Topics in Medicinal Chemistry 17

Jacob Schwarz *Editor*

Atypical Elements in Drug Design

Springer

17
Topics in Medicinal Chemistry

Aims and Scope

Drug research requires interdisciplinary team-work at the interface between chemistry, biology and medicine. Therefore, the new topic-related series Topics in Medicinal Chemistry will cover all relevant aspects of drug research, e.g. pathobiochemistry of diseases, identification and validation of (emerging) drug targets, structural biology, drugability of targets, drug design approaches, chemogenomics, synthetic chemistry including combinatorial methods, bioorganic chemistry, natural compounds, high-throughput screening, pharmacological in vitro and in vivo investigations, drug-receptor interactions on the molecular level, structure-activity relationships, drug absorption, distribution, metabolism, elimination, toxicology and pharmacogenomics.

In general, special volumes are edited by well known guest editors.

In references Topics in Medicinal Chemistry is abbreviated Top Med Chem and is cited as a journal.

More information about this series at http://www.springer.com/series/7355

Jacob Schwarz
Editor

Atypical Elements in Drug Design

With contributions by

R. Abdur · S. Dörrich · A. Draganov · Z. Huang · S.W. May · S.McN. Sieburth · R. Tacke · B. Wang · D. Wang

Springer

Editor
Jacob Schwarz
Genentech, Inc.
San Francisco, California
USA

ISSN 1862-2461 ISSN 1862-247X (electronic)
Topics in Medicinal Chemistry
ISBN 978-3-319-27740-0 ISBN 978-3-319-27742-4 (eBook)
DOI 10.1007/978-3-319-27742-4

Library of Congress Control Number: 2016937752

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

The chemistry of drug discovery revolves primarily around the use of carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus. Over time, nontraditional elements have been incorporated into drug-like compounds for a number of purposes, for instance to slow metabolism or to impart novelty. As a demonstration of the validity of this approach, several examples of compounds containing nontraditional elements have advanced into human clinical trials. This volume will survey three nontraditional elements (B, Si, Se), providing perspective not only on the general properties they impart to organic molecules but also on their utility in specific drug classes.

South San Francisco, CA, USA Jacob Schwarz

Contents

Top Med Chem (2016) 17: 1–28
DOI: 10.1007/7355_2014_65

Published online: 30 July 2014

The Future of Boron in Medicinal Chemistry: Therapeutic and Diagnostic Applications

Alexander Draganov, Danzhu Wang, and Binghe Wang

Abstract Because of its unique electronic structure, boron has special properties useful in designing new diagnostic and therapeutic agents. Specifically, boron's strong Lewis acidity and ability to undergo fission under neutron bombardment form the foundation for boron-containing compounds to be developed as inhibitors of hydrolytic enzymes, chemosensors and artificial receptors for carbohydrates and other Lewis bases, and boron neutron capture agents. A number of boron-containing compounds have been evaluated in animal and human studies for diagnostics and therapeutic applications. One compound in particular, Velcade, has been approved as an anticancer agent. This chapter highlights some of the most significant contributions in the field.

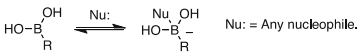
Keywords Boronic acid, Bortezomib, Chemosensor, Diagnostics, Imaging mass spectrometry, MALDI-IMS, Velcade

Contents

A. Draganov, D. Wang, and B. Wang (✉)
Department of Chemistry and the Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30303, USA
e-mail: wang@gsu.edu

1 Introduction

Although boron is ubiquitous and is implicated in various biological processes, its potential in the development of pharmaceutically relevant therapeutic agents had not been widely explored until about two decades ago [1, 2]. Recent activities, however, have shown boron's exciting potential in developing both therapeutics and diagnostics [3–6]. Velcade, a US Food and Drug Administration (FDA)-approved anticancer drug by Millennium Pharmaceuticals, is the poster child of success in using a boron/boronic acid moiety in a pharmaceutical agent and there are several more in clinical trials [6, 7]. Much of the design in using boron/boronic acid for the development of pharmaceutical agents relies on at least one of the following chemical and radiochemical properties [1, 3–8]. First, boron is trivalent in its neutral form and has an open shell. Thus, boronic acid is a Lewis acid and can readily react with Lewis bases/nucleophiles (Scheme 1) such as alcohols [3, 9–25], amino alcohols [26–28], hydroxyl acids [29–32], amino acids [33], as well as cyanide [34, 35] and fluoride [35–39]. Second, because of its Lewis acidity, boronic acid can bind with diols [3, 4, 40–56], commonly found on carbohydrates, with high affinity and reversibility. Especially important is boronic acid's ability to bind diols or hydroxyl groups on carbohydrates because of their important roles in physiological and pathological processes such as in cancer development [57–64]. For example, different cell types carry a number of characteristic complex carbohydrates on the cellular surface that are part of signature structures [65]. Cell surface carbohydrates, such as sialyl Lewis X (sLe^x), sialyl Lewis A (sLe^a), Lewis X (Le^x), and Lewis Y (Le^y), are closely related with the development and progression of many types of cancers [66]. Furthermore, interactions between carbohydrates and other biomolecules are seen as "triggers" for different types of diseases [67–71]. Molecules such as boronic acid-containing compounds, capable of binding to carbohydrates have various biological and biomedical applications as diagnostics and/or therapeutics. Third, neutron bombardment of boron-10 leads to the formation of lithium nuclei with the concomitant emission of high-energy α-particles, which cause localized ionization and thus damages. Thus, boron has been explored for the development of neutron capture therapy (NCT) agents. There are a number of reviews that discuss in detail the developments of boron-containing pharmaceutically relevant agents; thus, this chapter only highlights some of the most important developments in the field.



Scheme 1 The reaction of a boronic acid with a Lewis base/nucleophile

2 Boronic Acid Compounds as Therapeutic Agents

Significant effort has been made in the development of pharmaceutically relevant boronic acid compounds as therapeutic agents [1, 4–6]. This section briefly summarizes the most recent and significant developments in the field, introducing FDA approved-drugs as well as compounds in phase II and III clinical trials [1, 5]. However, before discussing the development of boronic acid-based therapeutic agents, it is important to understand some basic physicochemical properties. Most commonly used arylboronic acids have a pK_a in the range of 4.5–8.8 depending on the nature of the aromatic rings and substituents [32, 72, 73]. It is important for one to understand that the acidity of boronic acids is not related to its deprotonation, but rather to its ability to react with water (or other protic solvents) with a concomitant release of proton and formation of the tetrahedral anionic species (Scheme 1). The design of many inhibitors of hydrolytic enzymes takes advantage of such properties not only because of covalent bond formation between boronic acid and an active site nucleophile but also because the tetrahedral species mimics the transition state of such reactions [8].

2.1 Boronic Acid Compounds as Anticancer Agents

A few promising boronic acid-containing inhibitors have been summarized in the literature [1, 4, 5]. Before discussing in more detail, major recent developments in the boronic acid anticancer field can be grouped into five sections based on their mechanism of actions: (1) proteasome inhibitors [74–86], (2) FAP (fibroblast activation protein) inhibitors [87, 88], (3) HDAC (histone deacetylase) inhibitors [89, 90], 4) HIF-1α (hypoxia-induced factor-1α) [91] and MDM2 (murine double minute 2 protein)/p53 inhibitors [92], and 5) ATX (autotaxin) inhibitors [93].

2.1.1 Proteasome Inhibitors

The proteasome is a eukaryotic enzymatic complex that is responsible for protein degradations in mammalian cells [94] and is a major regulator of enzymatic concentrations in the cytoplasm [95–97]. Proteins covalently tagged by ubiquitin are recognized by the protease complex and degraded to small peptides and amino acids. Thus, inhibition of this enzymatic machinery results in accumulation of proteins in the cell and consequently apoptosis. The importance of the proteasome function makes it a good target for the development of inhibitors, especially in

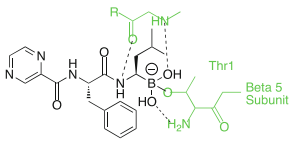


Fig. 1 Binding interactions between bortezomib and the proteosomal β5 subunit

cancer therapy because of the rapid proliferation of cancer cells [8, 98]. A number of boronic acid compounds targeting the proteasome have been developed with the most significant achievement in the area being bortezomib (trade name Velcade) [4, 5, 8, 85]. Peptide boronates appeared to be some of the most potent reversible inhibitors of the proteasome [5, 8, 85, 99]. Some of these peptide boronates are excellent drug candidates, possessing the chemical characteristics of a peptide and a boronic acid giving rise to soluble, physiologically stable compounds, with good bioavailability [1, 5, 8]. In 2003, Velcade was approved by the FDA for clinical use against multiple myeloma, and a year later against mantle cell lymphoma [8]; (http://bortezomib.org/). Additional studies showed that Velcade has potent activities against a number of solid tumors such as that of the breast, lung, colon, pancreas, and prostate [76–78, 80, 82, 84].

The success of Velcade lies not only in its physicochemical properties but also on its mechanism of action. Bortezomib is selective for the chymotrypsin-like activity of the proteasome, which, for the most part, is provided through the β5 subunit [8, 85, 99]. After extensive cytotoxicity studies in 1999, Adams and co-workers in partnership with the National Cancer Institute reported bortezomib as a proteasomal inhibitor with inhibitory potency (K_i) of ~0.6 nM and average GI_{50} (across 60 cell lines) of ~7 nM [85]. After screening 60 cell lines, it was established that the compound possesses activity against a number of solid cancer types. The presence of the boronic acid moiety allows for the formation of reversible covalent interactions of the available boron orbital and the nucleophillic threonine of the proteasome [85]. The crystal structure of the compound in complexation with the S20 yeast proteasome gave insights into the mechanism of actions of the drug and the specific interactions involved in the proteosome [19]. Threonine 1 from the β5 subunit covalently binds with the boron atom giving rise to the tetrahedral anionic form stabilized by a hydrogen donating glycine 47 and an additional hydrogen bonding interaction with the free amino group of threonine 1 (Fig. 1) [8]. Despite its utility, Velcade does have a number of side effects including cardiovascular disorders, nausea, and neurologic disorders (http://bortezomib.org/) [100]. It is important to note that mechanistic studies have shown that the toxicity of Velcade is not related to boron itself [1, 101]. Knowing the mechanistic details of the inhibition process has allowed for the development of other di- and tripeptide boronates as protease inhibitors, some of which are in advanced stages of clinical trials for various indications (compounds **2**, **3**, **5**–**7**, Fig. 2) [4, 5, 8, 102, 103]. There is still more work to be done in the development of boronic acid-based drugs with

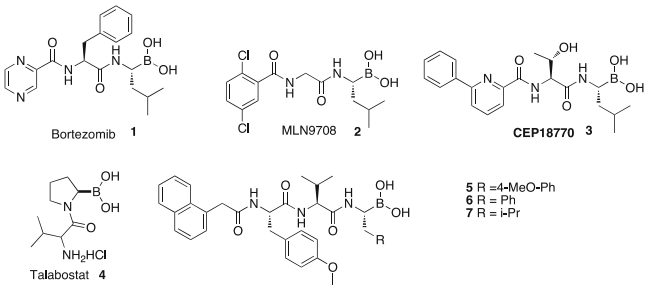


Fig. 2 Examples of boronic acid agents as proteasome inhibitors

low toxicity. Nevertheless, the development of bortezomib and others opened the doors for developing boronic acid-containing therapeutics.

2.1.2 FAP (Fibroblast Activation Protein) Inhibitors

Another peptide boronate compound (Talabostat, Fig. 2) was initially designed as a DPP4 (dipeptidyl peptidase 4) inhibitor for treatment of diabetes but also showed potency as an anticancer agent. It was studied in clinical trials against non-small cell lung cancer based on its inhibitory properties towards fibroblast activation protein (FAP) [1, 49]. FAP is a membrane protein from the serine protease family that is widely expressed in epithelial cancers, soft tissue sarcomas, and some bone cancers [104, 105]. The importance of this protein is related to the structural formation and stability of the cancerous cell. The protein has some structural similarities to DPP4 and a common exopeptidase activity [104]. The compound reached phase III clinical trials, but did not meet the required efficacy standards [1, 106]. Although single-dose tolerance was 500 μg, the plasma levels achieved top concentration within 2 h, and the compound was considered suitable for daily dosage; its lack of selectivity for FAP and efficacy forced termination of the studies [1, 106]. FAP is a transmembrane enzyme that possesses endo- and exopeptidase activity. The exopeptidase activity of that FAP is a dipeptidyl peptidase activity (DPP), common for enzymes such as DPP4 [49]. The specific endopeptidase activity that FAP has makes this enzyme a good therapeutic target. Despite the fact that Talabostat showed a lack of direct in vitro anticancer activity, phase I clinical trials showed reduction of tumor volume of up to 90% (WEHI 164 fibrosarcoma mouse model) at 5 μg dose [107]. FAP inhibitory studies during phase II clinical trials of Talabostat showed about 90% enzymatic inhibition of the exopeptidase activity of FAP; however, there was only a 21% decrease of the endopeptidase activity [49]. Thus, the lack of inhibitory selectivity of the compound towards the specific function of the enzyme makes the anticancer drug candidate inapplicable.

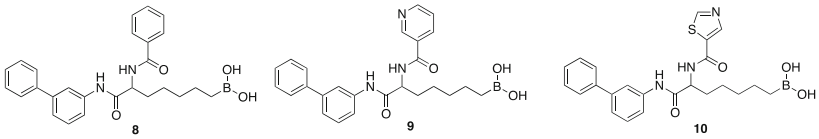


Fig. 3 Examples of boronic acids as HDAC inhibitors

2.1.3 HDAC (Histone Deacetylase) Inhibitors

The importance of histone deacetylases in the mammalian system has been extensively studied throughout the years, and the importance of HDACs as therapeutic targets has been well defined [89, 108]. Inhibition of HDAC results in histone overacetylation giving rise to activation of genes closely associated with growth arrest and apoptosis, such as p21 [108]. In 2009 Suzuki and co-workers reported the first boronic acid compounds bearing α-amino acid moiety that showed inhibitory effects on HDAC (Fig. 3) [90]. The HDAC inhibitory concentrations of the compounds were studied in MKN45 cells (stomach cancer) and were reported to be 2.0, 2.0, and 1.4 μM for compounds **8**, **9,** and **10,** respectively. Western blot studies confirmed that the anticancer activity (GI_{50} 9.3, 5.5, and 3.5 μM, respectively) of these compounds was in fact due to HDAC inhibition [90]. Interestingly enough, the S-isomers of the compounds showed low micromolar activity, while the R-isomers had no effect. The compounds showed selectivity towards HDAC2 and HDAC6 with IC_{50} as low as 0.1 μM [90]. The most potent compound of the series (**10**) was tested against a number of cancer cell lines and retained low micromolar IG_{50}, some of which are as low as 1.3 μM against LOX-IMVI (melanoma) cells [90]. Extensive computational studies showed that the boronic acid group of the active agents was indeed playing an important role in the HDAC binding pocket [90]. In fact the tetrahedral anionic form of the boronic acid is stabilized by a histidine and a tyrosine residue while interacting with the zinc ion in the active site [90]. The significance of this work is that never before had a boron-containing compound been recognized as an HDAC inhibitor. However, the designed compounds did not show desirable pharmacokinetic qualities for a drug candidate, and thus, there was no further development of these compounds.

2.1.4 HIF-1α and MDM2 Expression Inhibitors

During the past decade, HIF-1α and MDM2 have been identified as two important anticancer targets and a number of research groups and pharmaceutical companies have put substantial effort in the development of therapeutic agents targeting these two proteins [109–111]. Although extremely important and independent of each other, we are introducing them under one section because of the recent development of boronic acid compounds, which target these two proteins [91, 92]. Despite the lack of structural diversity and conclusive experimental data, the boronic acid

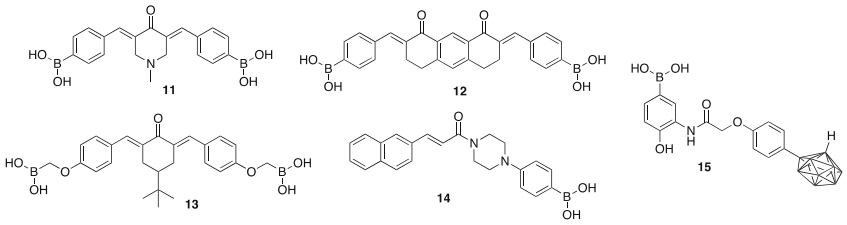


Fig. 4 Boronic acids as MDM2 (**11**–**14**) and HIF-1α (**15**) inhibitors

compounds targeting MDM2 and HIF-1α are worth noting as they may carry an answer to the design of successful anticancer agents.

MDM2 is a negative regulator of p53, a tumor suppressor protein or so-called "genome guardian" [112]. There are a number of ways that the p53 protein suppresses tumor development: apoptosis, autophagy, senescence, and promoting growth arrest [113, 114]. MDM2 regulates p53 in two distinct ways. The first and most obvious way of p53 regulation by MDM2 is through its E3 ubiquitin ligase activity, meaning that p53 is ubiquitinated by MDM2 leading to proteasomal degradation [115, 116]. The second method of p53 inactivation by MDM2 is shown to be through binding to the transactivation domain of p53 and blocking the transcription activity of the protein on its substrates [117, 118]. In either case, the interaction between the two proteins is extremely important for the cells. Designing an inhibitor that prevents the two proteins from interacting is a commonly employed strategy [110]. However, to our best knowledge only Khan and co-workers have reported boronic acid compounds inhibiting the interactions between MDM2 and p53 (Fig. 4, compounds **11**–**14**) [92]. The reported IC_{50} values are in the low micromolar range, which by itself does not mean that the reported compounds have true potential to be developed into drug candidates. However, these compounds are another example of boronic acid compounds used as potential therapeutic agents against cancer and a novel class of MDM2 inhibitors.

In 2010 Shimizu and co-workers reported boronic acid compound **15** that showed an IG_{50} around 16 μM in HeLa-based HRE reporter assays with an IC_{50} of 750 nM in its inhibition of HIF-1 transcriptional activities (Fig. 4) [91]. In addition, immunoblotting assays showed inhibition of HIF-1α expression, and RT-PCR showed that the expression of VEGF, induced under hypoxic conditions, decreased upon addition of compound **15** in a concentration-dependent manner [91]. Although no mechanistic data for the compound is present at this point, this new class of HIF-1 inhibitors seems to have good potential.

2.1.5 ATX (Autotaxin) Inhibitors

Autotaxin (ATX) is a nucleotide phosphodiesterase that produces lysophosphatidic acid (LPA) by hydrolysis of phospholipid lysophosphatidylcholine (LPC) [93, 119–123]. Many biological processes, such as chronic inflammation [124], fibrosis [125, 126], and tumor progression [127], are related to the ATX-LPA signaling

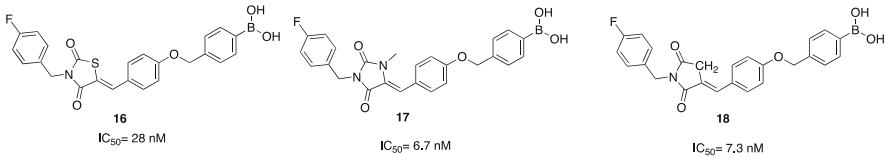


Fig. 5 Boronic acid compounds as ATX inhibitors. *IC_{50} values obtained through choline release assay

pathway. Since LPA acts on multiple receptors in the mammalian system [123, 128], direct targeting of LPA receptors is not a plausible strategy. Because of this drawback, targeting ATX has become an attractive method for addressing some of the related issues. In recent years, various synthetic boronic acids have been explored as ATX inhibitors [129–132]. Huib Ovaa and co-worker screened small-molecule libraries in 2009 to search for unique ATX inhibitors [93]. Thiazolidinedione analogues were found to selectively inhibit ATX activity. They optimized these compounds by using "active-site-targeted strategy," which was successfully used for the development of bortezomib [99, 133]. The results show that their boronic acid-based inhibitors potently inhibit ATX both in vitro and in vivo. Albers and co-workers have developed boronic acid compounds that showed single-digit nM activities (Fig. 5, compounds **17** and **18**) [134]. An almost fourfold decrease of the LPA levels in mouse blood plasma was achieved by compound **16** at serum concentration of 350 nM [93]. The reported in vivo data clearly indicates the relationship between ATX inhibition by the boron-containing compounds and a decrease of LPA in the blood plasma [93]. It can be safely concluded that boronic acid-based inhibitors hold promise as drug candidates to target the ATX-LPA axis in vivo [93]. In 2011, molecular docking and X-ray crystallographic studies were reported using a boronic acid-based inhibitor of ATX, compound **17** (Fig. 5) [134]. Crystal structural studies of the ATX complex with the inhibitor gave good insights explaining the compound's activity [134]. The exploration of structure–activity relationships and the study of the lipophilic pocket near the ATX active site should allow for the future design of more potent inhibitors.

The examples summarized above clearly show that boronic acid compounds can serve as good structural scaffold for anticancer drug discovery. However, cancer is not the only condition against which boronic acid compounds have shown potency; recently advances have been made in the development of successful serine protease inhibitors for treatment against hepatitis C virus (HCV) [4, 5, 8].

2.2 *Boronic Acid Compounds as NS3 Protease Inhibitors Targeting Hepatitis C Virus*

Hepatitis C is a contagious liver disease caused by HCV. According to the World Health Organization (WHO), between three and four million people are infected with HCV every year and about 150 million have chronic infection and live with the

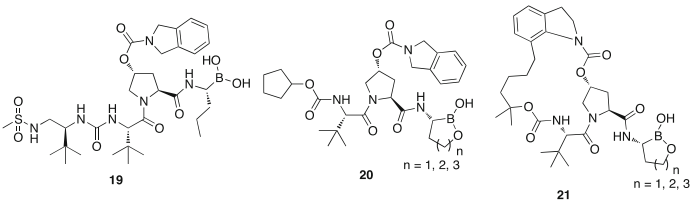


Fig. 6 Examples of boronic acid-based NS3 protease inhibitors

risk of developing liver cirrhosis (http://www.who.int/mediacentre/factsheets/fs164/en/). The fact that over 350,000 people die from HCV-related diseases every year is alarming and has stimulated interest in developing new therapeutic agents to target this virus (http://www.who.int/mediacentre/factsheets/fs164/en/). The most widely used treatment against HCV is a combination of interferon and ribavirin, which can cause serious side effects and in some cases lacks positive responses (http://www.who.int/mediacentre/factsheets/fs164/en/). The field of boronic acid-based therapeutics has a significant contribution to the battle against HCV. Efforts in targeting HCV and more specifically its vital NS3 serine protease, by utilizing boronic acid analogues, have been ongoing since the mid-1990s [135]. However, only in the recent years, significant progress has been made in the development of boronic acid-containing compounds (**19, 20)** as NS3 protease inhibitors. Some such compounds have gone into phase II and III clinical trials (Fig. 6) [136–139]. The main target, NS3 protease, is an important enzyme in the transcriptional process of the virus [140, 141]. The significance of the enzyme in the development of a mature HCV makes it an extremely important target [137]. The enzyme has been well studied, and its crystal structure revealed that the catalytic site possesses a classic catalytic triad aspartate–histidine–serine [137, 139]. The serine residue in the catalytic site has been shown to covalently bind to the boron of the peptide boronates, stabilize the tetrahedral anionic form of the boronic acid moiety, and thus inhibit the NS3 protease activity [139]. To understand the mechanistic details of action of the active boronic acid compounds, a number of boronopeptides were synthesized with enzymatic activity in the low to sub-nanomolar range [5, 139, 142]. The inhibitory effects of these compounds were evaluated and the cyclic boronopeptides were shown to have potential for further exploration as antiviral agents [67]. Structure–activity relationship analysis showed that the size of the boron-containing rings in compounds with the core structure resembling **20** and **21** (Fig. 6) significantly affects the activity. For example, the compound containing core **20**, where $n = 3$, possesses potent NS3 inhibitory activity (IC_{50}, ~24 nM), while the activity drops to 320 nM (almost tenfold) when $n = 2$ [139]. Although significant advancement in the development of boronic acid-based NS3 inhibitors has been made, there has not been a single compound that has been fully developed and reached the end of the pharmaceutical pipeline yet. The promising results in the past 5 years, however, bring optimism to this area.

2.3 Boronic Acid Compounds as Antibacterial and Antifungal Agents

Another major challenge of modern-day medicinal chemistry is the development of efficient antibacterial agents to counter the constant emergence of drug-resistant strains of bacteria. One of the most widely used classes of antimicrobials is β-lactam antibiotics [5, 8]. However, the biggest challenge in using these β-lactam antibiotics has been the rapid development of drug resistance due to the overproduction of four different classes of β-lactamases that catalyze the hydrolysis and thus inactivation of β-lactams [143, 144]. The classes of β-lactamases are A, C, D (serine hydrolases), and B (metallohydrolase) [143]. Two of the clinically most relevant classes are A and C with representatives such as TEM penicillinase and AmpC cephalosporinase, respectively [8, 143]. The β-lactam antibiotics achieve their effect by acting on enzymes responsible for the cell wall synthesis; more specifically they inactivate transpeptidases such as the penicillin-binding proteins (PBP) [5]. PBPs are attractive targets for developing new antibiotic agents because they catalyze unique steps of the biosynthesis of peptidoglycans in bacteria [145, 146]. A common strategy in the prevention of β-lactam-related drug resistance is the synergistic use of β-lactam antibiotics and β-lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam [147]. In search for new β-lactamase inhibitors, Grundstrom and co-workers conducted detailed studies of class C β-lactamase and simple aryl boronic acids including ortho-, meta-, and para-substituted methyl-, hydroxymethyl-, formyl-, and phenylboronic acids [148, 149]. Crystallographic studies showed evidence that the hydroxyl group of a serine (Ser-221) residue of AmpC acts as a nucleophile and forms a covalent bond with the boronic acid in the active site [20, 150]. As mentioned earlier, most of the clinically relevant β-lactamases are serine-based hydrolases. Compounds containing a boronic acid moiety are excellent candidates for inhibitor development against these β-lactamases because the electrophilic boron can readily interact with the nucleophilic serine, resulting in covalent tetrahedral anionic intermediate and thus enzyme inactivation [151]. For example, Pechenov and co-worker developed a series of boronic acid analogues, and the peptide boronic acid Boc-L-Lys(Cbz)-D-boroAla showed an inhibitory effect with an IC_{50} value of 370 nM [152]. Their crystal structural studies demonstrated that the boron atom is covalently attached to the serine. The complex is a transition state analogue of the enzyme-catalyzed reaction [153]. Strynadka [154], Prati [155], Shoichet [151, 156], and White [157] also reported highly potent, nanomolar boronic acid β-lactamase inhibitors (compounds **22**–**25**, Fig. 7). Despite the potent inhibition activities, no boronic acid-based inhibitors of β-lactamases have made to phase II or III clinical trials yet.

Diazaborines, first invented by Dewar and then further developed by Gronowitz, are another class of boron-containing compounds with antibacterial activities [158, 159]. However, due to toxicity problems in a number of analogues (**26**–**28**, Fig. 8), further development of this class of compounds was stopped [1].

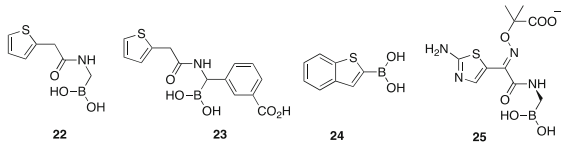


Fig. 7 Boronic acid-based inhibitors of β-lactamases

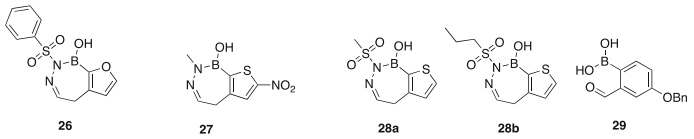


Fig. 8 Some boronic acid based compounds designed as antibacterial agents

In 2009 Wang and co-workers reported boronic acid-based antibacterial agents that interfere with bacterial quorum sensing and can be potentially used as antibacterial agents [160–163]. Briefly, bacterial quorum sensing is a term describing the bacterial ability to control gene expression and other biologically relevant processes through the detection of minimal concentrations of signal molecules called autoinducers [163]. There are a number of autoinducers in the bacterial quorum sensing process. Among them, autoinducer-2 (AI-2) is a signaling molecule that is universal for both Gram-positive and Gram-negative bacteria, thus an important therapeutic target. In *Vibrio harveyi,* AI-2 exists as a boric acid complex [163]. Wang and co-workers studied the idea of using phenyl boronic acid compounds that can mimic AI-2 in bacterial quorum sensing, thus inhibiting the signaling pathway. Compound **29** is one example from the library of quorum sensing inhibitors that carries sub-micromolar IC_{50} (0.7 μM, Fig. 8) [163]. To our best knowledge, this is the most potent boron-containing compound to inhibit the AI-2 signaling pathway.

In addition to the antibacterial boron-containing antibiotics, boronic acids with antifungal activity (dihydrobenzoxaboroles) were reported by Baker and co-workers in 2006 and were investigated in phase II and III clinical trials [164]. These compounds have been proven to target fungal leucyl tRNA synthetase [164, 165], which is an extremely important class of enzymes in protein synthesis. They are responsible for the attachment of specific amino acids to the corresponding tRNA. The importance of this class of enzymes makes them very attractive therapeutic targets. One major setback of this strategy is that these enzymes are present in all eukaryotic cells including the mammalian system; therefore, there is an issue for binding specificity. However, crystal structural analysis revealed that certain benzoxaborole compounds bind specifically to the fungal leucyl tRNA synthase and form a stable inhibitor–enzyme complex [164, 166]. The compounds (**30**–**33**) were tested against a number of fungal strains, and two of the most potent compounds **30** and **31** (Fig. 9) showed MIC as low as

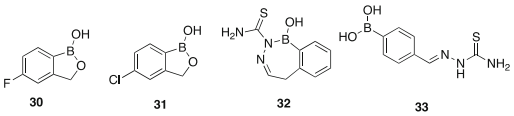


Fig. 9 Boronic acid derivatives as potential antifungal agents

0.25 μg/mL against *A. fumigatus* [164]. As of 2009 compounds **30** and **31** were being investigated in phase I and II clinical trials as topical agents for treatment of onychomycosis (fungal infection of the nail) [1].

2.4 Boronic Acids Compounds as Anticoagulant Agents

Cardiovascular diseases (CVD) are one of the major causes of mortality in the modern-day society. An estimate provided by the WHO states that over 17 million people per year die from cardiovascular diseases. Such a number makes CVDs the leading cause of death worldwide (http://www.who.int/mediacentre/factsheets/fs317/en/). Thromboembolitic diseases are one of the top three causes of CVD-related deaths [167]. Thrombin is a serine protease involved in the final steps of the blood coagulation cascade. This protein is an important target for the development of anticoagulant agents [4]. A major concern with using thrombin inhibitors as anticoagulant agents is the nonspecific inhibition of other enzymes. For example, DuPont have identified a boronic acid thrombin inhibitor DUP 714 with K_i of 40 pM (Fig. 10) [168, 169]. The compound appeared to be very potent in its inhibition of thrombin; however, it exhibited severe side effects in animal studies that were proven to be related to strong inhibition of complement factor I [169]. After careful analyses of the crystal structure of the inhibitor DUP 714 and its interactions with α-thrombin and the complement factor I homologue factor Xa, it was observed that the binding pockets were significantly different between these two proteins. Such information allowed for structural optimization of DUP 714 to afford a more selective fit, the α-thrombin pocket [170, 171]. The optimized compound **35** (Fig. 10) was more selective towards α-thrombin compared to factor Xa; however, the K_i values for both enzymes were at or below nanomolar concentrations (0.06 and 99 nM, respectively), which is undesirable. When designing an enzyme inhibitor, it is important not only to have high potency but also have selectivity. Although compound **35** is more selective for thrombin, the inhibitory concentration is too close to the one against fXa. Thus, the "inhibitory margin" is too narrow and does not allow for the development of a pharmaceutical agent. Ideally when designing an inhibitor, the "inhibitory margin" should be a few orders of magnitude larger. The designed boronic acid compounds exhibited potent inhibitory activity towards α-thrombin through covalent interaction of the boron with serine-195 in the P1 binding pocket and formation of a tetrahedral species,

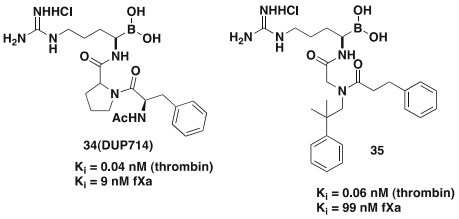


Fig. 10 Boronic acid-based thrombin inhibitors

which is considered a transition state analogue inhibitor. However, in this case, the boronic acid compounds never advance further as anticoagulant agents.

2.5 Boronic Acid Compounds as Diabetes Therapeutic Agents

Earlier in the chapter Talabostat was introduced as a promising anti-cancer agent by inhibiting FAP and DPP4. This compound was initially designed as a DPP4 inhibitor for the development of treatment options for diabetes. There have been a number of DPP4-targeting boronic acid compounds reported, and a few of them were reported to have been studied in clinical trials [1]. DPP4 is a membrane-bound serine protease that specifically cleaves two N-terminal amino acids followed by either a proline or alanine residue [172]. DPP4 has become a key target towards the development of diabetes therapeutics, because it plays such a crucial role in the degradation of the glucagon-like peptide 1(GLP-1) [173], which is a hormone responsible for the increases of insulin release from the pancreas upon elevation of blood glucose levels [173]. Inhibition of DPP4 results in an increase of GLP-1 levels and thus proper control of blood glucose concentrations. A number of non-boron-containing DPP4 inhibitors have been clinically used for treatment of diabetes; some examples of commercially available DPP4 inhibitors include saxagliptin (Onglyza, by BMS), vildagliptin (Galvus, by Novartis), and sitagliptin (Januvia, by Merck). Another interesting aspect about DPP4 as a drug target is that its catalytic triad is atypical for the serine proteases order: serine–aspartate–histidine. The presence of this catalytic triad suggests that boronic acid-containing compounds can have the potential to be developed as successful DPP4 inhibitors. A number of dipeptide boronates have been synthesized and have shown activity in the low nanomolar to picomolar range, which in principle makes them more potent than the clinically used DPP4 inhibitors [174]. However, a major downside of compounds such as **36** and **37** is that at physiological pH they can undergo intramolecular cyclization between the primary amine and the boronic acid moiety (Fig. 11). It is clear that the open-shell orbital of the boronic acid can easily accept electrons and interact with strong nucleophiles such as free amines or alcohols. The

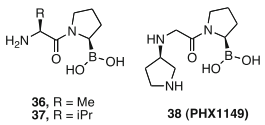


Fig. 11 Boronic acid-based DPP4 inhibitors

free amine (and a strong nucleophile at physiological pH) that is in proximity to the boron, as in compounds **36** and **37**, can easily undergo nucleophilic intramolecular cyclization to form a stable six-membered ring (Fig. 11). Later, analogues containing mono-alkyl-protected amino groups that carry nanomolar inhibition were developed. However, these small peptides carry low nano-molar activity against not only DPP4 but also DPP9 and FAP. Thus, the lack of selectivity made these small molecules unsuitable as drug candidates [175–177]. In 2008 after clinical trials, compound PHX1149 (**38**, Fig. 11) was reported to have not only just excellent activity (DPP4, $IC_{50} = 25$ nM) but also high patient tolerance via oral administration in doses up to 400 mg/kg [106, 178, 179]. This orally bioavailable, water-soluble small molecule reached phase III clinical trials and may possibly be the next boron-containing marketed drug because of its DPP4 selectivity.

