OH		14	169

[a]Abbreviations: Bn, benzyl; Dec, n-decyl; Hex, n-hexyl; αMeBn, α-methylbenzyl; Pht, phthaloyl; Pr, n-propyl. [b]Assayed at pH 6.5. [c]Assayed at pH 7.5.

inhibitor potency, especially when it is functionalized with polarizing groups ((74)–(76) and (77)–(79)). It has also been demonstrated that the P_2' and P_3' side-chains can be connected in a lactam ring as long as the ring size is large enough to accommodate a *trans* peptide bond conformation. The phosphinate inhibitor (79), optimized in this way, exhibits an IC_{50} of 17 nM against HFC. As predicted from the substrate specificity data discussed in

Table 8.9. PHOSPHINATE AND PHOSPHONATE INHIBITORS OF MAMMALIAN COLALGENASES

Examples

Ac-Pro-Leu(Gly-PO_2H)-(CH_2-L-Leu)-Leu-Ala-OEt (80)

No.	Inhibitor[a] P_3	P_2	P_1	P_1'	P_2'	P_3'	IC_{50} {K_i} (μM)[b]	Ref.
73		Ac	(Gly-PO_2H)	(CH_2-L-Leu)	Leu	NHMe	37	143
74		Pht	(Gly-PO_2H)	(CH_2-L-Leu)	Leu	NHMe	0.6	143
75		Npt	(Gly-PO_2H)	(CH_2-DL-Leu)	Leu	NHMe[c]	0.16	170
76		3-BrNpt	(Gly-PO_2H)	(CH_2-L-Leu)	Leu	NHMe	0.03	143
77		Npt	(Gly-PO_2H)	(CH_2-L-Leu)	Alm		0.2	143,170
78		3-BrNpt	(Gly-PO_2H)	(CH_2-L-Leu)	Alm		0.03	143
79		3-Br,4-HONpt	(Gly-PO_2H)	(CH_2-L-Leu)	Alm		0.017	171
80	Ac-Pro	Leu	(Gly-PO_2H)	(CH_2-L-Leu)	Leu	Ala-OEt	0.07	143
81	Cbz-Pro	Leu	(Gly-PO_2H)	(CH_2-DL-Leu)	Leu	Ala-OH[c]	0.056	170
82			(Hex-PO_2H)	(CH_2-DL-Leu)	Trp	NHMe[d]	{0.5}	167
83			(Hex-PO_2H)	(O-DL-Leu)	Trp	NHMe[d]	{300}	167
84			(EtO-PO_2H)	(CH_2-DL-Leu)	Trp	NHMe[d]	{4}	167
85		Cbz	(Gly-PO_2H)	(CH_2-DL-Leu)	Trp	NHMe[d]	{0.2}	167
86		Nph	(Gly-PO_2H)	(CH_2-DL-Leu)	Trp	NHMe[d]	{0.03}[e]	167
86		Nph	(Gly-PO_2H)	(CH_2-DL-Leu)	Trp	NHMe[d]	{0.11}[f]	167
87		Nph	(Gly-PO_2H)	(CH_2-DL-Leu)	Trp	NHBn[d]	{0.01}	167

[a]Abbreviations: Alm, (*S*)-3-aminolaurolactam; Bn, benzyl; Hex, n-hexyl; Nph, β-naphthoyl; Npt, 1,8-napthaloyl; Pht, phthaloyl. [b]HFC was used. [c]One diastereomer. [d]Mixture of diastereomers. [e]Assayed at pH 6.5. [f]Assayed at pH 7.5.

an earlier section, aromatic residues in subsites P_2' and P_3' are preferred. The most potent inhibitor of this series is the phosphinate (87), with a K_I value of 10 nM for the mixture of diastereomers. A pH dependence for K_I has been reported for phosphinate (86), which is approximately 3-fold less active at the higher pH. The phosphonates (83) and (84) are both much less effective inhibitors than the related phosphinate (82).

Sulphur-based inhibitors

Substrate analogues containing the mercaptan functionality have been extensively investigated as collagenase inhibitors, and some other sulphur-based functionalities have also been explored [1,161,172–185]. The mercaptans tend to be very potent inhibitors of all of the MMP, presumably due to the strong interaction between the active site Zn(II) and the mercaptide anion. Unfortunately, these compounds tend to undergo inactivation by oxidative disulphide formation. However, the rate at which this occurs varies widely and depends on the structure of the inhibitor. The most common synthetic route to these derivatives again leads to a diastereomeric mixture.

Mercaptan derivatives that have the SH group formally in-register with the C=O of the substrate scissile bond are tabulated in *Table 8.10*, while those with the SH group out-of-register are summarized in *Table 8.11*. Among the in-register derivatives, the slower eluting diastereomer of the tripeptide analogue HS-(CH_2-DL-Leu)-Phe-Ala-NH_2 (99) is approximately 10-fold more potent than is the faster eluting isomer (98). A subsequent asymmetric synthesis of the mercaptan-substituted P_1' residue led to the surprising demonstration that the more active stereoisomer of this tripeptide mimetic is in fact the D-Leu analogue (compare (100)–(101), *Table 8.10*). The Ala residue in subsite P'_3 can be replaced by a methyl group, and the resulting dipeptide analogue inhibitor has essentially the same potency and stereoselectivity ((100)–(101) vs. (103)–(104)). However, the incorporation of a D-Ala residue in subsite P_3' causes a substantial loss in potency ((99) vs. (102)). Analogues with aromatic residues in subsite P_2' are again preferred. The most potent inhibitor in this series has the 3-(2-naphthyl)alanyl residue in this subsite ((110), $IC_{50} \leqslant 14$ nM).

Among the out-of-register mercaptans, derivatives (115) and (116) are equivalent in potency to their in-register counterparts (90) and (91), but with a reversal in stereoselectivity as indicated by the modest preference for the L-Leu analogue stereoisomer in subsite P_1'. The thiophenol derivative (118) is very potent, at least in the presence of β-mercaptoethanol which presumably suppresses disulphide formation.

It has been reported that the incorporation of an alkyl group at the posi-

tion α to the sulphydryl moiety of an in-register mercaptan enhances inhibition [175]. Data for the inhibition of HSC by several such compounds are listed in *Table 8.12*. Comparison of the IC_{50} value of the α-methylmercaptan (121) in *Table 8.12* with that of the comparable unsubstituted mercaptan (105) in *Table 8.10*, indicates an approximate 10-fold enhancement of potency taking into account that the latter was assayed as the diastereomeric mixture. Although no substantiation was offered, it was claimed that one of the diastereomers with the *S* configuration at the methyl-substituted α-carbon is the more potent one [175].

A very dramatic increase in the effectiveness of an in-register mercaptan inhibitor is achieved by the attachment of a second chelating moiety at the carbon atom α to the mercaptan (*Table 8.13*). The mercapto-ester (130) can act as a bidentate ligand for the active site Zn(II) and, with an IC_{50} of 3.9 nM, it is some 50-fold more potent than the corresponding α-methylmercaptan (121); note, however, that the collagenase sources are different in the two cases.

Other sulphur-based scissile bond surrogate functionalities that have been explored include the sulphinate, sulphide, sulphoxide and sulphone moieties, but they have proved to be weak collagenase inhibitors, even when incorporating an amino acid residue in subsite P_1 (*Table 8.14*). The sulphide analogue (139) is the best inhibitor in the series shown. It is perhaps noteworthy that, with the sulphide-containing substrate analogue, the L-Leu diastereomer is the preferred stereoisomer ((139) vs. (140)), in contrast to what is observed with the analogous mercaptan inhibitors. It is clear from the behaviour of many of the stereoisomers discussed above that some of these substrate analogue inhibitors do not, in fact, bind in the same way as the analogous substrates.

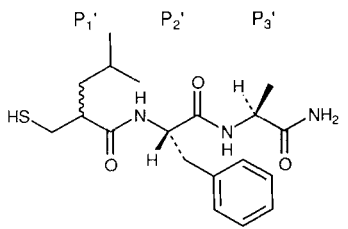
Hydroxamate inhibitors

The hydroxamate functionality is an effective scissile bond surrogate in collagenase inhibitors [1,186–203], probably because of its ability to serve as a bidentate ligand for the active site Zn(II). N-terminal tri- and tetrapeptide hydroxamate substrate analogues are only moderately potent inhibitors (*Table 8.15*). An intriguing observation with respect to possible binding modes is that the residues in subsites P_1 and P_2 can be replaced with their D-stereoisomer counterparts with essentially no loss of potency, as long as *both* are replaced with the D-isomers (compare (151), (152) and (153), and (154) vs. (157), in *Table 8.15*).

C-terminal hydroxamate analogues have proven to be very potent collagenase inhibitors (*Table 8.16*). The pseudotripeptide antibiotic actinonin

Table 8.10. IN-REGISTER MERCAPTAN INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



HS-(CH_2-DL-Leu)-Phe-Ala-NH_2 (98,99)

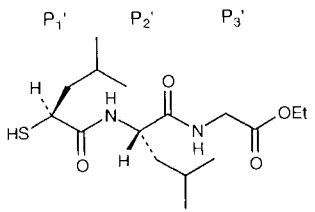
	Inhibitor[a]							
No.	P_1'	P_2'	P_3'	P_4'	*Diaster.*[b]	*Enz.*	$IC_{50}(\mu M)$	*Ref.*
88	HS-(CH_2-DL-Phe)	Ala	OEt		1	PSC	100	172
89	HS-(CH_2-DL-Leu)	Ala	OEt		1 or 2	PSC	4–10	172
90	HS-(CH_2-DL-Leu)	Ala	Gly	OEt	1	PSC	1–4	172
91	HS-(CH_2-DL-Leu)	Ala	Gly	OEt	2	PSC	4–10	172
92	HS-(CH_2-DL-Leu)	Ala	Gly	Gln-OH	1 or 2	PSC	4–10	172
93	HS-(CH_2-DL-Leu)	Leu	Gly	OEt	mix	HSC	1.3	1
94	HS-(CH_2-DL-Leu)	Trp	OMe		mix	PSC	3.6	173
95	HS-(CH_2-DL-Leu)	Tyr(OMe)-OEt			mix	PSC	1	173

96	HS-(CH_2-DL-Leu)	Phe	NH_2		1 or 2	PSC	3–4	173
97	HS-(CH_2-DL-Leu)	Phe	Phe	NH_2	mix	PSC	5	173
98	HS-(CH_2-DL-Leu)	Phe	Ala	NH_2	1	PSC	0.3	173
99	HS-(CH_2-DL-Leu)	Phe	Ala	NH_2	2	PSC	0.04	173
99	HS-(CH_2-DL-Leu)	Phe	Ala	NH_2	2	RCC	0.011	161
100	HS-(CH_2-L-Leu)	Phe	Ala	NH_2	–	HNC	0.36	174
100	HS-(CH_2-L-Leu)	Phe	Ala	NH_2	–	HFC	0.45	174
101	HS-(CH_2-D-Leu)	Phe	Ala	NH_2	–	HNC	0.042	174
101	HS-(CH_2-D-Leu)	Phe	Ala	NH_2	–	HFC	0.056	174
102	HS-(CH_2-DL-Leu)	Phe	D-Ala	NH_2	mix	PSC	2.6	173
103	HS-(CH_2-L-Leu)	Phe	NHMe		–	HNC	0.24	174
103	HS-(CH_2-L-Leu)	Phe	NHMe		–	HFC	0.45	174
104	HS-(CH_2-D-Leu)	Phe	NHMe		–	HNC	0.056	174
104	HS-(CH_2-D-Leu)	Phe	NHMe		–	HFC	0.072	174
105	HS-(CH_2-DL-Leu)	Tyr(OMe)	NHMe		mix	HSC	3.6	175
106	HS-(CH_2-DL-Leu)	Leu	NHMe		A	HSC	3.5	1
107	HS-(CH_2-DL-Leu)	Leu	NHMe		B	HSC	7.0	1
108	HS-(CH_2-DL-Leu)	Trp	Ala	NH_2	1	PSC	0.1	173
109	HS-(CH_2-DL-Leu)	Trp	Ala	NH_2	2	PSC	0.07	173
110	HS-(CH_2-DL-Leu)	Nal	Ala	NH_2	1	PSC	0.014[c]	173
111	HS-(CH_2-DL-Leu)	Nal	Ala	NH_2	2	PSC	0.1	173

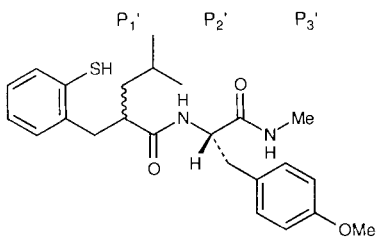
[a]Abbreviations: Nal, L-3-(2-naphthyl)alanine. [b]Diastereomers of unknown stereochemistry are characterized by chromatographic behavior: 1 = faster eluting, 2 = slower eluting, diastereomer in reverse-phase chromatography; A, B, etc. designate separated but uncharacterized diastereomers. [c]Upper limit because $[I]_{free} \approx$ [enzyme].

Table 8.11. OUT-OF-REGISTER MERCAPTAN INHIBITORS OF MAMMALIAN COLLAGENASES

Examples:



(HS-L-Leu)-Leu-Gly-OEt
(112)



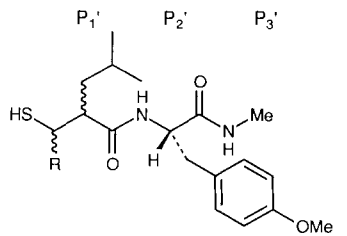
2-HSPh-(CH_2-DL-Leu)-Tyr(OMe)-NHMe
(117,118)

	Inhibitor[a]								
No.	P_1'	P_2'	P_3'	P_4'	P_5'	*Diaster.*[b]	*Enz.*	$IC_{50}(\mu M)$	*Ref.*
112	(HS-L-Leu)	Leu	Gly	OEt		–	HSC	8.5	1
113	(HS-L-Leu)	Ala	Gly	Gln	D-Arg-NH_2	–	TSC	10	176
114	(HS-L-Phe)	Ala	Gly	Gln	D-Arg-NH_2	–	TSC	70	176
115	$HSCH_2$-(CH_2-L-Leu)	Ala	Gly	OEt		–	HSC	4.0	1
116	$HSCH_2$-(CH_2-D-Leu)	Ala	Gly	OEt		–	HSC	9.0	1
117	*o*-HSPh-(CH_2-DL-Leu)	Tyr(OMe)NHMe				1	RBC	1.9	177
118	*o*-HSPh-(CH_2-DL-Leu)	Tyr(OMe)NHMe				2	RBC	1.4	177
118	*o*-HSPh-(CH_2-DL-Leu)	Tyr(OMe)NHMe				2	HFC	0.71	177
118	*o*-HSPh-(CH_2-DL-Leu)	Tyr(OMe)NHMe				2	HFC	0.024[c]	177

[a]Abbreviations: *o*-HSPh, *ortho*-mercaptophenyl. [b]Diastereomers of unknown stereochemistry are characterized by chromatographic behaviour: 1 = faster eluting, 2 = slower eluting diastereomer in normal-phase chromatography. [c]With β-mercaptoethanol in the assay medium.

Table 8.12. α-SUBSTITUTED IN-REGISTER MERCAPTAN INHIBITORS OF MAMMALIAN COLLAGENASES

Inhibitor[a]

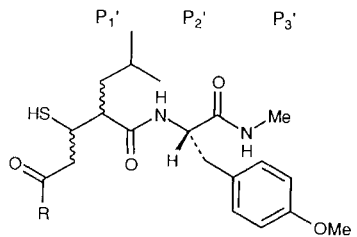


No.	*R*	*P_1'*	*P_2'*	*P_3'*	*Diaster.*[b]	*Enz.*	*IC_{50} (μM)*	*Ref.*
119	Me	HS-(CHR-DL-Leu)	Tyr(OMe)	NHMe	1	HSC	19	175
120	Me	HS-(CHR-DL-Leu)	Tyr(OMe)	NHMe	2	HSC	13	175
121	Me	HS-(CHR-DL-Leu)	Tyr(OMe)	NHMe	3	HSC	0.22	175
122	Me	HS-(CHR-DL-Leu)	Tyr(OMe)	NHMe	4	HSC	1.5	175
123	Ph	HS-(CHR-DL-Leu)	Tyr(OMe)	NHMe	1	HSC	0.27	175
124	Me	HS-(CHR-DL-Ahx)	Tyr(OMe)	NHMe	1+2	HSC	0.2	175
125	Me	HS-(CHR-DL-Ahx)	Tyr(OMe)	NHMe	3+4	HSC	1.0	175
126	Me	HS-(CHR-DL-Leu)	Thr(OBn)	NHMe	1+2	HSC	0.45	175
127	Me	HS-(CHR-DL-Leu)	Thr(OBn)	NHMe	3+4	HSC	4.9	175

[a]Abbreviations: Ahx, 2-aminohexanoyl; Bn, benzyl. [b]Diastereomers of unknown stereochemistry are characterized by chromatographic behavior: 1 = fastest eluting ... 4 = slowest eluting diastereomer in normal-phase chromatography.

Table 8.13. BIDENTATE α-SUBSTITUTED IN-REGISTER MERCAPTAN INHIBITORS OF MAMMALIAN COLLAGENASES

Inhibitor[a]

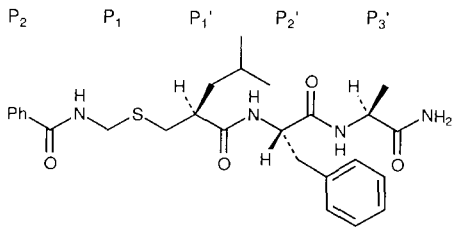


No.	*R*	*P_1'*	*P_2'*	*P_3'*	*Diaster.*[b]	*Enz.*	*IC_{50} (μM)*	*Ref.*
128	Me	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	A	HFC	0.014	178
129	MeO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	1	RBC	0.072	179
130	MeO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	2	RBC	0.0039	179
131	BnO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	A	RBC	0.031	179
132	BnO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	B	RBC	0.27	179
133	BnO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	C	RBC	0.23	179
134	BnO	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	D	RBC	12.6	179
135	MeNH	HS-(CHR'-DL-Leu)	Tyr(OMe)	NHMe	A	RBC	0.0047	179
136	MeO	HS-(CHR'-DL-Leu)	Phe	NHMe	A	RBC	0.0083	179

[a]Abbreviations: Bn, benzyl. [b]Diasteromers of unknown stereochemistry are characterized by chromatographic behaviour: 1 = faster eluting, 2 = slower eluting diastereomer in normal-phase chromatography; A, B, etc. designate separated but uncharacterized diastereomers.