2.6 BNCT (Boron Neutron Capture Therapy)

In addition to being enzymatic inhibitors and carbohydrate binders, some boron-containing compounds have been used in BNC therapies. This type of therapy is based on the fact that neutron irradiation of boron-10 leads to high-energy α-particles and lithium nuclei, which cause localized ionization and thus tissue damage. There is one critical aspect to consider when designing a BNCT agent [4]. Due to the highly damaging effect of high-energy α-particles on tissue, cancer-selective compounds that can deliver boron-10 precisely to the cancer tissue and at high density need to be utilized. A number of BCN agents have been developed throughout the years, some of which are currently being clinically used for cancer therapy outside of the USA. Studies in this area have been summarized in a number of reviews and therefore are not to be discussed in detail [4, 5, 180].

3 Boronic Acids as Carbohydrate Binders/Receptors

Carbohydrates in the form of oligosaccharides and polysaccharides are a major class of biomolecules in the mammalian system. A well-known fact is that mammalian cells are decorated with complex carbohydrates such as glycolipids and glycoproteins, playing various functional roles in biological systems [3, 181–183]. Carbohydrates are involved in a number of major physiological events in

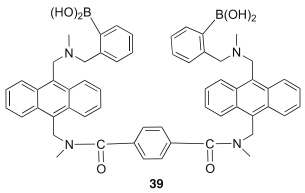
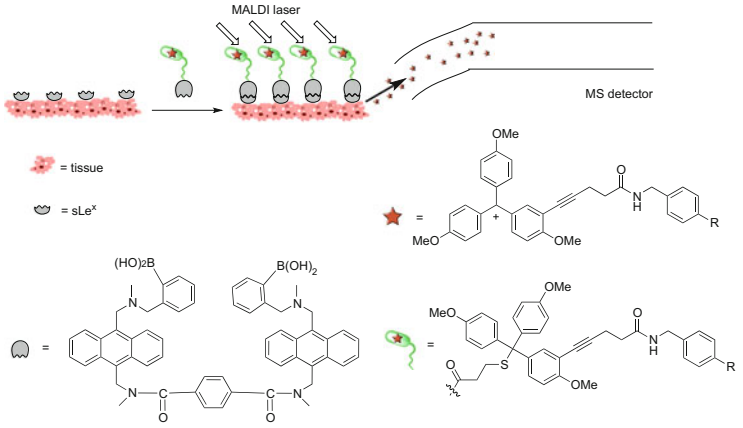


Fig. 12 sLe^x selective bisboronic acid

the mammalian system [3]. In cellular adhesion and inflammation processes, it is the interactions between carbohydrates and carbohydrate-binding proteins (lectins) that trigger the leucocyte response [184]. Another example of the importance of carbohydrate functions in the mammalian system is HIV infection, which is mediated by the binding of a glycoprotein to the cellular surface receptor [185, 186]. Furthermore, the surface of cancer cells is coated with glycoproteins; interactions of lectins with these carbohydrates can trigger biological events such as metastasis, cell adhesion, apoptosis, etc [187–189]. Understanding the interactions between carbohydrates and other molecules has also inspired the development of a number of therapeutically relevant compounds [3, 4]. Therefore, the physiological importance of saccharides and polysaccharides brings the need for the development of molecular probes that can be used as tools and potential diagnostics and therapeutics. In this section, we focus on the progress made in the development of boronic acids as carbohydrate binders and sensors as well as their applications as diagnostic and potential therapeutic agents. Non-boronic acid-based sensors for carbohydrates have been discussed in various reviews [190–195] and thus are not discussed here in detail.

3.1 BisBoronic Acid Sensors for sLe^x as a Cancer Diagnostic Tool

Lectin mimics capable of selective and high affinity recognitions of carbohydrate biomarkers can be very useful tools in the diagnostics field [3]. This is especially true in the field of cancer and diabetes. For example, the development of gastrointestinal, pancreatic, and breast cancer has been associated with overexpression of various sLe^x-containing glycoproteins [196]. Therefore, the development of sLe^x-selective sensors could aid in the diagnosis and early detection of these forms of cancer. In 2002, Wang and co-workers first reported a series of anthracene-based bisboronic acids, some of which possess selectivity for sLe^x (**39**, Fig. 12). Among the same series, there was also a sensor with selectivity for glucose over fructose. When compared to normal arylboronic acids, the bisboronic fluorescent sensor showed selectivity improvement of over 1,400-fold [197]. sLe^x-selective sensor has been used in various applications.



Scheme 2 The general concept of Boronolectin–MS tag conjugation for imaging applications

3.1.1 MALDI-IMS Imaging for Histological Analysis of Cancer Tissues

In modern histological work, especially in cancer, there is a need for the development of methods for the simultaneous detection of multiple biomarkers, i.e., multiplexing. With the traditional histochemistry work, fluorescent and/or visible dyes are used in staining tissues. One drawback with this approach is the issue of spectral signal overlap. With the spectral window of 500–700 nm and the normal spectral bandwidth of more than 50 nm, possible signal overlap issues severely limit the ability to simultaneously detect multiple biomarkers [198]. In addition, the issue of dye stability, photobleaching, and difficulty in quantitation are all issues associated with the traditional histological work [198]. Recently, the development of matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) allowed for the direct examination of tissue biopsies without the need for microdissection and biomarker solubilization prior to analysis (Scheme 2) [198]. MALDI-IMS can generate images correlating exact locations within a thin tissue section and exact molecular mass [199–202]. Very importantly, one can monitor a large number of ions simultaneously because of the extremely high resolution of mass spectrometry, allowing for multiplexing.

In the very first example of this field, Wang and co-workers conjugated sLe^x-selective **39** with a trityl-based tag through a thioether linkage for matrix-free MALDI-IMS analysis of cancer tissues that contain detectable levels of sLe^x. Because the trityl cation is very stable, MALDI laser can ionize the conjugate by cleaving the thiol linkage to give the trityl carbocation [203]. Using kidney cancer as an example, the conjugate was used to treat the tissue and then subjected to systematic analysis by a grid of MALDI laser shots. The mass tag peaks were observed in the cancer tissue region (expressing sLe^x) and the MALDI-IMS results were consistent with that of tissue immunostaining [198]. Because of the

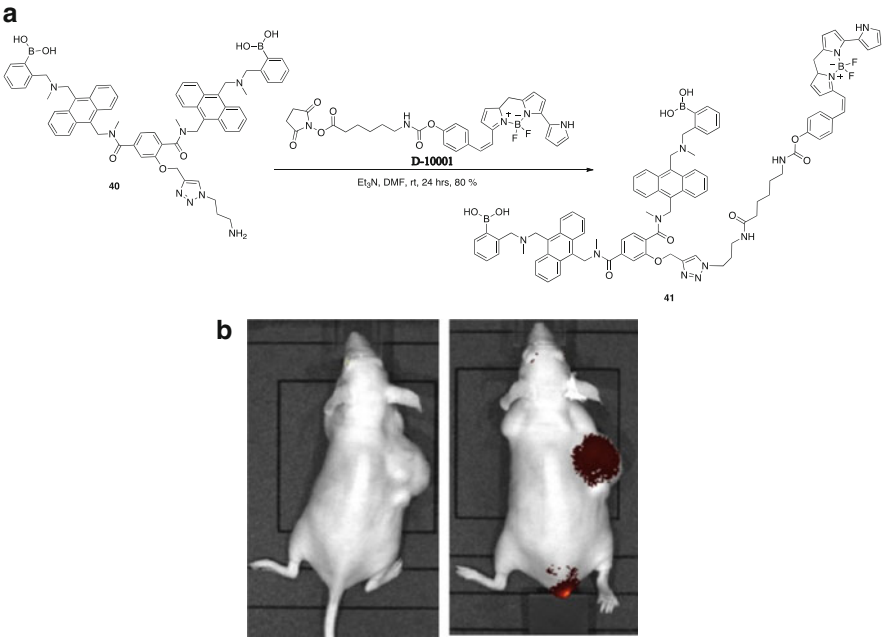


Fig. 13 (**a**) Structures of conjugate **41.** (**b**) Optical (fluorescent) imaging of tumor by targeting cell surface carbohydrate biomarker (*left*: mouse before imaging agent injection; *right*: mouse 24 hours after tail vein injection of the contrast agent showing almost exclusive delivery to the tumor site)

widespread presence of carbohydrate biomarkers in cancer, it is hoped that the MALDI-IMS methods will find increasing utility in the diagnosis of cancer.

3.1.2 In Vivo Fluorescent Imaging Application

Multiple glycosyltransferase genes responsible for the further glycosylation of glycoconjugates were found to be upregulated in many forms of cancer, resulting in the high-level expression of various glycobiomarkers. Furthermore, high concentrations of sLe^x have been found in the sera of patients with colon cancer, presumably due to the shedding of such carbohydrates by cancer cells [204]. In terms of the pathological relevance for the observed high levels of carbohydrate biomarkers, sLe^x has also been implicated in mediating the metastasis and invasiveness of colon and other forms of cancer [205–208].

Wang and co-worker conjugated Le^x-selective bisboronic acid **40** to a fluorophore (BODIPY) to examine the feasibility of using such a conjugate to image tumor in vivo [209]. Thus, conjugate **41** (Fig. 13) was synthesized and evaluated. Specifically, fluorescent agent **41** was injected into mice with implanted tumor, which overexpress sLe^x. After a 24-h washout period, it was found that the

fluorescent agent was concentrated in the tumor area, allowing for ready detection of the tumor (Fig. 13; some injury probably occurred during tail vein injection, which led to diffusion and retention of the fluorescent agent in the tail area) [209]. This work represents the very first example that a small molecule capable of recognizing a carbohydrate biomarker was used for optical imaging applications and demonstrates the tremendous potential of this area of studies.

3.2 Boronic Acids as a Tool for Bacterial Detection

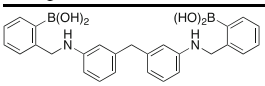
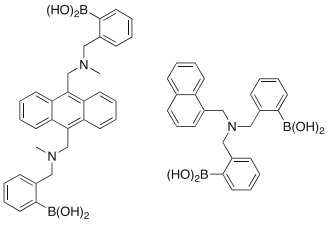
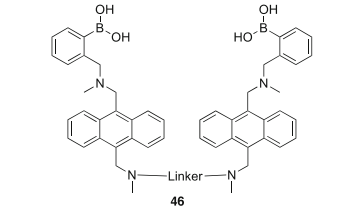
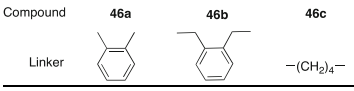
Carbohydrates are one of the major constituents of the bacterial cell wall and surface, which are important for the immunological properties of microorganisms, signal transduction, and cell–cell recognition. Boronic acids have been used for the recognition of bacterial surface carbohydrates and thus bacterial detection [210–215].

One example in bacterial detection is 3-aminophenylboronic acid (3-APBA). Thavarungkul and co-workers were the first group to detect bacteria in drinking water by using 3-APBA [212]. Briefly, 3-APBA was immobilized on a gold electrode via a self-assembled monolayer. The binding between 3-APBA and bacteria in a flow system resulted in the change in capacitance of the sensing surface. The results showed good performance with a wide linear range 1.5×10^2–1.5×10^6 CFU mL^{-1} and low detection limit 100 CFU mL^{-1}. Compared with the normal detection limit, which is set at 500 CFU mL^{-1}, the new method increased detection sensitivity of total bacteria in drinking water by fivefold. 3-APBA was shown to bind with several types of bacteria; however, it is only well suited for a rapid analysis of total bacteria in water samples without any selectivity for a particular type of bacteria. This work could serve as a model for the further development of rapid and accurate water contamination detection methods.

3.3 Boronic Acids as a Tool for Viral Infection Detections

Boronic acids not only can be used for bacterial detection, it also studied for the rapid, accurate, sensitive, and low-cost detection of viruses [216, 217]. For example, Say and co-workers developed a detection method for influenza virus based on boronic acid–carbohydrate interactions and applied this method to quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) sensing [218]. 4-Aminophenyl boronic acid (4-APBA) can bind to sialic acid (SA), which has an important role in viral binding to hemagglutinin (HA) and thus infection. In these studies, QCM and/or SPR chips were modified by immobilizing 4-APBA. Viral binding, through interactions between boronic acid and SA, would give a signal in the sensogram, and thus allow for viral detection. In using this method, the authors

Table 1 Selected examples of potent boronic acid glucose binders

Compound	Binding affinity	Author and References
 **42**	$K_a = 140\ M^{-1}$	James [243]
 **43** **44** **45**	$K_a = 3.6\ M^{-1}$ (**43**)	Shinkai [43, 225]
 **46**	$K_a = 178\ M^{-1}$ (**46a**) $K_a = 1{,}472\ M^{-1}$ (**46b**) $K_a = 638\ M^{-1}$ (**46c**)	Wang [197]
		

were able to calculate the detection limit by QCM (4.7×10^{-2} μM) and affinity constant of SA immobilized sensor for HA ($1.79 \times 10^{6}\ M^{-1}$) by SPR. Atomic force microscopy (AFM) was used to show that the HA molecule was homogeneously bound to 4-APBA–SA on the sensor surface [219–221]. This method has comparable detection limit to other reported HA detection methods. More importantly, the developed method could help overcome certain issues related to influenza detection, such as protein denaturation, requiring lengthy time, tedious labor work, and expensive agents such as antibodies [218].

3.4 Fluorescent Boronic Acids as a Tool for Glucose Detection

Diabetes is a disease that afflicts millions of people all over the world. One of the key issues in diabetes management is the monitoring of blood glucose concentrations. Commercially available glucose monitoring systems rely on redox chemistry and color reactions. These approaches work very well in blood sample analysis,

which gives a snap shot of glucose concentration at a given time point. One of the trends in diabetes management is the development of continuous glucose monitoring systems, which can be coupled with an insulin delivery device. In addition, in hospital settings and intensive care units (ICU), there are also needs for continuous glucose concentration monitoring. Thus, this has been an area of intense interest. For example, there have been interests in developing contact lens-type of glucose sensors for easy concentration determinations. Along these lines, chemosensing systems provide tremendous advantages over existing methods. In developing chemosensors for glucose, boronic acid-based compounds occupy a special place. The field of boronic acid-based glucose chemosensing development is a very active one. Along this line, the labs of Czarnik [33, 45], Shinkai [43, 222–226], Heagy [47, 227–230], Strongin [231–235], Singaram [29], Lakowicz [35, 236–239], James [54, 138, 240–246], and Wang [3, 53, 56, 67, 73, 197] have reported many such chemosensors. However, because this field has been heavily reviewed, readers are referred to these excellent review articles and selected examples are only listed in a table format (Table 1).

4 Conclusions

Because of its unique chemistry, boron has shown exciting potential in the development of both therapeutics and diagnostics. As we move into the future, there is an increasing emphasis on therapeutic approaches that are either guided by or coupled with biomarker detection. Boronic acid-based compounds are uniquely positioned for the development of theranostics because of their utilities as enzyme inhibitors, sensors, targeting molecules, and BNCT agents. We hope this chapter will stimulate more interest in boron chemistry and its application in biomedical sciences.

References

1. Baker SJ, Ding CZ, Akama T, Zhang YK, Hernandez V, Xia Y (2009) Future Med Chem 1:1275
2. Rainey CJ, Nyquist LA, Christensen RE, Strong PL, Culver BD, Coughlin JR (1999) J Am Diet Assoc 99:335
3. Yan J, Fang H, Wang BH (2005) Med Res Rev 25:490
4. Yang WQ, Gao XM, Wang BH (2003) Med Res Rev 23:346
5. Das BC, Thapa P, Karki R, Schinke C, Das S, Kambhampati S, Banerjee SK, Van Veldhuizen P, Verma A, Weiss LM, Evans T (2013) Future Med Chem 5:653
6. Zhu Y, Hosmane NS (2013) Future Med Chem 5:705
7. Baker SJ, Tomsho JW, Benkovic S (2011) J Chem Soc Rev 40:4279
8. Touchet S, Carreaux F, Carboni B, Bouillon A, Boucher JL (2011) Chem Soc Rev 40:3895
9. Yang W, Gao S, Wang B (2005) In: Hall D (ed) Organoboronic acids. Wiley, New York, p 481
10. Dowlut M, Hall DG (2006) J Am Chem Soc 128:4226

11. Hiratake J, Oda J (1997) Biosci Biotech Biochem 61:211
12. Gallardo-Williams MT, Maronpot RR, Wine RN, Brunssen SH, Chapin RE (2003) Prostate 54:44
13. Adams J, Kauffman M (2004) Can Invest 22:304
14. Bacha U, Barrila J, Velazquez-Campoy A, Leavitt SA, Freire E (2004) Biochemistry 43:4906
15. Holyoak T, Wilson MA, Fenn TD, Kettner CA, Petsko GA, Fuller RS, Ringe D (2003) Biochemistry 42:6709
16. Snow RJ, Bachovchin WW, Barton RW, Campbell SJ, Coutts SJ, Freeman DM, Gutheil WG, Kelly TA, Kennedy CA, Krolikowski DA, Leonard SF, Pargellis CA, Tong I, Adams J (1994) J Am Chem Soc 116:10860
17. Geele G, Garrett E, Hageman H (1975) J Am Soc Micro 1975:391
18. Farr-Jones S, Smith SO, Kettner CA, Griffin RG, Bachovchin WW (1989) Proc Natl Acad Sci U S A 86:6922
19. Groll M, Berkers CR, Ploegh HL, Ovaa H (2006) Structure 14:451
20. Matthews DA, Alden RA, Birktoft JJ, Freer ST, Kraut J (1975) J Biol Chem 250:7120
21. Powers RA, Blazquez J, Weston GS, Morosini MI, Baquero F, Shoichet BK (1999) Protein Sci 8:2330
22. Powers RA, Shoichet BK (2002) J Med Chem 45:3222
23. Stoll VS, Eger BT, Hynes RC, Martichonok V, Jones JB, Pai EF (1998) Biochemistry 37:451
24. Zhong S, Jordan F, Kettner CA, Polgar L (1991) J Am Chem Soc 113:9429
25. Bone R, Frank D, Kettner CA, Agard DA (1989) Biochemistry 28:7600
26. Aharoni R, Bronstheyn M, Jabbour A, Zaks B, Srebnik M, Steinberg D (2007) Bioorg Med Chem. doi:10.1016/j.bmc.2007.11.032
27. Cho BT (2005) In: Hall DG (ed) Boronic acids. Wiley-VCH, Weinheim, p 411
28. Jabbour A, Steinberg D, Dembitsky VM, Moussaieff A, Zaks B, Srebnik M (2004) J Med Chem 47:2409
29. Gamsey S, Miller A, Olmstead MM, Beavers CM, Hirayama LC, Pradhan S, Wessling RA, Singaram B (2007) J Am Chem Soc 129:1278
30. Gray CW Jr, Houston TA (2002) J Org Chem 67:5426
31. Wang Z, Zhang DQ, Zhu DB (2005) J Org Chem 70:5729
32. Wiskur SL, Lavigne JL, Ait-Haddou H, Lynch V, Chiu YH, Canary JW, Anslyn EV (2001) Org Lett 3:1311
33. Mohler LK, Czarnik AW (1994) J Am Chem Soc 116:2233
34. Badugu R, Lakowicz JR, Geddes CD (2004) Anal Chem 327:82
35. Badugu R, Lakowicz JR, Geddes CD (2005) Sens Actuators B Chem 104:103
36. DiCesare N, Lakowicz JR (2002) Anal Biochem 301:111
37. Oehlke A, Auer AA, Jahre I, Walfort B, Ruffer T, Zoufala P, Lang H, Spange S (2007) J Org Chem 72:4328
38. Swamy KMK, Lee YJ, Lee HN, Chun J, Kim Y, Kim SJ, Yoon J (2006) J Org Chem 71:8626
39. Cooper CR, Spencer N, James TD (1998) Chem Commun 1998:1365
40. Asher SA, Alexeev VL, Goponenko AV, Sharma AC, Lednev IK, Wilcox CS, Finegold DN (2003) J Am Chem Soc 125:3322
41. Bielecki M, Eggert H, Norrild JC (1999) J Chem Soc, Perkin Trans 2 1999:449
42. Eggert H, Frederiksen J, Morin C, Norrild JC (1999) J Org Chem 64:3846
43. James TD, Sandanayake KRAS, Iguchi R, Shinkai S (1995) J Am Chem Soc 117:8982
44. Yang W, Fan H, Gao S, Gao X, Ni W, Karnati V, Hooks WB, Carson J, Weston B, Wang B (2004) Chem Biol 11:439
45. Yoon J, Czarnik AW (1992) J Am Chem Soc 114:5874
46. Badugu R, Lakowicz JR, Geddes CD (2005) Curr Anal Chem 1:157
47. Cao HS, Heagy MD (2004) J Fluor 14:569
48. James TD, Shinkai S (2002) Top Curr Chem 218:159

49. Narra K, Mullins SR, Lee H-O, Strzemkowski-Brun B, Magalong K, Christiansen VJ, McKee PA, Egleston B, Cohen SJ, Weiner LM, Meropol NJ, Cheng JD (2007) Cancer Biol Ther 6:1691
50. Mader HS, Wolfbeis OS (2008) Microchim Acta 162:1
51. Pickup JC, Hussain F, Evans ND, Rolinski OJ, Birch DJS (2005) Biosens Bioelectr 20:2555
52. Striegler S (2003) Curr Org Chem 7:81
53. Wang W, Gao X, Wang B (2002) Curr Org Chem 6:1285
54. Norrild JC, Eggert H (1995) J Am Chem Soc 117:1479
55. Springsteen G, Wang B (2001) Chem Commun 2001:1608
56. Yan J, Springsteen G, Deeter S, Wang B (2004) Tetrahedron 60:11205
57. Alavi A, Axford JS (eds) (1995) Glycoimmunology, vol 376. Plenum, New York
58. Timmer M, Stocker B, Seeberger P (2007) Curr Opin Chem Biol 11:59
59. Alvarez-Manilla G, Warren NL, Abney T, Atwood J, Azadi P, York WS, Pierce M, Orlando R (2007) Glycobiology 17:677
60. Fukuda M (1994) In: Fukuda M, Hindsgaul O (eds) Molecular glycobiology. Oxford Univeristy Press, New York, p 1
61. Dennis JW (1992) In: Fukuda M (ed) Cell surface carbohydrates and cell development. CRC, Boca Raton, p 161
62. Gabius H-J, Gabius S (eds) (1993) Lectins and glycobiology. New York, Springer
63. Taylor M, Drickamer K (2006) Introduction to glycobiology. Oxford University Press, New York
64. Fukuda M, Hindsgaul O (eds) (1994) Molecular glycobiology. Oxford Univeristy Press, New York
65. Fukuda M (1992) Cell surface carbohydrates and cell development. CRC, Boca Raton
66. Jin S, Cheng Y, Reid S, Li M, Wang B (2010) Med Res Rev 30:171
67. Cheng Y, Li M, Wang S, Peng H, Reid S, Ni N, Fang H, Xu W, Wang B (2010) Sci China Ser B Chem 2010:53
68. Andreotti AH, Kahne D (1993) J Am Chem Soc 115:3352
69. Bosques CJ, Tschampel SM, Woods RJ, Imperiali B (2004) J Am Chem Soc 126:8421
70. Brisson J-R, Uhrinova S, Woods RJ, van der Zwan M, Jarrell HC, Paoletti LC, Kasper DL, Jennings H (1997) J Biochem 36:3278
71. Dennis JW, Granovsky M, Warren CE (1999) BioEssays 21:412
72. Liu XC, Hubbard JL, Scouten WH (1995) J Organomet Chem 493:91
73. Springsteen G, Wang BH (2002) Tetrahedron 58:5291
74. Kane RC, Bross PF, Farrell AT, Pazdur R (2003) Oncologist 8:508
75. San Miguel J, Blade J, Boccadoroe M, Cavenagh J, Glasmacher A, Jagannath S, Lonial S, Orlowski RZ, Sonneveld P, Ludwig H (2006) Oncologist 11:51
76. Codony-Servat J, Tapia MA, Bosch M, Oliva C, Domingo-Domenech J, Mellado B, Rolfe M, Ross JS, Gascon P, Rovira A, Albanell J (2006) Mol Cancer Ther 5:665
77. Schmid P, Kuehnhardt D, Kiewe P, Lehenbauer-Dehm S, Schippinger W, Greil R, Lange W, Preiss J, Niederle N, Brossart P, Freier W, Kuemmel S, de Velde HV, Regierer A, Possinger K (2008) Ann Oncol 19:871
78. Schenkein DP (2005) Clin Lung Cancer 7(Suppl 2):S49
79. Davies AM, Lara PN Jr, Mack PC, Gandara DR (2007) Clin Cancer Res 13:4647S
80. Voutsadakis IA, Patrikidou A, Tsapakidis K, Karagiannaki A, Hatzidaki E, Stathakis NE, Papandreou CN (2010) Int J Color Dis 25:795
81. Cusack JC, Liu R, Houston M, Abendroth K, Elliott PJ, Adams J, Baldwin AS (2001) Cancer Res 61:3535
82. Papandreou CN, Logothetis C (2004) J Cancer Res 64:5036
83. Hu W, Zheng R-R, Cui H-X, Yue D, Wang Y, Jiang Y-H (2012) Asian J Androl 14:695
84. Nawrocki ST, Carew JS, Pino MS, Highshaw RA, Dunner K, Huang P, Abbruzzese JL, McConkey D (2005) J Cancer Res 65:11658

85. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott P (1999) J Cancer Res 59:2615
86. Adams J (2004) Nat Rev Cancer 4:349
87. Gallwitz B (2010) Pharm Unserer Zeit 39:114
88. Gallwitz B (2007) Drugs Today 43:13
89. Matsuzaki Y, Sowa Y, Hirose T, Yokota T, Sakai T (2003) Environ Health Prev Med 8:157
90. Suzuki N, Suzuki T, Ota Y, Nakano T, Kurihara M, Okuda H, Yamori T, Tsumoto H, Nakagawa H, Miyata N (2009) J Med Chem 52:2909
91. Shimizu K, Maruyama M, Yasui Y, Minegishi H, Ban HS, Nakamura H (2010) Bioorg Med Chem Lett 20:1453
92. Khan SRMO (2011) US 2011/0,105,431 A1
93. Albers HMHG, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, Morris AJ, Smyth SS, Moolenaar WH, Ovaa H (2010) Proc Natl Acad Sci U S A 107:7257
94. Orlowski M (1994) J Cell Biochem 1994:120
95. Rose I (2005) Angew Chem Int Ed 44:5926
96. Hershko A (2005) Angew Chem Int Ed 44:5932
97. Ciechanover A (2005) Angew Chem Int Ed 44:5944
98. Genin E, Reboud-Ravaux M, Vidal J (2010) Curr Top Med Chem 10:232
99. Adams J, Behnke M, Chen SW, Cruickshank AA, Dick LR, Grenier L, Klunder JM, Ma YT, Plamondon L, Stein RL (1998) Bioorg Med Chem Lett 8:333
100. Richardson PG, Sonneveld P, Schuster MW, Stadtmauer EA, Facon T, Harousseau J-L, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, Blade J, Boccadoro M, Cavenagh JD, Boral AL, Esseltine D-L, Wen PY, Amato AA, Anderson KC, San Miguel J (2009) Br J Haematol 144:895
101. Adams JM, Cory S (2007) Oncogene 26:1324
102. Kupperman E, Lee EC, Cao Y, Bannerman B, Fitzgerald M, Berger A, Yu J, Yang Y, Hales P, Bruzzese F, Liu J, Blank J, Garcia K, Tsu C, Dick L, Fleming P, Yu L, Manfredi M, Rolfe M, Bolen J (2010) Cancer Res 2010:70
103. Zhu Y, Yao S, Xu B, Ge Z, Cui J, Cheng T, Li R (2009) Bioorg Med Chem 17:6851
104. Mathew S, Scanlan MJ, Raj BKM, Murty V, Garinchesa P, Old LJ, Rettig WJ, Chaganti RSK (1995) Genomics 25:335
105. Scanlan MJ, Raj BKM, Calvo B, Garinchesa P, Sanzmoncasi MP, Healey JH, Old LJ, Rettig W (1994) J Proc Natl Acad Sci U S A 91:5657
106. O'Farrell AM, van Vliet A, Abou Farha K, Cherrington JM, Campbell DA, Li X, Hanway D, Li J, Guler H-P (2007) Clin Ther 29:1692
107. Adams S, Miller GT, Jesson MI, Watanabe T, Jones B, Wallner BP (2004) Cancer Res 64:5471
108. Sambucetti LC, Fischer DD, Zabludoff S, Kwon PO, Chamberlin H, Trogani N, Xu H, Cohen D (1999) J Biol Chem 274:34940
109. Burroughs SK, Kaluz S, Wang D, Wang K, Van Meir EG, Wang B (2013) Future Med Chem 5:553
110. Zak K, Pecak A, Rys B, Wladyka B, Doemling A, Weber L, Holak TA, Dubin G (2013) Expert Opin Ther Pat 23:425
111. Carry J-C, Garcia-Echeverria C (2013) Bioorg Med Chem Lett 23:2480
112. Miriam Shadfan VL-P (2012) Zhi-Min Yuan Transl Cancer Res 1:88
113. Vousden KH, Prives C (2009) Cell 137:413
114. Donehower LA, Lozano G (2009) Nat Rev Cancer 9:830
115. Haupt Y, Maya R, Kazaz A, Oren M (1997) Nature 387:296
116. Honda R, Tanaka H, Yasuda H (1997) FEBS Lett 420:25
117. Momand J, Zambetti GP, Olson DC, George D, Levine A (1992) J Cell 69:1237
118. Oliner JD, Pietenpol JA, Thiagalingam S, Gvuris J, Kinzler KW, Vogelstein B (1993) Nature 362:857

119. Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J (2002) J Cell Biol 158:227
120. Moolenaar WH (2002) J Cell Biol 158:197
121. Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K (2002) J Biol Chem 277:39436
122. Stefan C, Jansen S, Bollen M (2005) Trends Biochem Sci 30:542
123. van Meeteren LA, Moolenaar WH (2007) Prog Lipid Res 46:145
124. Kanda H, Newton R, Klein R, Morita Y, Gunn MD, Rosen SD (2008) Nat Immunol 9:415
125. Pradère J-P, Klein J, Grès S, Guigné C, Neau E, Valet P, Calise D, Chun J, Bascands J-L, Saulnier-Blache J-S (2007) J Am Soc Nephrol 18:3110
126. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah BA, Kim ND (2007) Nat Med 14:45
127. Pamuklar Z, Federico L, Liu S, Umezu-Goto M, Dong A, Panchatcharam M, Fulerson Z, Berdyshev E, Natarajan V, Fang X (2009) J Biol Chem 284:7385
128. Noguchi K, Herr D, Mutoh T, Chun J (2009) Curr Opin Pharmacol 9:15
129. Baker DL, Fujiwara Y, Pigg KR, Tsukahara R, Kobayashi S, Murofushi H, Uchiyama A, Murakami-Murofushi K, Koh E, Bandle RW (2006) J Biol Chem 281:22786
130. Cui P, McCalmont WF, Tomsig JL, Lynch KR, Macdonald TL (2008) Bioorg Med Chem 16:2212
131. Ferry G (2008) J Pharmacol Exp Ther 327:809
132. Van Meeteren LA, Brinkmann V, Saulnier-Blache JS, Lynch KR, Moolenaar WH (2008) Cancer Lett 266:203
133. Kropff M, Bisping G, Schuck E, Liebisch P, Lang N, Hentrich M, Dechow T, Kröger N, Salwender H, Metzner B (2007) Br J Haematol 138:330
134. Albers HM, Hendrickx LJ, van Tol RJ, Hausmann J, Perrakis A, Ovaa H (2011) J Med Chem 54:4619
135. Hoffmann F (1998) WO98022496
136. Perni RB, Almquist SJ, Byrn RA, Chandorkar G, Chaturvedi PR, Courtney LF, Decker CJ, Dinehart K, Gates CA, Harbeson SL, Heiser A, Kalkeri G, Kolaczkowski E, Lin K, Luong YP, Rao BG, Taylor WP, Thomson JA, Tung RD, Wei YY, Kwong AD, Lin C (2006) Antimicrob Agents Chemother 50:899
137. Venkatraman S, Bogen SL, Arasappan A, Bennett F, Chen K, Jao E, Liu Y-T, Lovey R, Hendrata S, Huang Y, Pan W, Parekh T, Pinto P, Popov V, Pike R, Ruan S, Santhanam B, Vibulbhan B, Wu W, Yang W, Kong J, Liang X, Wong J, Liu R, Butkiewicz N, Chase R, Hart A, Agrawal S, Ingravallo P, Pichardo J, Kong R, Baroudy B, Malcolm B, Guo Z, Prongay A, Madison V, Broske L, Cui X, Cheng K-C, Hsieh Y, Brisson J-M, Prelusky D, Korfmacher W, White R, Bogdanowich-Knipp S, Pavlovsky A, Bradley P, Saksena AK, Ganguly A, Piwinski J, Girijavallabhan V, Njoroge FG (2006) J Med Chem 49:6074
138. Boloor A, Hanway D, Joshi M, Winn DT, Mendez G, Walls M, Wei P, Qian F, Zhang X, Zhang Y, Hepperle ME, Li X, Campbell DA, Betancort JM (2009) Bioorg Med Chem Lett 19:5708
139. Li XF, Zhang YK, Liu Y, Ding CZ, Li Q, Zhou Y, Plattner JJ, Baker SJ, Qian XL, Fan DZ, Liao L, Ni ZJ, White GV, Mordaunt JE, Lazarides LX, Slater MJ, Jarvest RL, Thommes P, Ellis M, Edge CM, Hubbard JA, Somers D, Rowland P, Nassau P, McDowell B, Skarzynski TJ, Kazmierski WM, Grimes RM, Wright LL, Smith GK, Zou WX, Wright J, Pennicott LE (2010) Bioorg Med Chem Lett 20:3550
140. Llinas-Brunet M, Bailey MD, Bolger G, Brochu C, Faucher AM, Ferland JM, Garneau M, Ghiro E, Gorys V, Grand-Maitre C, Halmos T, Lapeyre-Paquette N, Liard F, Poirier M, Rheaume M, Tsantrizos YS, Lamarre D (2004) J Med Chem 47:1605
141. Llinas-Brunet M, Bailey MD, Goudreau N, Bhardwaj PK, Bordeleau J, Boes M, Bousquet Y, Cordingley MG, Duan J, Forgione P, Garneau M, Ghiro E, Gorys V, Goulet S, Halmos T, Kawai SH, Naud J, Poupart M-A, White PW (2010) J Med Chem 53:6466