Table 8.14. OTHER SULPHUR-BASED INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



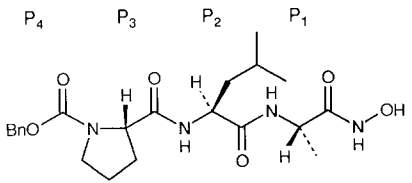
Bz-(Gly-S)-CH_2-L-Leu)-Phe-Ala-NH_2
(139)

	Inhibitor				*IC_{50} (μM)*		
No.	*P_1*	*P_1'*	*P_2'*	*P_3'*	*HFC*	*HNC*	*Ref.*
137	HO_2S	(CH_2-L-Leu)	Phe	Ala-NH_2	220	79	174
138	HO_2S	(CH_2-D-Leu)	Phe	Ala-NH_2	1100	710	174
139	Bz-(Gly-S)	(CH_2-L-Leu)	Phe	Ala-NH_2	36	25	174
140	Bz-(Gly-S)	(CH_2-D-Leu)	Phe	Ala-NH_2	210	110	174
141	Bz-(Gly-SO)	(CH_2-L-Leu)	Phe	Ala-NH_2[a]	240	130	174
142	Bz-(Gly-SO)	(CH_2-L-Leu)	Phe	Ala-NH_2[b]	710	580	174
143	Bz-(Gly-SO)	(CH_2-D-Leu)	Phe	Ala-NH_2[a]	1500	1100	174
144	Bz-(Gly-SO)	(CH_2-D-Leu)	Phe	Ala-NH_2[b]	79	560	174
145	Bz-(Gly-SO_2)	(CH_2-L-Leu)	Phe	Ala-NH_2	350	140	174
146	Bz-(Gly-SO_2)	(CH_2-D-Leu)	Phe	Ala-NH_2	500	300	174

[a]Faster-eluting diastereomer (normal-phase chromatography). [b]Slower-eluting diastereomer (normal-phase chromatography)

Table 8.15. N-TERMINAL HYDROXYAMATE INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



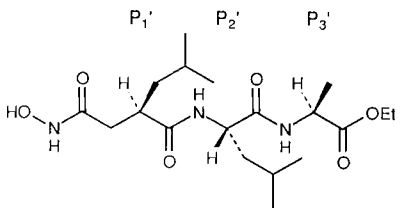
Cbz-Pro-Leu-Ala-NHOH
(151)

	Inhibitor[a]				*IC_{50} (μM)*		
No.	*P_4*	*P_3*	*P_2*	*P_1*	*HFC*	*TSC*	*Ref.*
147	Cbz	Pro	Leu	Gly-NHOH	40		186,187
148	Ac	Pro	Leu	Gly-NHOH	10		1
149	Cbz	Leu	Leu	Gly-NHOH	300		187
150	Cbz	Pro	Leu	Leu-NHOH	27	35	188
151	Cbz	Pro	Leu	Ala-NHOH	2.6	7.3	188
152	Cbz	Pro	D-Leu	Ala-NHOH		1500	188
153	Cbz	Pro	D-Leu	D-Ala-NHOH	4.1	1.3	188
154	Bz-Gly	Pro	Leu	Ala-NHOH	2.7	7.7	189,190
155	Boc-Gly	Pro	Leu	Ala-NHOH		8.9	190
156	Boc-Gly	Pro	Leu	Val-NHOH	3.1	9.8	189,190
157	Bz-Gly	Pro	D-Leu	D-Ala-NHOH	6.4	3.1	189,190
158	4-HOBz-Gly	Pro	D-Leu	D-Ala-NHOH	1.2		189
159	4-NH_2Bz-Gly	Pro	D-Leu	D-Ala-NHOH	1.3		189

[a]Abbreviations: 4-HOBz, 4-hydroxybenzoyl; 4-NH_2Bz, 4-aminobenzoyl.

Table 8.16. C-TERMINAL HYDROXAMATE INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



HONHCO-(CH_2-L-Leu)-Leu-Ala-OEt (180)

	Inhibitor[a]					
No.	P_1'	P_2'	P_3'	*Diaster.*[b]	IC_{50} {K_I} (μM)[c]	*Ref.*
160	HONHCO-(CH_2-L-Ahp)	Val	(Pro-CH_2)-OH	–	{1.4}[d]	191
161	HONHCO-(CH_2-DL-Ahp)	Val	NMph	mix	1[d]	192
161	HONHCO-(CH_2-DL-Ahp)	Val	NMph	mix	{0.35}[d]	193
162	HONHCO-(CH_2-DL-Leu)	Val	NHBn	A	{0.013}[d]	194,195
163	HONHCO-(CH_2-DL-Leu)	Val	NHBn	B	{6}[d]	194,195
164	HONHCO-(CH_2-DL-Leu)	Val	Gly-NHEt	mix	0.2	1

165	HONHCO-(CH_2-DL-Leu)	Leu	Gly-NH_2	mix	0.2	1
166	HONHCO-(CH_2-DL-Leu)	Leu	NHMe	mix	0.05	1
167	HONHCO-(CH_2-DL-Leu)	Ala	NHMe	A	0.15	196
168	HONHCO-(CH_2-DL-Leu)	Tyr	NHMe	A	0.037	196
169	HONHCO-(CH_2-DL-Leu)	Tyr	NHMe	B	0.52	196
170	HONHCO-(CH_2-DL-Leu)	Tyr(OMe)NHMe		A	0.02	196
171	HONHCO-(CH_2-DL-Leu)	Tyr(OMe)NHMe		B	4	196
172	HONHCO-(CH_2-DL-Leu)	Phe	NHMe	A	0.02	196
173	HONHCO-(CH_2-DL-Leu)	Phe	NHMe	B	1.0	196
174	HONHCO-(CH_2-L-Leu)	Phe	$NHCH_2$-3-Pyr	–	0.020	197
175	HONHCO-(CH_2-L-Leu)	Phe	$NH(CH_2)_2$NMph	–	0.015	197
176	HONHCO-(CH_2-L-Leu)	Leu	Gly-OEt	–	0.025	198
177	HCON(OH)-(CH_2-DL-Leu)	Leu	Gly-OEt	mix	0.30	198
178	HONHCO-(CH_2-L-Nle)	Leu	Gly-OEt	–	0.070	1
179	HONHCO-(CH_2-L-Phe)	Leu	Gly-OEt	–	>1	1
180	HONHCO-(CH_2-L-Leu)	Leu	Ala-OEt	–	0.0085	198
180	HONHCO-(CH_2-L-Leu)	Leu	Ala-OEt	–	{0.005}	1
181	HONHCO-(CH_2-L-Leu)	DL-Alm		mix	0.038	198
182	HONHCO-(CH_2-L-Leu)	DL-Alm		A	0.026	1

[a]Abbreviations: Ahp, 2-aminoheptanoyl; Alm, 3-aminolaurolactam; Bn, benzyl; CH_2-3-Pyr, 3-pyridinylmethyl; NMph, 1-morpholinyl. [b]A and B designate separated but uncharacterized diastereomers. [c]HSC was used unless otherwise noted. [d]PSC was used.

(160) is a moderately effective inhibitor, while modification by substitution of Leu in subsite P_1' and a benzyl moiety in P_3' affords the potent inhibitor (162) with a K_I value of 13 nM. Connection of the P_2' and P_3' side-chains to form a cyclic lactam, as was done in the phosphinate series, is again tolerated with little loss in potency ((181) and (192)). The most potent inhibitor in the series shown is the tripeptide analogue HONHCO-(CH_2-L-Leu)-Leu-Ala-OEt (180), which exhibits a K_I value of 5 nM againt HSC. The incorporation of another substituent at the carbon atom α to the hydroxamate moiety leads to an only modest enhancement of potency in most cases (*Table 8.17*), in contrast to the success of analogous substitutions for mercaptan inhibitors. For example, the phthalimidobutyl substituted hydroxamate (185) is only slightly more active than its unsubstituted counterpart ((180), *Table 8.16*). However, the mercaptomethyl substituted hydroxamate (188), with an IC_{50} value of 3 nM, is some 7-fold more potent than its unsubstituted analogue (172). The possibility that this enhancement is a result of tridentate binding of the hydroxamate and mercaptide moieties to the Zn(II) of the enzyme seems unlikely, because the analogous (4-*t*-butylphenyl)thiomethyl derivative (193) is equivalently potent.

N-*Carboxyalkyl inhibitors*

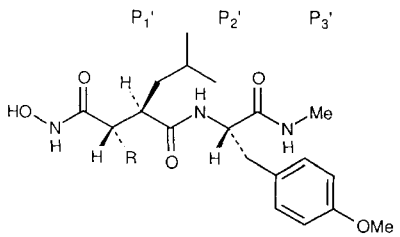
A number of *N*-carboxyalkyl and *N*-phosphonoalkyl substituted substrate analogue inhibitors have been examined [161,204–208]. These derivatives contain both the acidic carboxylate (or phosphonate) and basic amine functionalities in the vicinity of the scissile bond. Thus, they are capable both of electrostatic interaction with the active site Zn(II) and hydrogen bonding interactions with other active site residues. They are, however, only moderately potent collagenase inhibitors (*Table 8.18*). The stereochemistry at the carbon atom to which the carboxylate moiety is bonded markedly influences the inhibitory potency of these derivatives ((197) vs. (198)). The phosphonate analogues of this class of derivatives have also been evaluated (*Table 8.18*), but are not substantially better inhibitors than the carboxylates.

Miscellaneous inhibitors

Included in this category of compounds are one example of a substrate analogue containing an *N*-ketoalkyl scissile bond surrogate moiety (213), and a number of non-substrate analogue peptides (*Table 8.19*) [176, 209–214]. Some of the latter compounds contain a Cys residue whose side-chain may be interacting with the active site Zn(II) atom. All of these compounds are

Table 8.17. α-SUBSTITUTED C-TERMINAL HYDROXAMATE INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



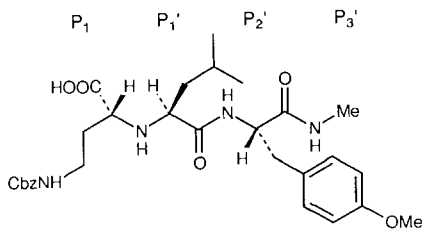
R = Me; HONHCO-((*S*)CHMe-L-Leu)-Tyr(OMe)-NHMe
(186)

	Inhibitor[a]					
No.	*R*	*P_1'*	*P_2'*	*P_3'*	*IC_{50} (μM)*[b]	*Ref.*
183	Me	HONHCO-((*S*)CHR-L-Leu)	Leu	Gly-OEt	0.02	1
184	PhtN$(CH_2)_4$	HONHCO-((*S*)CHR-L-Leu)	Leu	Gly-OEt	0.012	198
185	PhtN$(CH_2)_4$	HONHCO-((*S*)CHR-L-Leu)	Leu	Ala-OEt	0.005	1
186	Me	HONHCO-((*S*)CHR-L-Leu)	Tyr(OMe)	NHMe	0.005	196,199
187	Me	HONHCO-((*S*)CHR-D-Leu)	Tyr(OMe)	NHMe	>1	196,199
188	$HSCH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	0.003	200
189	$BzSCH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	1	200
190	$PhSCH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	0.008	201
191	$PhS(O)CH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	0.015	201
192	4-$HOPhSCH_2$	HONHCO-((*S*)CHR-L- Leu)	Phe	NHMe	0.010	201
193	4-t-$BuPhSCH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	0.004	201
194	2-$ThiSCH_2$	HONHCO-((*S*)CHR-L-Leu)	Phe	NHMe	0.006	201

[a]Abbreviations: Pht, phthaloyl; Thi, thiophene. [b]HSC was used.