142. Venkatraman S, Wu WL, Prongay A, Girijavallabhan V, Njoroge FG (2009) Bioorg Med Chem Lett 19:180
143. Hall BG, Barlow M (2005) J Antimicrob Chemother 55:1050
144. Livermore DM (1998) J Antimicrob Chemother 41:25
145. Hartman BJ, Tomasz A (1984) J Bacteriol 158:513
146. Zervosen A, Sauvage E, Frère J-M, Charlier P, Luxen A (2012) Molecules 17:12478
147. Bush K, Jacoby GA, Medeiros AA (1995) Antimicrob Agents Chemother 39:1211
148. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley S, Jaurin B, Grundstrom T (1983) Biochem J 209:229
149. Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, Grundstrom T (1982) Biochem J 207:315
150. Coudron PE (2005) J Clin Microbiol 43:4163
151. Weston GS, Blazquez J, Baquero F, Shoichet BK (1998) J Med Chem 41:4577
152. Pechenov A, Stefanova ME, Nicholas RA, Peddi S, Gutheil WG (2003) Biochemistry 42:579
153. Nicola G, Peddi S, Stefanova M, Nicholas RA, Gutheil WG, Davies C (2005) Biochemistry 44:8207
154. Ness S, Martin R, Kindler AM, Paetzel M, Gold M, Jensen SE, Jones JB, Strynadka NC (2000) J Biochem 39:5312
155. Morandi F, Caselli E, Morandi S, Focia PJ, Blazquez J, Shoichet BK, Prati F (2003) J Am Chem Soc 125:685
156. Chen Y, Shoichet B, Bonnet R (2005) J Am Chem Soc 127:5423
157. Bailey PJ, Cousins G, Snow GA, White A (1980) J Antimicrob Agents Chemother 17:549
158. Dewar M (1964) Progress in boron chemistry, vol 1. Macmillan, New York
159. Gronowitz S, Dahlgren T, Namtvedt J, Roos C, Rosen G, Sjoberg B, Forsgren U (1971) Acta Pharm Suec 8:623
160. Ni N, Choudhary G, Peng H, Li M, Chou H-T, Lu C-D, Gilbert ES, Wang B (2009) Chem Biol Drug Des 74:51
161. Ni N, Choudhary G, Li M, Wang B (2009) Biochem Biophys Res Commun 382:153
162. Ni N, Li M, Wang J, Wang B (2009) Med Res Rev 29:65
163. Peng H, Cheng Y, Ni N, Li M, Choudhary G, Chou H, Lu C-D, Tai PC, Wang B (2009) Abstracts of Papers of the American Chemical Society, p 238
164. Baker SJ, Zhang YK, Akama T, Lau A, Zhou H, Hernandez V, Mao WM, Alley MRK, Sanders V, Plattner JJ (2006) J Med Chem 49:4447
165. Seiradake E, Mao W, Hernandez V, Baker SJ, Plattner JJ, Alley MRK, Cusack S (2009) J Mol Biol 390:196
166. Rock FL, Mao WM, Yaremchuk A, Tukalo M, Crepin T, Zhou HC, Zhang YK, Hernandez V, Akama T, Baker SJ, Plattner JJ, Shapiro L, Martinis SA, Benkovic SJ, Cusack S, Alley MRK (2007) Science 316:1759
167. Melnikova I (2009) Nat Rev Drug Discov 8:353
168. Quan ML, Pruitt JR, Ellis CD, Liauw AY, Galemmo RA, Stouten PFW, Wityak J, Knabb RM, Thoolen MJ, Wong PC, Wexler RR (1997) Bioorg Med Chem Lett 7:2813
169. Quan ML, Wityak J, Dominguez C, Duncia JV, Kettner CA, Ellis CD, Liauw AY, Park JM, Santella JB, Knabb RM, Thoolen MJ, Weber PC, Wexler RR (1997) Bioorg Med Chem Lett 7:1595
170. Fevig JM, Cacciola J, Alexander RS, Knabb RM, Lam GN, Wong PC, Wexler RR (1998) Bioorg Med Chem Lett 8:3143
171. Fevig JM, Buriak J, Cacciola J, Alexander RS, Kettner CA, Knabb RM, Pruitt JR, Weber PC, Wexler RR (1998) Bioorg Med Chem Lett 8:301
172. Leiting B, Pryor KD, Wu JK, Marsilio F, Patel RA, Craik CS, Ellman JA, Cummings RT, Thornberry NA (2003) Biochem J 371:525
173. von GTW, Trevillyan JM (2006) Drug Dev Res 67:627
174. Coutts SJ, Kelly TA, Snow RJ, Kennedy CA, Barton RW, Adams J, Krolikowski DA, Freeman DM, Campbell SJ et al. (1996) J Med Chem 39:2087

175. Hu Y, Ma L, Wu M, Wong MS, Li B, Corral S, Yu Z, Nomanbhoy T, Alemayehu S, Fuller SR, Rosenblum JS, Rozenkrants N, Minimo LC, Ripka WC, Szardenings AK, Kozarich JW, Shreder KR (2005) Bioorg Med Chem Lett 15:4239
176. Tran T, Quan C, Edosada CY, Mayeda M, Wiesmann C, Sutherlin D, Wolf BB (2007) Bioorg Med Chem Lett 17:1438
177. Lai JH, Wu W, Zhou Y, Maw HH, Liu Y, Milo LJ Jr, Poplawski SE, Henry GD, Sudmeier JL, Sanford DG, Bachovchin WW (2007) J Med Chem 50:2391
178. Garcia-Soria G, Gonzalez-Galvez G, Argoud GM, Gerstman M, Littlejohn TW III, Schwartz SL, O'Farrell AM, Li X, Cherrington JM, Bennett C, Guler HP (2008) Diabetes Obes Metab 10:293
179. Gupta R, Walunj SS, Tokala RK, Parsa KVL, Singh SK, Pal M (2009) Curr Drug Targets 10:71
180. Marepally SR, Yao M-L, Kabalka GW (2013) Future Med Chem 5:693
181. Krzeslak A, Pomorski L, Gaj Z, Lipinska A (2003) Cancer Lett 196:101
182. Srinivas U, Pahlsson P, Lundblad A (1996) Scand J Immunol 44:197
183. Mannori G, Crottet P, Cecconi O, Hanasaki K, Aruffo A, Nelson RM, Varki A, Bevilacqua MP (1995) Cancer Res 55:4425
184. Goodman JL, Nelson CM, Klein MB, Hayes SF, Weston BW (1999) J Clin Invest 103:407
185. Grundner C, Pancera M, Kang JM, Koch M, Sodroski J, Wyatt R (2004) Virology 330:233
186. Azevedo-Pereira JM, Santos-Costa Q, Mansinho K, Moniz-Pereira J (2003) Virology 313:136
187. Clark GF, Oehninger S, Patankar MS, Koistinen R, Dell A, Morris HR, Koistinen H, Seppala M (1996) Hum Reprod 11:467
188. Fukumori T, Takenaka Y, Yoshii T, Kim HRC, Hogan V, Inohara H, Kagawa S, Raz A (2003) Cancer Res 63:8302
189. Grassadonia A, Tinari N, Iurisci I, Piccolo E, Cumashi A, Innominato P, D'Egidio M, Natoli C, Piantelli M, Iacobelli S (2002) Glycoconj J 19:551
190. Gifford R (2013) ChemPhysChem 14:2032
191. Hatano K, Matsuoka K, Terunuma D (2013) Chem Soc Rev 42:4574
192. Becker B, Cooper MA (2011) J Mol Recognit 24:754
193. Gabius HJ, Andre S, Jimenez-Barbero J, Romero A, Solis D (2011) Trends Biochem Sci 36:298
194. Lei JP, Ju HX (2012) Chem Soc Rev 41:2122
195. Zeng XQ, Andrade CAS, Oliveira MDL, Sun XL (2012) Anal Bioanal Chem 402:3161
196. Ye C, Kiriyama K, Mistuoka C, Kannagi R, Ito K, Watanabe T, Kondo K, Akiyama S, Takagi H (1995) Int J Cancer 61:455
197. Karnati VV, Gao XM, Gao SH, Yang WQ, Ni WJ, Sankar S, Wang BH (2002) Bioorg Med Chem Lett 12:3373
198. Dai C, Cazares LH, Wang L, Chu Y, Wang SL, Troyer DA, Semmes OJ, Drake RR, Wang B (2011) Chem Commun 47:10338
199. Thiery G, Anselmi E, Audebourg A, Darii E, Abarbri M, Terris B, Tabet J-C, Gut IG (2008) Proteomics 8:3725
200. Rauser S, Marquardt C, Balluff B, Deininger SR-O, Albers C, Belau E, Hartmer R, Suckau D, Specht K, Ebert MP, Schmitt M, Aubele M, Höfler H, Walch A (2010) J. Proteome Res 9:1854
201. Schwamborn K, Caprioli RM (2010) Nat Rev Cancer 10:639
202. Chaurand PSM, Caprioli RM (1999) Anal Chem 71:5263
203. Shchepinov M, Korshun V (2003) Chem Soc Rev 32:170
204. Zhang K, Baeckstrom D, Brevinge H, Hansson GC (1996) J Cell Biochem 60:538
205. Yamada N, Chung YS, Maeda K, Sawada T, Ikehara T, Nishino H, Okuno M, Sowa M (1995) Invasion Metastasis 15:95
206. Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T (1997) Dis Colon Rectum 40:420

207. Tanaka K, Togo S, Nanko M, Ishikawa T, Ichikawa Y, Yamaoka H, Shimada H (1999) Hepatogastroenterol 46:875
208. Zhang J, Nakayama J, Ohyama C, Suzuki M, Suzuki A, Fukuda M, Fukuda MN (2002) Cancer Res 62:4194
209. Chu Y, Wang D, Wang K, Liu ZL, Weston B, Wang B (2013) Bioorg Med Chem Lett 23:6307
210. Liu S, Wollenberger U, Halámek J, Leupold E, Stöcklein W, Warsinke A, Scheller FW (2005) Chem Eur J 11:4239
211. Song W, Jeong SH, Kim JS, Kim HS, Shin DH, Roh KH, Lee KM (2007) Diagn Microbiol Infect Dis 57:315
212. Wannapob R, Kanatharana P, Limbut W, Numnuam A, Asawatreratanakul P, Thammakhet C, Thavarungkul P (2010) Biosens Bioelectron 26:357
213. Saito S, Massie TL, Maeda T, Nakazumi H, Colyer CL (2012) Anal Chem 84:2452
214. Saito S, Maeda T, Nakazumi H, Colyer CL (2012) Anal Sci 29:157
215. Upadhyay S, Sen MR, Bhattacharjee A (2011) J Med Microbiol 60:691
216. Rosi NL, Mirkin CA (2005) Chem Rev 105:1547
217. Iqbal SS, Mayo MW, Bruno JG, Bronk BV, Batt CA, Chambers JP (2000) Biosens Bioelectron 15:549
218. Diltemiz SE, Ersöz A, Hür D, Keçili R, Say R (2013) Mater Sci Eng C Mater Biol Appl 33(2): 824–830
219. Amano Y, Cheng Q (2005) Anal Bioanal Chem 381:156
220. Schofield D, Dimmock N (1996) J Virol Methods 62:33
221. Sato T, Ishii M, Ohtake F, Nagata K, Terabayashi T, Kawanishi Y, Okahata Y (1999) Glycoconj J 16:223
222. Takeuchi M, Yamamoto M, Shinkai S (1997) Chem Commun 1997:1731
223. Suenaga H, Mikami M, Sandanayake KRAS, Shinkai S (1995) Tetrahedron Lett 36:4825
224. Takeuchi M, Mizuno T, Shinmori H, Nakashima M, Shinkai S (1996) Tetrahedron 52:1195
225. Kijima H, Takeuchi M, Shinkai S (1998) Chem Lett 1998:781
226. Imada T, Kijima H, Takeuchi M, Shinkai S (1996) Tetrahedron 52:2817
227. Adhikiri DP, Heagy MD (1999) Tetrahedron Lett 40:7893
228. Cao H, Diaz DI, DiCesare N, Lakowicz JR, Heagy MD (2002) Org Lett 4:1503
229. Cao H, McGill T, Heagy MD (2004) J Org Chem 69:2959
230. Cao Z, Nandhikonda P, Heagy MD (2009) J Org Chem 74:3544
231. Davis CJ, Lewis PT, McCarroll ME, Read MW, Cueto R, Strongin RM (1999) Org Lett 1:331
232. Lewis PT, Davis CJ, Cabell LA, He M, Read MW, McCarroll ME, Strongin RM (2000) Org Lett 2:589
233. He M, Johnson RJ, Escobedo JO, Beck PA, Kim KK, St Luce NN, Davis CJ, Lewis PT, Fronczek FR, Melancon BJ, Mrse AA, Treleaven WD, Strongin RM (2002) J Am Chem Soc 124:5000
234. Kim KK, Escobedo JO, St Luce NN, Rusin O, Wong D, Strongin RM (2003) Org Lett 5:5007
235. Lim S, Escobedo JO, Lowry M, Strongin RM (2011) Chem Commun 47:8295
236. Badugu R, Lakowicz JR, Geddes CD (2005) Talanta 66:569
237. DiCesare N, Lakowicz JR (2001) Chem Commun 2001:2022
238. DiCesare N, Lakowicz JR (2001) J Phys Chem A 105:6834
239. Di Cesare N, Lakowicz JR (2001) J Photoch Photobio A 143:39
240. James TD, Shinmori H, Shinkai S (1997) Chem Commun 1997:71
241. Cooper CR, James TD (1998) Chem Lett 27:883
242. Arimori S, Bosch LI, Ward CJ, James TD (2001) Tetrahedron Lett 42:4553
243. Arimori S, Bosch LI, Ward CJ, James TD (2002) Tetrahedron Lett 43:911
244. Scrafton DK, Taylor JE, Mahon MF, Fossey JS, James TD (2008) J Org Chem 73:2871
245. Zhao J, Davidson MG, Mahon MF, Kociok-Köhn G, James TD (2004) J Am Chem Soc 126:16179
246. Bosch LI, Mahon MF, James TD (2004) Tetrahedron Lett 45:2859

Top Med Chem (2016) 17: 29–60
DOI: 10.1007/7355_2014_55

Published online: 2 December 2014

Drug Design Based on the Carbon/Silicon Switch Strategy

Reinhold Tacke and Steffen Dörrich

Abstract Silicon chemistry has been demonstrated to be a novel source of chemical diversity in drug design. The carbon/silicon switch strategy, i.e., the strategic replacement of a carbon atom with a silicon atom (sila-substitution) within a well-known drug, with the rest of the molecule being identical, is one of the methods that are currently used for the design and development of new silicon-based drugs. Some of the fundamental differences between carbon and silicon (e.g., differences in the covalent radii and electronegativities) can lead to marked alterations in the physicochemical and biological properties of the sila-drugs. In general, the sila-analogues share the same mode of action as the parent carbon compounds but may have altered biological properties. Incorporation of silicon into a drug can affect and, ideally, improve the pharmacological potency and selectivity, the pharmacodynamics, and the pharmacokinetics. Examples resulting from the carbon/silicon switch strategy are sila-venlafaxine, sila-haloperidol, and disila-bexarotene, the silicon analogues of the serotonin/noradrenaline reuptake inhibitor venlafaxine, the dopamine antagonist haloperidol, and the retinoid agonist bexarotene, respectively. Using these particular examples, the basic principles of the carbon/silicon strategy in drug design are illustrated in this review.

Keywords Bexarotene, Bioisosterism, Carbon/silicon switch, Drug design, Haloperidol, Sila-drugs, Silicon, Synthesis and SAR, Venlafaxine

R. Tacke (✉) and S. Dörrich
Institute of Inorganic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
e-mail: r.tacke@uni-wuerzburg.de

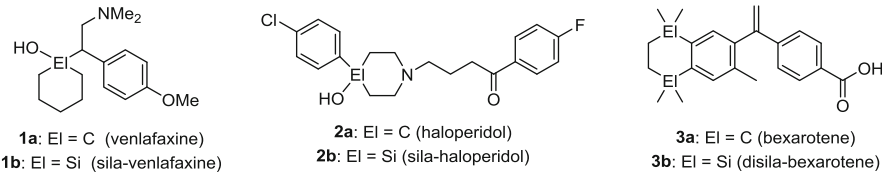
Contents

1 Introduction

Over the past five decades, many biologically active organosilicon compounds have been synthesized, and silicon chemistry has been demonstrated to be a novel source of chemical diversity in drug design (for reviews, see [1–17]). There are two different approaches that can be used for the design of silicon-based drugs: (1) synthesizing a silicon analogue of a known drug in which at least one carbon atom has been replaced by a silicon atom, with the rest of the molecule being identical (carbon/silicon exchange, carbon/silicon switch, sila-substitution) and (2) synthesizing completely new silicon-based classes of compounds (the carbon analogues of which are unknown or do not exist for principal reasons) that exploit specific features of silicon chemistry to address a well-validated target. This contribution is focused exclusively on the first approach, the carbon/silicon switch strategy. The second approach is discussed in [18] of this book.

Almost 50 years ago, Fessenden et al. reported on the first silicon analogues of known organic drugs [19–22]. Following this pioneering work, many sila-drugs have been synthesized and pharmacologically characterized over the past five decades, and nowadays the carbon/silicon switch strategy is an established method in drug design, not only in academia but also in pharmaceutical industry.

As there are many reviews dealing with the carbon/silicon switch strategy, this contribution has not been written as another, updated overview. Instead, this article is focused on the discussion of a few selected examples of this approach to demonstrate the strategic background and to highlight the huge potential of the carbon/silicon switch strategy for drug design. The examples chosen are selected studies of our group and refer to sila-venlafaxine (**1b**), sila-haloperidol (**2b**), and disila-bexarotene (**3b**) and derivatives. These compounds are silicon analogues of the serotonin/noradrenaline reuptake inhibitor venlafaxine (**1a**), the dopamine antagonist haloperidol (**2a**), and the retinoid agonist bexarotene (**3a**), respectively. The chemical structures of the C/Si pairs **1a/1b**, **2a/2b**, and **3a/3b** are shown in Fig. 1.



1a: El = C (venlafaxine)
1b: El = Si (sila-venlafaxine)

2a: El = C (haloperidol)
2b: El = Si (sila-haloperidol)

3a: El = C (bexarotene)
3b: El = Si (disila-bexarotene)

Fig. 1 Chemical structures of the C/Si pairs **1a/1b**–**3a/3b**

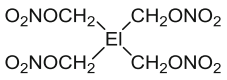
2 Sila-Substitution of Drugs: The Concept

Silicon is similar to carbon in that it forms four covalent bonds with many other elements. Like carbon, silicon is a Group 14 element, possessing many similarities to carbon but also presenting some crucial differences. The following are some of the fundamental differences that can be used to provide a benefit in drug design by using the carbon/silicon switch strategy [1, 4, 8–12, 14, 15, 17].

Silicon (1.17 Å) has a larger covalent radius than carbon (0.77 Å), resulting in the formation of longer silicon–element bonds compared with the analogous carbon–element bonds. For example, the average C–C bond length is 1.54 Å, whereas the Si–C bond length is 1.87 Å. This increase in bond lengths leads to an increase in the size and conformational flexibility of the sila-analogue and also affects its shape. These subtle changes in size, shape, and conformational flexibility can lead to changes in the way the sila-analogue interacts with specific proteins when compared with its parent carbon counterpart, with consequential effects on its pharmacodynamic and pharmacokinetic properties. In the case of lipophilic organic substituents attached to a central carbon atom of a given drug, the corresponding sila-analogue is more lipophilic due to the different covalent radii of carbon and silicon. This change in lipophilicity can modify in many ways the in vivo properties of a drug.

Silicon (1.74, Allred–Rochow) is less electronegative than carbon (2.50), leading to different polarizations of analogous carbon–element and silicon–element bonds. These differences can also affect the pharmacodynamics and pharmacokinetics. A special effect is an increase in acidity of a silanol (R_3Si–OH) compared with that of an analogous alcohol (R_3C–OH), particularly in those cases where electron-withdrawing substituents are bound to the silicon atom. As a result, the hydrogen bond strength of the silanol will be more favorable as a donor than that of the analogous alcohol. In pharmacophores, in which the COH group functions as a hydrogen bond donor, the carbon/silicon switch (→ SiOH group) can be beneficial in providing improved ligand–receptor binding.

However, there are also some striking differences between carbon and silicon that lead to stability-driven limitations of the carbon/silicon switch strategy. Unlike carbon, silicon can form thermodynamically stable five- or six-coordinate compounds that can be isolated. Conversely, coordination numbers two and three are disfavored over the coordination number four in the case of silicon. Generally,



4a: El = C
4b: El = Si

Fig. 2 Chemical structures of the C/Si analogues **4a** and **4b**

silicon–element double and triple bonds are thermodynamically unstable and can only be kinetically stabilized by very bulky substituents. Thus, replacement of an sp- or sp^2-hybridized carbon atom in a drug by a silicon atom is not possible.

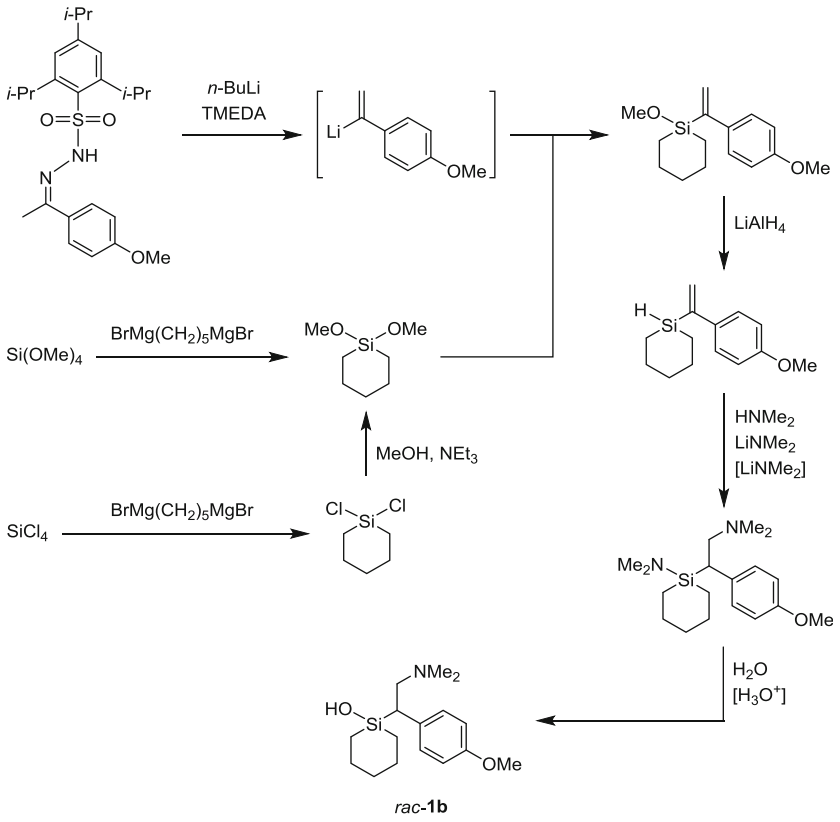
Another limitation concerns the hydrolytic instability of many silicon–element bonds, such as the Si–H, Si–O, Si–S, Si–N, and Si–P bond. Under physiological conditions, these bonds undergo hydrolysis ($R_3Si–H + H_2O \rightarrow R_3Si–OH + H_2$; $R_3Si–XR + H_2O \rightarrow R_3Si–OH + HXR$ (X=O, S); $R_3Si–XR_2 + H_2O \rightarrow R_3Si–OH + HXR_2$ (X=N, P)). On the other hand, this hydrolytic instability offers possibilities for the development of short-acting drugs that undergo a controlled hydrolytic decomposition. Also, this hydrolytic lability could be useful for prodrug strategies.

In this context, another stability issue (although very special) should be briefly mentioned. Some years ago, the silicon analogue of the coronary vasodilator pentaerythritol tetranitrate (**4a**), sila-pentaerythritol tetranitrate (**4b**), was successfully synthesized but then turned out to be a very dangerous explosive that did not allow any biological characterization (Fig. 2) [23]. The carbon compound **4a** is also shock-sensitive and is a well-known commercial explosive (nitropenta, PENT), but it can be handled relatively easily. In contrast, the silicon analogue **4b** is an extremely shock-sensitive material that is very difficult to handle (for sila-analogues of other explosives, see [24, 25]). This different behavior of the C/Si analogues **4a** and **4b** once again demonstrates that, in spite of the many similarities of carbon and silicon, the carbon/silicon switch can significantly affect the chemical and physicochemical properties.

3 Sila-Substitution of Drugs: Selected Examples

3.1 Sila-Venlafaxine: A Silicon Analogue of the Serotonin/Noradrenaline Reuptake Inhibitor Venlafaxine

Racemic venlafaxine (*rac*-**1a**) is a serotonin/noradrenaline reuptake inhibitor that is in clinical use as an antidepressant. *rac*-Sila-venlafaxine (*rac*-**1b**), a silicon analogue of *rac*-**1a**, was synthesized in multistep syntheses, starting from $SiCl_4$ or $Si(OMe)_4$ (Scheme 1) [26, 27]. An alternative synthesis of *rac*-**1b**, starting from $SiCl_4$, is shown in Scheme 2 [28, 29]. The single enantiomers of sila-venlafaxine, (*R*)-**1b** and (*S*)-**1b**, were obtained by resolution of *rac*-**1b**, using (+)- and (−)-10-camphorsulfonic acid as resolving agents [26]. The absolute configuration of (*R*)-**1b**

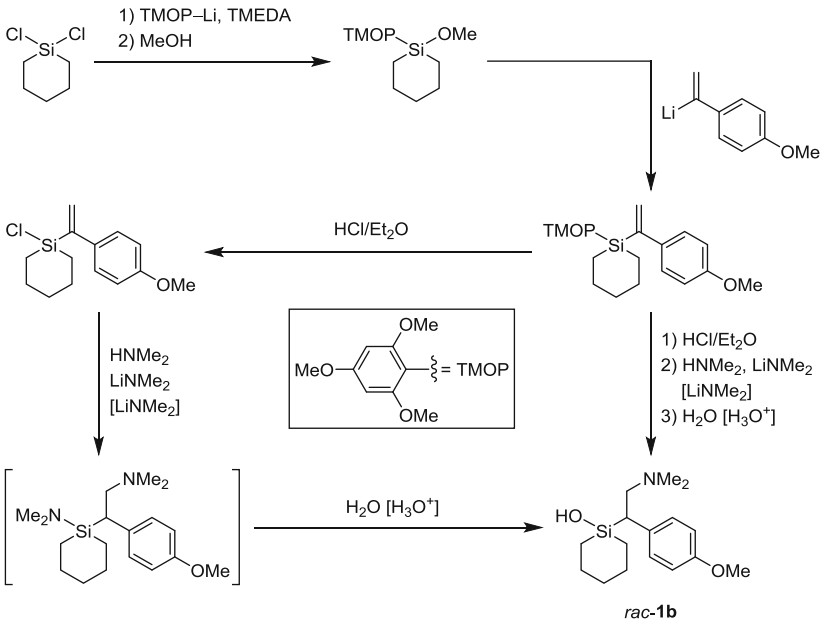


Scheme 1 Synthesis of *rac*-**1b**

was determined by a crystal structure analysis of the corresponding hydrobromide (*R*)-**1b**·HBr [26]. The molecular structure of the cation of (*R*)-**1b**·HBr is depicted in Fig. 3 [26].

Figure 4 shows a superposition of the cyclohexane skeleton of (*S*)-**1a**·HBr and the 1-silacyclohexane skeleton of the (*S*)-enantiomer of *rac*-**1b**·HCl (structures determined by single-crystal X-ray diffraction) [26]. Due to the longer covalent radius of the silicon atom, the 1-silacyclohexane ring is more flat than the cyclohexane ring, leading to different relative orientations of the OH and NH groups of the two C/Si analogues. These different structures may affect the ligand–receptor interactions of venlafaxine (**1a**) and sila-venlafaxine (**1b**).

To get information about their physicochemical properties, compounds *rac*-**1a** and *rac*-**1b** were studied for their pK_a, log*P*, and log*D* (log*P* at pH 7.4) values [26]. As can be seen from Table 1, very similar physicochemical profiles were observed for the two C/Si analogues, suggesting similar brain penetration profiles of venlafaxine (**1a**) and sila-venlafaxine (**1b**). At pH 7.4 (physiological pH), both compounds exist predominantly in their protonated form (degree of protonation ca. 90%).



Scheme 2 Synthesis of *rac*-**1b**

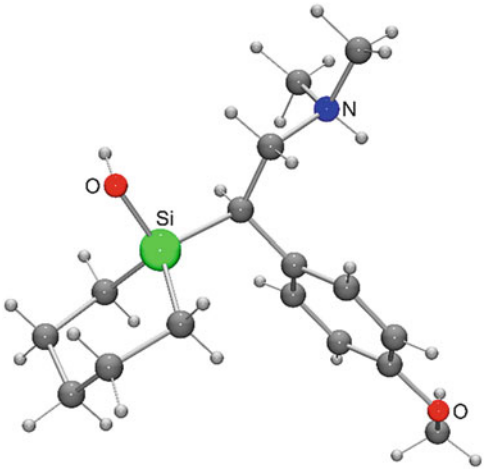


Fig. 3 Molecular structure of the cation of (*R*)-**1b**·HBr in the crystal [26]

Compounds (*R*)-**1a**, (*S*)-**1a**, (*R*)-**1b**, and (*S*)-**1b** were studied for their efficacy in serotonin, noradrenaline, and dopamine reuptake inhibition assays [26]. Human HEK-293 (serotonin), MDCK (noradrenaline), and CHO-K1 (dopamine) cell lines and the radioligands [^{3}H]serotonin, [^{3}H]noradrenaline, and [^{3}H]dopamine,

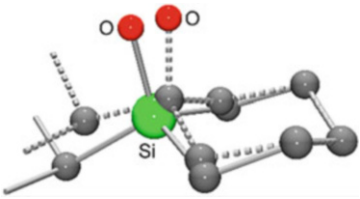


Fig. 4 Superposition of the cyclohexane skeleton of (*S*)-**1a**·HBr (*dashed bonds*) and the 1-silacyclohexane skeleton of the (*S*)-enantiomer of *rac*-**1b**·HCl (*solid bonds*) (hydrogen atoms are omitted for clarity) [26]

Table 1 pK_a, log*P*, and log*D* (log*P* at pH 7.4) data for *rac*-**1a** and *rac*-**1b** [26]

Compound	pK_a	log*P*	log*D*
rac-**1a**	9.7	3.13	0.88
rac-**1b**	9.7	3.21	0.92

Table 2 Serotonin, noradrenaline, and dopamine transporter inhibition profiles of (*R*)-**1a**, (*S*)-**1a**, (*R*)-**1b**, and (*S*)-**1b** (IC_{50}, μM) [26]

Compound	Serotonin	Noradrenaline	Dopamine
(*R*)-**1a**	0.030	0.061	19.600
(*S*)-**1a**	0.006	0.754	6.670
(*R*)-**1b**	3.168	0.251	5.270
(*S*)-**1b**	0.791	4.715	36.350

The data represent the mean of at least two determinations

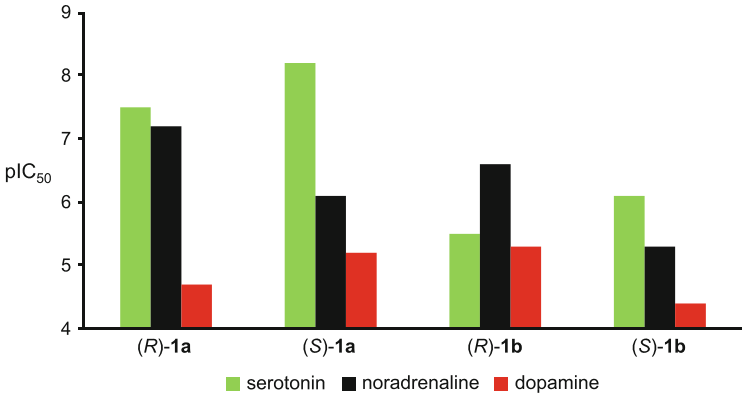


Fig. 5 In vitro efficacy of (*R*)-**1a**, (*S*)-**1a**, (*R*)-**1b**, and (*S*)-**1b** regarding serotonin, noradrenaline, and dopamine reuptake inhibition [26]. pIC_{50} denotes the negative decadic logarithm of the half-maximum effect concentration (M). The data represent the mean of at least two determinations

respectively, were used in these studies. The results obtained are shown in Table 2 and Fig. 5.

As can be seen from Table 2 and Fig. 5, the sila-venlafaxine enantiomers (*R*)-**1b** and (*S*)-**1b** exhibit a substantially altered monoamine reuptake inhibition profile when compared with the respective venlafaxine enantiomers (*R*)-**1a** and (*S*)-**1a**.

While activity at the noradrenaline and dopamine transporters is only slightly affected by sila-substitution, the potency at the serotonin transporters is reduced by two orders of magnitude, resulting in totally altered selectivity profiles. (*S*)-Venlafaxine ((*S*)-**1a**) is a potent and selective serotonin reuptake inhibitor, being about 100- and 1,000-fold more potent at serotonin transporters than noradrenaline and dopamine transporters, respectively. Sila-substitution of (*S*)-**1a** (→ (*S*)-**1b**) makes this compound a rather weak mixed serotonin/noradrenaline reuptake inhibitor, with only about 50- and 6-fold, respectively, selectivity over dopamine and noradrenaline transporters. (*R*)-Venlafaxine ((*R*)-**1a**) is also a mixed serotonin/noradrenaline reuptake inhibitor, with about 500-fold selectivity over dopamine transporters and a significantly higher potency than that of (*S*)-**1b**. Sila-substitution of (*R*)-**1a** (→ (*R*)-**1b**) results in a substantially altered selectivity profile. (*R*)-Sila-venlafaxine ((*R*)-**1b**) is a selective noradrenaline reuptake inhibitor being about 10-fold more potent at noradrenaline transporters than at serotonin and dopamine transporters. This is one of the most impressive examples demonstrating that sila-substitution can affect both pharmacological potency and pharmacological selectivity.

To evaluate the potential of the noradrenaline selectivity of (*R*)-sila-venlafaxine ((*R*)-**1b**) for the treatment of various CNS disorders, the compound was profiled further and was tested in vitro across a panel of 68 common receptors/channels and 16 enzymes [30]. In the majority of assays, (*R*)-**1b** was found to be inactive (<50% inhibition at 10 μM), including opioid receptors. Only weak affinity at Ca^{2+} and Na^{+} channels was observed. (*R*)-Sila-venlafaxine ((*R*)-**1b**) was also shown to display only low in vitro activity at the cytochrome P450 enzymes CYP2D6, CYP1A2, CYP2C19, and CYP3A4 (<50 % inhibition at 10 μM). In conclusion, (*R*)-**1b** can be indeed regarded as a selective noradrenaline reuptake inhibitor.

Following the idea that this particular selectivity profile may have utility in the treatment of emesis, (*R*)-**1b** was evaluated in a ferret model of morphine-induced emesis [30]. As the C/Si analogues *rac*-**1a** and *rac*-**1b** have almost identical physicochemical properties (Table 1), and based on the evidence that *rac*-**1a** achieves high clinical efficacy in the treatment of depression, one would have high confidence that (*R*)-**1b** would also readily reach its site of action in the CNS. (*R*)-Sila-venlafaxine ((*R*)-**1b**) was dosed orally 2 h prior to the emetogen morphine (0.125 mg/kg, s.c.), and the animals were monitored for retching and vomiting events for up to 2 h following the administration of morphine. At 50 mg/kg, (*R*)-**1b** completely abolished emetic episodes, and almost complete inhibition (93 %) was achieved at 5 mg/kg. In male ferrets, dosed at 5 mg/kg orally, (*R*)-**1b** displayed a plasma half-life of 1.1 h, with a C_{max} of 225 ng/ml. In conclusion, the selective noradrenaline reuptake inhibitor (*R*)-sila-venlafaxine ((*R*)-**1b**) has been demonstrated to effectively inhibit emetic episodes caused by an emetogen in a well-characterized in vivo model.