Table 8.18. *N*-CARBOXYLALKYL AND *N*-PHOSPHONOALKYL INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



$CbzNH(CH_2)_2(R)CH(CO_2H)$-Leu-Tyr(OMe)-NHMe (197)

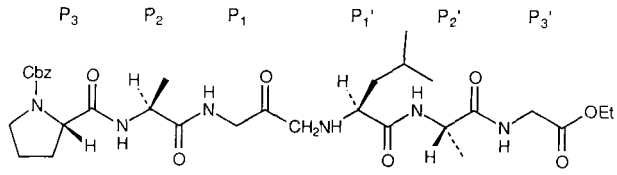
	Inhibitor[a]						
No.	P_1	P_1'	P_2'	P_3'	*Diaster.*[b]	IC_{50} {K_I} (μM)[c]	*Ref.*
195	$Pr(RS)CH(CO_2H)$	Leu	Phe	$Ala\text{-}NH_2$	mix	25[d]	161
195	$Pr(RS)CH(CO)_2H)$	Leu	Phe	$Ala\text{-}NH_2$	mix	1–10[e]	204
196	$CbzNH(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OH)	NHMe	–	1.2	205
197	$CbzNH(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OMe)	NHMe	–	0.81	205,206
197	$CbzNH(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OMe)	NHMe	–	{0.3}	207
198	$CbzNH(CH_2)_2(S)CH(CO_2H)$	Leu	Tyr(OMe)	NHMe	–	200	207
199	$CbzNH(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OBn)	NHMe	–	0.91	205

200	$CbzNH(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OPn)	NHMe	–	0.54	205
201	$MeS(CH_2)_2(R)CH(CO_2H)$	Leu	Tyr(OMe)	NHMe	–	5.5	206
202	$Me(R)CH(CO_2H)$	Leu	Tyr(OBn)	NHMe	–	1.7	206
203	$Me(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	1+2	0.51[f]	208
203	$Me(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	1+2	1[g]	208
204	$Me(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	3+4	>100[g]	208
205	$Me(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	1	1.2[g]	208
206	$Me(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	2	3.1[g]	208
207	$CH_2(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	mix	27[g]	208
208	$Et(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	1+2	0.33[f]	208
209	$Et(RS)CH(PO_3H_2)$	DL-Leu	Tyr(OMe)	NHMe	3+4	5.4[f]	208
210	$Me(RS)CH(PO_3H_2)$	DL-Leu	Trp	NHMe	1	0.30[f]	208
211	$Me(RS)CH(PO_3H_2)$	DL-Leu	Trp	NHMe	2	0.43[f]	208
211	$Me(RS)CH(PO_3H_2)$	DL-Leu	Trp	NHMe	2	0.69[g]	208
212	$Me(RS)CH(PO_3H_2)$	DL-Leu	Trp	NHMe	3+4	22[f]	208

[a]Abbreviations: Bn, benzyl; Pn, pentyl; Pr, propyl. [b]Diastereomers of unknown stereochemistry are characterized by chromatographic behaviour: 1 = fastest eluting ... 4 = slowest eluting diastereomer in reverse-phase chromatography. [c]HSC was used unless otherwise specified. [d]RCC was used. [e]PSC was used. [f]HFC was used. [g]RBC was used.

Table 8.19. MISCELLANEOUS INHIBITORS OF MAMMALIAN COLLAGENASES

Example:



Cbz-Pro-Ala-(Gly-$COCH_2$)-Leu-Ala-Gly-OEt
(213)

No.	*Inhibitor*[a]	*IC_{50} {K_I} (μM) or % Inhibition*[b]	*Ref.*
213	Cbz-Pro-Ala-(Gly-$COCH_2$)-Leu-Ala-Gly-OEt	{60}	209
214	Ac-Cys-Ala-Gly-Gln-D-Arg-NH_2	70[c]	176
215	Cys-Ala-Gly-Gln-D-Arg-NH_2	50[c]	176
216	Cbz-Glu-Leu-Ala-Gly-OH	450[c]	210
217	Cys-Ile-NH_2	35% @ 2 mM	211
218	pGlu-Cys-Ile-NH_2	75% @ 2 mM	211
219	Cpc-Gly-Pro-Glu-DL-Val(SH)-NH_2	86% @ 2 mM	212
220	Ac-Gly-Cys-Gly-Glu-Glu-NH_2	80% @ 2 mM	213
221	Boc-Gly-Leu-Pro-NH(n-$C_{12}H_{26}$)	44% @ 50 μM	214
222	Boc-Pro-Leu-Gly-NH(n-$C_{12}H_{26}$)	82% @ 50 μM	214
223	Boc-Val-NH(n-$C_{12}H_{26}$)	68% @ 50 μM	214
224	Boc-Val-Val-NH(n-$C_{12}H_{26}$)	100% @ 1 μM	214

[a]Abbreviations: Cpc, cyclopentylcarbonyl; pGlu, pyroglutamatic acid; Val(SH), β-mercaptovaline. [b]HFC was used unless otherwise specified. [c]TSC was used.

relatively ineffective collagenase inhibitors, with the exception of the highly hydrophobic dipeptide derivative (224), which appears to be quite potent; since the latter is not a substrate analogue, the reason for its effectiveness is not at all clear.

PHYSIOLOGICAL ASSAYS AND TESTS OF EFFICACY

A number of studies have used synthetic collagenase inhibitors in physiological assays with live tissue or whole animals for the purposes of either establishing the role of a collagenase in a physiological event, or as a test of the efficacy of the inhibitor. Since these represent two of the most important

goals of collagenase inhibitor development, we conclude this chapter by mentioning several of these reports.

The ability of collagenase inhibitors to reduce or prevent cartilage damage in arthritic conditions is, and will continue to be, a key testing ground for the efficacy of collagenase inhibitors. A SmithKline & French/British Biotechnology collaboration has recently investigated the anti-inflammatory and anti-arthritic effects of inhibitor BB16 (compound (186) in *Table 8.17*) using the less potent diastereomer BB15 (compound (187) in *Table 8.17*) as a control [199,215]. Intraperitonal injections of BB16 (0.6 to 50 mg/kg) significantly inhibited (>68%) hindpaw swelling of rats with arthritis induced by intradermal injections of Freund's complete adjuvant, while BB15 produced a similar effect only at much higher doses. These substrates did not inhibit eicosanoid production, suggesting that the beneficial effects were due instead to inhibition of one or more MMP. A number of other hydroxamate-type collagenase inhibitors have also been shown to reduce oedema in the adjuvant arthritic rat [201]. The arthritic lesions in the rats were evaluated radiographically and histologically to assess the degree of skeletal tissue loss. Moderate decreases in the severity of bone and cartilage loss paralleled the decrease in tissue swelling. Interestingly, parallel experiments with indomethacin showed that it reduced the oedema, but did not inhibit skeletal loss.

The rat air-pouch model was also used to assess *in vivo* collagen degradation by injecting collagen into preformed dorsal, subcutaneous air pouches and quantitating urinary hydroxyproline as a measure of collagen breakdown. Intraperitoneal injection of BB16 (100 mg/kg) before (1 h) and after (5 h) collagen injection caused a moderate inhibition of the elevation in urinary hydroxyproline concentrations, while BB15 had no effect. These observations are consistent with an anti-arthritic effect of BB16 related to its ability to inhibit one or both collagenases or another MMP. In a somewhat related study, the daily intraperitoneal injection of 2 mg of recombinant human TIMP-1 has been shown to reduce the incidence of joint erosion in type II collagen-induced arthritic mice [216]. While not a synthetic inhibitor, TIMP-1 is a potent protein (28.5 kDa) inhibitor of all of the MMP, and this observation supports a role for one or more of these enzymes in this arthritic model. Other studies have used TIMP or increased TIMP expression to implicate MMP in tumour cell invasion [217–220].

In another study, a group at Roche investigated the effect of two collagenase inhibitors on the degradation of both collagen and proteoglycan in interleukin-1-α-stimulated bovine nasal cartilage slices [171]. Ro 31-4724 (compound (180) in *Table 8.16*) is a hydroxamate inhibitor that inhibits fibroblast-type collagenase, stromelysin and gelatinase almost equally, while

Ro 31-7467 (compound (79) in *Table 8.9*) is a phosphinate fibroblast-type collagenase inhibitor that is at least 10-fold less potent against stromelysin and gelatinase. Explants of bovine nasal cartilage exhibit a dose-dependent release first of proteoglycan followed by hydroxyproline on stimulation by interleukin-1-α. Explants cultured in the presence of 0.3–100 μM Ro 31-7467 for up to 30 days showed a marked dose-dependent inhibition of collagen degradation, while the reduction in proteoglycan release over 12 days was minimal. In contrast, low micromolar concentrations of Ro 31-4724 inhibited both proteoglycan and collagen release in this system. These results are consistent with the view that fibroblast-type collagenase is responsible for the loss in cartilage collagen in this model, while a different MMP is responsible for the loss of proteoglycan.

Tetracycline antibiotics have at times been prescribed for the treatment of rheumatoid arthritis. Although the efficacy of this treatment has been questioned [221], there is some evidence that tetracyclines are effective in treating rheumatoid arthritis and that this is associated with the inhibition of collagenases. Several tetracyclines have been shown to inhibit mammalian collagenases *in vitro* [161]. In one *in vivo* study, synovial tissue was collected from 7 patients undergoing bilateral total joint replacements before and after twice-daily oral administration of 100 mg of minocycline (compound (60) in *Table 8.7*) [222]. Relative to the pretreatment values, the post-minocycline trypsin-activated collagenase activities of the tissue samples were 67% lower. A more recent study of 10 patients receiving 200–400 mg daily of oral minocycline reported a significant improvement in several efficacy variables over pretreatment values [223]. The therapeutic properties of tetracycline antibiotics in the treatment of periodontal disease has also been attributed to their collagenase inhibitory properties [224–228]. The efficacy of these substances had generally been attributed to their ability to suppress micro-organisms believed to be periodontal pathogens. However, their administration has also been shown to reduce the collagenase activity in the gingival and crevicular fluids of patients with periodontitis, implying that this is at least a partial basis for their efficacy.