Following this proof of principle, the antiemetic potential of (*R*)-sila-venlafaxine ((*R*)-**1b**) was further evaluated. For this purpose, the potential of (*R*)-**1b** to antagonize cisplatin (15 mg/kg, i.p.)-induced acute and delayed emesis in the ferret was studied [31]. At 5 and 15 mg/kg/4 h (i.p.), (*R*)-**1b** was highly effective in reducing

emetic episodes during the 0–24-h period (acute emesis), and at 15 mg/kg/4 h (i.p.), (*R*)-**1b** was also active to reduce emesis during the 24–72-h period (delayed emesis). These studies provided evidence for an antiemetic potential of the selective noradrenaline reuptake inhibitor (*R*)-sila-venlafaxine ((*R*)-**1b**) to reduce chemotherapy-induced acute and delayed emesis.

3.2 Sila-Haloperidol: A Silicon Analogue of the Dopamine Antagonist Haloperidol

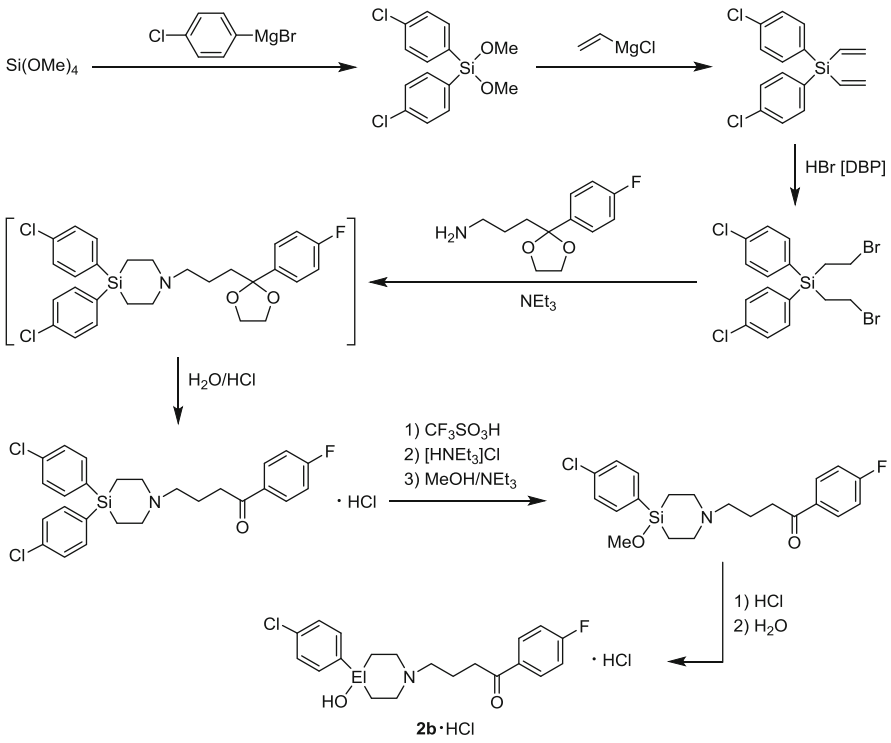
Haloperidol (**2a**) is a dopamine (D_2) antagonist that is in clinical use as an antipsychotic agent for the treatment of schizophrenia. Sila-haloperidol (**2b**), a silicon analogue of **2a**, was synthesized in a multistep synthesis, starting from $Si(OMe)_4$ (Scheme 3) [32, 33]. An alternative synthesis, also starting from $Si(OMe)_4$, is shown in Scheme 4 [34]. In both cases, sila-haloperidol (**2b**) was isolated as the hydrochloride **2b**·HCl.

Compound **2b**·HCl was structurally characterized by single-crystal X-ray diffraction [32]. The molecular structure of the cation of **2b**·HCl is depicted in Fig. 6.

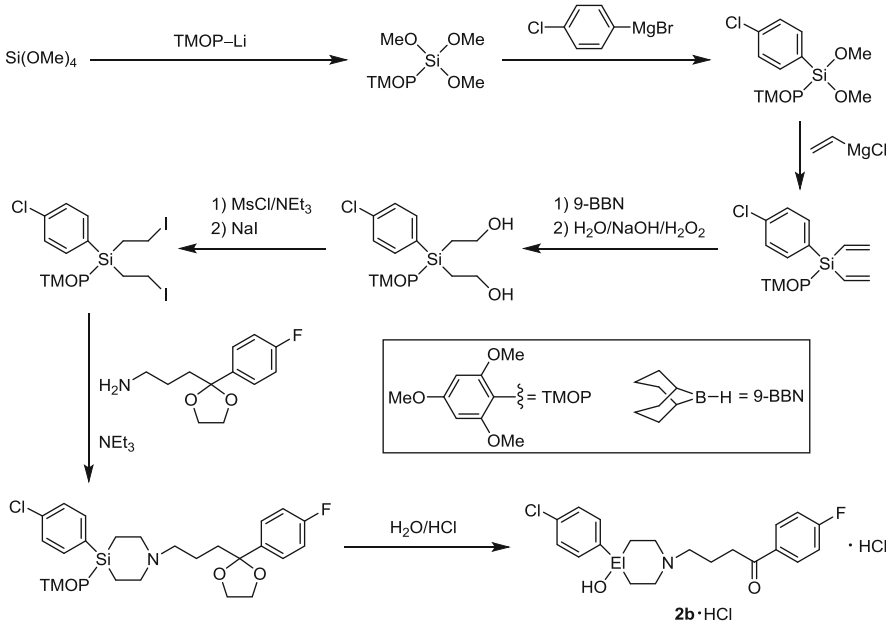
Figure 7 shows a superposition of the piperidinium skeleton of **2a**·HCl and the 4-silapiperidinium ring of **2b**·HCl (structures determined by single-crystal X-ray diffraction) [32]. Due to the longer covalent radius of the silicon atom, the 4-silapiperidinium ring is more flat than the piperidinium skeleton, leading to different relative orientations of the *N*-organyl side chain toward the hydroxy and 4-chlorophenyl groups. These different structural features may affect the ligand–receptor interactions of haloperidol (**2a**) and sila-haloperidol (**2b**).

As shown by NMR spectroscopic studies in DMSO at room temperature, two conformers of the piperidinium cation of **2a**·HCl exist in solution (molar ratio ca. 1:13) [32]. This ratio differs significantly from that found for the 4-silapiperidinium cation of **2b**·HCl (molar ratio ca. 1:2), indicating considerable differences in the energies of the respective two conformers of the piperidinium and 4-silapiperidinium ring systems [32]. The structures of the two conformers α and β are depicted in Fig. 8. The different populations of these two conformers may affect the pharmacological potency of haloperidol (**2a**) and sila-haloperidol (**2b**).

To get information about their physicochemical properties, compounds **2a** and **2b** were studied as hydrochlorides for their pK_a and log*D* (log*P* at pH 7.4) values and for their solubility in HBSS buffer (pH 7.4) [34]. As can be seen from Table 3, similar physicochemical profiles were observed for the two C/Si analogues. The apparent permeability (P_{app}) of **2a** and **2b** was also similar (studied in a human Caco-2 model) [34]. In both cases, the P_{app} values were high (**2a**, $P_{app} = 18.9 \times 10^{-6}$ cm s^{-1}; **2b**, $P_{app} = 19.8 \times 10^{-6}$ cm s^{-1}), and the recovery rate was around 90%, indicating that both compounds passed the cellular barrier more or less unchanged. As haloperidol (**2a**) is known to possess good bioavailability in humans, these results suggest a high permeability for sila-haloperidol (**2b**) as well.



Scheme 3 Synthesis of **2b**·HCl



Scheme 4 Synthesis of **2b**·HCl

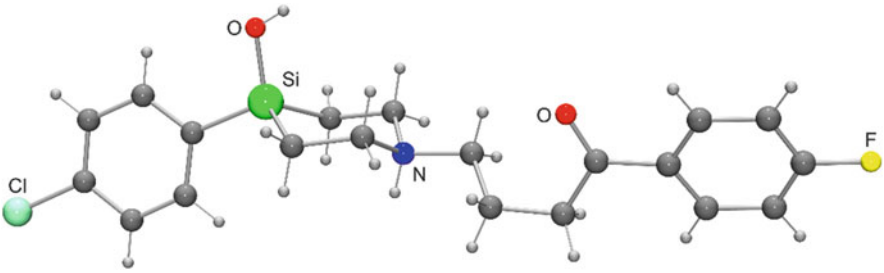


Fig. 6 Molecular structure of the cation of **2b**·HCl in the crystal [32]

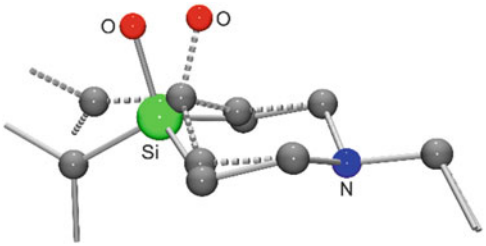


Fig. 7 Superposition of the piperidinium skeleton of **2a**·HCl (*dashed bonds*) and the 4-silapiperidinium skeleton of **2b**·HCl (*solid bonds*) (hydrogen atoms are omitted for clarity) [32]

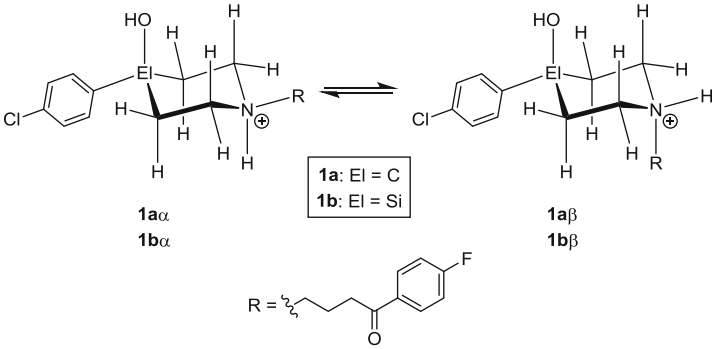


Fig. 8 Conformers α and β of the piperidinium skeleton of **2a**·HCl and the 4-silapiperidinium skeleton of **2b**·HCl [32]

Haloperidol (**2a**) and sila-haloperidol (**2b**) were studied for their affinities at all five human dopamine receptors in competitive radioligand receptor binding assays [34]. The results obtained are shown in Table 4 and Fig. 9. All Hill slopes were not significantly different from unity, thus assuming a single binding site for **2a** and **2b** at all receptors.

Table 3 pK_a, log$D$ (log$P$ at pH 7.4), and solubility (in HBSS buffer at pH 7.4) data for **2a** and **2b** [34]

Compound	pK_a	log$D$	Solubility (μM)
2a	9.07 ± 0.07	2.42 ± 0.03	90 ± 2
2b	9.27 ± 0.10	2.77 ± 0.13	78 ± 7

Table 4 Affinities of **2a** and **2b** at human dopamine receptors [34]

Receptor subtype	K_i (nM) for **2a**	K_i (nM) for **2b**
hD_1	107 ± 22.0	162 ± 34.5
hD_2	2.84 ± 0.26	0.55 ± 0.06
hD_3	10.4 ± 1.47	4.73 ± 0.51
hD_4	7.94 ± 0.69	14.1 ± 1.09
hD_5	38.0 ± 4.72	16.6 ± 3.20

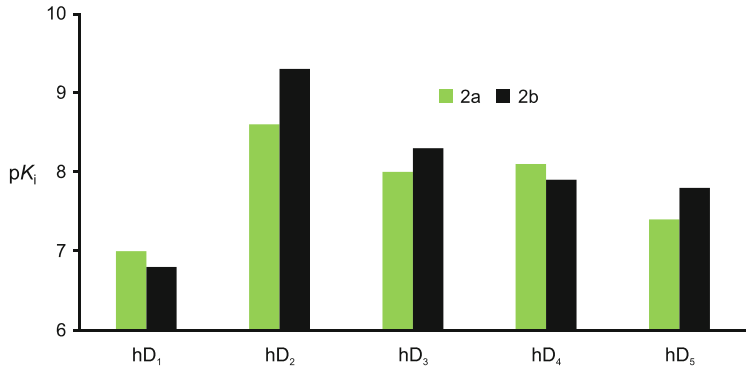


Fig. 9 Affinities of **2a** and **2b** at human dopamine receptors [34]

As can be seen from Table 4 and Fig. 9, sila-haloperidol (**2b**) shows a significantly higher affinity for hD_2 receptors than haloperidol (5.1-fold), whereas the silicon compound **2b** is approximately equipotent to its carbon analogue **2a** at all the other dopamine receptors (differences are less than 2.3-fold). As a result, the subtype selectivity of **2b** for hD_2 over the other dopamine receptors is somewhat higher than that of **2a** (Fig. 10). Sila-haloperidol (**2b**) has an approximately 2-fold higher selectivity than haloperidol (**2a**) for D_2 over D_3 and D_2 over D_5 but an 8- to 9-fold higher selectivity for D_2 over D_1 and D_2 over D_4 relative to **2a**.

The C/Si analogues **2a** and **2b** were also characterized by functional studies at human dopamine D_1 and D_2 receptors using a calcium fluorimetric functional assay [35]. The functional data for **2a** (hD_1, $K_i = 398$ nM; hD_2, $K_i = 0.36$ nM) and **2b** (hD_1, $K_i = 194$ nM; hD_2, $K_i = 0.12$ nM) were similar to the binding data (Table 4). None of the compounds displayed any agonist activity at D_1 or D_2 receptors.

To further characterize the pharmacodynamic properties of **2a** and **2b**, the two C/Si analogues were also studied for their σ_1 (guinea pig brain) and σ_2 (rat liver) receptor affinities using competitive radioligand receptor binding assays (Table 5)

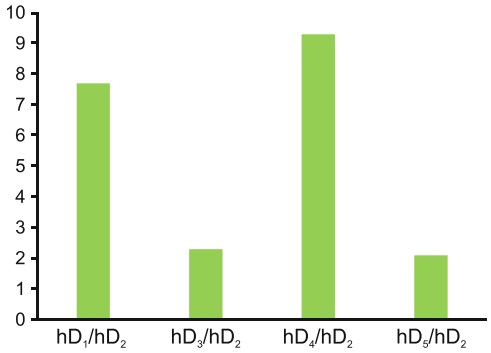


Fig. 10 Ratios of the subtype selectivities hD_1/hD_2, hD_3/hD_2, hD_4/hD_2, and hD_5/hD_2 of **2a** and **2b**, shown as **2a**/**2b** ratios [34]

Table 5 Affinities of **2a** and **2b** at σ_1 (guinea pig brain) and σ_2 receptors (rat liver) [34]

Receptor subtype	K_i (nM) for **2a**	K_i (nM) for **2b**
σ_1	1.9 ± 0.4	3.4 ± 0.4
σ_2	78.1 ± 2.4	309 ± 55

[34]. At the σ_1 receptor, **2a** and **2b** are approximately equipotent, whereas **2a** exhibits a 4-fold higher affinity for the σ_2 receptor than the silicon analogue **2b**. As a result, the subtype selectivity of sila-haloperidol (**2b**) for the σ_1 over the σ_2 receptor is approximately 3-fold higher than that of haloperidol (**2a**).

In conclusion, in comparison with haloperidol (**2a**), sila-haloperidol (**2b**) shows a higher potency at dopamine D_2 receptors and a higher subtype selectivity at both dopamine and σ receptors.

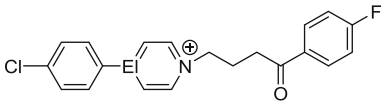
To determine the rate of decomposition of haloperidol (**2a**) and sila-haloperidol (**2b**) in vitro, the intrinsic clearance and half-lives were measured in human and female rat liver microsomes [34]. As can be seen from Table 6, the C/Si analogues **2a** and **2b** showed a similar moderate stability in the presence of human liver microsomes. However, decomposition in the presence of rat liver microsomes was significantly increased by sila-substitution.

Haloperidol (**2a**) and sila-haloperidol (**2b**) were also studied for their CYP inhibition of the five major isoforms of cytochrome P450 [34]. No CYP inhibition of either **2a** or **2b** against CYP1A2, CYP2C9, and CYP2C19 could be detected ($IC_{50} > 20$ μM). However, the silicon compound **2b** showed an almost 3-fold increased inhibitory potency against CYP3A4 ($IC_{50} = 9.6 \pm 1.4$ μM) compared to the carbon analogue **2a** ($IC_{50} = 26.2 \pm 7.9$ μM). CYP2D6 was also inhibited by **2a** and **2b**, with almost identical IC_{50} values (**2a**, 2.1 ± 0.6 μM; **2b**, 1.7 ± 0.9 μM).

Haloperidol (**2a**) and sila-haloperidol (**2b**) were also investigated for their in vitro metabolism [34, 36]. It is known that one of the major metabolites of **1a**, the pyridinium species HPP^+ (**5a**, Fig. 11), has neurotoxic properties and is suspected to cause severe extrapyramidal side effects, including Parkinsonism and tardive dyskinesia. As a matter of principle, an analogous metabolite of the

Table 6 Intrinsic clearance Cl_{int} (μL min^{-1} mg^{-1}) and half-life $t_{1/2}$ (min) of **2a** and **2b** in human and rat liver microsomes [34]

Compound	$Cl_{int\ hu}$	$Cl_{int\ rat}$	$t_{1/2\ hu}$	$t_{1/2\ rat}$
2a	20 ± 2	21 ± 5	51	65
2b	21 ± 2	78 ± 7	65	18



5a: El = C
5b: El = Si

Fig. 11 Chemical structures of the C/Si analogues **5a** and **5b**

silicon analogue **2b**, the silapyridinium species sila-HPP^+ (**5b**, Fig. 11), cannot be formed, because the silicon–carbon double bond is unstable [32].

The phase I metabolism of haloperidol (**2a**) and sila-haloperidol (**2b**) was investigated in rat and human liver microsomes [34, 36]. The phase II metabolism of **2a** and **2b** was studied in rat, dog, and human hepatocytes and also in rat and human liver microsomes supplemented with UDP-glucuronic acid (UDPGA) [36]. These studies were performed using mass-spectrometric techniques. The structures of the proposed metabolites were assigned based on accurate mass measurements and interpretation of MS–MS spectra. The relative metabolite amounts were estimated by integration of extracted ion chromatograms and refer to the respective fractions metabolized.

The phase I and II metabolism of sila-haloperidol (**2b**) differs significantly from that of haloperidol (**2a**). This is demonstrated exemplarily in Figs. 12 and 13, where the major metabolites in human liver microsomes (incubation time 60 min) and human hepatocytes (incubation time 120 min) are shown.

For the carbon compound **2a**, the pyridinium metabolite **5a** was identified as a major metabolite in the microsomal incubations. The analogous silapyridinium metabolite **5b** was not formed. Instead, two ring-opened metabolites, the silanediols **6** and **7**, were observed. These two metabolites were the major metabolites of sila-haloperidol (**2b**) in human liver microsomes, followed by the *N*-dealkylated metabolite **8b**. The corresponding carbon analogue **8a** was also observed in microsomal incubations of haloperidol (**2a**).

Figure 14 shows the proposed pathway for the metabolic ring opening of sila-haloperidol (**2b**) to give the silanediols **6** and **7** [36]. By analogy to the pathway leading to the pyridinium metabolite of haloperidol (**2a**), the metabolism of the silapiperidine ring of the silicon analogue **2b** is most likely initiated by an enzymatic hydroxylation at the β-carbon atom (β to the silicon atom), followed by ring opening (C–N bond cleavage) to form an intermediate with an aldehyde and a secondary amine functionality. This intermediate, an α-silylaldehyde, is very reactive and easily undergoes hydrolytic cleavage of the Si–C bond. The proposed

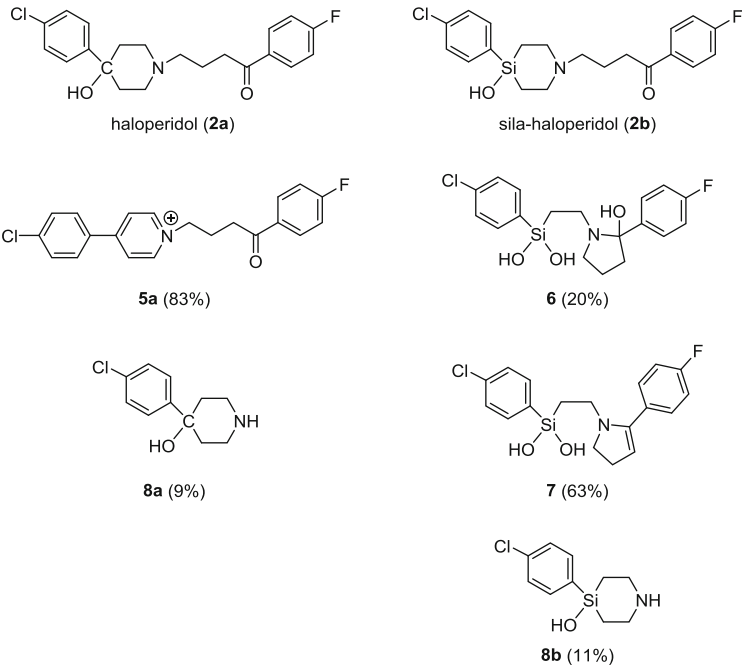


Fig. 12 Major metabolites of the C/Si analogues **2a** and **2b** in human liver microsomes. Relative metabolite amounts of the respective fraction metabolized (**2a**, 41%; **2b**, 35%) are given in percent [36]

cyclization following the elimination of acetaldehyde then yields the metabolite **6**, which upon water elimination affords **7**.

The major metabolites of haloperidol (**2a**) in hepatocytes were the pyridinium metabolite **5a**, the *N*-dealkylated metabolite **8a**, reduced haloperidol (**2a**) (metabolite **9a**), and the glucuronidation metabolite **10** (not observed in dog hepatocytes). Reduced sila-haloperidol (**2b**) (metabolite **9b**) was the major metabolite in the dog and human hepatocytes, whereas the *N*-dealkylated metabolite **8b** and a metabolite originating from hydroxylation and glucuronidation were the major metabolites in rat hepatocytes. The metabolites **8b** and **9b** were found in all hepatocytes, and the ring-opened metabolites **6** and **7** were only formed in dog and human hepatocytes.

While haloperidol (**2a**) undergoes a direct glucuronidation of the OH group in rat and human hepatocytes and in rat and human liver microsomes supplemented with UDPGA, an analogous glucuronide conjugation was not observed for sila-haloperidol (**2b**). One could speculate that the direct glucuronidation of the SiOH group of **2b** occurs but that the resulting conjugate with its hydrolytically sensitive Si–OC bond then undergoes a spontaneous hydrolysis to give **2b** and free glucuronic acid. In this context, it is important to note that the chemical reactivity of the thermodynamically very stable (but against water kinetically labile) Si–OC bond of alkoxysilanes (alkyl silyl ethers) differs significantly from that of the C–OC bond of analogous dialkyl ethers. An alternative explanation for the absence of

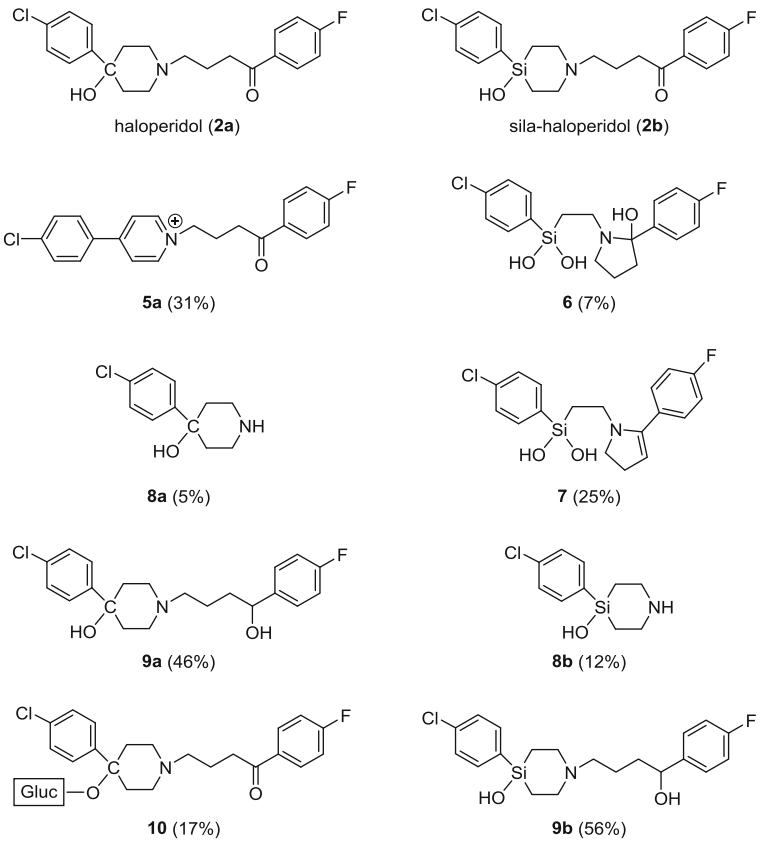


Fig. 13 Major metabolites of the C/Si analogues **2a** and **2b** in human hepatocytes. Relative metabolite amounts of the respective fraction metabolized (**2a**, 14%; **2b**, 6%) are given in percent [36]

glucuronidation of the SiOH group of sila-haloperidol (**2b**) might be that **2b** is a poor substrate for the UDP-glucuronosyltransferases. Anyway, these results clearly demonstrate that sila-substitution of haloperidol (**2a**) strongly affects the phase II metabolism.

In conclusion, haloperidol (**2a**) and sila-haloperidol (**2b**) differ significantly in their phase I and II metabolism. These differences can be directly correlated with the silicon-specific reactivity profile of **2b**. Studies on the in vitro metabolism of the structurally related dopamine (D_2) antagonists trifluperidol (**11a**) and sila-trifluperidol (**11b**) led to similar results (Fig. 15) [36].

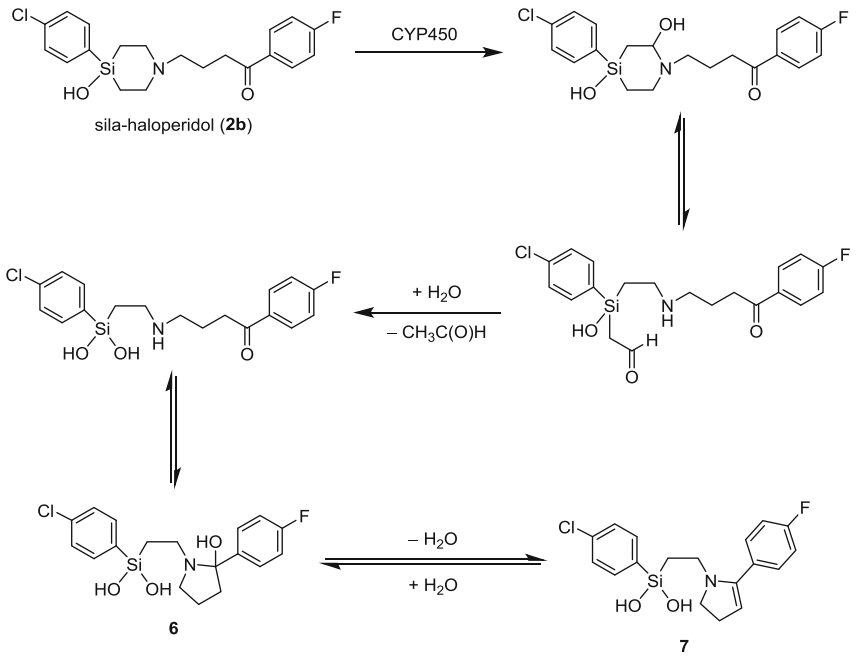


Fig. 14 Proposed mechanism for the metabolic ring opening of sila-haloperidol (**2b**) to give the silanediol metabolites **6** and **7** [36]

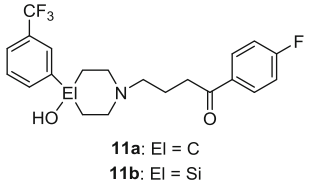
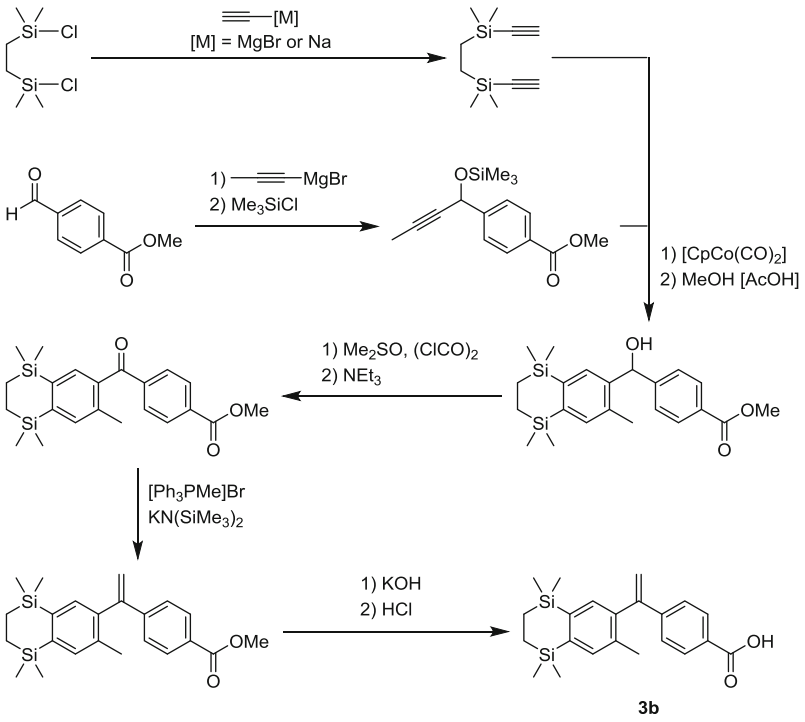


Fig. 15 Chemical structures of the C/Si analogues **11a** and **11b**

3.3 Disila-Bexarotene: A Silicon Analogue of the Retinoid Agonist Bexarotene

Bexarotene (**3a**) is an RXR-selective retinoid agonist that is in clinical use for the treatment of cutaneous T-cell lymphoma. Disila-bexarotene (**3b**), a silicon analogue of **3a**, was synthesized in a multistep synthesis, starting from $ClMe_2Si(CH_2)_2SiMe_2Cl$ (Scheme 5) [37, 38]. An alternative synthesis, also starting from $ClMe_2Si(CH_2)_2SiMe_2Cl$, is shown in Scheme 6 [39].

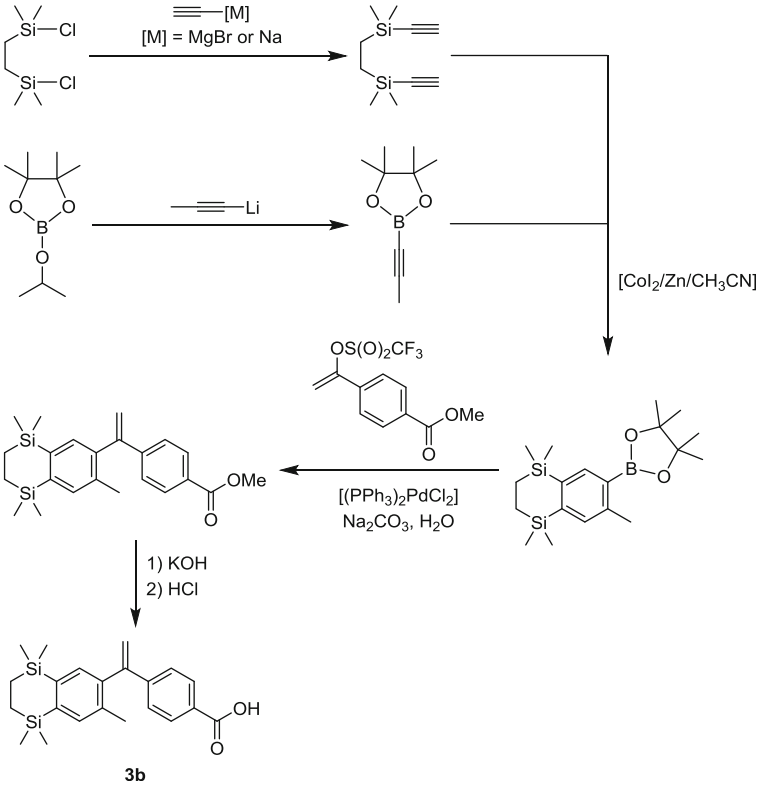
Compound **3b** was structurally characterized by single-crystal X-ray diffraction [37]. The molecular structure of **3b** is depicted in Fig. 16.



Scheme 5 Synthesis of **3b**

Figure 17 shows a superposition of the tetrahydronaphthalene skeleton of **3a** and the tetrahydrodisilanaphthalene skeleton of **3b** (structures determined by single-crystal X-ray diffraction) [37]. Due to the longer covalent radius of the silicon atom, the two molecular frameworks differ in their size and shape. As shown by experimental [40] and computational [41] studies, disila-substitution of bexarotene (**3a**) also affects the electronic structure (electrostatic potential) of the bicyclic skeleton. As the tetrahydronaphthalene ring of **3a** and structurally related retinoid agonists is known to play a crucial role in receptor binding, the structural and electronic differences between bexarotene (**3a**) and disila-bexarotene (**3b**) may affect the ligand–receptor interactions of these two C/Si analogues.

As a proof of principle, compounds **3a** and **3b** were studied for their RXRβ agonistic potency in a HeLa cell-based receptor assay [37]. The cell line was engineered to express a chimeric receptor composed of the DNA binding domain of the GALA4 yeast transcription factor and the ligand binding domain of human RXRβ (GALA4-hRXRβ). In these studies, the natural ligand 9-*cis*-retinoic acid (9-*cis*RA) served as the positive control. As can be seen from Fig. 18, bexarotene (**3a**) is more potent than 9-*cis*-retinoic acid, and disila-bexarotene (**3b**) has a profile comparable to that of its carbon analogue **3a**. Thus, despite of the structural and electronic changes upon C/Si exchange, very similar RXRβ agonistic potencies of the C/Si analogues **3a** and **3b** were observed.



Scheme 6 Synthesis of **3b**

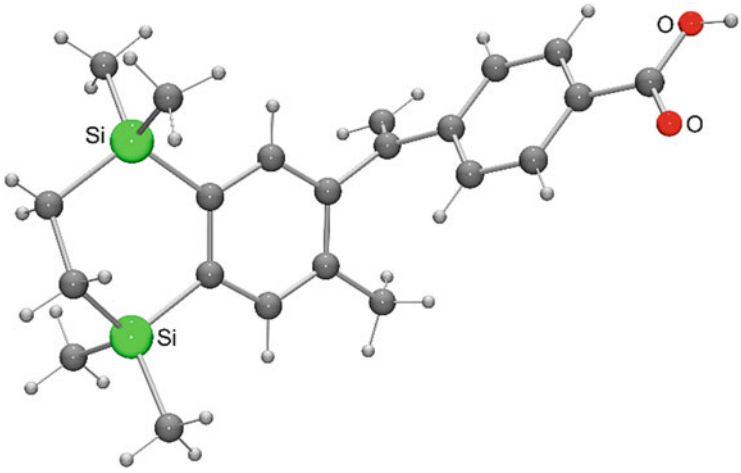


Fig. 16 Molecular structure of **3b** in the crystal [37]

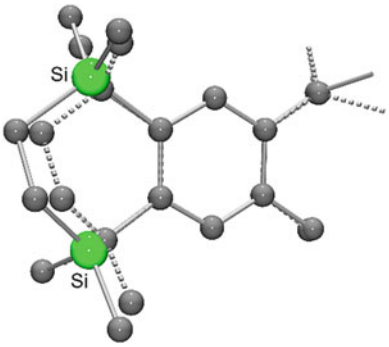


Fig. 17 Superposition of the tetrahydronaphthalene skeleton of **3a** (*dashed bonds*) and the tetrahydrodisilanaphthalene skeleton of **3b** (*solid bonds*) (hydrogen atoms are omitted for clarity) [37]

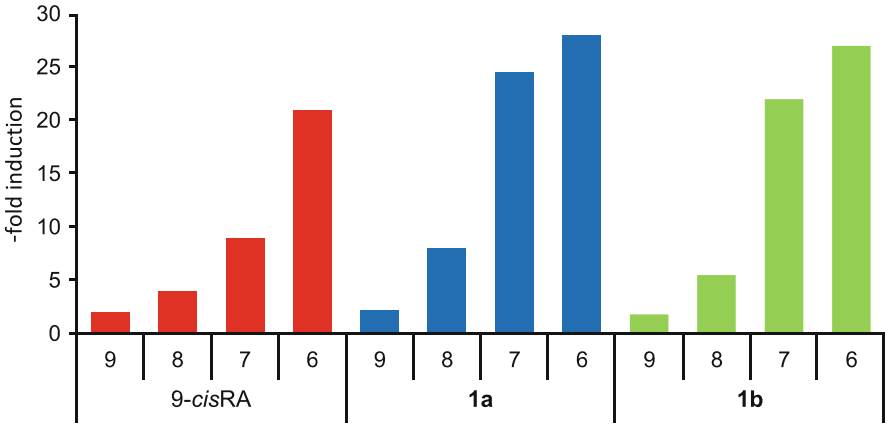


Fig. 18 Concentration-dependent RXRβ agonistic potency of 9-*cis*-retinoic acid (9-*cis*RA), bexarotene (**3a**), and disila-bexarotene (**3b**). The data were obtained using a HeLa cell-based receptor assay [37]

As shown in a more recent study, bexarotene (**3a**) may partially act to induce cell differentiation and cell death pathways in pluripotent TERA2.cl.SP12 stem cells [42]. However, disila-bexarotene (**3b**) did not show the ability to induce stem cell differentiation in this study but may possess some enhanced functions over **3a**, such as cell death induction and regulation of cell numbers [42].

As disila-substitution of bexarotene (**3a** → **3b**) did not lead to any detrimental effects on the biological activity in terms of its ability to activate target genes through the RXR receptor [37], these studies were extended, and a series of further disila-analogues of retinoid agonists (both RXR and RAR agonists) was synthesized and studied for their biological activity: disila-TTNPB (**12b**) [43], disila-3-methyl-TTNPB (**13b**) [43], disila-SR11237 (**14b**) [41], disila-tamibarotene (disila-AM80, **15b**) [44], disila-AM580 (**16b**) [44], disila-EC23 (**17b**) [45], and disila-TTNN (**18b**)

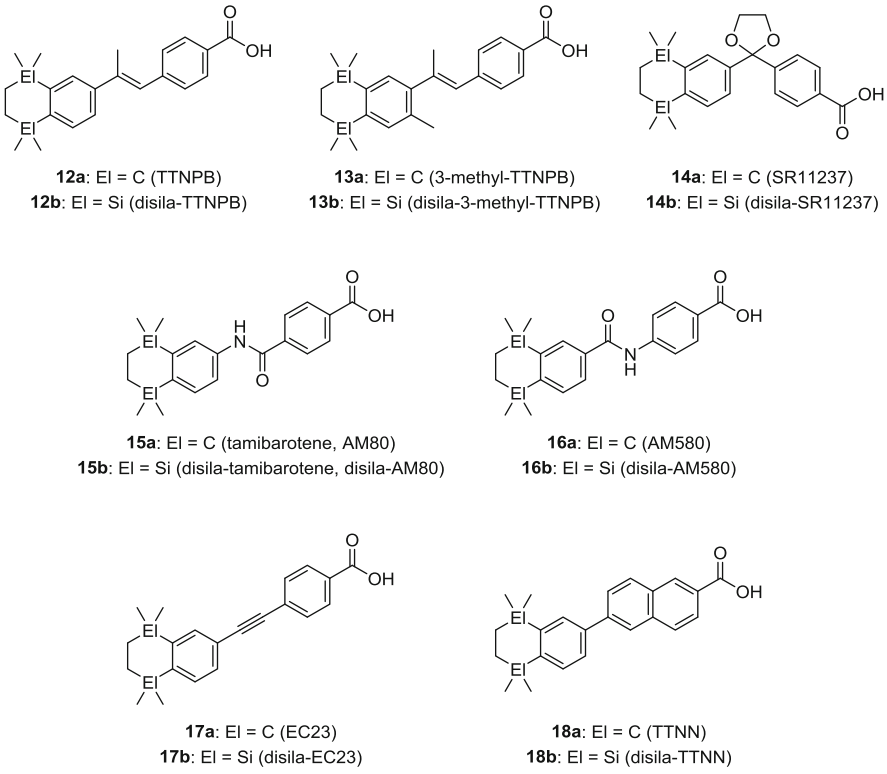


Fig. 19 Chemical structures of the C/Si pairs **12a/12b–18a/18b**

[45] – the silicon analogues of the retinoid agonists TTNPB (**12a**), 3-methyl-TTNPB (**13a**), SR11237 (**14a**), tamibarotene (AM80, **15a**), AM580 (**16a**), EC23 (**17a**), and TTNN (**18a**), respectively (Fig. 19).

In these studies, some of the tested silicon compounds showed an enhanced agonistic potency compared to the parent carbon compounds. For example, as can be seen from Fig. 20, 2-fold sila-substitution of the pan-RXR-selective retinoid agonist SR11237 (**14a** → **14b**) resulted in an increased RXRβ agonistic potency [41]. Whereas the dose–response curves of the C/Si analogues **14a** and **14b** are very similar up to 10 nM, there is a stronger activation by the silicon compound **14b** at higher concentrations. This difference in the activities is even more pronounced for the related C/Si analogues **19a** and **19b** (Fig. 21) [41]. As shown in Fig. 20, 2-fold sila-substitution of **19a** (→ **19b**) resulted in a 10-fold increased RXRβ agonistic potency. It is worth noting that at low concentrations **19b** is even more potent than the natural ligand 9-*cis*RA, which served as the positive control. The 10-fold higher activity of the silicon compound **19b** could be explained with an increased receptor affinity of **19b**, which is in line with the results of crystal structure analyses of the ternary complexes formed by **19a** and **19b**, respectively, with the ligand binding domain of hRXRα and a peptide of the co-activator TIF2/GRIP1 [41]. These

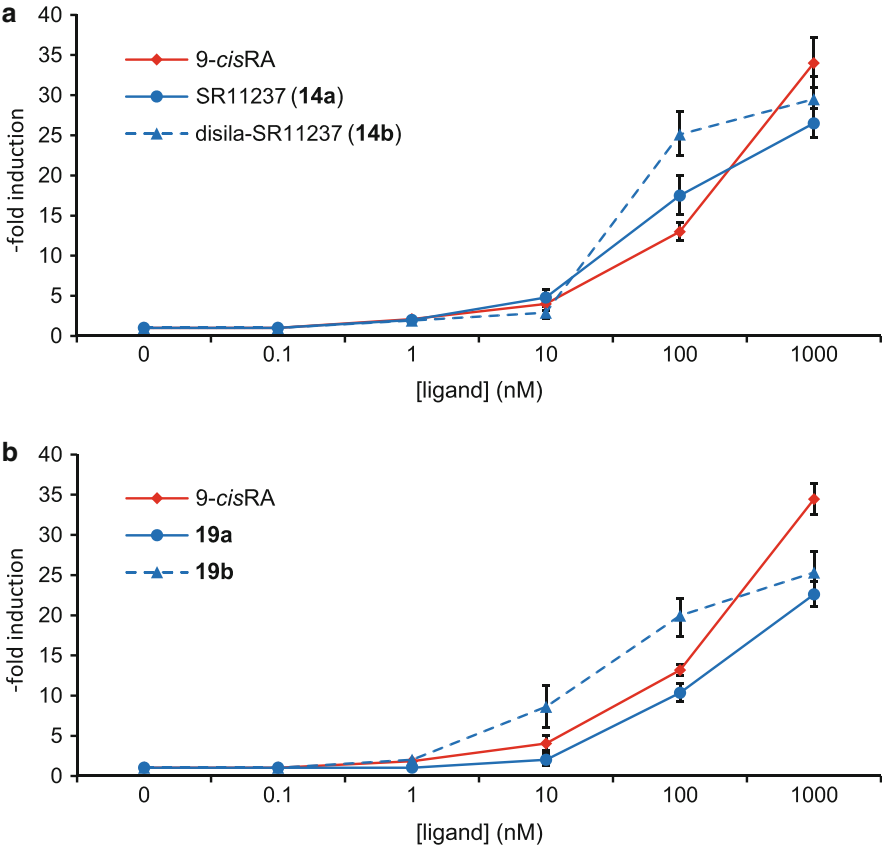
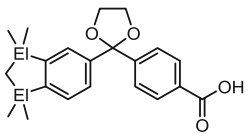


Fig. 20 Dose–response curves for the transcription activation of RXRβ by (**a**) SR11237 (**14a**) and disila-SR11237 (**14b**) and by (**b**) **19a** and **19b** [38]. 9-*cis*-Retinoic acid (9-*cis*RA) served as the positive control. For the HeLa cell-based receptor assay, see [37]



19a: El = C
19b: El = Si

Fig. 21 Chemical structures of the C/Si analogues **19a** and **19b**

structural investigations revealed additional interactions of **19b** with the H7 and H11 residues. In contrast, crystal structure analyses of the ternary complexes formed by TTNPB (**12a**) and disila-TTNPB (**12b**), respectively, with the ligand binding domain of hRARβ and a peptide of the co-activator SRC-1 revealed nearly

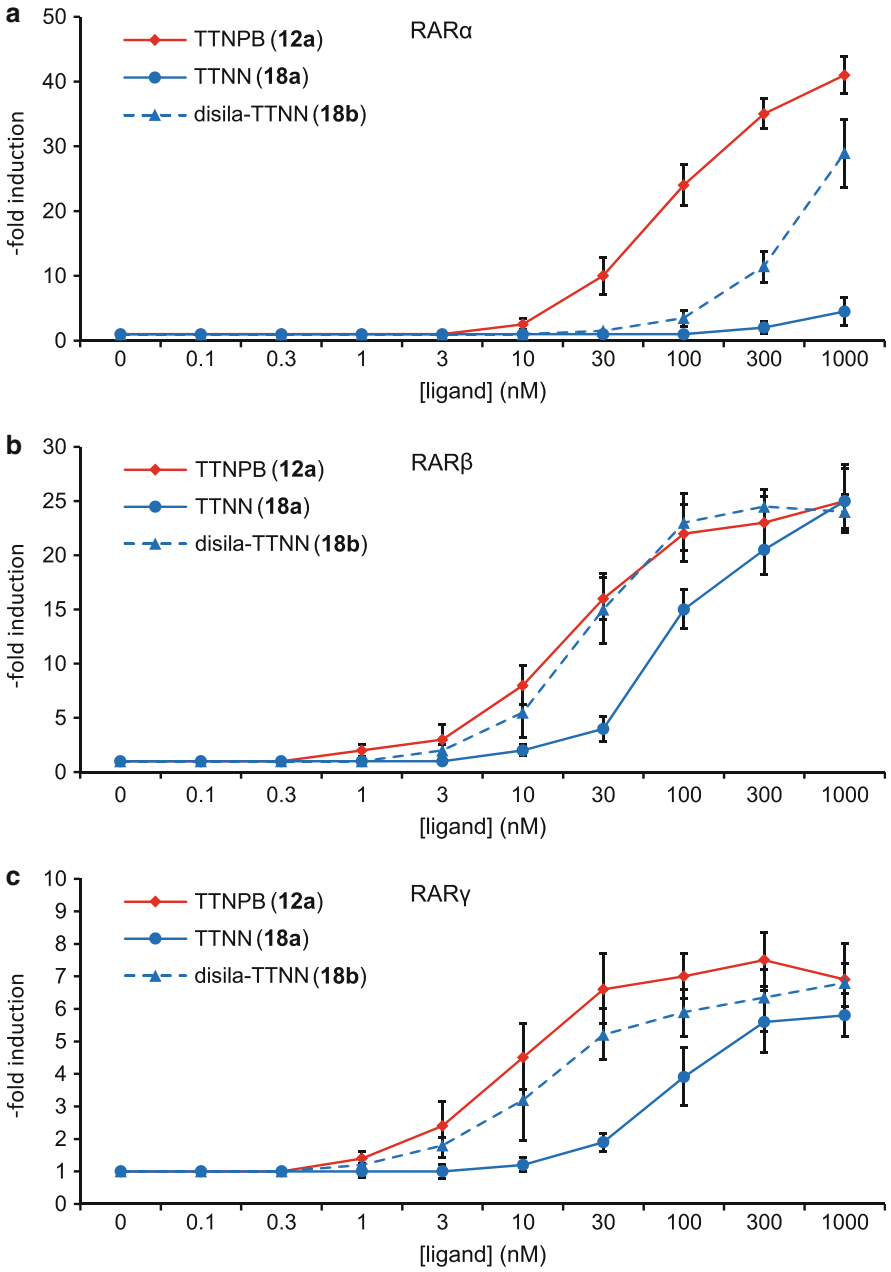


Fig. 22 Dose–response curves for the transcription activation of (**a**) hRARα, (**b**) hRARβ, and (**c**) hRARγ by TTNN (**18a**) and disila-TTNN (**18b**) [45]. TTNPB (**12a**) served as the positive control

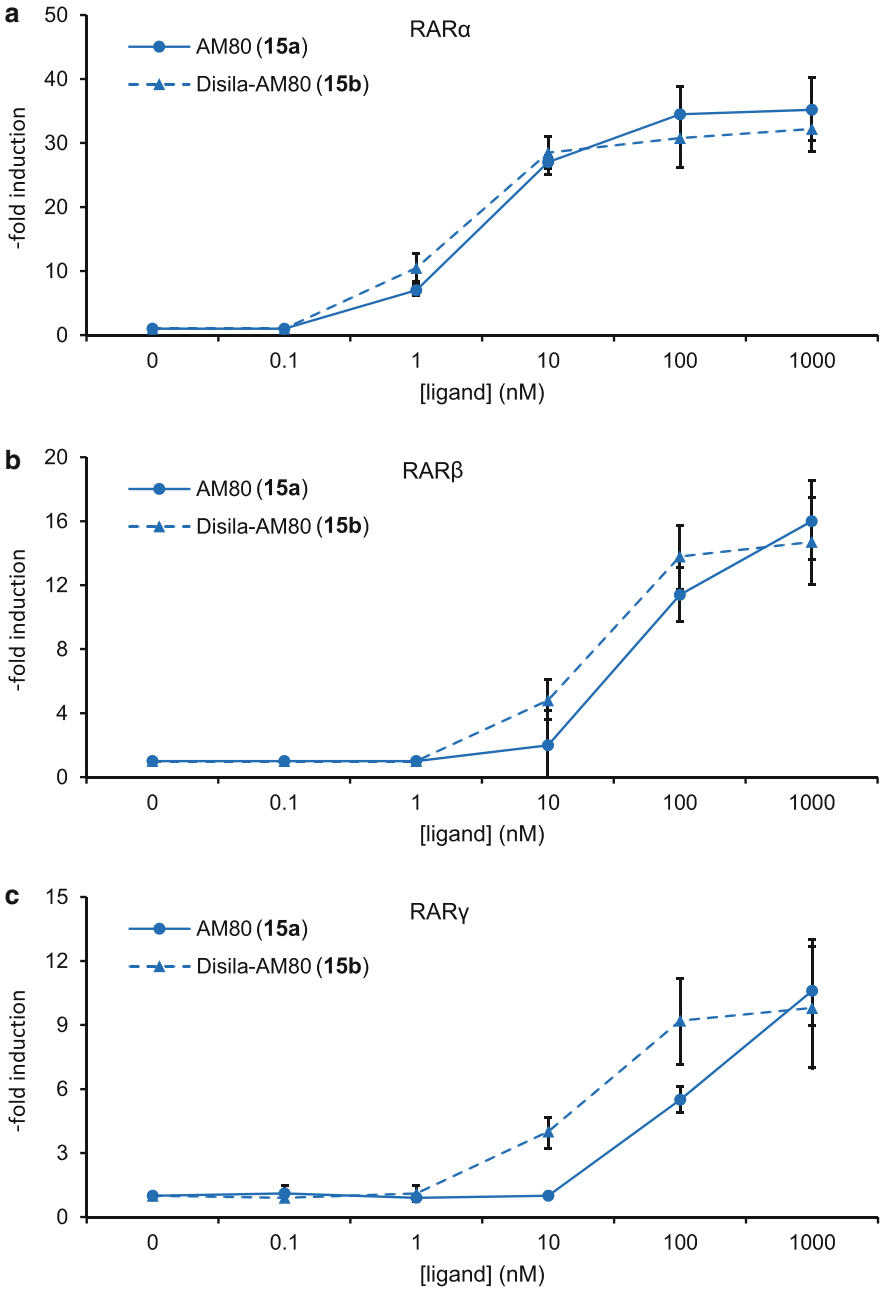


Fig. 23 Dose–response curves for the transcription activation of (**a**) hRARα, (**b**) hRARβ, and (**c**) hRARγ by tamibarotene (AM80, **15a**) and disila-tamibarotene (disila-AM80, **15b**) [44]

isomorphous structures [43]. This finding correlates with the very similar agonistic potencies of the C/Si analogues **12a** and **12b** at RARα, RARβ, and RARγ, respectively. Compounds **12a** and **12b** were also studied as differentiation- and apoptosis-inducing agents on the basis of their RARα receptor-activation potential [43]. Upon exposure of NB4 acute promyelocytic leukemia cells to TTNPB (**12a**) and disila-TTNPB (**12b**), respectively, the same strong induction of differentiation and apoptosis of these cells was observed for **12a** and **12b**, which is again in line with the results of the abovementioned structural investigations.

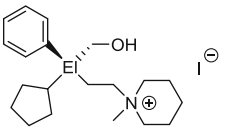
As can be seen from Fig. 22, disila-substitution of the weak retinoid agonist TTNN (**18a** → **18b**) resulted in a significant gain in transcription activation potential for hRARα, hRARβ, and hRARγ [45]. Disila-TTNN (**18b**) can be regarded as a powerful RARβ,γ-selective retinoid agonist, displaying a similar potency as the powerful reference compound TTNPB (**12a**), which served as the positive control.

In the case of the RARα-selective retinoid agonist tamibarotene (AM80, **15a**), disila-substitution (→ **15b**) did affect not only the agonistic potency but also the retinoid receptor selectivity [44]. Figure 23 shows the dose–response curves of **15a** and **15b** in RARα, RARβ, and RARγ reporter cells. In the case of RARα, both compounds inhibited virtually identical profiles, with EC_{50} values around 5 nM. However, in the case of RARβ and even more pronounced for RARγ, there was a significant left shift of the dose–response curves of the silicon compound **15b**. In the case of RARγ, disila-tamibarotene (disila-AM80, **15b**) displayed an up to 10-fold higher activity. As a result, the RARα selectivity of tamibarotene (AM80, **15a**) is reduced upon disila-substitution. Similar results were also observed for the C/Si analogues AM580 (**16a**) and disila-Am580 (**16b**) [44].

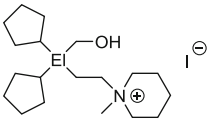
3.4 Structures of Other Sila-Drugs

To demonstrate the structural diversity of other sila-analogues of drugs that have been synthesized and biologically characterized in recent years, a series of selected C/Si pairs is compiled in Fig. 24: the muscarinic antagonists **20a/20b** [46], **21a/21b** [46], and **22a/22b** [47]; the σ receptor ligands **23a/23b**–**26a/26b** [48]; the calcium channel antagonists niguldipine (**27a**) and sila-niguldipine (**27b**) [49]; the histaminic H_1 antagonists terfenadine (**28a**), sila-terfenadine-A (**28b**), sila-terfenadine-B (**28c**), disila-terfenadine (**28d**), fexofenadine (**29a**), and sila-fexofenadine (**29b**) [50]; the σ receptor ligands panamesine (**30a**) and sila-panamesine (**30b**) [51]; the GnRH antagonists AG-045572 (**31a**) and disila-AG-045572 (**31b**) [52]; the antiviral agents **32a/32b**–**35a/35b** [53]; the antiallergic agents **36a/36b** [54]; the σ receptor ligands **37a/37b**–**40a/40b** [55]; the estrogenic agents **41a/41b** [56]; and the androgen receptor antagonists LG190178 (**42a**), sila-LG190178 (**42b**), LG190176 (**43a**), and sila-LG190176 (**43b**) [57].

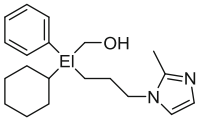
The collection of these compounds shall emphasize the high synthetic potential of modern organosilicon chemistry. This field is rapidly developing, and a huge variety of new classes of organosilicon compounds have been synthesized in recent



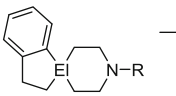
20a: El = C
20b: El = Si



21a: El = C
21b: El = Si

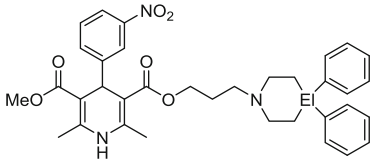


22a: El = C
22b: El = Si

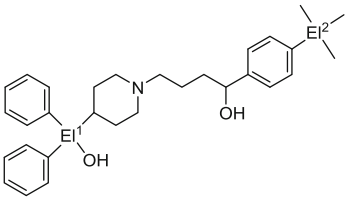


a: El = C
b: El = Si

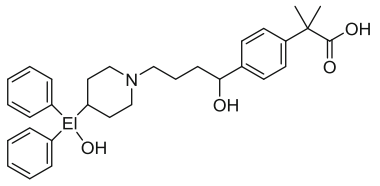
	R
23	CH_2Ph
24	p-CH_2-C_6H_4-OMe
25	$(CH_2)_2Ph$
26	$CH_2CH{=}CMe_2$



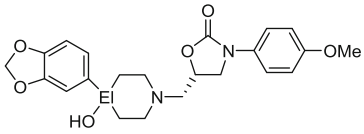
27a: El = C (niguldipine)
27b: El = Si (sila-niguldipine)



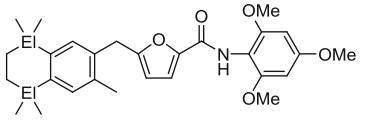
28a: El^1 = C, El^2 = C (terfenadine)
28b: El^1 = Si, El^2 = C (sila-terfenadine-A)
28c: El^1 = C, El^2 = Si (sila-terfenadine-B)
28d: El^1 = Si, El^2 = Si (disila-terfenadine)



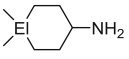
29a: El = C (fexofenadine)
29b: El = Si (sila-fexofenadine)



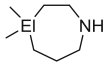
30a: El = C (panamesine)
30b: El = Si (sila-panamesine)



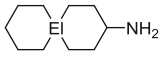
31a: El = C (AG-045572)
31b: El = Si (sila-AG-045572)



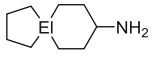
32a: El = C
32b: El = Si



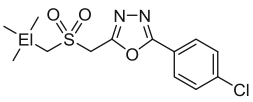
33a: El = C
33b: El = Si



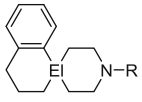
34a: El = C
34b: El = Si



35a: El = C
35b: El = Si

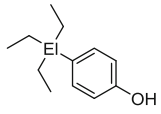


36a: El = C
36b: El = Si



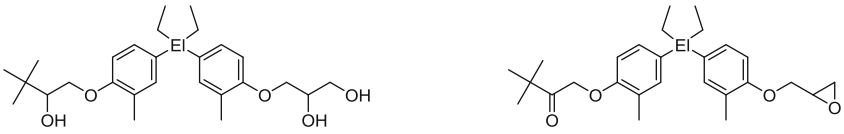
a: El = C
b: El = Si

	R
37	CH_2Ph
38	p-CH_2-C_6H_4-OMe
39	$(CH_2)_2Ph$
40	$CH_2CH{=}CMe_2$



41a: El = C
41b: El = Si

Fig. 24 (continued)



42a: El = C (LG190178)
42b: El = Si (sila-LG190178)

43a: El = C (LG190176)
43b: El = Si (sila-LG190176)

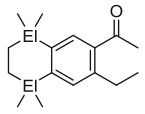
Fig. 24 Chemical structures of the C/Si pairs **20a/20b–43a/43b**, **28a/28c**, and **28a/28d**

years, and many novel silicon-based molecular scaffolds for drug design will be seen in the near future. In this context, it should also be mentioned that there is an efficient chemical industry, with a special expertise in organosilicon chemistry, that supports this development and can supply pharmaceutical industry with the silicon-based fine chemicals needed.

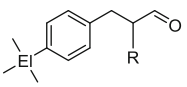
4 Appendix: Sila-Substitution of Odorants

In context with the studies reported in this article, it should be mentioned that the carbon/silicon switch strategy has also successfully been used for the design of silicon-based odorants. As odorant recognition is also based on ligand–receptor interactions (in this case between odorants and olfactory receptors), this approach follows similar principles as described for the design of sila-drugs. To demonstrate the structural diversity of sila-analogues of odorants that have been synthesized and olfactorily characterized in recent years, a series of selected C/Si pairs is compiled in Fig. 25: the musk odorants versalide (**44a**) and disila-versalide (**44b**) [58]; the lily-of-the-valley odorants bourgeonal (**45a**), sila-bourgeonal (**45b**), lilial (**46a**), and sila-lilial (**46b**) [59]; the ambergris odorants okoumal (**47a**) and disila-okoumal (**47b**) [60]; the woody-ambery odorant **48a** and its sila-analogues **48b–48d** [61]; the floral odorants coranol (**49a**), sila-coranol (**49b**), dimetol (**50a**), and sila-dimetol (**50b**) [62]; the musk odorant **51a** and its sila-analogues **51b–51d** [62]; the musk odorants phantolide (**52a**) and disila-phantolide (**52b**) [63]; the patchouli odorants **53a** and **53b** [64]; the musk odorant **54a** and its sila-analogues **54b–54d** [65]; the musk odorants galaxolide (**55a**) and disila-galaxolide (**55b**) [66]; and the grapefruit odorants rhubafuran (**56a**), sila-rhubafuran (**56b**) [67], methyl pamplemousse (**57a**), and sila-methyl pamplemousse (**57b**) [68].

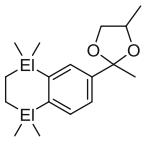
Sila-substitution of odorants can alter and, ideally, optimize the olfactory properties by affecting the binding affinity to the olfactory receptors, which can change both the olfactory profile and the odor threshold. These are quite promising perspectives for fragrance industry, which indeed has started to use the high potential of the carbon/silicon switch strategy for odorant design and development.



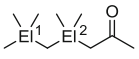
44a: El = C (versalide)
44b: El = Si (disila-versalide)



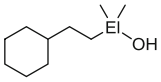
45a: El = C, R = H (bourgeonal)
45b: El = Si, R = H (sila-bourgeonal)
46a: El = C, R = Me (lilial)
46b: El = Si, R = Me (sila-lilial)



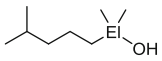
47a: El = C (okoumal)
47b: El = Si (disila-okoumal)



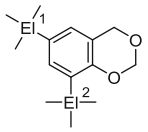
48a: El^1 = C, El^2 = C
48b: El^1 = Si, El^2 = C
48c: El^1 = C, El^2 = Si
48d: El^1 = Si, El^2 = Si



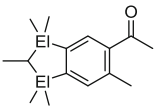
49a: El = C
49b: El = Si



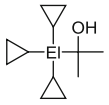
50a: El = C
50b: El = Si



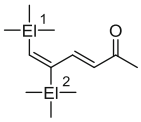
51a: El^1 = C, El^2 = C
51b: El^1 = Si, El^2 = C
51c: El^1 = C, El^2 = Si
51d: El^1 = Si, El^2 = Si



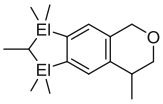
52a: El = C (phantolide)
52b: El = Si (disila-phantolide)



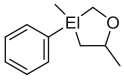
53a: El = C
53b: El = Si



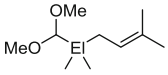
54a: El^1 = C, El^2 = C
54b: El^1 = Si, El^2 = C
54c: El^1 = C, El^2 = Si
54d: El^1 = Si, El^2 = Si



55a: El = C (galaxolide)
55b: El = Si (disila-galaxolide)



56a: El = C (rhubafuran)
56b: El = Si (disila-rhubafuran)



57a: El = C (methyl pamplemousse)
57b: El = Si (sila-methyl pamplemousse)

Fig. 25 Chemical structures of the C/Si pairs **44a/44b–57a/57b**, **48a/48c**, **48a/48d**, **51a/51c**, **51a/51d**, **54a/54c**, and **54a/54d**

5 Summary and Outlook

Following the first pioneering studies on biologically active organosilicon compounds five decades ago, silicon chemistry is nowadays accepted to be a novel source of chemical diversity in drug design. The carbon/silicon switch strategy, i.e., the strategic replacement of a carbon atom with a silicon atom (sila-substitution) within a well-known drug, with the rest of the molecule being identical, is one of the methods that are currently used for the design and development of new silicon-based drugs. As members of Group 14 of the periodic table, the elements carbon and silicon show many similarities in their chemical properties, but there are also some fundamental differences that can lead to striking differences in the physicochemical

and biological properties of drugs and their corresponding sila-analogues. In general, the sila-analogues share the same mode of action as the parent carbon compounds (carbon/silicon bioisosterism) but may have altered biological properties. Generally, sila-substitution can affect and, ideally, improve the pharmacological potency and selectivity, the pharmacodynamics, and the pharmacokinetics. Thus, the strategic sila-substitution of existing drugs is a very promising approach for the search of new drug candidates that have beneficial properties. The carbon/silicon switch strategy is a very powerful tool for both (1) the evaluation of structure–activity relationships in basic research and (2) the development of new silicon-based drugs for clinical applications. In this context, it is important to note that up to now there are no indications for a silicon-specific toxicity associated with organosilicon compounds, in contrast to the element-associated toxicity of organogermanium, tin, and lead compounds. This lack of inherent toxicological issues with silicon and the increasing clinical experience with organosilicon compounds will stimulate the search for new silicon-based drugs for clinical applications.

When assessing the potential of the carbon/silicon switch strategy for the design and development of new silicon-based drugs, some fundamental limitations have also to be mentioned. There are chemistry-driven rules in terms of both synthetic accessibility and chemical stability that dictate whether or not and where a silicon atom can be introduced into a given drug scaffold. Synthetic organosilicon chemistry is a very rapidly developing field, and therefore, the issue "synthetic accessibility" will constantly change depending on the future developments in synthetic organosilicon chemistry, whereas the issue "chemical stability" is of course a constant factor.

The perspectives of the carbon/silicon switch strategy for pharmaceutical industry look quite promising. Generally, incorporation of silicon into a known drug can affect the pharmacological potency and selectivity, the pharmacodynamics, and the pharmacokinetics and therefore can lead to beneficial biological properties that can be used with a clear IP position. The carbon/silicon switch strategy allows to follow in the development footsteps of the already optimized parent carbon compound and thereby to speed up the development process of the sila-analogue. There is a very efficient chemical industry, with a special expertise in organosilicon chemistry, that can supply pharmaceutical industry with the silicon-based fine chemicals needed. Nowadays, the carbon/silicon switch strategy is already an established tool in drug design and development in pharmaceutical industry, and it is anticipated that this approach will play an increasingly important role in the future.