A role for a collagenase, presumably fibroblast-type collagenase, in bone resorption has been indicated by studies employing the Searle *N*-carboxyl alkyl synthetic collagenase inhibitor CI-1 (compound (197) in *Table 8.18*) and its less potent stereoisomer CI-2 (compound (198) in *Table 8.18*) [207]. Cultured embryonic mouse calvaria treated with parathyroid hormone exhibit loss of calcium and show pronounced collagen resorption. CI-1 inhibited the collagen resorption in a dose-dependent manner at significantly lower concentrations than CI-2, but had only a small effect on calcium loss. This inhibitory effect was reversible and not due to inhibitor cytotoxicity.

Several tetracyclines with collagenase inhibitory activity have also been shown to inhibit parathyroid-induced bone resorption in organ culture [229]. Subsequent experiments with CI-1 have reinforced the view that a collagenase is involved in bone resorption and, in fact, the resorption of demineralized collagen by collagenase may be the rate-limiting step [230,231].

Collagenase inhibitors have also been used to implicate collagenases in a number of other physiological states characterized by collagen catabolism. The involvement of a collagenase in the ulceration following alkali-induced corneal burns has been investigated in rabbits [232]. The fate of 10 untreated controls was compared with 10 animals treated topically six times daily and subconjunctivally once daily with a 1 mM solution of a mercaptan collagenase inhibitor (compound (99) in *Table 8.10*). All of the control eyes developed ulcers and 10 progressed to deep ulcers. In the treated group, 4 eyes developed ulcers and only one a deep ulcer. None of the treated eyes, but 7 of 10 control eyes progressed to perforation. Since this inhibitor is active against most of the MMP including both fibroblast- and neutrophil-type collagenases, no definitive conclusion regarding the involvement of a particular MMP can be drawn. Tetracyclines with collagenase inhibitory properties have also been shown to be effective in the treatment of alkali-induced corneal burns [233], as well as in the corneal degeneration associated with epithelial defects [234] and infectious keratitis induced by *Pseudomonas aeruginosa* [235]. Inhibitory tetracyclines may also be effective in the treatment of epidermolysis bullosa [236,237]. Finally, the Searle inhibitor CI-1 has been shown to suppress ovulation in the perfused rat ovary [113].

CONCLUDING COMMENTS

The recognition that the members of the MMP gene family are intimately involved in the turnover of various components of the extracellular matrix has greatly stimulated interest in virtually every aspect of these enzymes. The interstitial collagenases were the first members of this family to be discovered and are the only two human enzymes capable of catabolizing interstitial collagens at an appreciable rate under physiological conditions. Since collagen is the most abundant protein in humans and its breakdown in a variety of pathological conditions has profound negative consequences, the development of collagenase inhibitors is currently a high priority in many pharmaceutical companies and academic centres. This is evidenced by the rapid increase in new patents and reports of new inhibitors, as described herein. Despite this burst of current activity, however, collagenase inhibitor development is still in its early stages. From the therapeutic viewpoint, the

key issues that remain are potency, selectivity and bioavailability. Thus, no subnanomolar collagenase inhibitors have yet been reported, and the issue of selectivity is just beginning to be addressed. Moreover, the important task of establishing efficacy in experiments at the tissue or whole animal level requires pharmokinetic studies that are still in their initial stages. Once these issues are resolved, specific potent inhibitors of collagenases and the other MMP will be critical tools in elucidating the role of these enzymes *in vivo* and, hopefully, these substances will fulfil their potential as therapeutic agents toward a variety of human diseases.

ACKNOWLEDGEMENT

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REFERENCES

1 Johnson, W.H., Roberts, N.A. and Borkakoti, N. (1987) J. Enzyme Inhib. 2, 1–22.
2 Hay, E.D. (1981) Cell Biology of the Extracellular Matrix, Plenum Press, New York.
3 Van Wart, H.E. and Mookhtiar, K.A. (1990) in Biological Response Modifiers for Tissue Repair (Grotendorst, G., Hjelmeland, L.M. and Gills, J.P., eds.), pp. 23–50, Portfolio, The Woodlands, TX.
4 Miller, E.J. and Gay, S. (1982) Methods Enzymol. 82, 3–32.
5 Miller, E.J. and Gay, S. (1987) Methods Enzymol. 144, 3–41.
6 Piez, K.A. (1984) in Extracellular Matrix Biochemistry (Piez, K.A. and Reddi, A.H., eds.), pp. 1–39, Elsevier, New York.
7 Fasman, G.D. (1977) Handbook of Biochemistry and Molecular Biology, Proteins – Vol. III, 3rd Edn., pp. 474–489, CRC Press, Cleveland.
8 Seyer, J.M., Hasty, K.A. and Kang, A.H. (1989) Eur. J. Biochem. 181, 159–173.
9 Glanville, R.W. (1987) in Structure and Function of Collagen Types (Mayne, R. and Burgeson, R.E., eds.), pp. 43–79, Academic Press, New York.
10 Birkedal-Hansen, H., Taylor, R.E., Bhown, A.S., Katz, J., Lin, H.-Y. and Wells, B.R. (1985) J. Biol. Chem. 260, 16411–16417.
11 Seifter, S. and Harper, E. (1971) in The Enzymes (Boyer, P.D., ed.), 3rd Edn., pp. 649–697, Academic Press, New York.
12 Tong, N.T., Tsugita, A. and Keil-Dlouha, V. (1986) Biochim. Biophys. Acta 874, 296–304.
13 Welton, R.L. and Woods, D.R. (1975) Biochim. Biophys. Acta 384, 228–234.
14 Bond, M.D. and Van Wart, H.E. (1984) Biochemistry 23, 3077–3085.
15 Van Wart, H.E. and Steinbrink, D.R. (1985) Biochemistry 24, 6520–6526.
16 Bond, M.D. and Van Wart, H.E. (1984) Biochemistry 23, 3085–3091.
17 Bond, M.D. and Van Wart, H.E. (1984) Biochemistry 23, 3092–3099.
18 Steinbrink, D.R., Bond, M.D. and Van Wart, H.E. (1985) J. Biol. Chem. 260, 2771–2776.
19 Mookhtiar, K.A., Steinbrink, D.R. and Van Wart, H.E. (1985) Biochemistry 24, 6527–6533.

20 Mookhtiar, K.A., Grobelny, D., Galardy, R.E. and Van Wart, H.E. (1988) Biochemistry 27, 4299–4304.
21 Angleton, E.L. and Van Wart, H.E. (1988) Biochemistry 27, 7406–7412.
22 Angleton, E.L. and Van Wart, H.E. (1988) Biochemistry 27, 7413–7418.
23 French, M.F., Mookhtiar, K.A. and Van Wart, H.E. (1987) Biochemistry 26, 681–687.
24 Schechter, I. and Berger, A. (1967) Biochem. Biophys. Res. Commun. 27, 157–162.
25 Mandl, I., Keller, S. and Manahan, J. (1964) Biochemistry 3, 1737–1741.
26 Mallya, S.K., Mookhtiar, K.A., Gao, Y., Brew, K., Dioszegi, M., Birkedal-Hansen, H. and Van Wart, H.E. (1990) Biochemistry 29, 10628–10634.
27 Goldberg, G.I., Wilhelm, S.M., Kronberger, A., Bauer, E.A., Grant, G.A. and Eisen, A.Z. (1986) J. Biol. Chem. 261, 6600–6605.
28 Knauper, V., Kramer, S., Reinke, H. and Tschesche, H. (1990) Eur. J. Biochem. 189, 295–300.
29 Devarajan, P., Mookhtiar, K.A., Van Wart, H.E. and Berliner, N. (1991) Blood, 77, 2731–2738.
30 Hasty, K.A., Pourmotabbed, T.F., Goldberg, G.I., Thompson, J.P., Spinella, D.G., Stevens, R.M. and Mainardi, C.L. (1990) J. Biol. Chem. 265, 11421–11424.
31 Collier, I.E., Wilhelm, S.M., Eisen, A.Z., Marmer, B.L., Grant, G.A., Seltzer, J.L., Kronberger, A., He, C., Bauer, E.A. and Goldberg, G.I. (1988) J. Biol. Chem. 263, 6579–6587.
32 Wilhelm, S.M., Collier, I.E., Marmer, B.L., Eisen, A.Z., Grant, G.A. and Goldberg, G.I. (1989) J. Biol. Chem. 264, 17213–17221.
33 Wilhelm, S.M., Collier, I.E., Kronberger, A., Eisen, A.Z., Marmer, B.L., Grant, G.A., Bauer, E.A. and Goldberg, G.I. (1987) Proc. Natl. Acad. Sci. USA 84, 6725–6729.
34 Whitham, S.E., Murphy, G., Angel, P., Rahmsdorf, H.-J., Smith, B.J., Lyons, A., Harris, T.J.R., Reynolds, J.J., Herrlich, P. and Docherty, A.J.P. (1986) Biochem. J. 240, 913–916.
35 Saus, J., Quinones, S., Otani, Y., Nagase, H., Harris, E.D., Jr. and Krukinen, M. (1988) J. Biol. Chem. 263, 6742–6745.
36 Muller, D., Quantin, B., Gesnel, M.-C., Millon-Collard, R., Abecassis, J. and Breathnach, R. (1988) Biochem. J. 253, 187–192.
37 Basset, P., Bellocq, J.P., Wolf, C., Stoll, I., Hutin, P., Limacher, J.M., Podhajcer, O.L., Chenard, M.P., Rio, M.C. and Chambon, P. (1990) Nature (London) 348, 699–704.
38 Quinn, C.O., Scott, D.K., Brinckerhoff, C.E., Matrisian, L.M., Jeffrey, J.J. and Partridge, N.C. (1990) J. Biol. Chem. 265, 22342–22347.
39 Clarke, N.J., O'Hare, M.C., Cawston, T.E. and Harper, G.P. (1990) Nucleic Acids Res. 18, 6703.
40 Fini, M.E., Plucinska, I.M., Mayer, A.S., Gross, R.H. and Brinckerhoff, C.E. (1987) Biochemistry 26, 6156–6165.
41 Fini, M.E., Karmilowicz, M.J., Ruby, P.L., Beeman, A.M., Borges, K.A. and Brinckerhoff, C.E. (1987) Arthritis Rheum. 30, 1254–1264.
42 Matrisian, L.M., Glaichenhaus, N., Gesnel, M.-C. and Breathnach, R. (1985) EMBO J. 4, 1435–1440.
43 Breathnach, R., Matrisian, L.M., Gesnel, M.-C., Staub, A. and Leroy, P. (1987) Nucleic Acids Res. 15, 1139–1151.
44 Okada, Y., Nagase, H. and Harris, E.D., Jr. (1986) J. Biol. Chem. 261, 14245–14255.
45 Nagase, H., Barrett, A.J. and Woessner, J.F., Jr. (1990) in Proceedings of the Matrix Metalloproteinase Conference (Birkedal-Hansen, H., Van Wart, H.E., Wlegus, H. and Werb, Z., eds.), Matrix, in press.
46 Gross, J. and Lapiere, C.M. (1962) Proc. Natl. Acad. Sci. USA 48, 1014–1022.

47 Gross, J. and Nagai, Y. (1965) Proc. Natl. Acad. Sci. USA 54, 1197–1204.
48 Seltzer, J.L., Eisen, A.Z., Bauer, E.A., Morris, N.P., Glanville, R.W. and Burgeson, R.E. (1989) J. Biol. Chem. 264, 3822–3826.
49 Schmid, T.M., Mayne, R., Jeffrey, J.J. and Linsenmayer, T.F. (1986) J. Biol. Chem. 261, 4184–4189.
50 Gadher, S.J., Eyre, D.R., Duance, V.C., Wotton, S.F., Heck, L.W., Schmid, T.M. and Woolley, D.E. (1988) Eur. J. Biochem. 175, 1–7.
51 Gadher, S.J., Schmid, T.M., Heck, L.W. and Woolley, D.E. (1989) Matrix 9, 109–115.
52 Salo, T., Liotta, L.A. and Tryggvason, K. (1983) J. Biol. Chem. 258, 3058–3063.
53 Okada, Y., Morodomi, T., Enghild, J.J., Suzuki, K., Yasui, A., Nakanishi, I., Salvesen, G. and Nagase, H. (1990) Eur. J. Biochem., 194, 727–730.
54 Takahashi, H., Nukiwa, T., Basset, P. and Crystal, R.G. (1988) J. Biol. Chem. 263, 2543–2547.
55 Chin, J.R., Murphy, G. and Werb, Z. (1985) J. Biol. Chem. 260, 12367–12376.
56 Okada, Y., Konomi, H., Yada, T., Kimata, K. and Nagase, H. (1989) FEBS Lett. 244, 473–476.
57 Woolley, D.E. (1984) in Ref. 6, pp. 119–157.
58 Harris, E.D., Jr. and Cartwright, E.C. (1977) in Proteinases in Mammalian Cells and Tissues, (Barrett, A.J., ed.), pp. 249–283, Elsevier, Amsterdam.
59 Brinckerhoff, C.E., Ruby, P.L., Austin, S.D., Fini, M.E. and White, H.D. (1987) J. Clin. Invest. 79, 542–546.
60 Campbell, E.J., Cury, J.D., Lazarus, C.J. and Welgus, H.G. (1987) J. Biol. Chem. 262, 15862–15868.
61 Lin, H.-Y., Wells, B.R., Taylor, R.E. and Birkedal-Hansen, H. (1987) J. Biol. Chem. 262, 6823–6831.
62 Otsuka, K., Sodek, J. and Limeback, H. (1984) Eur. J. Biochem. 145, 123–129.
63 Enghild, J.J., Salvesen, G., Brew, K. and Nagase, H. (1989) J. Biol. Chem. 264, 8779–8785.
64 Welgus, H.G., Jeffrey, J.J., Stricklin, G.P and Eisen, A.Z. (1982) J. Biol. Chem. 257, 11534–11539.
65 Welgus, H.G., Fliszar, C.J., Seltzer, J.L., Schmid, T.M. and Jeffrey, J.J. (1990) J. Biol. Chem. 265, 13521–13527.
66 Welgus, H.G., Jeffrey, J.J. and Eisen, A.Z. (1981) J. Biol. Chem. 256, 9511–9515.
67 Sottrup-Jensen, L. and Birkedal-Hansen, H. (1989) J. Biol. Chem. 264, 393–401.
68 Fields, G.B., Netzel-Arnett, S.J., Windsor, L.J., Engler, J.A., Birkedal-Hansen, H. and Van Wart, H.E. (1990) Biochemistry 29, 6670–6677.
69 Stricklin, G.P., Bauer, E.A., Jeffrey, J.J. and Eisen, A.Z. (1977) Biochemistry 16, 1607–1615.
70 Petersen, M.J., Woodley, D.T., Stricklin, G.P. and O'Keefe, E.J. (1987) J. Biol. Chem. 262, 835–840.
71 Welgus, H.G., Campbell, E.J., Bar-Shavit, Z., Senior, R.M. and Teitelbaum, S.L. (1985) J. Clin. Invest. 76, 219–224.
72 Welgus, H.G., Connolly, N.L. and Senior, R.M. (1986) J. Clin. Invest. 77, 1675–1681.
73 Moscatelli, D., Jaffe, E. and Rifkin, D.B. (1980) Cell 20, 343–351.
74 Horwitz, A.L., Hance, A.J. and Crystal, R.G. (1977) Proc. Natl. Acad. Sci. USA 74, 897–901.
75 Hasty, K.A., Jeffrey, J.J., Hibbs, M.S. and Welgus, H.G. (1987) J. Biol. Chem. 262, 10048–10052.
76 Desrochers, P.E. and Weiss, S.J. (1988) J. Clin. Invest. 81, 1646–1650.
77 Vissers, M.C.M., George, P.M., Bathurst, I.C., Brennan, O. and Winterbourn, C.C. (1988) J. Clin. Invest. 82, 706–711.

78 Knauper, V., Reinke, H. and Tschesche, H. (1990) FEBS Lett. 263, 355–357.
79 Mookhtiar, K.A. and Van Wart, H.E. (1990) Biochemistry 29, 10620–10627.
80 Hibbs, M.S., Hasty, K.A., Seyer, J.M., Kang, A.H. and Mainardi, C.L. (1985) J. Biol. Chem. 260, 2493–2500.
81 Emonard, H. and Grimaud, J.-A. (1990) Cell. Mol. Biol. 36, 131–153.
82 Woessner, J.F., Jr. (1991) FASEB J. 5, 2145–2154.
83 Birkedal-Hansen, H. (1987) Methods Enzymol. 144, 140–171.
84 Mookhtiar, K.A., Wang, F. and Van Wart, H.E. (1986) Arch. Biochem. Biophys. 246, 645–649.
85 Matrisian, L.M., Leroy, P., Ruhlmann, C., Gesnel, M.-C. and Breathnach, R. (1986) Mol. Cell Biol. 6, 1679–1686.
86 Sanchez-Lopez, R., Nicholson, R., Gesnel, M.-C., Matrisian, L.M. and Breathnach, R. (1988) J. Biol. Chem. 263, 11892–11899.
87 Birkedal-Hansen, B., Moore, W.G.I., Taylor, R.E., Bhown, A.S. and Birkedal-Hansen, H. (1988) Biochemistry 27, 6751–6758.
88 Stricklin, G.P., Jeffrey, J.J., Roswit, W.T. and Eisen, A.Z. (1983) Biochemistry 22, 61–68.
89 Wilhelm, S.M., Eisen, A.Z., Teter, M., Clark, S.D., Kronberger, A. and Goldberg, G.A. (1986) Proc. Natl. Acad. Sci. USA 83, 3756–3760.
90 Weiss, S.J., Peppin, G., Ortiz, X., Ragsdale, C. and Test, S.T. (1985) Science (Washington) 227, 747–749.
91 Gross, J., Harper, E., Harris, E.D., Jr., McCroskery, P.A., Highberger, J.H., Corbett, C. and Kang, A.H. (1974) Biochem. Biophys. Res. Commun. 61, 605–612.
92 Miller, E.J., Harris, E.D., Jr., Chung, E., Finch, J.E., Jr., McCroskery, P.A. and Butler, W.T. (1976) Biochemistry 15, 787–792.
93 Fields, G.B., Van Wart, H.E. and Birkedal-Hansen, H. (1987) J. Biol. Chem. 262, 6221–6226.
94 Netzel-Arnett, S.J., Fields, G.B., Birkedal-Hansen, H. and Van Wart, H.E. (1991) J. Biol. Chem. 266, 6747–6755.
95 Springman, E.B., Angleton, E.L., Birkedal-Hansen, H. and Van Wart, H.E. (1990) Proc. Natl. Acad. Sci. USA 87, 364–368.
96 Van Wart, H.E. and Birkedal-Hansen, H. (1990) Proc. Natl. Acad. Sci. USA 87, 5578–5582.
97 Murphy, G., Cockett, M.I., Stephens, P.E., Smith, B.J. and Docherty, A.J.P. (1987) Biochem. J. 248, 265–268.
98 Ito, A. and Nagase, H. (1988) Arch. Biochem. Biophys. 267, 211–216.
99 Nagase, H., in Ref. 45, in press.
100 Mookhtiar, K.A., Mallya, S.K. and Van Wart, H.E. (1986) Anal. Biochem. 158, 322–333.
101 Mallya, S.K., Mookhtiar, K.A. and Van Wart, H.E. (1986) Anal. Biochem. 158, 334–345.
102 Weingarten, H. and Feder, J. (1985) Anal. Biochem. 147, 437–440.
103 Weingarten, H., Martin, R. and Feder, J. (1985) Biochemistry 24, 6730–6734.
104 Stack, M.S. and Gray, R.D. (1989) J. Biol. Chem. 264, 4277–4281.
105 Netzel-Arnett, S., Mallya, S.K., Nagase, H., Birkedal-Hansen, H. and Van Wart, H.E. (1991) Anal. Biochem., 195, 86–92.
106 Grillo, H.C. and Gross, J. (1967) Dev. Biol. 15, 300–317.
107 Grillo, H.C., McLennan, J.E. and Wolfort, F.G. (1969) in Repair and Regeneration: The Scientific Basis of Surgical Practice (Dunphy, J.E., ed.), pp. 185–197, McGraw-Hill, New York.