References

1. Fessenden RJ, Fessenden JS (1967) Adv Drug Res 4:95
2. Voronkov MG, Lukevics E (1969) Russ Chem Rev 38:975
3. Garson LR, Kirchner LK (1971) J Pharm Sci 60:1113
4. Tacke R, Wannagat U (1979) Top Curr Chem 84:1

5. Voronkov MG (1979) Top Curr Chem 84:77
6. Tacke R, Zilch H (1986) Endeavour. New Series 10:191
7. Tacke R, Becker B (1987) Main Group Met Chem 10:169
8. Tacke R, Linoh H (1989) Bioorganosilicon chemistry In: Patai S, Rappoport Z (eds) The chemistry of organic silicon compounds, part 2. Wiley, Chichester, p 1143
9. Bains W, Tacke R (2003) Curr Opin Drug Discovery Dev 6:526
10. Showell GA, Mills JS (2003) Drug Discovery Today 8:551
11. Mills JS, Showell GA (2004) Expert Opin Investig Drugs 13:1149
12. Pooni PK, Showell GA (2006) Mini-Rev Med Chem 6:1169
13. Sieburth SMcN, Chen C-A (2006) Eur J Org Chem 311
14. Gately S, West R (2007) Drug Dev Res 68:156
15. Franz AK (2007) Curr Opin Drug Discovery Dev 10:654
16. Meanwell NA (2011) J Med Chem 54:2529
17. Franz AK, Wilson SO (2013) J Med Chem 56:388
18. Sieburth SMcN (2014) Top Med Chem. doi:10.1007/7355_2014_80
19. Fessenden RJ, Coon MD (1965) J Med Chem 8:604
20. Fessenden RJ, Ahlfors C (1967) J Med Chem 10:810
21. Fessenden RJ, Rittenhouse R (1968) J Med Chem 11:1070
22. Fessenden RJ, Hartman RA (1970) J Med Chem 13:52
23. Klapötke TM, Krumm B, Ilg R, Troegel D, Tacke R (2007) J Am Chem Soc 129:6908
24. Evangelisti C, Klapötke TM, Krumm B, Nieder A, Berger RJF, Hayes SA, Mitzel NW, Troegel D, Tacke R (2010) Inorg Chem 49:4865
25. Klapötke TM, Krumm B, Nieder A, Richter O, Troegel D, Tacke R (2012) Z Anorg Allg Chem 638:1075
26. Daiss JO, Burschka C, Mills JS, Montana JG, Showell GA, Warneck JBH, Tacke R (2006) Organometallics 25:1188
27. Tacke R, Daiss J (2003) International Patent Application WO2003 037905
28. Daiss JO, Penka M, Burschka C, Tacke R (2004) Organometallics 23:4987
29. Showell GA, Miller D, Mandal AK, Tacke R, Daiss J (2004) International Patent Application WO2004 094436
30. Showell GA, Barnes MJ, Daiss JO, Mills JS, Montana JG, Tacke R, Warneck JBH (2006) Bioorg Med Chem Lett 16:2555
31. Warneck JB, Cheng FHM, Barnes MJ, Mills JS, Montana JG, Naylor RJ, Ngan M-P, Wai M-K, Daiss JO, Tacke R, Rudd JA (2008) Toxicol Appl Pharmacol 232:369
32. Tacke R, Heinrich T, Bertermann R, Burschka C, Hamacher A, Kassack MU (2004) Organometallics 23:4468
33. Tacke R, Heinrich T (2003) UK Patent Application GB2003 2 382 575
34. Tacke R, Popp F, Müller B, Theis B, Burschka C, Hamacher A, Kassack MU, Schepmann D, Wünsch B, Jurva U, Wellner E (2008) ChemMedChem 3:152
35. Tacke R, Nguyen B, Burschka C, Lippert WP, Hamacher A, Urban C, Kassack MU (2010) Organometallics 29:1652
36. Johansson T, Weidolf L, Popp F, Tacke R, Jurva U (2010) Drug Metab Dispos 38:73
37. Daiss JO, Burschka C, Mills JS, Montana JG, Showell GA, Fleming I, Gaudon C, Ivanova D, Gronemeyer H, Tacke R (2005) Organometallics 24:3192
38. Montana JG, Showell GA, Fleming I, Tacke R, Daiss J (2004) International Patent Application WO2004 048390
39. Büttner MW, Nätscher JB, Burschka C, Tacke R (2007) Organometallics 26:4835
40. Luger P, Weber M, Hübschle C, Tacke R (2013) Org Biomol Chem 11:2348
41. Lippert WP, Burschka C, Götz K, Kaupp M, Ivanova D, Gaudon C, Sato Y, Antony P, Rochel N, Moras D, Gronemeyer H, Tacke R (2009) ChemMedChem 4:1143
42. Bauer JB, Lippert WP, Dörrich S, Tebbe D, Burschka C, Christie VB, Tams DM, Henderson AP, Murray BA, Marder TB, Przyborski SA, Tacke R (2011) ChemMedChem 6:1509

43. Büttner MW, Burschka C, Daiss JO, Ivanova D, Rochel N, Kammerer S, Peluso-Iltis C, Bindler A, Gaudon C, Germain P, Moras D, Gronemeyer H, Tacke R (2007) ChemBioChem 8:1688
44. Tacke R, Müller V, Büttner MW, Lippert WP, Bertermann R, Daiß JO, Khanwalkar H, Furst A, Gaudon C, Gronemeyer H (2009) ChemMedChem 4:1797
45. Gluyas JBG, Burschka C, Dörrich S, Vallet J, Gronemeyer H, Tacke R (2012) Org Biomol Chem 10:6914
46. Tacke R, Kornek T, Heinrich T, Burschka C, Penka M, Pülm M, Keim C, Mutschler E, Lambrecht G (2001) J Organomet Chem 640:140
47. Tacke R, Handmann VI, Kreutzmann K, Keim C, Mutschler E, Lambrecht G (2002) Organometallics 21:3727
48. Tacke R, Handmann VI, Bertermann R, Burschka C, Penka M, Seyfried C (2003) Organometallics 22:916
49. Heinrich T, Buschka C, Warneck J, Tacke R (2004) Organometallics 23:361
50. Tacke R, Schmid T, Penka M, Burschka C, Bains W, Warneck J (2004) Organometallics 23:4915
51. Ilg R, Burschka C, Schepmann D, Wünsch B, Tacke R (2006) Organometallics 25:5396
52. Barnes MJ, Burschka C, Büttner MW, Conroy R, Daiss JO, Gray IC, Hendrick AG, Tam LH, Kuehn D, Miller DJ, Mills JS, Mitchell P, Montana JG, Muniandy PA, Rapley H, Showell GA, Tebbe D, Tacke R, Warneck JBH, Zhu B (2011) ChemMedChem 6:2070
53. Wang J, Ma C, Wu Y, Lamb RA, Pinto LH, DeGrado WF (2011) J Am Chem Soc 133:13844
54. Reddy GD, Park S-J, Cho HM, Kim T-J, Lee ME (2012) J Med Chem 55:6438
55. Tacke R, Bertermann R, Burschka C, Dörrich S, Fischer M, Müller B, Meyerhans G, Schepmann D, Wünsch B, Arnason I, Bjornsson R (2012) ChemMedChem 7:523
56. Fujii S, Miyajima Y, Masuno H, Kagechika H (2013) J Med Chem 56:160
57. Nakamura M, Makishima M, Hashimoto Y (2013) Bioorg Med Chem 21:1643
58. Büttner MW, Penka M, Doszczak L, Kraft P, Tacke R (2007) Organometallics 26:1295
59. Doszczak L, Kraft P, Weber H-P, Bertermann R, Triller A, Hatt H, Tacke R (2007) Angew Chem 119:3431; (2007) Angew Chem Int Ed 46:3367
60. Büttner MW, Burschka C, Junold K, Kraft P, Tacke R (2007) ChemBioChem 8:1447
61. Büttner MW, Metz S, Kraft P, Tacke R (2007) Organometallics 26:3925
62. Tacke R, Metz S (2008) Chem Biodiversity 5:920
63. Metz S, Nätscher JB, Burschka C, Götz K, Kaupp M, Kraft P, Tacke R (2009) Organometallics 28:4700
64. Sunderkötter A, Lorenzen S, Tacke R, Kraft P (2010) Chem Eur J 16:7404
65. Geyer M, Bauer J, Burschka C, Kraft P, Tacke R (2011) Eur J Inorg Chem 2769
66. Dörrich S, Bauer JB, Lorenzen S, Mahler C, Schweeberg S, Burschka C, Baus JA, Tacke R, Kraft P (2013) Chem Eur J 19:11396
67. Förster B, Bertermann R, Kraft P, Tacke R (2014) Organometallics 33:338
68. Friedrich J, Dörrich S, Berkefeld A, Kraft P, Tacke R (2014) Organometallics 33:796

Top Med Chem (2016) 17: 61–86
DOI: 10.1007/7355_2014_80

Published online: 12 December 2014

Silicon Mimics of Unstable Carbon

Scott McN. Sieburth

Abstract Silicon–carbon bonds are unknown in nature, yet the search for bioactive organosilanes has a rich and successful history. Substitution of silicon for a stable quaternary carbon in biologically active molecules often leads to bioactive organosilanes. An alternative approach is the substitution of silicon for an unstable carbon, such as a hydrated carbonyl. The proclivity of carbon to favor a carbonyl over a 1,1-diol is reversed for silicon. Tetrahedral, hydrated carbonyls are ubiquitous intermediates in the reactivity of carbonyl compounds including many enzymatic reactions, and hydrolase enzymes are critical mediators of a broad range of biological processes. Proteases, one group of hydrolase enzymes, can be potently inhibited by silanediol-based peptide mimics when the silanediol substitutes for the hydrated carbonyl of amide bond hydrolysis. This chapter outlines the key parameters for bioactive organosilanes, the evolution of the silanediols as protease inhibitors, the supporting chemistry, and the current status.

Keywords Angiotensin-converting enzyme, Chymotrypsin, Enzyme inhibitor, Factor XIa, HIV, Hydrolase inhibitor, Hydrosilylation, Protease inhibitor, Silanediol, Silyl anion, Sulfinimine, Thermolysin

Contents

S. McN. Sieburth (✉)
Department of Chemistry, Temple University, 1901 N. 13th Street, Philadelphia, PA 19122, USA
e-mail: scott.sieburth@temple.edu

1 Introduction

1.1 Discovery of Bioactive Organosilanes

Among the elements most closely associated with bioactive molecules – carbon, hydrogen, nitrogen, and oxygen – only oxygen and hydrogen are present in the Earth's crust at substantial levels. Oxygen is the most abundant element by weight at 47 % and hydrogen tenth most abundant at 0.14 %. Carbon is 16th (0.03 %) and nitrogen 21st (0.005 %). Silicon is the second most abundant element (28 % of the Earth's crust), and while silicon is the element most similar to carbon, virtually all naturally occurring silicon is in the form of silicates, bonded only to oxygen atoms (silicon carbide, which has no Si–O bonds, is naturally occurring but rare substance, discovered in the 1950s [1]). Following the emergence of organosilicon chemistry more than a century ago [2], a search for biologically active organosilanes emerged, a search that continues today ([3]; for reviews, see [4–13]). The absence of naturally occurring substances with bonds between silicon and carbon makes bioactive organosilanes all the more interesting.

There are two fundamental approaches for discovery of novel bioactive molecules: design and random screening. Random screening is an extraordinarily useful approach for discovering breakthrough bioactive molecules and uses for otherwise arbitrary compounds, such as the laundry-additive-turned-billion-dollar-herbicide Roundup [14]. Random screening will discover bioactive structures that could not be derived through a design process, but for the most part, intellectual involvement by chemists comes only after identification of a lead molecule.

Random screening presumably was responsible for discovery of bioactive organosilanes for which there are no carbon analogs, such as cisobitan (**1**) [15], SAN 58–112 (**2**) [16], and phenyl silatrane (**3**) (Fig. 1) [17].

1.2 Silicon as a Substitute for Carbon

In contrast, the design process requires, by its very nature, involvement of chemists from the outset. One of the most reliable ways to prepare a biologically active organosilane is replacement of a carbon atom by a silicon atom (see reference

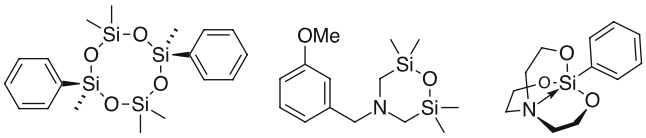


Fig. 1 Bioactive organosilanes without carbon analogy, identified by random screening

[4–13], especially [7] and [12]). This is arguably the most subtle change one can make in terms of size and electronegativity (outside of hydrogen–deuterium exchange), but not all carbons are replaceable by silicon. Silicon does not form stable double bonds [18, 19]. The Si–O, Si–N, and Si–halogen bonds are strong, but they hydrolyze readily [20]. Silicon–hydrogen bonds are readily oxidized in vivo [21]. Replacement of a quaternary carbon by silicon is therefore the most readily realized approach to bioactive organosilane discovery, yet even swapping quaternary carbon with silicon has limitations. These parameters are nicely illustrated by the naturally occurring pyrethrin insecticides and the evolution of the synthetic, commercial analogs of these natural products (Fig. 2).

1.3 The Pyrethrin Pathway

Pyrethrin I is an insecticidal compound isolated from the common chrysanthemum flower (Fig. 2) [22]. The pyrethins, however, are not light stable and therefore unsuitable for field use. Cypermethrin is a potent, light-stable commercial analog that has been used in the field with great success [23]. In both of these molecules, the sole quaternary carbon (black dot) cannot be replaced by silicon because silicon in a three-membered ring is extremely unstable [24, 25]. In addition, this quaternary carbon is alpha to a carbonyl, and silicon alpha to a carbonyl is also unstable [26]. In the third pyrethroid, esfenvalerate [27], the dimethylcyclopropane has been replaced by an isopropyl group, but this structure remains unsuitable for a C to Si exchange (at the black dot) for two reasons: the resulting Si–H bond would be rapidly oxidized [21] and the silane remains adjacent to a carbonyl. In the fourth pyrethroid etofenprox, only the gem-dimethyl group and the central oxygen atom remain from the natural pyrethrin I [28]. Etofenprox does retain the quaternary carbon, not in a cyclopropane, and therefore was the first analog of the natural product in which silicon substitution was possible. Replacement of this quaternary carbon with silicon retained insecticidal properties, and the resulting silafluofen is a commercial insecticide [29, 30]. Silafluofen and the agricultural fungicide flusilazole [31] are currently the only organosilanes manufactured because of their biological activity.

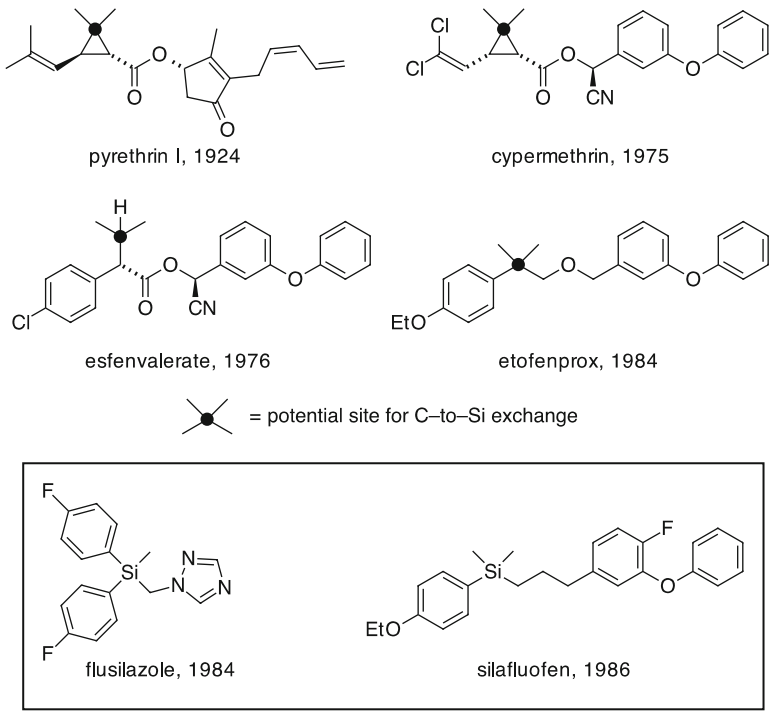


Fig. 2 Naturally occurring insecticide pyrethrin I and three carbon-based commercial analogs. Years of discovery are given. Only etofenprox is compatible with silicon substitution. Two organosilanes are produced commercially for their biological activity: fungicide flusilazole and insecticide silafluofen

1.4 Silanols

Silicon ethers, the Si–O–C linkage, have stability that is related to the nature of the component alcohol and the silane components [32]. Some silane-derivatized alcohols and amines have been found to enhance the bioactivity of the parent compounds, presumably by hydrolysis in vivo to the alcohol/amine and the corresponding silanol (see reference [4–13], especially [7] and [12]). Some silanols have been found to have very intriguing biological properties, most notably the hypnotic properties of diphenylsilanediol **4** [33] and sila-haloperidol **5** [34, 35] (Fig. 3). The hydroxyls of silanols exchange rapidly in the presence of water, but they remain silanols (see Fig. 12). The primary instability of silanols is dehydration and formation of siloxanes, structures containing the Si–O–Si bonding arrangement (see Fig. 4). Siloxanes are generally more stable than their Si–O–C counterparts (see Fig. 1). The many useful properties of siloxanes form the basis of the large organosilane industry [36].

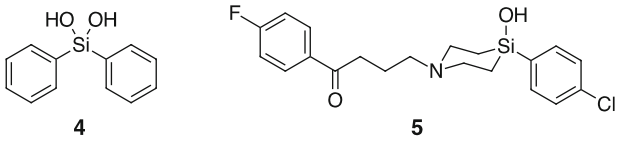


Fig. 3 Bioactive organosilanols

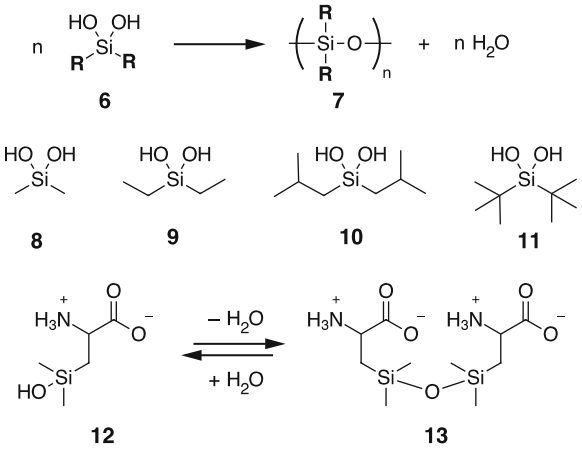


Fig. 4 Silanediols can readily dehydrate to form siloxanes (silicones), but steric hindrance can slow or prevent oligomerization

1.5 Silanols, Silanones, and Siloxanes

Silanediols (**6**) are best known as precursors of siloxanes **7** (aka silicones), the product of a condensation reaction that is catalyzed by both acid and base (Fig. 4). The polymerization of dimethylsilanediol **8** is reported to be spontaneous, with even very pure **8** being unstable [37]. Steric hindrance plays a significant role here; dimethylsilanediol **9** polymerizes at one tenth the rate of **8** [38, 39]. Diisobutylsilanediol **10** is a liquid crystal, illustrating both stability and the outstanding ability of silanols to be hydrogen bond donors and acceptors [40, 41]. With sufficient steric bulk, as in di-*tert*-butylsilanediol **11**, even forcing conditions do not lead to polymerization. Solvation of silanols can also play a significant role as well. Tacke found that dimethylsilanol-substituted alanine **12** formed siloxane **13** when it was isolated, but dissolving **13** in water gave a spontaneous, quantitative hydrolysis to yield **12** [42, 43]. This finding suggests that the insolubility of most siloxanes in water adds to their hydrolytic stability.

The stability of simple silanediols like **10** provided encouragement for efforts to design biologically active analogs that would remain monomeric [44, 45]. An early example of a bioactive silanediol is **4** (Fig. 3) which has barbiturate-like properties [33].

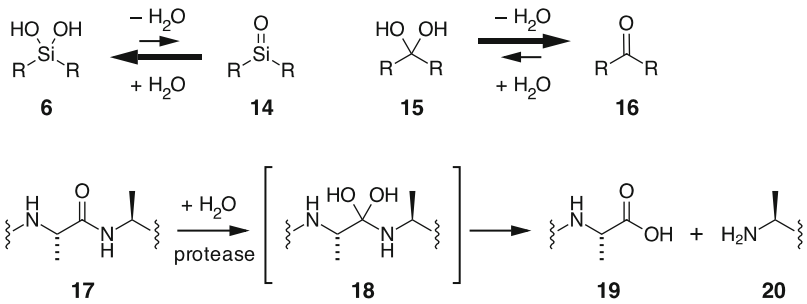


Fig. 5 Silanones **14** are very unstable toward hydration, whereas ketones **16** are more stable than their hydrates. Amides such as **17**, much less prone toward hydration than ketones, are substrates for protease enzymes

Silicones (e.g., **7**) were first prepared more than 100 years ago in an attempt to make silicon analogs of ketones, inadvertently illustrating the instability of double bonds to silicon (**14**) [46]. This contrasts with the proclivity of 1,1-diols **15** to dehydrate and form carbon–oxygen double bonds **16**. The stability of carbonyls relative to their hydrates is particularly pronounced for carboxylic acid derivatives, especially amides such as **17**. Peptides **17** are polyamides that can be hydrolyzed by protease enzymes to their respective acid **19** and amine **20** under mild physiological conditions. Proteases catalyze the addition of water (or alcohol or thiol) to the amide carbonyl, in part by stabilizing the tetrahedral intermediate **18** [47]. Tight binding, nonhydrolyzable mimics of this tetrahedral group can be effective inhibitors of these enzymes and therefore pharmaceuticals [48–50].

1.6 Protease Enzymes and Their Inhibition

Protease enzymes mediate many important biological processes and are pharmaceutical targets for a broad range of diseases [48–50]. Protease inhibitors as pharmaceuticals were first introduced to treat hypertension nearly 40 years ago [51]. Figure 6 shows four examples of commercial protease inhibitors, with gray boxes highlighting the functional group that replaces and mimics the tetrahedral intermediate of amide hydrolysis **18**. Fosinoprilate utilizes a phosphinic acid to replace the hydrated carbonyl and interact with the active site zinc ion of angiotensin-converting enzyme [52, 53]. Inhibiting this metalloprotease lowers blood pressure [54]. Atazanavir is an inhibitor of the HIV protease, an aspartic protease, and prevents reproduction of the HIV virus [55, 56]. In this case, a hydroxyethylene group replaces the hydrated amide and hydrogen bonds to the active site aspartic acids. The boronic acid bortezomib is a treatment for cancer; the electrophilic boron binds to the nucleophilic threonine hydroxyl group at the active site [57]. Sitagliptin binds to the active site of a serine protease (DPP-4) and is a treatment for diabetes [58].

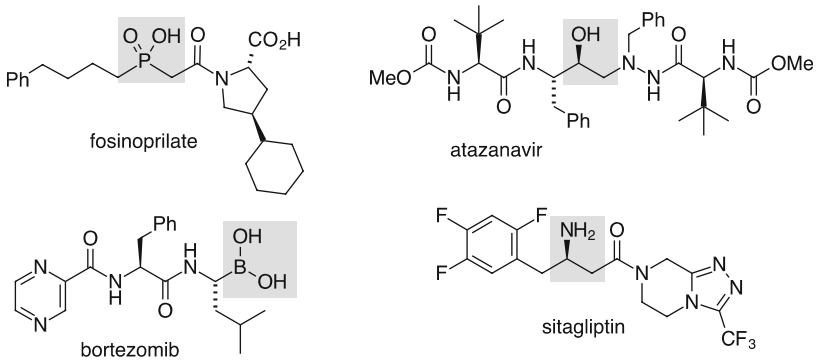


Fig. 6 Examples of commercial protease inhibitor drugs used for the treatment of hypertension (fosinoprilate), HIV infection (atazanavir), cancer (bortezomib), and diabetes (sitagliptin)

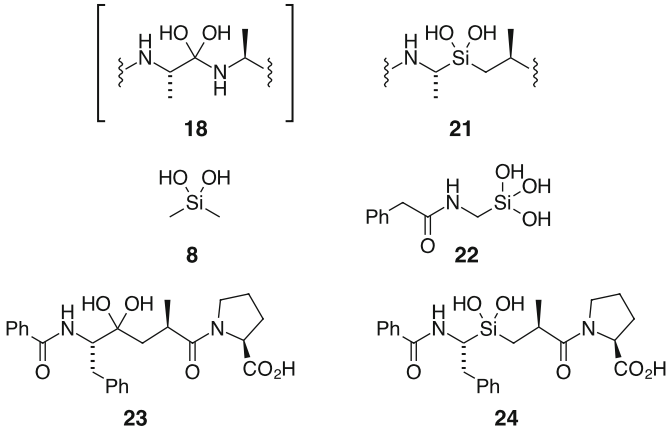


Fig. 7 Silanediol **21** as an isostere of hydrated amide **18**. Compounds **8** and **22** had been found to be ineffective as hydrolase inhibitors. Redesigning protease inhibitor **23** (shown as the ketone hydrate) gave the silanediol inhibitor **24**

2 Silanediols in Drug Design

Silanediols **21** seemed nearly ideal isosteres of the hydrated amide carbonyl **18,** but at the beginning of our investigations, this idea had only been investigated sparingly (Fig. 7) [59]. The testing of silanediols as hydrated carbonyl mimics was largely suppressed by the general knowledge of the instability of silanediols toward polymerization (Fig. 5) and a dearth of methods for preparing silanediols with functionality and stereochemistry.

Prior to our studies, two silanols had been evaluated as inhibitors of hydrolase enzymes, **8** and **22**. An aqueous solution of dimethylsilanediol **8** was found to be ineffective as an inhibitor of the metalloprotease angiotensin-converting enzyme at

300 mM [60]. In addition, silanetriol **22** was prepared as a potential inhibitor of a beta-lactamase and was inactive at the highest concentration tested, 0.1 mM [61].

Our first vehicle for testing structure **21** as a nonhydrolyzable analog of **18** and the centerpiece of a protease inhibitor was silanediol **24**, by analogy with **23**. Structure **23** is shown as the hydrate of a ketone that was tested as a replacement for an Ala-Phe dipeptide and was found to be a 1 nM inhibitor of angiotensin-converting enzyme [62]. The hydrate **23** is presumably the form that binds at the ACE active site [63]. The silanediol in **23** was anticipated to have a steric environment that would inhibit oligomerization, shielded on each side by branched alkyl substituents, not unlike diisobutylsilanediol **10** (Fig. 4).

2.1 Synthesis of Silanediol Peptidomimetics

2.1.1 First-Generation Silanediol Syntheses

A synthesis of **24** was needed, including a strategy for carrying the silanediol through the synthetic manipulations that would be required. Diethers of silanes (silyl acetals) are rather unstable toward hydrolysis and were rejected as a viable form of protected silanediol [64]. Acid-catalyzed hydrolysis, "protodesilylation," appeared to be an interesting alternative [65]. Acidic cleavage of Si–C bonds of aryl silanes is a classic electrophilic aromatic substitution reaction and therefore electronically tunable if simple phenyl groups proved to be less than perfect (see Fig. 9) [66]. The precursor of silanediol **24** therefore was diphenylsilane **25** (Fig. 8).

In this initial exploration of silanediol synthesis, difluorodiphenylsilane **30** was coupled with metalated dithiane **29** followed by the reaction with enantiomerically pure lithium reagent **28** [67]. The resulting silane **32** contains all the carbons of dipeptide mimic **24** but with functional group elaboration required. Lithium reagent (*R*)-**28** was prepared from commercially available (*S*)-ester **26**.

The dithiane in **32** was hydrolyzed to the corresponding ketone and then reduced to the corresponding alcohol. The nitrogen of **33** was introduced as a phthalimide using Mitsunobu chemistry, followed by hydrolysis and conversion of the amine to benzamide **33**.

Following cleavage of the benzyl ether of **33**, the diastereomers were separated and the alcohol of the (*S*,*R*) stereoisomer was oxidized to acid **34**. Condensation with the *tert*-butyl ester of (*S*)-proline gave silane **35** [67].

2.1.2 Silanediols from Diphenylsilanes

In the ultimate synthetic step, deprotection of **35** (Fig. 8) was accomplished by treatment with triflic acid, serving to remove the *tert*-butyl ester and both phenyl groups on silicon. The optimized sequence for isolation of silanediol **24** involved neutralization of the mixture with ammonium hydroxide and then conversion of the

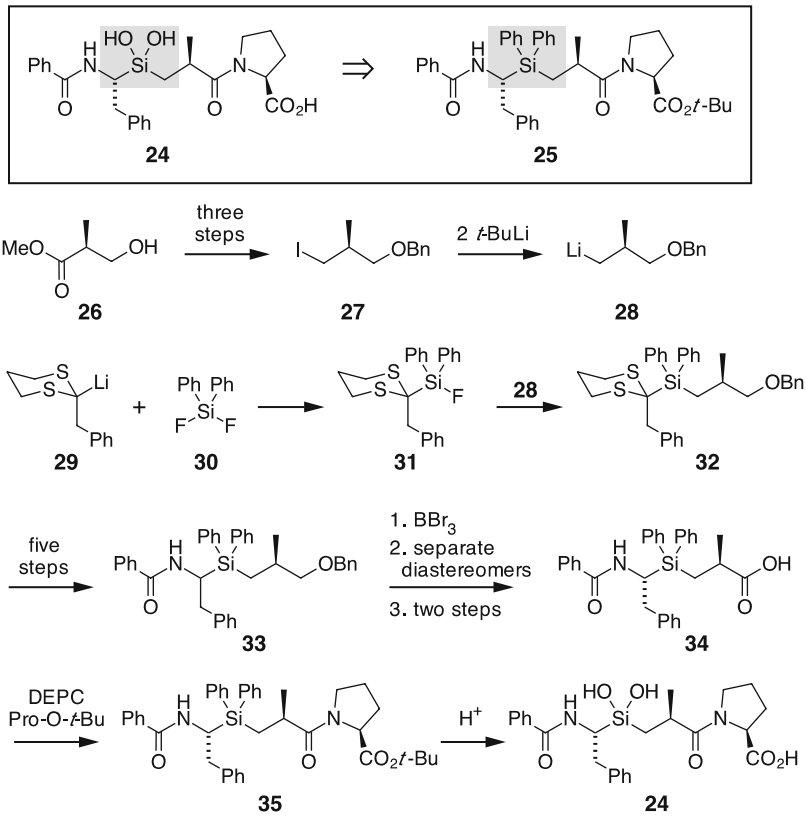


Fig. 8 ACE inhibitor **24** and its synthesis beginning with difluorodiphenylsilane **30**

silane to a crystalline difluorosilane with aqueous hydrofluoric acid [68]. Hydrolysis of the difluorosilane to the silanediol was readily accomplished by treatment with aqueous base.

Direct conversion of diarylsilanes to silane ditriflates using triflic acid is well known [69]; however, acidic hydrolysis of the diphenylsilane **35** and related compounds is believed to involve participation of the flanking amide carbonyls (Fig. 9). Beginning with *ipso*-protonation of the aromatic ring (**36**), loss of benzene requires a nucleophile to attack silicon, allowing departure of the benzene, a role readily fulfilled by either of the flanking amides. A second protonation–nucleophilic attack leads to **38**. Five-membered silyl ethers are strained, and therefore, these undergo a facile hydrolysis to the silanediol [70]. Participation of the amides is consistent with our observations of intermediates by ^{1}H NMR [71] and the work of Nielsen and Skrydstrup with monoamide **39** [72]. Whereas triflic acid hydrolysis of diamide **35** leads to **38** within minutes, and thus the silanediol **24**, treatment of monoamide **39** under similar conditions results in rapid loss of the first phenyl group and a very slow cleavage of the second [72]. Recent work by the Skrydstrup

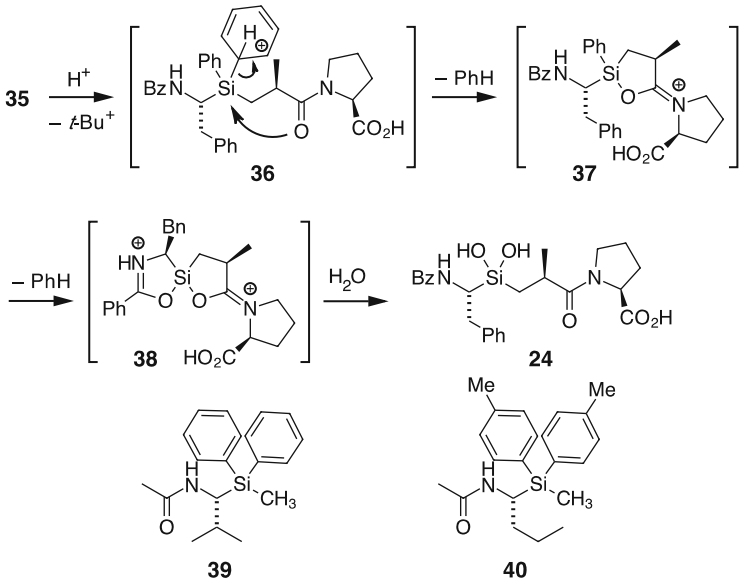


Fig. 9 Acid-catalyzed hydrolysis of the diphenylsilane involves participation of the adjacent amides

group has shown that activation of the aromatic rings by alkyl substitution, as in **40**, allows for hydrolysis of both Si–aryl bonds with trifluoroacetic acid instead of triflic acid, presumably by lowering the activation energy for the *ipso*-protonation step (see also Fig. 20) [73].

2.1.3 Inhibition of Metallo- and Aspartic Proteases

Using chemistry related to the initial investigation (Fig. 8), a set of silanediols were prepared as analogs of other known protease inhibitors (Fig. 10). Silanediol **24**, an isostere of hydrated **23a** (see Fig. 7), was found to be an effective inhibitor of angiotensin-converting enzyme (ACE), a metalloprotease with a zinc ion at the active site [74]. Compound **23a** inhibits ACE with an IC_{50} of 1 nM, and silanediol **24** was found to inhibit this enzyme with a K_i of 3.8 nM [62]. Similarly, silanediol **42** was prepared as an analog of phosphinic acid **41**, an inhibitor of thermolysin [75, 76]. Silane **42** and phosphinic acid **41** are constructed around second row elements. As such, the silane and phosphorus units are closer in size than silicon is to carbon, but are also dramatically different in their acidities. Silanediols have pK_a values of approximately 12 [44, 77] and would be unionized at physiological pH, whereas phosphinic acids have a pK_a in the range of 3–4 and are anionic at physiological pH. Ionized phosphinic acid **41** would be expected to benefit from a Coulombic attraction to the active site zinc cation and silanediol would not. Nevertheless, phosphinic acid **41** inhibits thermolysin with an IC_{50} of 10 nM, and silane **42**

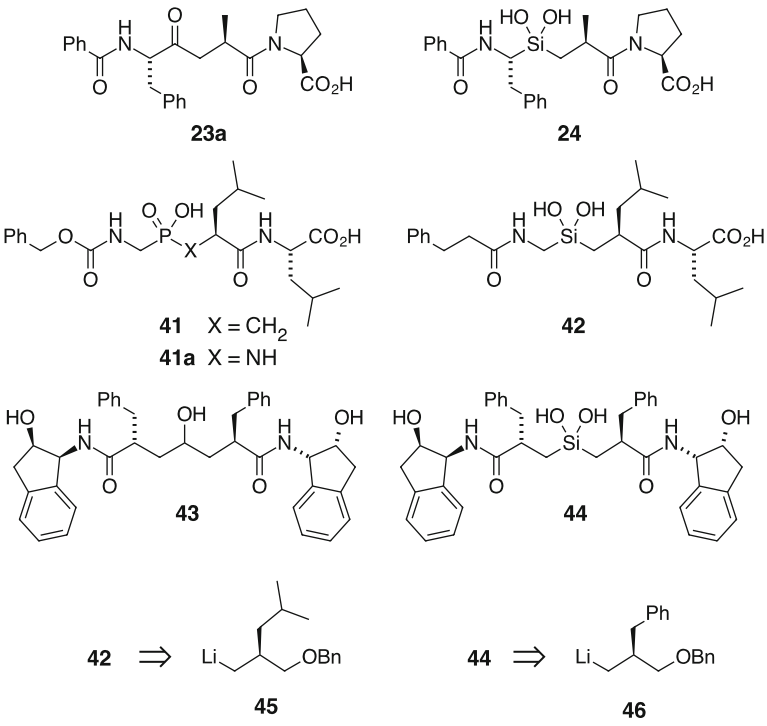


Fig. 10 Silanediol inhibitors by analogy: known inhibitors (*left*) and their silane analogs (*right*)

inhibits thermolysin with a very similar IC_{50} of 40 nM. A crystal structure of **42** bound to thermolysin has been obtained; see Fig. 11.

A single example of a silanediol aspartic protease inhibitor has been studied, compound **44**, prepared by analogy to HIV protease inhibitor **43** [78]. Carbinol **43** inhibits the HIV protease with an IC_{50} of 0.38 nM, and silanediol **44** is sevenfold less potent, 2.7 nM. Side-by-side testing of **43** and **44** and the similarly potent and commercial HIV inhibitor indinavir [79] found that they all cross cell membranes with equal efficiency, as determined by their ability to prevent infection of cells by the HIV virus. These studies demonstrated that silanediol **44** has drug-like properties [78].

Syntheses of silanediols **42** and **44** followed paths similar to the preparation of **24** (Fig. 8), using enantiomerically pure lithium reagents **45** and **46**. While effective, this strategy was not efficient nor was it readily scalable. More efficient and catalytic methods have been developed recently and have been utilized for the silanediols that have followed the examples described below.