108 Ryan, J.N. and Woessner, J.F., Jr. (1971) Biochem. Biophys. Res. Commun. 44, 144–149.
109 Jeffrey, J.J. and Gross, J. (1970) Biochemistry 9, 268–273.
110 Lee, A. and Langer, R. (1983) Science (Washington, DC) 221, 1185–1187.
111 Langer, R., Conn, H., Vacanti, J., Haudenschild, C. and Folkman, J. (1980) Proc. Natl. Acad. Sci. USA 77, 4331–4335.
112 Rifkin, D.B., Gross, J.L., Moscatelli, D. and Jaffe, E. (1982) in Pathobiology of the Endothelial Cell (Nossel, M.L. and Vogel, H.J., eds.), pp. 191–197, Academic Press, New York.
113 Brannstrom, M., Woessner, J.F., Jr., Koos, R.D., Sear, C.H.J. and LeMaire, W.J. (1988) Endocrinology 122, 1715–1721.
114 Woessner, J.F., Jr. and Cahill, D.R. (1974) Arch. Oral. Biol. 19, 1195–1201.
115 Harris, E.D., Jr., Cohen, G.L. and Krane, S.M. (1969) Arthritis Rheum. 12, 92–102.
116 Harris, E.D., Jr., Evanson, J.M., DiBona, D.R. and Krane, S.M. (1970) Arthritis Rheum. 13, 83–95.
117 Woolley, D.E., Crossley, M.J. and Evanson, J.M. (1977) Arthritis Rheum. 20, 1231–1239.
118 Krane, S.M. (1981) Ann. Rheum. Dis. 40, 433–448.
119 Robertson, P.B. and Simpson, J. (1976) J. Periodontol. 47, 29–33.
120 Birkedal-Hansen, H. (1980) in Collagenases in Normal and Pathological Connective Tissue (Woolley, D.E. and Evanson, J.M., eds.), pp. 127–140, Wiley and Sons, New York.
121 Birkedal-Hansen, H., Taylor, R.E., Zambon, J.J., Barwa, P.K. and Neiders, M.E. (1988) J. Periodont. Res. 23, 258–264.
122 Vaes, G. (1980) in Ref. 120, pp. 185–207.
123 Gardner, B., Wallach, S., Gray, H. and Baker, R.K. (1971) Surg. Forum 22, 435–437.
124 Abramson, M. (1969) Ann. Otol. Rhinol. Laryngol. 78, 112–124.
125 Sakamoto, S., Sakamoto, M., Goldhaber, P. and Glimcher, M.J. (1975) Biochem. Biophys. Res. Commun. 63, 172–177.
126 Griffith, G.C., Nichols, G., Jr., Asher, J.D. and Flanagan, B. (1965) J. Am. Med. Assoc. 193, 91–94.
127 Eeckhout, Y., Delaisse, J.-M. and Vaes, G. (1986) Biochem. J. 239, 793–796.
128 Heath, J.K., Atkinson, S.J., Meikle, M.C. and Reynolds, J.J. (1984) Biochim. Biophys. Acta 802, 151–154.
129 Sakamoto, S. and Sakamoto, M. (1984) Biomed. Res. 5, 39–46.
130 Strauli, P., Barrett, A.J. and Baici, A. (1980) Proteinases and Tumor Invasion, Raven Press, New York.
131 Liotta, L.A., Garbisa, S. and Tryggvason, K. (1991) Tumor Invation and Metastasis, pp. 319–333, Martinus Nijhoff, Dordrecht.
132 Blood, C.H. and Zetter, B.R. (1990) Biochim. Biophys. Acta 1032, 89–118.
133 Liotta, L.A., Rao, C.N. and Barsky, S.H. (1983) Lab. Invest. 49, 636–649.
134 Liotta, L.A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C.M. and Shafie, S. (1980) Nature (London) 284, 67–68.
135 Brown, S.I., Weller, C.A. and Wasserman, H.E. (1969) Arch. Ophthalmol. 81, 370–373.
136 Berman, M.B. (1980) in Ref. 120, pp. 141–174.
137 Eisen, A.Z. (1969) J. Invest. Dermatol. 52, 449–453.
138 Ondetti, M.A., Rubin, B. and Cushman, D.W. (1977) Science (Washington, DC) 196, 441–444.
139 Ondetti, M., Williams, N.J., Sabo, E.F., Pluscec, J., Weaver, E.R. and Kocy, O. (1971) Biochemistry 10, 4033–4039.

140 Ondetti, M.A. and Cushman, D.W. (1982) Annu. Rev. Biochem. 51, 283–308.
141 Matthews, B.W. (1988) Acc. Chem. Res. 21, 333–340.
142 Lloyd, L.F., Skarzynski, T., Wonacott, A.J., Cawston, T.E., Clark, I.M., Mannix, C.J. and Harper, G.P. (1989) J. Mol. Biol. 210, 237–238.
143 Borkakoti, N., Broadhurst, M.J., Brown, P.A., Handa, B.K., Johnson, W.H., Lawton, G., Machin, P.J. and Roberts, N.A. (1988) Peptides as Targets for Drug Research Meeting, University of York, July, 1988.
144 Dive, V., Yiotakis, A. and Fermandjian, S. (1986) Ann. Biol. Clin. 44, 176–180.
145 Yiotakis, A. and Dive, V. (1986) Eur. J. Biochem. 160, 413–418.
146 Yiotakis, A., Hatgiyannacou, A., Dive, V. and Toma, F. (1988) Eur. J. Biochem. 172, 761–766.
147 Vencill, C.F., Rasnick, D., Crumley, K.V., Nishino, N. and Powers, J.C. (1985) Biochemistry 24, 3149–3157.
148 Grobelny, D. and Galardy, R.E. (1985) Biochemistry 24, 6145–6152.
149 Grobelny, D., Teater, C. and Galardy, R.E. (1989) Biochem. Biophys. Res. Commun. 159, 426–431.
150 Galardy, R.E. and Grobelny, D. (1983) Biochemistry 22, 4556–4561.
151 Dive, V., Yiotakis, A., Nicolaou, A. and Toma, F. (1990) Eur. J. Biochem. 191, 685–693.
152 Siffert, O. and Pasquet, M. (1977) Chem. Pharm. Bull. 25, 2763–2767.
153 Toma, F., Dive, V. and Yiotakis, A. (1988) in Pept.: Chem. Biol., Proc. Am. Pept. Symp. 10th, (Marshall, G.R. ed.), pp. 87–89, ESCOM, Leiden.
154 Galardy, R.E. and Grobelny, D. (1985) U.S. Patent 4558034; (1986) Chem. Abstr. 105, 6826.
155 Grant, N.H. and Clark, D.E. (1982) U.S. Patent 4361574; (1983) Chem. Abstr. 98, 101200.
156 Clark, D.E. and Grant, N.H. (1983) U.S. Patent 4367233; (1983) Chem. Abstr. 99, 133651.
157 Clark, D.E. and Grant, N.H. (1986) U.S. Patent 4609667; (1987) Chem. Abstr. 106, 607.
158 Clark, D.E., Wei, P. and Grant, N.H. (1985) Life Sci. 37, 575–578.
159 Conrow, R.B. and Bernstein, S. (1981) U.S. Patent 4275076; (1981) Chem. Abstr. 95, 150269.
160 Siuta, G.J. and Bernstein, S. (1983) Eur. Patent 70972; (1983) Chem. Abstr. 99, 53410.
161 Burns, F.R., Stack, M.S., Gray, R.D. and Paterson, C.A. (1989) Invest. Ophthalmol. Vis. Sci. 30, 1569–1575.
162 Poletto, J.F. and Bernstein, S. (1986) U.S. Patent 4599204; (1986) Chem. Abstr. 105, 172071.
163 Quadro, G. (1990) Eur. Patent 348541; (1990) Chem. Abstr. 113, 23895.
164 Bernstein, S. (1981) U.S. Patent 4297372.
165 Oda, M., Ino, Y., Nakamura, K., Kuramoto, S., Shinamura, K., Iwaki, M. and Fujii, S. (1990) Jpn. J. Pharmacol. 52, 23–34.
166 Kortylewicz, Z.P. and Galardy, R.E. (1990) J. Med. Chem. 33, 263–273.
167 Galardy, R.E., Grobelny, D., Kortylewicz, Z.P. and Poncz, L., in Ref. 45, in press.
168 Kortylewicz, Z.P. and Galardy, R.E. (1989) J. Enzyme Inhib. 3, 159–162.
169 Mookhtiar, K.A., Marlowe, C.K., Bartlett, P.A. and Van Wart, H.E. (1987) Biochemistry 26, 1962–1965.
170 Broadhurst, M.J., Handa, B.K., Johnson, W.H., Lawton, G. and Machin, P.J. (1988) Eur. Patent 276436; (1988) Chem. Abstr. 110, 24085.
171 Marley, J., Bottomley, K.M.K., Broadhurst, M.J., Brown, P.A., Johnson, W.H., Lawton, G., Nixon, J.S., Sedgwick, A.D. and Wilkinson, S.E. (1990) Abstract: Cell Biology

of Cartilage and Bone Meeting of the British Connective Tissue Society, Christ Church, Oxford.
172 Gray, R.D., Miller, R.B. and Spatola, A.F. (1986) J. Cell. Biochem. 32, 71–77.
173 Darlak, K., Miller, R.B., Stack, M.S., Spatola, A.F. and Gray, R.D. (1990) J. Biol. Chem. 265, 5199–5205.
174 Schwartz, M.A., Venkataraman, S., Ghaffari, M.A., Libby, A., Mookhtiar, K.A., Mallya, S.K., Birkedal-Hansen, H. and Van Wart, H.E. (1991) Biochem. Biophys. Res. Commun. 176, 173–179.
175 Donald, D.K., Hann, M.M., Saunders, J. and Wadsworth, H.J. (1986) U.S. Patent 4595700; (1986) Chem. Abstr. 105, 209393.
176 Gray, R.D., Saneii, H.H. and Spatola, A.F. (1981) Biochem. Biophys. Res. Commun. 101, 1251–1258.
177 Hughes, I. (1990) Eur. Patent 358305; (1990) Chem. Abstr. 113, 132810.
178 Markwell, R.E., Smith, S.A. and Gaster, L.M. (1989) Eur. Patent 322184; (1990) Chem. Abstr. 112, 36457.
179 Markwell, R.E., Smith, S.A. and Hughes, I. (1988) Eur. Patent 273689; (1989) Chem. Abstr. 110, 232070.
180 Gray, R.D., Spatola, A.F. and Miller, R.B. (1988) World Patent 8806890; (1989) Chem. Abstr. 110, 173673.
181 Sundeen, J.E. and Dejneka, T. (1982) Eur. Patent 67429; (1983) Chem. Abstr. 98, 179923.
182 Sundeen, J.E. and Dejneka, T. (1982) U.S. Patent 4327111.
183 Sundeen, J.E. and Dejneka, T. (1981) U.S. Patent 4297275; (1982) Chem. Abstr. 96, 104789.
184 Sundeen, J.E. and Dejneka, T. (1981) U.S. Patent 4263293; (1981) Chem. Abstr. 95, 61542.
185 Sundeen, J.E. and Dejneka, T. (1980) U.S. Patent 4235885; (1981) Chem. Abstr. 94, 103838.
186 Moore, W.M. and Spilburg, C.A. (1986) Biochem. Biophys. Res. Commun. 136, 390–395.
187 Spilburg, C.A. and Moore, W.M. (1987) U.S. Patent 4687841; (1988) Chem. Abstr. 108, 150971.
188 Odake, S., Okayama, T., Obata, M., Morikawa, T., Hattori, S., Hori, H. and Nagai, Y. (1990) Chem. Pharm. Bull. 38, 1007–1011.
189 Odake, S., Okayama, T., Obata, M., Morikawa, T. and Nagai, Y. (1989) World Patent 8905819; (1990) Chem. Abstr. 112, 7933.
190 Odake, S., Okayama, T., Obata, M., Morikawa, T., Hattori, S., Hori, H. and Nagai, Y. (1991) Chem. Pharm. Bull., in press.
191 Faucher, D.C., Lelievre, Y. and Cartwright, T. (1987) J. Antibiot. 40, 1757–1761.
192 Bouboutou, R., Lelievre, Y., Boziau, J. and Cartwright, T. (1989) in Second Forum on Peptides (Aubry, A., Marraud, M. and Vitoux, B., eds.) 174, 341–344.
193 Lelievre, Y., Bouboutou, R., Boiziau, J. and Cartwright, T. (1989) Pathologie Biologie 37, 43–46.
194 Lelièvre, Y., Bouboutou, R., Boioziau, J., Faucher, D., Achard, D. and Cartwright, T. (1990) Matrix 10, 292–299.
195 Cartwright, T., Bouboutou-Tello, R., Lelievre, Y. and and Fournie-Zaluski, M.C. (1988) Eur. Patent 274453; (1989) Chem. Abstr. 110, 193394.
196 Dickens, J.P., Donald, D.K., Kneen, G. and McKay, W.R. (1987) Eur. Patent 214639; (1987) Chem. Abstr. 106, 214381.
197 Davidson, A.H., Dickens, J.P. and Crimmin, M.J. (1990) World Patent 9005716; (1990) Chem. Abstr. 113, 190468.

198 Handa, B.K., Johnson, W.H. and Machin, P.J. (1987) Eur. Patent 236872; (1988) Chem. Abstr. 108, 167973.
199 DiMartino, M.J., Wolff, C.E., Marshall, P.J., Mong, S., Crimmin, M.J. and Galloway, W.A. (1990) Abstract: Inflammation Research Association, 5th International Conference, White Haven, Pennsylvania.
200 Campion, C., Davidson, A.H., Dickens, J.P. and Crimmin, M.J. (1990) World Patent 9005719; (1990) Chem. Abstr. 113, 212677.
201 Crimmin, M.J., Galloway, W.A. and DiMartino, M.J., in Ref. 199.
202 Kotake, S., Okayama, T., Obata, M., Morikawa, T. and Nagai, Y. (1989) Jpn. Patent 1146896; (1989) Chem. Abstr. 111, 233688.
203 Dickens, J.P., Donald, D.K., Kneen, G. and McKay, W.R. (1986) U.S. Patent 4599361; (1986) Chem. Abstr. 105, 153550.
204 Gray, R.D., Darlak, K., Miller, R.B., Stack, M.S. and Spatola, A.F. (1988) FASEB J., 2, A345.
205 McCullagh, K., Wadsworth, H. and Hann, M. (1985) Eur. Patent 159396; (1986) Chem. Abstr. 104, 168866.
206 McCullagh, K., Wadsworth, H. and Hann, M. (1984) Eur. Patent 126974; (1985) Chem. Abstr. 102, 221199.
207 Delaisse, J.-M., Eeckhout, Y., Sear, C., Galloway, A., McCullagh, K. and Vaes, G. (1985) Biochem. Biophys. Res. Commun. 133, 483–490.
208 Hunter, D.J., Markwell, R.E. and Ward, R.W. (1989) Eur. Patent 320118; (1990) Chem. Abstr. 112, 56704.
209 Wallace, D.A., Bates, S.R.E., Walker, B., Kay, G., White, J., Guthrie, D.J.S., Blumsom, N.L. and Elmore, D.T. (1986) Biochem. J. 239, 797–799.
210 Maruyama, S., Tanaka, H., Tomizuka, N., Mitsuyoshi, S. and Fukui, F. (1990) Jpn. Patent 2115198; (1990) Chem. Abstr. 113, 153054.
211 McGregor, W.H. (1983) U.S. Patent 4371466; (1983) Chem. Abstr. 98, 179928.
212 McGregor, W.H. (1983) U.S. Patent 4371465; (1983) Chem. Abstr. 98, 179930.
213 McGregor, W.H. (1983) U.S. Patent 4374765; (1983) Chem. Abstr. 98, 198753.
214 Ternai, B. (1990) World Patent 9005141; (1990) Chem. Abstr. 113, 212689.
215 High, W.B., DiMartino, M.J. and Crimmin, M. (1990) in Ref. 199.
216 Carmichael, D.F., Stricklin, G.P. and Stuart, J.M. (1989) Agents Actions 27, 378–379.
217 Thorgeirsson, U.P., Liotta, L.A., Kalebic, T., Margulies, I.M., Thomas, K., Rios-Candelore, M. and Russo, R.G. (1982) J. Natl. Cancer Inst. 69, 1049–1054.
218 Schultz, R.M., Silberman, S., Persky, B., Bajkowski, A.S. and Carmichael, D.F. (1988) Cancer Res. 48, 5539–5545.
219 Khokha, R., Waterhouse, P., Yagel, S., Lala, P.K., Overall, C.M., Norton, G. and Denhardt, D.T. (1989) Science (Washington, DC) 243, 947–950.
220 Kubota, S., Fridman, R. and Yamada, Y. (1991) Biochem. Biophys. Res. Commun. 176, 129–136.
221 Skinner, M., Cathcart, E.S., Mills, J.A. and Pinals, R.S. (1971) Arthritis Rheum. 14, 727–732.
222 Greenwald, R.A., Golub, L.M., Lavietes, B., Ramamurthy, N.S., Gruber, B., Laskin, R.S. and McNamara, T.F. (1987) J. Rheumatol. Res. 14, 28–32.
223 Breedveld, F.C., Dijkmans, B.A.C. and Mattie, H. (1990) J. Rheumatol. 17, 43–46.
224 Golub, L.M., Ramamurthy, N., McNamara, T.F., Gomes, B., Wolff, M., Casino, A., Kapoor, A., Zambon, J., Ciancio, S., Schneir, M. and Perry, H. (1984) J. Periodont. Res. 19, 651–655.
225 Golub, L.M., Wolff, M., Lee, H.M., McNamara, T.F., Ramamurthy, N.S., Zambon, J. and Ciancio, S. (1985) J. Periodont. Res. 20, 12–23.

226 Golub, L.M., McNamara, T.F., D'Angelo, G., Greenwald, R.A. and Ramamurthy, N.S. (1987) J. Dent. Res. 66, 1310–1314.
227 McCulloch, C.A.G., Birek, P., Overall, C., Aitken, S., Lee, W. and Kulkarni, G. (1990) J. Clin. Periodontol 17, 616–621.
228 Golub, L.M., Ciancio, S., Ramamurthy, N.S., Leung, M. and McNamara, T.F. (1990) J. Periodont. Res. 25, 321–330.
229 Gomes, B.C., Golub, L.M. and Ramamurthy, N.S. (1984) Experientia 40, 1273–1275.
230 Delaisse, J.-M., Eeckhout, Y. and Vaes, G. (1988) Endocrinology 123, 264–276.
231 Delaisse, J.M., Eeckhout, Y. and Vaes, G. (1988) Calc. Tis. Int. 42, A15.
232 Burns, F.R., Gray, R.D. and Paterson, C.A. (1990) Invest. Ophthalmol. Vis. Sci. 31, 107–114.
233 Seedor, J.A., Perry, H.D., McNamara, T.F., Golub, L.M., Buxton, D.F. and Guthrie, D.S. (1987) Arch. Ophthalmol. 105, 268–271.
234 Perry, H.D., Kenyon, K.R., Lamberts, D.W., Foulks, G.N., Seedor, J.A. and Golub, L.M. (1986) Ophthalmol. 93, 1320–1322.
235 Levy, J.H. and Katz, H.R. (1990) Ann. Ophthalmol. 22, 179–183.
236 Humbert, P., Renaud, A., Laurent, R. and Agache, P. (1989) Lancet ii, 277.
237 White, J.E. (1989) Lancet i, 966.

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