A crystal structure of silanediol **42** (PDB #1Y3G) bound to the active site of thermolysin found that the silanol oxygens are arrayed about the active site zinc in precisely the same way that the phosphorous oxygens are for the related phosphinamide **41a** (PDB #5TMN) (Fig. 11) [80]. Phosphinic acid **41** and

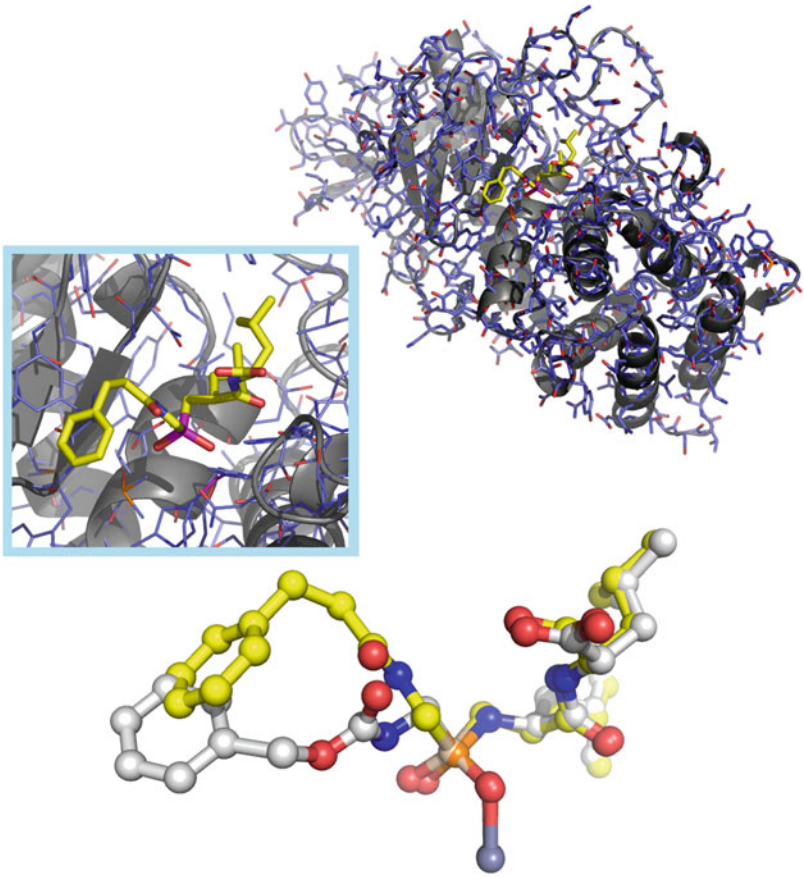


Fig. 11 Crystal structure of silanediol **42** bound to thermolysin (PDB #1Y3G) and its overlay with **41b** also bound to thermolysin (PDB #5TMN). Silanediol **42** in yellow and **41b** in light gray. The overlay also shows the active site zinc in dark gray illustrating that they each touch the zinc in the same way

phosphinamide **41a** are both 10 nM inhibitors of thermolysin, but only the latter has been crystallized at the active site of thermolysin [75, 76, 81].

2.1.4 Inhibition of Serine Proteases

Two of the more recent subjects of investigation are **48** and **50** (Fig. 12). Both of these structures target serine proteases, which are fundamentally different from the metallo- and aspartic proteases targeted initially. Metalloproteases have an active site zinc ion to coordinate with and activate an amide carbonyl. This zinc ion also delivers the water nucleophile (see inhibitors **23**, **24**, **41,** and **42**). Aspartic proteases have two aspartic acid residues at the active site, delivering the nucleophilic water

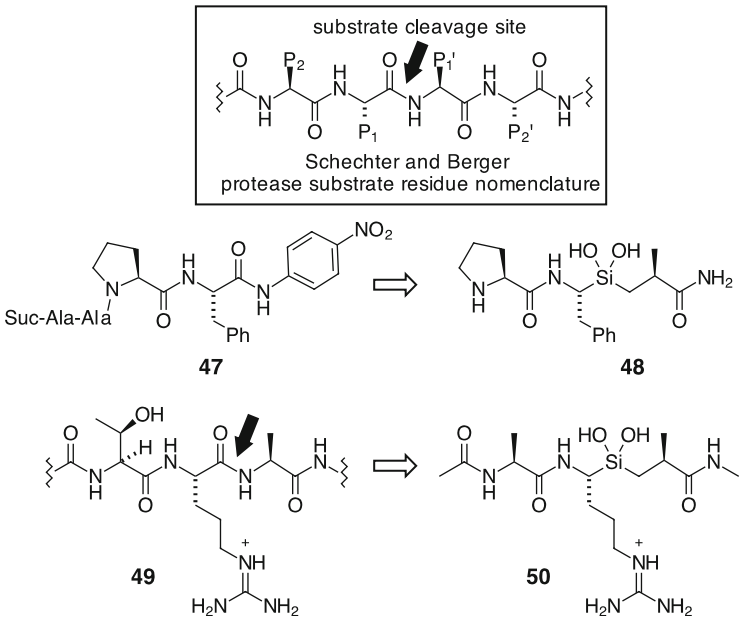


Fig. 12 Protease substrate residue definitions and silanediol inhibitors by substrate analogy

molecule and stabilizing the hydrated carbonyl by hydrogen bonding (inhibitors **43** and **44**).

In contrast to the metallo- and aspartic proteases, serine proteases use a serine residue of the enzyme as the nucleophile. This reaction leads to departure of the amine portion of the peptide substrate, leaving the carbonyl esterified to the serine residue. In a second step, water hydrolyzes the ester, freeing the acid and regenerating the active site. Many serine protease inhibitors have electrophilic functionality that interact with the serine alcohol, but only carry recognition groups on one side of the active site nucleophile [82]. Using the Schechter and Berger descriptors [83], only the non-prime residues are represented in many serine protease inhibitors (Fig. 12). To adequately protect the silanediol moiety from oligomerization, we desired substitution at both P_1 and P_1' sites to provide steric shielding. From the perspective of protease inhibitor selectivity – a major consideration in protease inhibitor design [84] – accessing recognition centers on both sides of the enzyme active site can be advantageous.

For the classic serine protease chymotrypsin, an enzyme that requires an aromatic side chain at P_1 for substrate recognition, we modeled the silanediol inhibitor after the chromogenic substrate **47** and utilized a methyl group at P_1' (Fig. 12) [85]. Silanediol **48** was found to inhibit chymotrypsin with an IC_{50} of 107 nM [85].

A medically important serine protease, Factor XIa, was chosen as a second test case for study. Silanediol **50** was prepared as a potential inhibitor of Factor XIa, which is a key component of the coagulation cascade [86]. Silanediol **50** is a mimic of Factor IX, the substrate for Factor XIa, which is cleaved between Ala^{146} and

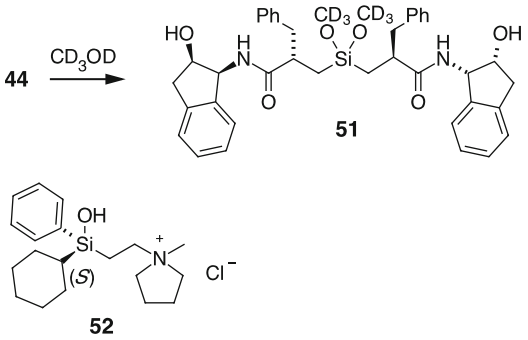


Fig. 13 Hydroxyls on silicon rapidly exchange with water and alcohols as evidenced by substitution by deutero-methanol in **44** and the racemization of **52** in the presence of water

Arg^{145} (**49**). The P_1 guanidinium ion of **49** is required for recognition by Factor XIa [87].

Electrophilic coordination to an active site serine nucleophile by a silanediol is inherently different from hydrogen bonding of the silanediol group in an aspartic protease active site or zinc ion coordination in metalloproteases. There was, however, ample experimental evidence for this kind of electrophilic role for silanols. We had observed a rapid exchange of silanediol hydroxyls by two equivalents of methanol-d_4 when **44** was dissolved in this solvent (Fig. 13) [71]. Additional evidence for rapid exchange of hydroxyls in silanols came from the work of Tacke. For example, enantiomerically pure silanol **52** was found to be stable in aprotic solvents, but when exposed to aqueous solutions **52** rapidly racemized [88].

2.1.5 Second- and Third-Generation Silanediol Syntheses

Initially, syntheses of **48** and **50** used an approach similar to that used earlier (Fig. 8), but these syntheses were superseded by better techniques for assembly and control of the two stereogenic centers flanking the silanediol [89, 90]. In all cases, a diphenylsilane was carried through the synthesis sequence and hydrolyzed to the silanediol by strong acid as the last step.

The synthesis of **59**, precursor of inhibitor **48**, began with cycloaddition of dichlorodiphenylsilane and isoprene to yield dihydrosilole **53** (Fig. 14). This reaction can easily produce hundreds of grams in a single run [91]. Asymmetric hydroboration of **53** with pinene-derived isopinocamphylborane gave **54** in >90% ee [85, 92]. Warming **54** with aqueous HF leads to Peterson-like fragmentation and formation of fluorosilane **55** with the methyl stereogenic center in place. Fluorosilanes are relatively moisture insensitive and also react efficiently with nucleophiles [93]. Conversion of fluorosilane **55** to ketone **56** was followed by asymmetric reduction of the ketone using stoichiometric CBS-borane reagent to give **57** with >90% diastereoselectivity [94]. Mitsunobu inversion of the alcohol by phthalimide installed the amine in **58** with the correct stereochemistry. Oxidative

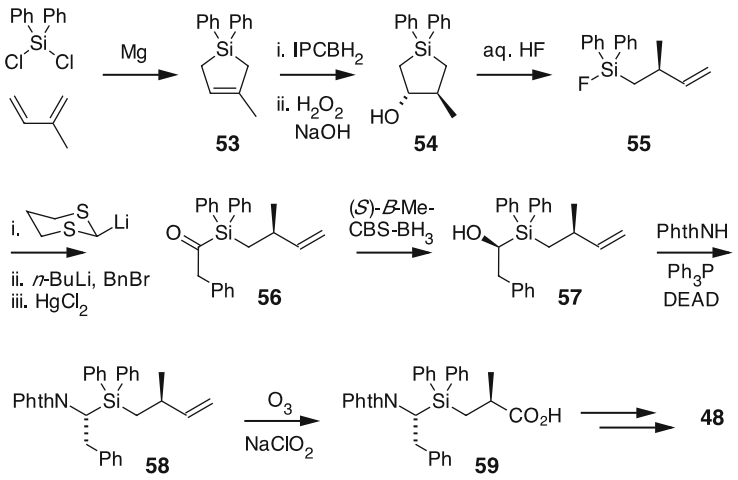


Fig. 14 The stereogenic centers of **59** were installed using asymmetric hydroboration reagents

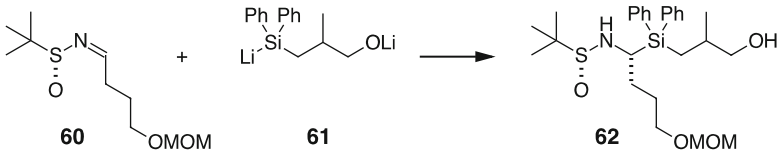


Fig. 15 Using the Skrydstrup protocol, silyllithium reagent **61** added to sulfinimine **60** to give **62** with complete stereocontrol

cleavage of the alkene then gave the amino acid **59** full control of both stereocenters. Conversion of **59** to **48** then followed standard protocols [85].

A direct and efficient method for preparation of the α-aminosilane group with control of enantioselectivity was introduced by Nielsen and Skrydstrup using silyllithium reagents and sulfinimines [72]. This method was applied to the synthesis of silanediol precursor **62** (Fig. 15). Silyllithium reagent **61** added to the enantiomerically pure **60** to give **62** with excellent diastereocontrol [90]. Use of racemic **61** demonstrated that the methyl stereogenic center does not influence the diastereoselectivity of the reaction with **60**.

The coupling of Davis–Ellman sulfinimines [95] with silicon nucleophiles by Skrydstrup was an excellent solution for stereocontrol to the α-aminosilane stereogenic center, but the stereochemistry of the β-silyl acid portion of these inhibitors (see **61** in Fig. 15) was in need of a better solution as well. The asymmetric hydroboration approach for control of this stereocenter, illustrated in Fig. 14 via **53**, was limited by the lack of readily available 2-substituted-1,3-dienes [96]. In the original syntheses of the inhibitors shown in Fig. 8, an enantiomerically pure lithium reagent was used, either prepared from the Roche ester **26** or using Evans chiral auxiliary technology **63** (Fig. 16).

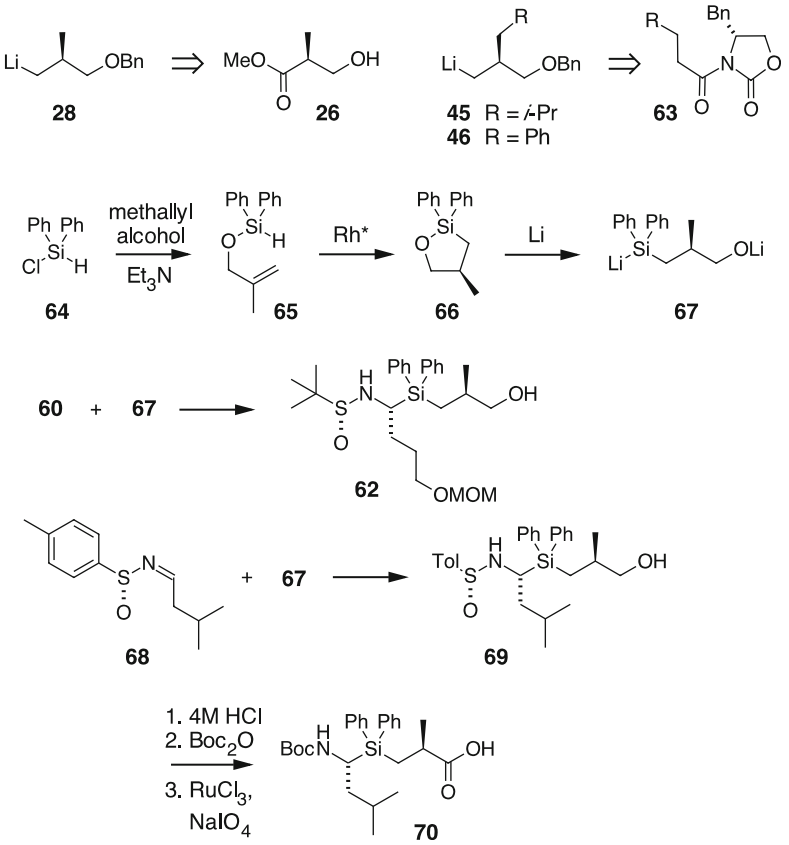


Fig. 16 Asymmetric hydrosilylation, reductive opening of **66,** and addition to sulfinimines yield advanced silanediol intermediates rapidly and with complete control of stereochemistry

One of the most efficient methods for forming Si–C bonds is hydrosilylation [97–100]. Intramolecular hydrosilylation has been extensively studied, but the asymmetric transformation of **65** into **66**, needed for our efforts, had only been achieved with low enantioselectivity [101, 102]. In a survey of commercially available rhodium ligands, it was found that (*S*,*S*)-diethylferrotane gave >90 % ee for this transformation and a similarly high enantioselectivity for homologs of **65** carrying groups other than methyl. A second breakthrough was the discovery that treatment of **66** with lithium directly and quantitatively generated the dianion **67**. In a reaction similar to that shown in Fig. 15, addition of **67** to sulfinimine **60** gave **62** in good yield and with complete control of stereochemistry (Fig. 16). Coupling of **67** and **68** gave **69**. Hydrolysis of the sulfonamide **69**, protection of the amine with a Boc group, and oxidation of the alcohol to an acid completed the preparation of key intermediate **70** in short order [103].

The combination of intramolecular hydrosilylation, direct reductive opening of the silyl ether, and coupling of the resulting silyl anion with a sulfinimine (Fig. 16)

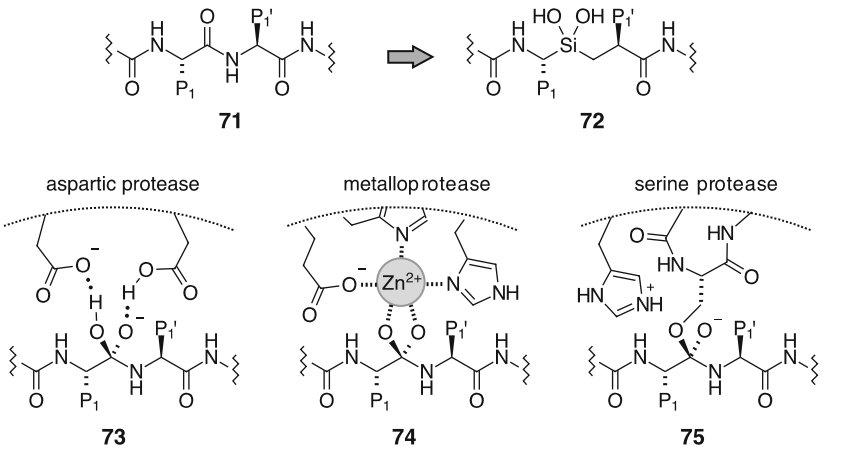


Fig. 17 First-generation silanediol protease inhibitors by substitution of the hydrated amide with a methylene silane group and the active sites of three protease classes for which silanediols are effective inhibitors

has proven to be a very efficient way to assemble the core components of the silanediol precursors such as **62** and **70**. Compound **70** can be directly coupled with amino acids and hydrolyzed to the silanediol, providing protease inhibitor candidates. Challenges remain, however. The key intermediate for assembly of Factor XIa inhibitor **50** (Fig. 12) is the readily prepared **62** (Fig. 16). The conversion of **62** to **50**, however, was a hard slog, requiring extensive functional group manipulation and illustrating the opportunities for improvement that remain [90].

3 Summary and Future Prospects

Silanediols embedded in a peptide-like structure were conceived as mimics of the tetrahedral intermediate of amide hydrolysis, with the potential for inhibiting protease enzymes. Starting with a protease substrate, **71** (Fig. 17), a first-generation inhibitor can be designed by replacing the scissile amide bond with a methylene-silanediol unit, e.g., **72**. This concept has been tested for three of the four classes of protease enzymes and found to be very effective.

3.1 Mechanistic Considerations and Future Challenges

Aspartic proteases, enzymes that contain two aspartic acids at the active site, mediate proteolysis through hydrogen bonding, **73** (Fig. 17). The aspartic acid units are generally considered to have one ionized and one unionized at the active

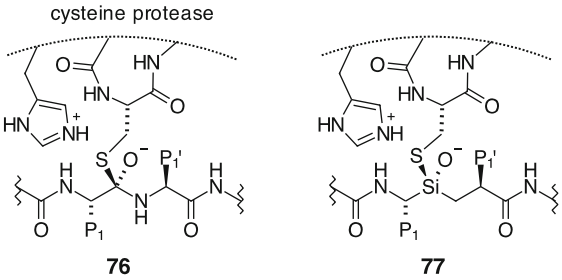


Fig. 18 Cysteine proteases utilize a nucleophilic sulfur to hydrolyze amides. Inhibition of this important class of proteases by silanediols has not been demonstrated

site, and these stabilize the amide hydrate [104]. A silanediol replacement of this hydrated peptide is expected to readily interact with the active site carboxylic acids because silanols are outstanding hydrogen bond donors and acceptors [44, 45, 105, 106]. This potential has been evaluated using the HIV protease, taking advantage of the C_2 symmetry of the enzyme with a C_2 symmetric inhibitor **44** (Fig. 10). Inhibitor **44** was prepared by analogy to a published inhibitor (**43**) that contained a hydroxyethylene replacement for the amide bond. This silanediol-for-carbinol switch, without reoptimization, gave an effective inhibitor with an IC_{50} under 3 nM. More importantly, this study found that the silanediol was as effective as the commercial drug indinavir at crossing cell membranes and protecting cells from infection [78]. Additional examples of silanediol-based inhibitors of aspartic proteases would be desirable.

Two metalloprotease enzymes have been potently inhibited by silanediols. Metalloproteases have a zinc ion, held at the active site by a glutamate and two histidine ligands, **74**. This zinc ion stabilizes the amide hydrate by coordination to the geminal hydroxyl groups of the hydrated carbonyl. Silanols can ligate and chelate metal ions [107–109]. Silanediol **42** (Fig. 10) was prepared as an analog of a phosphinic acid **41** and phosphinamide, **41a**, and was found to inhibit the metalloprotease thermolysin with an IC_{50} of 40 nM. Moreover, both **41a** and **42** were examined by X-ray spectroscopy and found to coordinate to the zinc active site with precisely the same arrangement of their two oxygen atoms. An additional example of metalloprotease inhibition by a silanediol is the 4 nM inhibition of ACE by silanediol **24** (Fig. 10). This silanediol was patterned after the ketone inhibitor **23a** and its presumed inhibitor form, the ketone hydrate **23** (Fig. 7).

These metallo- and aspartic protease inhibitors bind to the active site of the enzyme through noncovalent interactions. In contrast, most inhibitors of serine proteases incorporate an electrophilic group that covalently engages the active site serine. In the case of silanediol inhibitors **48** and **50,** the electrophilic group is a silanediol. A crystal structure of these inhibitors bound to the active sites and demonstrating the covalent attachment of the silane to the serine nucleophile has not yet been achieved.

The fourth class of proteases is the cysteine proteases [110], for which inhibition by a silanediol has not been examined (Fig. 18). The use of silanediols for these

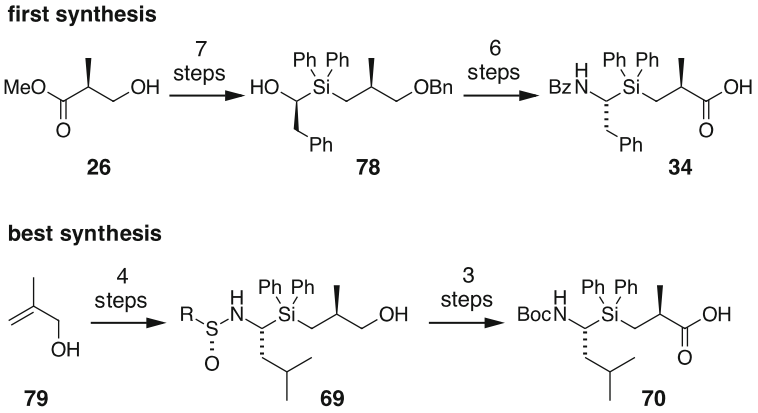


Fig. 19 Comparison of two routes to advanced silanediol precursors **34** and **70**

important targets implies the formation of a silicon–sulfur bond, e.g., **77** [111]. Should the strength of the bond of the enzyme thiol to silicon be important, the lower bond strength for Si–S relative to Si–O (see **79**) may limit the use of silanediols as inhibitors of these enzymes.

For all of the potential inhibitors discussed, the specificity and potency of the inhibition may, in large part, be determined by the side chain substituents that flank the central silicon atom (see Fig. 12). On the other hand, the silanediol imparts a polarity that lies somewhere between a phosphinic acid and a carbinol. This intermediate polarity has the potential to produce bioactive substances with useful log P values [112].

Preparation of silanediols as protease inhibitors, assembling the functionality and stereogenic centers in a general and efficient way, can now be routinely accomplished, but some targets remain a challenge. The original path to a silanediol required many functional group transformations, from the Roche ester **26** to the penultimate product **34** (Fig. 19). In the currently most efficient path, a 2-substituted allyl alcohol is converted to an intermediate like **69** in three steps, requiring only exchange of nitrogen substituents and oxidation of the primary alcohol. This route enjoys full control of stereochemistry using asymmetric hydrosilylation and a Davis–Ellman sulfinimine. Despite these advances, incorporation of the guanidine functional group for Factor XIa inhibitor **50** (Fig. 12) required many functional group manipulations [90].

An additional synthesis challenge is optimization of the ultimate deprotection step. There have been contributions from several labs [113], modifying the phenyl groups on silicon with a goal of avoiding the use of triflic acid in the deprotection. A variety of electrophiles can be utilized for this transformation, including bromine and mercury (Fig. 20) [114]. Protons are the most desirable of these; bromine can add to alkenes and activated aromatics, and mercury is a toxic heavy metal that has the potential to complicate enzyme assays if it is not fully removed from the inhibitor product.

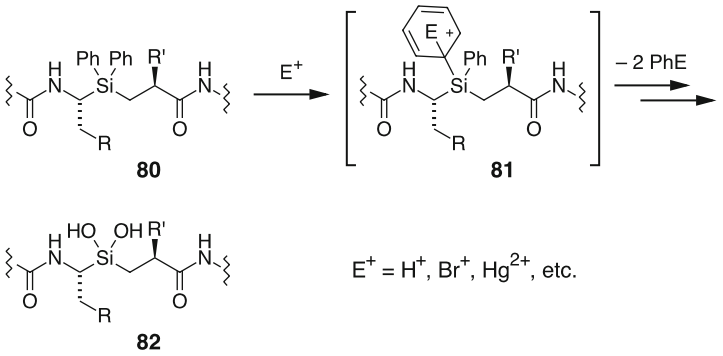


Fig. 20 The challenges of silanediol deprotection and the use of electrophiles other than protons

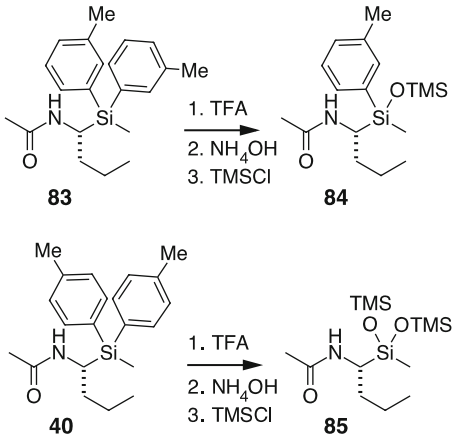


Fig. 21 Skyrdstrup's substituted phenyl silanes allow the use of milder acid for hydrolysis, particularly for *para* substitution

The most successful of the efforts to optimize the acid deprotection procedure has been from the Skrydstrup lab and the addition of methyl substituents to the phenyl groups (Fig. 21) ([73]; for the use of methoxy substitution to promote ease of protodesilylation reactions, see [115] and references therein). This work beautifully illustrates the utility of having electron-donating substitution appropriately placed. Methyl groups at the *meta* position (**83**) do not adequately facilitate aryl hydrolysis (see Fig. 9), but *para* substitution allows for removal of both toluene groups using only trifluoroacetic acid, which is far more mild than triflic acid.

The future of silanediols as pharmaceutical agents will also require a detailed understanding of the pharmacokinetics of these silanediols in peptide-like environments. Much is left to explore!

References

1. Bauer J, Fiala J, Hrichova R (1963) Natural α-silicon carbide. Am Mineral 48:620–634
2. Kipping FS, Lloyd LL (1901) XLVII.-organic derivatives of silicon. Triphenylsilicol and alkyloxysilicon chlorides. J Chem Soc Trans 79:449–459
3. Tacke R, Dörrich S (2014) Drug design based on the carbon/silicon switch strategy. Top Med Chem. doi:10.1007/7355_2014_55
4. Sieburth SMcN (2013) Bioactive organosilanes. Gelest Catalog 55–62
5. Franz AK, Wilson SO (2012) Organosilicon molecules with medicinal applications. J Med Chem 56:388–405
6. Franz AK (2007) The synthesis of biologically active organosilicon small molecules. Curr Opin Drug Disc Dev 10:654–671
7. Bains W, Tacke R (2003) Silicon chemistry as a novel source of chemical diversity in drug design. Curr Opin Drug Disc Dev 6:526–543
8. Englebienne P, Hoonacker AV, Herst CV (2005) The place of the bioisosteric sila-substitution in drug design. Drug Des Rev Online 2:467–483
9. Tacke R, Wagner SA (1998) Chirality in bioorganosilicon chemistry. In: Apeloig Y, Rappoport Z (eds) The chemistry of organic silicon compounds, vol 2. Wiley, New York, pp 236–240
10. Tacke R, Linoh H (1989) Bioorganosilicon chemistry. In: Patai S, Rappoport Z (eds) The chemistry of organic silicon compounds. Wiley, New York, pp 114–120
11. Fessenden RJ, Fessenden JS (1980) Trends in organosilicon biological research. Adv Organomet Chem 18:275–299
12. Tacke R, Wannagat U (1979) Syntheses and properties of bioactive organo-silicon compounds. Top Curr Chem 84:1–75
13. Fessenden RJ, Fessenden JS (1967) The biological properties of silicon compounds. Adv Drug Res 4:95–132
14. Franz JE, Mao MK, Sikorski JA (1996) Glyphosate: a unique global herbicide, vol 189, ACS Monograph. American Chemical Society, Washington, DC
15. Hayden JF, Barlow SA (1972) Structure-activity relationships of organosiloxanes and the female reproductive system. Toxicol Appl Pharmacol 21:68–79
16. Tse FLS, Jaffe JM, Dain JG (1984) Pharmacokinetics of compound 58–112, a potential skeletal muscle relaxant, in Man. J Clin Pharmacol 24:47–57
17. Voronkov MG (1979) Biological activity of silatranes. Top Curr Chem 84:77–135
18. Raabe G, Michl J (1989) Multiple bonds to silicon. In: Patai S, Rappoport Z (eds) The chemistry of organic silicon compounds, 2nd edn. Wiley, New York, pp 1015–1142
19. Ottosson H, Steel PG (2006) Silylenes, silenes, and disilenes: novel silicon-based reagents for organic synthesis? Chemistry 12:1576–1585
20. Fleming I (1979) Organic silicon chemistry. In: Ollis WD, Barton D (eds) Comprehensive organic chemistry, 3rd edn. Pergamon, New York, pp 541–686
21. Fessenden RJ, Hartman RA (1970) Metabolic fate of phenyltrimethylsilane and phenyldimethylsilane. J Med Chem 13:52–54
22. Staudinger H, Ruzicka L (1924) Insektentötende Stoffe I. Über Isolierung und Konstitution des wirksamen Teiles des dalmatinischen Insektenpulvers. Helv Chim Acta 7:177–201
23. Elliott M, Farnham AW, Janes NF, Needham PH, Pulman DA (1975) Insecticidal activity of the pyrethrins and related compounds. VII. Insecticidal dihalovinyl analogues of cis and trans chrysanthemates. Pestic Sci 6:537–542
24. Seyferth D, Annarelli DC (1975) Hexamethylsilirane. Simple, isolable silacyclopropane. J Am Chem Soc 97:2273–2275
25. Franz AK, Woerpel KA (2000) Development of reactions of silacyclopropanes as new methods for stereoselective organic synthesis. Acc Chem Res 33:813–820
26. Larson GL (1996) The chemistry of α-silyl carbonyl compounds. Adv Silicon Chem 3:105–271

27. Miura T, Takahashi RM (1976) Effects of a synthetic pyrethroid, SD43775, on nontarget organisms when utilized as a mosquito larvicide. Mosq News 36:322–326
28. Svendsen A, Pedersen L-EK, Klemmensen PD (1986) Synthesis and insecticidal activities of unsymmetrical bis-arylalkyl ketones. A new structural concept in pyrethroids. Pestic Sci 17:93–102
29. Sieburth SMcN, Manly CJ, Gammon DW (1990) Organosilane insecticides. Part I: biological and physical effects of isosteric replacement of silicon for carbon in Etofenprox and MTI-800. Pestic Sci 28:289–307
30. Katsuda Y (2012) Progress and future of pyrethroids, vol 314, Topics in current chemistry. Springer-Verlag, Berlin
31. Moberg WK, Basarab GS, Cuomo J, Liang PH, Greenhalgh R, Roberts TR (1986) Biologically active organosilicon compounds: silicon-containing triazole fungicides, Pesticide science and biotechnology. Blackwell Scientific, Boston, pp 57–60
32. Wuts PGM (2014) Greene's protective groups in organic synthesis, 5th edn. Wiley, Hoboken, NJ
33. Cunningham JG, Ford RB, Gifford JA, Hulce VD, Chandler ML, LeVier RR (1981) Clinical evaluation of the new compound diphenylsilanediol for anti-epileptic efficacy and toxicity. Am J Vet Res 42:2178–2181
34. Tacke R, Popp F, Müller B, Theis B, Burschka C, Hamacher A, Kassack MU, Schepmann D, Wünsch B, Jurva U, Wellner E (2008) Sila-haloperidol, a silicon analogue of the dopamine (D_2) receptor antagonist haloperidol: synthesis, pharmacological properties, and metabolic fate. ChemMedChem 3:152–164
35. Johansson T, Weidolf L, Popp F, Tacke R, Jurva U (2010) In vitro metabolism of haloperidol and sila-haloperidol: new metabolic pathways resulting from carbon/silicon exchange. Drug Metab Disp 38:73–83
36. Brown SS, Kendrick TC, McVie J, Thomas DR (1995) Silicones. In: Abel EW, Stone FGA, Wilkinson G (eds) Comprehensive organometallic chemistry II, vol 2. Pergamon, New York, pp 111–135
37. Takiguchi T (1959) Preparation of some organosilanediols and phenylsilanetriol by direct hydrolysis using aniline as hydrogen chloride acceptor 1. J Am Chem Soc 81:2359–2361
38. Dejak B, Lasocki Z, Mogilnicki W (1969) Rates of condensation of some diorganosilanediols in methanol with potassium hydroxide as catalyst. B Acad Pol Sci-Chim 17:7–12
39. Dejak B, Lasocki Z, Mogilnicki W (1969) Rates of condensation of some diorganosilanediols in methanol with HCl as catalyst. B Acad Pol Sci-Chim 17:571–574
40. Eaborn C, Hartshorne NH (1955) The mesomorphism of diisobutylsilanediol. J Chem Soc 549–555
41. Bunning JC, Lydon JE, Eaborn C, Jackson PM, Goodby JW, Gray GW (1982) Classification of the mesophase of di-isobutylsilanediol. J Chem Soc Faraday Trans 1(78):713–724
42. Tacke R, Schmid T, Merget M (2005) The SiOH-containing a-amino acid HOMe2SiCH2CH (NH2)CO2H and its immobilization on silica via an Si-O-Si linkage. Organometallics 24:1780–1783
43. Kim JK, Sieburth SMcN (2012) Synthesis and properties of a sterically unencumbered delta-silanediol amino acid. J Org Chem 77:2901–2906
44. Lickiss PD (1995) The synthesis and structure of organosilanols. Adv Inorg Chem 42:147–262
45. Lickiss PD, Rappoport Z, Apeloig Y (2001) Polysilanols, vol 3, The chemistry of organic silicon compounds. Wiley, New York, pp 695–744
46. Kipping FS (1912) CCXXIII.-organic derivatives of silicon. Part XVI. The preparation and properties of diphenylsilicanediol. J Chem Soc Trans 101:2108–2125
47. Mader MM, Bartlett PA (1997) Binding energy and catalysis: the implications for transition-state analogs and catalytic antibodies. Chem Rev 97:1281–1301
48. Babine RE, Bender SL (1997) Molecular recognition of protein-ligand complexes: applications to drug design. Chem Rev 97:1359–1472

49. Leung D, Abbenante G, Fairlie DP (2000) Protease inhibitors: current status and future prospects. J Med Chem 43:305–341
50. Turk B (2006) Targeting proteases: successes, failures and future prospects. Nat Rev Drug Discov 5:785–799
51. Ondetti MA, Rubin B, Cushman DW (1977) Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. Science 196:441–444
52. Murdoch D, McTavish D (1992) Fosinopril. Drugs 43:123–140
53. Sica DA, Gehr TWB, Kelleher N, Blumenthal M (1998) Fosinopril: emerging considerations and implications for angiotensin-converting enzyme inhibitor therapy. Cardiovasc Drug Rev 16:319–345
54. Matchar DB, McCrory DC, Orlando LA, Patel MR, Patel UD, Patwardhan MB, Powers B, Samsa GP, Gray RN (2008) Systematic review: comparative effectiveness of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treating essential hypertension. Ann Intern Med 148:16–29
55. Bold G, Faessler A, Capraro H, Cozens R, Klimkait T, Lazdins J, Mestan J, Poncioni B, Roesel J, Stover D, Tintelnot-Blomley M, Acemoglu F, Beck W, Boss E, Eschbach M, Huerlimann T, Masso E, Roussel S, Ucci-Stoll K, Wyss D, Lang M (1998) New Aza-dipeptide analogs as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development. J Med Chem 41:3387–3401
56. Havlir DV, O'Marro SD (2004) Atazanavir: new option for treatment of HIV infection. Clin Infect Dis 38:1599–1604
57. Groll M, Berkers CR, Ploegh HL, Ovaa H (2006) Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. Structure 14:451–456
58. Miller SA, Onge ELS (2006) Sitagliptin: a dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Ann Pharmacother 40:1336–1343
59. Sieburth SMcN, Chen C-A (2006) Silanediol protease inhibitors: from conception to validation. Eur J Org Chem 2:311–322
60. Galardy RE, Kortylewicz ZP (1985) Inhibitors of angiotensin-converting enzyme containing a tetrahedral arsenic atom. Biochem J 226:447–454
61. Curley K, Pratt RF (1997) Effectiveness of tetrahedral adducts as transition-state analogs and inhibitors of the class C b-lactamase of enterobacter cloacae P99. J Am Chem Soc 119:1529–1538
62. Almquist RG, Chao W-R, Ellis ME, Johnson HL (1980) Synthesis and biological activity of a ketomethylene analog of a tripeptide inhibitor of angiotensin converting enzyme. J Med Chem 23:1392–1398
63. Imperiali B, Abeles RH (1986) Inhibition of serine proteases by peptidyl fluoromethyl ketones. Biochemistry 25:3760–3767
64. Trost BM, Caldwell CG (1981) The di-t-butylsilylene protecting group for diols. Tetrahedron Lett 22:4999–5002
65. Fleming I, Henning R, Plaut H (1984) The phenyldimethylsilyl group as a masked form of the hydroxy group. Chem Commun 29–31
66. Deans FB, Eaborn C (1959) Aromatic reactivity. Part III. Cleavage of substituted phenyltrimethylsilanes by sulphuric acid in acetic acid-water. J Chem Soc 445:2299–2303
67. Kim J, Hewitt G, Carroll P, Sieburth SMcN (2005) Silanediol inhibitors of angiotensin-converting enzyme. Synthesis and evaluation of four diastereomers of Phe[Si]Ala dipeptide analogues. J Org Chem 70:5781–5789
68. Kim J (2004) Research notes, Temple University
69. Uhlig W (1996) Silyl triflates – valuable synthetic materials in organosilicon chemistry. Chem Ber 129:733–739
70. Corriu RJP, Guerin C, Guiraud G (1979) Analogies between nucleophilic substitutions at silicon and phosphorus: kinetic and stereochemical studies of five- and six-membered ring oxasilacycloalkanes. Chem Commun 8–9

71. Chen C-A (1997) Research notes, SUNY Stony Brook
72. Nielsen L, Lindsay KB, Faber J, Nielsen NC, Skrydstrup T (2007) Stereocontrolled synthesis of methyl silanediol peptide mimics. J Org Chem 72:10035–10044
73. Hernández D, Mose R, Skrydstrup T (2011) Reductive lithiation of methyl substituted diarylmethylsilanes: application to silanediol peptide precursors. Org Lett 13:732–735
74. Cushman DW, Cheung HS, Sabo EF, Ondetti MA (1977) Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxalkanoyl and mercaptoalkanoyl amino acids. Biochemistry 16:5484–5491
75. Grobelny D, Goli UB, Galardy RE (1989) Binding energetics of phosphorus-containing inhibitors of thermolysin. Biochemistry 28:4948–4951
76. Morgan BP, Bartlett PA, Rivier JE, Marshall GR (1990) Phosphinates as transition-state analog inhibitors of thermolysin: the importance of hydrophobic and hydrogen bonding effects. Peptides: Chemistry, Structure and Biology, [ESCOM] Leiden, pp 371–372
77. Liu M, Tran NT, Franz AK, Lee JK (2011) Gas-phase acidity studies of dual hydrogen-bonding organic silanols and organocatalysts. J Org Chem 76:7186–7194
78. Chen C-A, Sieburth SMcN, Glekas A, Hewitt GW, Trainor GL, Erickson-Viitanen S, Garber SS, Cordova B, Jeffry S, Klabe RM (2001) Drug design with a new transition state analog of the hydrated carbonyl: silicon-based inhibitors of the HIV protease. Chem Biol 8:1161–1166
79. Plosker GL, Noble S (1999) Indinavir - a review of its use in the management of HIV infection. Drugs 58:1165–1203
80. Juers DH, Kim J, Matthews BW, Sieburth SMcN (2005) Structural analysis of silanediols as transition-state-analogue inhibitors of the benchmark metalloprotease thermolysin. Biochemistry 44:16524–16528
81. Bartlett PA, Marlowe CK (1983) Phosphonamidates as transition-state analogue inhibitors of thermolysin. Biochemistry 22:4618–4624
82. Hedstrom L (2002) Serine protease mechanism and specificity. Chem Rev 102:4501–4524
83. Schechter I, Berger A (1967) On the size of the active site in proteases. I. Papain. Biochem Biophys Res Commun 27:157–162
84. Tyndall JDA, Nall T, Fairlie DP (2005) Proteases universally recognize beta strands in their active sites. Chem Rev 105:973–1000
85. Singh S, Sieburth SMcN (2012) Serine protease inhibition by a silanediol peptidomimetic. Org Lett 14:4422–4425
86. Schumacher WA, Luettgen JM, Quan ML, Seiffert DA (2010) Inhibition of factor XIa as a new approach to anticoagulation. Arterioscler Thromb Vasc Biol 30:388–392
87. Navaneetham D, Sinha D, Walsh PN (2010) Mechanisms and specificity of factor XIa and trypsin inhibition by protease nexin 2 and basic pancreatic trypsin inhibitor. J Biochem 148:467–479
88. Tacke R, Linoh H, Ernst L, Moser U, Mutschler E, Sarge S, Cammenga HK, Lambrecht G (1987) Sila-pharmaca. 37. Preparation and properties of the enantiomers of the antimuscarinic agents sila-procyclidine and sila-tricyclamol iodide: optically active silanols with silicon as the centre of chirality. Chem Ber 120:1229–1237
89. Singh S (2012) Research notes, Temple University
90. Duong HQ (2013) Research notes, Temple University
91. Mignani S, Damour D, Bastart J, Manuel G (1995) A convenient large scale synthesis of 1,1-diphenyl-1-silacyclopent-3-ene. Synth Commun 25:3855–3861
92. Sen S, Purushotham M, Qi Y, Sieburth SMcN (2007) Efficient asymmetric synthesis of silanediol precursors from 1,5-dihydrosiloles. Org Lett 9:4963–4965
93. Eaborn C (1952) Organosilicon compounds. III. Some sterically hindered compounds. J Chem Soc 2840–2846
94. Corey EJ, Helal CJ (1998) Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: a new paradigm for enantioselective catalysis and a powerful new synthetic method. Angew Chem Int Ed Engl 37:1986–2012

95. Zhou P, Chen B, Davis FA (2004) Recent advances in asymmetric reactions using sulfinimines (N-sulfinyl imines). Tetrahedron 60:8003–8030
96. Sen S, Singh S, Sieburth SMcN (2009) A practical, two-step synthesis of 2-substituted 1,3-butadienes. J Org Chem 74:2884–2886
97. Marciniec B (2009) Hydrosilylation, Advances in silicon chemistry. Springer, Heidelberg
98. Pagliaro M, Ciriminna R, Pandarus V, Béland F (2013) Platinum-based heterogeneously catalyzed hydrosilylation. Eur J Org Chem 2013:6227–6235
99. Troegel D, Stohrer J (2011) Recent advances and actual challenges in late transition metal catalyzed hydrosilylation of olefins from an industrial point of view. Coord Chem Rev 255:1440–1459
100. Ojima I, Li Z, Zhu J (1998) Recent advances in the hydrosilylation and related reactions. In: Apeloig Y, Rappoport Z (eds) The chemistry of organic silicon compounds, vol 2. Wiley, New York, pp 1687–1792
101. Bergens SH, Noheda P, Whelan J, Bosnich B (1992) Asymmetric catalysis. Production of chiral diols by enantioselective catalytic intramolecular hydrosilation of olefins. J Am Chem Soc 114:2121–2128
102. Bergens SH, Noheda P, Whelan J, Bosnich B (1992) Asymmetric catalysis. Mechanism of asymmetric catalytic intramolecular hydrosilation. J Am Chem Soc 114:2128–2135
103. Bo Y, Singh S, Duong HQ, Cao C, Sieburth SMcN (2011) Efficient, enantioselective assembly of silanediol protease inhibitors. Org Lett 13:1787–1789
104. Eder J, Hommel U, Cumin F, Martoglio B, Gerhartz B (2007) Aspartic proteases in drug discovery. Curr Pharm Des 13:271–285
105. Tran NT, Wilson SO, Franz AK (2014) Supramolecular hydrogen-bonding assembly of silanediols with bifunctional heterocycles. Chem Commun 50:3738–3740
106. Kondo S-I, Bie Y, Yamamura M (2013) Ratiometric fluorescence detection of anions by silanediol-based receptors bearing anthryl and pyrenyl groups. Org Lett 15:520–523
107. Su K, Tilley TD, Sailor MJ (1996) Molecular and polymer precursor routes to manganese-doped zinc orthosilicate phosphors. J Am Chem Soc 118:3459–3468
108. Basset J-M, Lefebvre F, Santini C (1998) Surface organometallic chemistry: some fundamental features including the coordination effects of the support. Coord Chem Rev 178–180:1703–1723
109. Ignatyev IS, Montejo M, Rodriguez OPG, Gonzalez JJL (2013) Quantum chemical study of silanediols as metal binding groups for metalloprotease inhibitors. J Mol Model 19:1819–1834
110. Reiser J, Adair B, Reinheckel T (2010) Specialized roles for cysteine cathepsins in health and disease. J Clin Invest 120:3421–3431
111. Walsh R (1989) Thermochemistry. In: Patai S, Rappoport Z (eds) The chemistry of organic silicon compounds, vol 1. Wiley, New York, pp 371–391
112. Edwards MP, Price DA (2010) Role of physicochemical properties and ligand lipophilicity efficiency in addressing drug safety risks. In: Macor JE (ed) Annu Rep Med Chem, vol 45. Academic, New York, NY, pp 380–391
113. Anderson TF, Statham MAJ, Carroll MA (2006) Bis(2-thienyl)silanes: new, versatile precursors to arylsilanediols. Tetrahedron Lett 47:3353–3355
114. Fleming I, Henning R, Parker DC, Plaut HE, Sanderson PEJ (1995) The phenydimethylsilyl group as a masked hydroxy group. J Chem Soc Perkin Trans 1:317–337
115. Fischer M, Burschka C, Tacke R (2014) Synthesis of 4-silacyclohexan-1-ones and (4-silacyclohexan-1-yl)amines containing the silicon protecting groups MOP (4-methoxyphenyl), DMOP (2,4-dimethoxyphenyl), or TMOP (2,4,6-trimethoxyphenyl): versatile Si- and C-functional building blocks for synthesis. Organometallics 33:1020–1029

Top Med Chem (2016) 17: 87–118
DOI: 10.1007/7355_2015_86

Published online: 6 June 2015

Selenium-Based Drug Design

Sheldon W. May

Abstract The biochemistry, pharmacology and epidemiology of selenium and selenium-containing compounds continue to be subjects of considerable interest from the viewpoint of public health. Selenium has a long history of association with human health and disease, and we now recognize that this element is an essential nutrient that is critical to key cellular processes. We now know that the selenoproteins constituting the human selenoproteome are encoded by 25 genes in the human genome, and much progress is being made in our understanding of selenium metabolism and the health effects of selenium metabolites in normal and disease states. The idea that selenium-containing dietary supplements might be effective in preventing disease has gone through both optimistic and pessimistic phases in recent years, and the future prospects for such a nutritional approach are unclear at the present time. In contrast, a significant number of promising efforts are underway that are aimed at designing and developing pharmaceutical agents that are selenium-based or that target specific aspects of selenium metabolism. This chapter focuses on some of these efforts to develop new selenium-based anticancer, antioxidant, antihypertensive, antiviral, immunosuppressive, and antimicrobial agents. While most of the efforts that entail designed organoselenium compounds – as opposed to inorganic selenium metabolites – are still at the preclinical stage, evidence is emerging that selenium-based compounds can operate via several beneficial biochemical and pharmacological mechanisms. Since our understanding of the biology, biochemistry and pharmacology of selenium and selenoproteins is rapidly expanding, we can anticipate that the coming years will bring further development of new selenium-based pharmaceutical agents with therapeutic potential against human diseases.

S.W. May (✉)
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
e-mail: sheldon.may@chemistry.gatech.edu

Keywords Anticancer agents, Antihypertensives, Antimicrobials, Antioxidants, Antiviral agents, Ebselen, Glutathione, Phenylaminoethyl selenides, Selenazolidines, Selenium, Selenium-based drugs, Selenium-containing metabolites, Selenoproteins, Therapeutic use of organoselenium compounds, Thioredoxin reductase

Contents

1 Introduction

The biochemistry, pharmacology, and epidemiology of selenium and selenium-containing compounds continue to be a subject of considerable interest, particularly from the viewpoint of public health. Selenium has a long history of association with human health and disease [1, 2], and specific populations have historically suffered from certain diseases that are now recognized to be associated with selenium deficiency. A widely known example is Keshan disease, which is characterized by congestive heart failure [3, 4]; healers in Keshan, China, where the disease was discovered, have traditionally treated it with the common herb, *Astragalus*, several species of which accumulate selenium from the soil. Ironically, *Astragalus* may be the very plant seen by Marco Polo in the "Succuir" district of China; the explorer describes how beasts of burden unaccustomed to the area would feast on this plant, thereby causing the animals to become so ill that their hooves dropped off [5]. Polo's description fits what is now known to be an equestrian form of selenosis. Thus, in retrospect, it is likely that even this very early report underscores the critical importance of managing the proper dose and molecular form of selenium when considering therapeutic applications.

It is worth noting that the notion that selenium may exert a protective effect against human cancer was actually discussed in the mainstream scientific literature more than four decades ago [6, 7]. Early geographical studies suggested an inverse correlation between selenium levels and cancer incidence, and cancer mortality rates were found to be significantly lower in US counties with intermediate or high

selenium levels as compared to counties with low selenium levels [8]. Similarly, low selenium concentrations were found in the sera of patients with pancreatic carcinoma and in the plasma of breast cancer patients, and selenium compounds have exhibited antitumorigenic activities in a number of animal studies over the years [5].

Turning to the present time, we now know that selenium (as selenocysteine) is an essential component of the active sites of the enzymes glutathione peroxidase and mammalian thioredoxin reductase and is also present in a variety of other mammalian selenoproteins [9–13]. Both glutathione peroxidase and thioredoxin reductase catalyze reactions that are essential to the protection of cellular components against oxidative and free radical damage. In epidemiological studies, a low concentration of selenium in plasma was identified as a risk factor for several diseases including cancer, cardiovascular disease, osteoarthritis, and AIDS [3, 14–19]. In the United States, the National Academies of Science Institute of Medicine has issued a dietary reference intake report on selenium and other antioxidants [20], with the current recommended dietary allowance (RDA) for selenium being 55 micrograms for adult men and women. It should be noted that symptoms of selenosis appear when dietary levels of selenium exceed 1 milligram/day, whereas a selenium dietary intake below 1 microgram/kg can lead to selenium deficiency diseases such as the aforementioned Keshan disease.

Over the past three decades, there have been a number of efforts aimed at designing and developing pharmaceutical agents that are selenium based or that target specific aspects of selenium metabolism (for some relevant reviews, see [5, 9–12, 21]). These efforts have focused on the design of selenium-based antihypertensive, anticancer, antiviral, immunosuppressive, and antimicrobial agents. There has also been much interest in the development of organoselenium compounds capable of reducing oxidative tissue damage and edema. This chapter will focus on some of the more promising of these efforts to develop new selenium-based therapeutic agents.

It is very important to mention at the outset that concurrent with these drug design and development efforts, some findings that looked initially quite promising emerged from nutritional trials and epidemiological studies. In 1996, Clark and coworkers [22] reported that their long-term, double-blind, placebo-controlled study demonstrated significant reductions in incidences of lung, colorectal, and prostate cancers in patients receiving daily 200 microgram oral doses of "selenium yeast." Subsequently, a prospective study of 33,737 men reported [14] that individuals with the highest selenium levels had only about one-third the likelihood of developing advanced prostate cancer as did individuals with low selenium levels, and similar results were reported in other prospective cancer studies (see, e.g., van den Brandt et al. [15]). In view of findings such as these, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was launched [23] by the National Cancer Institute. SELECT was designed to be a very large, randomized, placebo-controlled trial of selenium (200 μg/day L-selenomethionine) and/or vitamin E (400 IU/day racemic α-tocopheryl acetate) supplementation for a minimum of 7 years (maximum of 12 years) focusing on the prevention of prostate cancer by these agents.

Unfortunately, SELECT was discontinued since an independent review of the data being collected showed that selenomethionine and vitamin E, taken together or alone, were not preventing prostate cancer [24, 25]. Nevertheless, despite this disappointing result, there still remains much interest from a nutritional perspective in the development of effective selenium supplementation protocols, if only the proper selenium formulation and dosage could be identified (see, e.g., the "post-SELECT" perspective by Ledesma et al. [24]). A detailed discussion of epidemiological and nutritional aspects of selenium supplementation is beyond the scope of this review.

2 Phenylaminoalkyl Selenides

This author's laboratory has developed a family of phenylaminoalkyl selenides [26–31] and we have shown that these selenides possess the ability to propagate a cycle that depletes reduced ascorbate within adrenergic vesicles. Initially, dopamine-β-monooxygenase (DBM) present within the vesicle converts these selenides to the corresponding selenoxides; however, the product selenoxides are then nonenzymatically reduced back to the selenides, with the concomitant and stoichiometric oxidation of reduced ascorbate present in the vesicle to fully oxidized ascorbate. This selenide/ascorbate cycle is a localized process since DBM is present only in these vesicles and reduced ascorbate does not cross the vesicle membrane. While adrenergic vesicles possess a cytochrome b561-dependent ascorbate recycling system, this native system can only recycle semidehydroascorbate, which is generated during DBM turnover, and cannot recycle the fully oxidized ascorbate produced by the nonenzymatic selenoxide/ascorbate reaction. Thus, the net result of selenide processing in the vesicle is the effective local depletion of reduced ascorbate – an essential cofactor for DBM – and the consequent diminution of norepinephrine production. We demonstrated this turnover-dependent ascorbate depletion process both in vitro and in chromaffin granule ghosts, and we confirmed cellular and vesicular uptake of the selenides. Moreover, as predicted on the basis of the redox potentials of selenoxides versus sulfoxides, we showed that while sulfur-containing analogs undergo DBM-catalyzed sulfoxidation, they are not capable of propagating such a cycle of ascorbate depletion. Thus, it is clear that the ability of phenylaminoalkyl selenides to effect turnover-dependent depletion of local reduced ascorbate is a direct consequence of the redox chemistry of the selenium moiety present in these compounds (Fig. 1).

The pharmacological testing of phenylaminoethyl selenide (PAESe) confirmed that this compound exhibits dose-dependent antihypertensive activity when administered i.p. to spontaneously hypertensive rats, and we have provided evidence that the adrenergic nerve terminal is indeed the pharmacological site of action of PAESe in vivo. However, as is true for other peripherally acting pharmacological agents, the CNS permeability of PAESe is a significant concern, since undesirable side effects can often result from CNS penetration. Moreover, it is highly desirable from

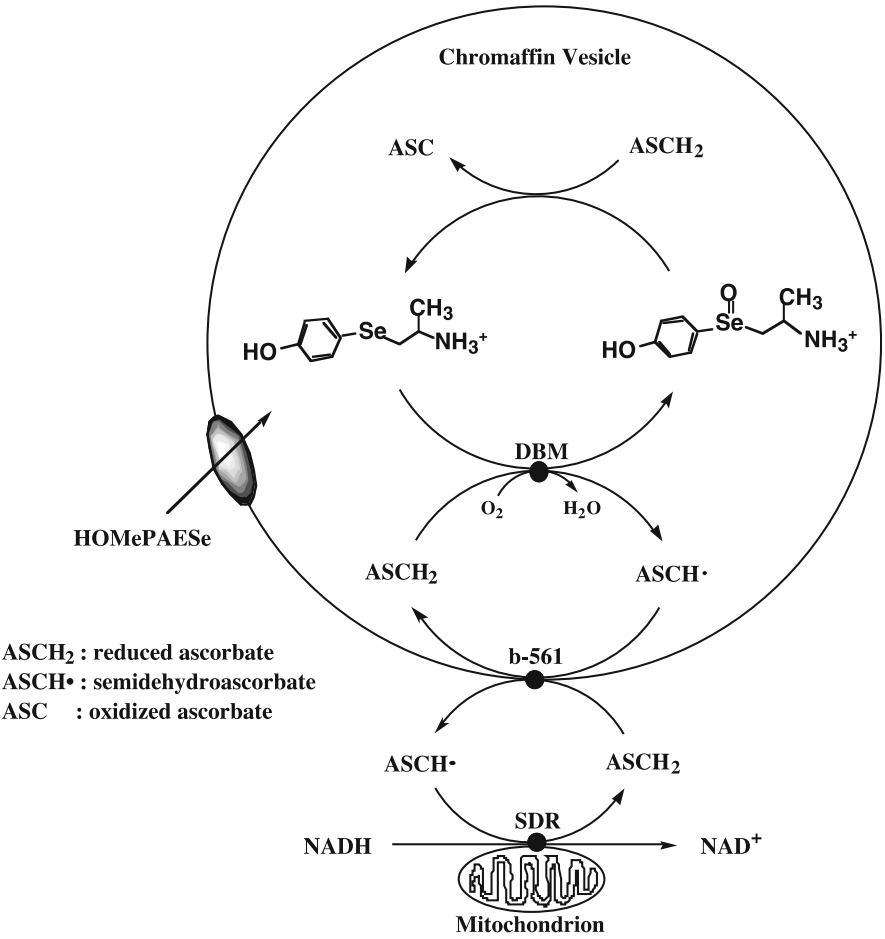


Fig. 1 Dopamine β-monooxygenase-catalyzed selenoxidation of 4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe), followed by nonenzymatic recycling of the selenoxide product. This results in the *local* depletion of reduced ascorbate within the chromaffin vesicle even in the presence of the b-561-dependent ascorbate recycling system, since the native b561 system can only recycle the semidehydroascorbate generated during DBM turnover but not the fully oxidized ascorbate produced by the nonenzymatic selenoxide/ascorbate reaction

a therapeutic perspective that pharmacological agents used to treat chronic diseases such as hypertension be orally active. We therefore proceeded to develop a PAESe derivative that would exhibit both restricted CNS permeability and oral antihypertensive activity [32].

Briefly, our approach was as follows: First, we employed inductively coupled plasma/mass spectroscopic (ICP/MS) analysis of plasma samples to determine the pharmacokinetic parameters for our selenide compounds. Next, an oxidative procedure for the digestion and processing of tissue samples was then developed in

order to obtain ICP/MS data on the tissue distributions of selenium-containing metabolites following the administration of selenide compounds. The results clearly demonstrated that the aromatic-ring hydroxylation of the selenides results in a marked reduction in brain levels of selenium-containing metabolites. We also demonstrated that α-methylation abolishes the activity of the enzyme MAO toward these compounds and markedly enhances their oral activity; since the intestinal mucosa possesses a considerable amount of MAO, resistance to MAO-catalyzed degradation is an essential characteristic for drugs to exhibit good oral bioavailability. Finally, we investigated the comparative effects of PAESe derivatives on locomotor activity and operant behavior. The results fully corroborated our analytical data, thus confirming the pharmacological relevance of the ICP/MS results and providing a compelling basis for drug design. On the basis of these biochemical, pharmacological, inductively coupled plasma/mass spectrometry, and behavioral studies, we successfully demonstrated that the novel compound (*S*)-4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe) exhibits both restricted CNS permeability and oral antihypertensive activity [32]. Indeed, this compound is the first orally active selenium-based antihypertensive agent ever reported (Fig. 2).

We carried out a series of experiments designed to probe the hemodynamic mechanism of action of these phenylaminoethyl selenide antihypertensives [33]. In these experiments, a noninvasive pulsed Doppler ultrasound probe was used to measure peak blood flow velocity in the aortic arch from the right second intercostal space. PAESe was found to increase peak aortic blood flow velocity (+44%), heart rate (+16%), and blood flow acceleration (+105%), while decreasing left ventricular ejection time (LVET) (−37%) concomitant with a decrease in mean arterial pressure (−54%). These results were compared with the known vasodilator, hydralazine, which had similar effects on mean arterial pressure (MAP) and peak velocity but caused an increase in LVET (+42%) and a decrease in heart rate (−18%). Taken together, these results indicate that PAESe decreases blood pressure via a decrease in peripheral resistance, which overcomes an initial increase in heart rate and acceleration to give a net decrease in mean arterial pressure.

More recent work in the author's laboratory has focused on elucidating and harnessing the redox cycling and antioxidant activity of phenylaminoethyl selenides [34–37]. We established that PAESe reacts rapidly with many cellular

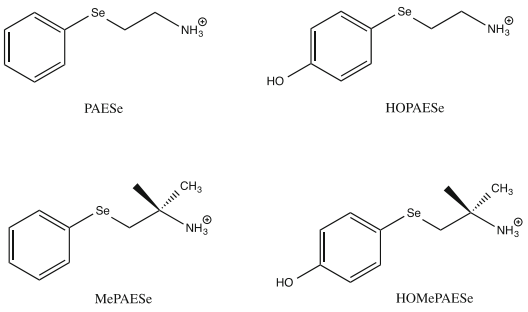


Fig. 2 Structures of phenylaminoethyl selenides investigated in the author's laboratory. It is noteworthy that the compound (*S*)-4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe) exhibits both restricted CNS permeability and oral antihypertensive activity

oxidants, with the selenoxide product generated from this antioxidant activity being readily recycled back to the selenide by cellular reductants such as ascorbate and glutathione with no complex side reactions. One of the most predominant of such cellular oxidants is peroxynitrite, a powerful oxidizing and nitrating agent formed from a diffusion-controlled reaction between nitric oxide and superoxide in endothelial cells, macrophages, and neutrophils. Reactions of peroxynitrite with cellular components have been linked to oxidative stress and inflammatory tissue damage, as, for example, in atherosclerosis and ischemia–reperfusion injury. Peroxynitrite also reacts readily with DNA where it causes base modification and induction of double- and single-strand breaks. We showed that phenylaminoalkyl selenides protect plasmid DNA from peroxynitrite-mediated damage by scavenging this powerful cellular oxidant and forming phenylaminoethyl selenoxides as the sole selenium-containing product. Moreover, on the basis of kinetic studies, potentiometric titrations, cyclic voltammetry, and MatLab simulations, we demonstrated that glutathione-based selenoxide redox cycling enhances these protective effects of the selenides against peroxynitrite-induced DNA damage.

The above results provide support for the idea that exogenously supplied or metabolically generated organoselenium compounds, capable of propagating a selenium redox cycle, might supplement natural cellular defenses against the oxidizing agents generated during metabolism. While several such organoselenium compounds are under active investigation as potential therapeutic agents, the chemical characterization of reaction intermediates involved in selenium redox cycling has been problematical. Among the proposed selenium intermediates in the redox reactions of organoselenium compounds are species such as selenenic and seleninic acids, selenones, spirodioxaselenanonanes, and thiolseleninates. Thus, for example, evidence has been reported for the formation of a thiolseleninate intermediate in the reactions between ebselen oxide and certain thiols [38, 39] and for a spirodioxaselenanonane intermediate in the reaction between di(3-hydroxypropyl) selenide and *tert*-butyl hydroperoxide [40]. The reactivity and instability of some tetra-coordinate selenium compounds, coupled with fast reaction rates for the reactions of organoselenium compounds with oxidants or with reducing agents such as thiols, have made it difficult for several investigators to clearly characterize thioselenurane-like intermediates (see Cowan et al. [36] for further discussion of this point).

Work in the author's laboratory has provided direct evidence that the reaction between phenylaminoalkyl selenoxides and glutathione (GSH) proceeds through the intermediacy of a thioselenurane species [36]. The results of stopped-flow kinetic experiments were consistent with a rapid and stoichiometric initial reaction of GSH with selenoxide to generate a kinetically detectable intermediate, followed by a slower reaction of this intermediate with a second molecule of GSH to produce the final selenide and GSSG products. Flow injection ESI-MS and ESI-MS/MS experiments confirmed that the reaction intermediate is indeed a thioselenurane. Final structural characterization of the thioselenurane intermediate was obtained from the analysis of the daughter ions produced in flow injection ESI-MS/MS experiments. These results elucidate the chemical nature of the redox cycling of

phenylaminoalkyl selenides and represent, to our knowledge, the first evidence for the intermediacy of a thioselenurane species in the reaction of thiols with selenoxides. Previously, Kumar et al. [38] had proposed the formation of a thioselenurane in the reaction between benzenethiol and a substituted phenyl benzyl selenoxide, since they observed via ESI-MS a cyclization product that could arise from such a species; however, they were unable to obtain any structural evidence for this proposed intermediate (Fig. 3).

Hydrogen peroxide, produced in living cells by oxidases and by other biochemical reactions, plays an important role in cellular processes such as signaling and cell cycle progression [41, 42]. Nevertheless, hydrogen peroxide is capable of inducing damage to cellular components, and it can be converted through Fenton chemistry to even more reactive molecules, such as hydroxyl and peroxide radicals. Indeed, oxidative stress – the cellular state arising from overproduction of hydrogen peroxide and other reactive oxygen species (ROS) – has been linked to cellular pathologies ranging from DNA and cellular membrane damage to more complex disorders such as inflammatory diseases and cancer [43]. In experiments carried out in the author's laboratory [37], we made use of peroxalate nanoparticle methodology to achieve chemiluminescent imaging of hydrogen peroxide consumption by phenylaminoethyl selenides. Further, we demonstrated that phenylaminoethyl selenides decrease lipopolysaccharide (LPS)-induced oxidative stress in human

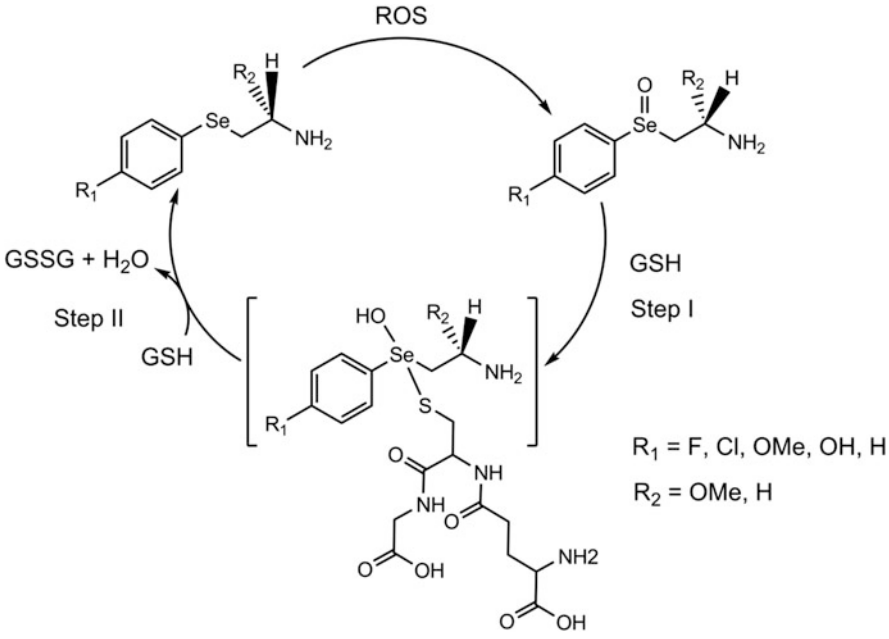


Fig. 3 Identification of a thioselenurane intermediate in the reaction between phenylaminoalkyl selenoxides and glutathione

embryonic kidney cells. We also encapsulated PAESe within poly(lactide-co-glycolide) nanoparticles, and we showed that these selenide-loaded nanoparticles exhibit antioxidant activity in a mammalian cell line. Taken together, these results significantly enhance the attractiveness of phenylaminoethyl selenides as potential agents for supplementing cellular defenses against reactive oxygen species.

Anthracyclines such as doxorubicin (DOX) and daunorubicin are widely used anticancer agents that are effective in the treatment of acute leukemia, non-Hodgkin's lymphomas, and breast, ovarian, and lung cancers [44]. However, the clinical use of anthracycline is limited by severe dose-limiting cardiotoxicities, such as cardiomyopathy and congestive heart failure. This cardiotoxicity is now generally believed to result primarily from the generation of reactive free radicals such as superoxide anion, hydroxyl radical, and peroxynitrite [45, 46]. Dexrazoxane, a potent iron chelator that interrupts anthracycline-iron-mediated free radical generation, is in clinical use to decrease this free radical-associated toxicity of DOX [47, 48]. However, dexrazoxane is known to cause myelosuppression, and recent studies suggest that its use may lead to acute myeloid leukemia and myelodysplastic syndrome. The FDA has noted (http://www.fda.gov/Drugs/DrugSafety/ucm263729.htm) that the European Medicines Agency has announced restrictions on dexrazoxane usage to certain types of patients [48].

In collaboration with the laboratory of Dr. Robert Arnold, we have carried out a series of studies [49] aimed at determining the potential of PAESe to mitigate anthracycline-induced cardiotoxicity. First, we determined the effects on the growth of human prostate carcinoma (PC-3) cells of combined administration of PAESe with DOX, vincristine, or *tert*-butyl hydroperoxide (TBHP). DOX and vincristine are, of course, used clinically as anticancer drugs, whereas *tert*-butylhydroperoxide is a potent oxidizing agent known to exert oxidative-mediated cytotoxicity on PC-3 cells. We found that while PAESe had little effect on the activities of DOX or vincristine, the selenide significantly decreased the cytotoxic effect of TBHP in a dose-dependent manner. Moreover, using the cell-permeable indicator, CM-H_2DCFDA, we showed that PAESe decreased the formation of intracellular reactive oxygen species from either TBHP or DOX. We then proceeded to determine the effect of PAESe on the antitumor activity of DOX in an in vivo xenograft tumor model of human prostate cancer in NCr nude mice. We found that PAESe did not alter DOX antitumor activity and also showed evidence of direct antitumor activity relative to controls. Most importantly, PAESe decreased DOX-mediated infiltration of neutrophil and macrophages into the myocardium, which are early signs of cardiotoxicity. In addition, recent experiments in H9C2 cells have shown that concomitant treatment with PAESe significantly reduced DOX-mediated expression of ANP and MHC-β, which are markers of cardiac hypertrophy (unpublished observations). These findings suggest that selenides such as PAESe may be quite attractive agents for mitigating the cardiotoxicity associated with clinical use of DOX and related anticancer drugs.

3 Ebselen and BXT-51072

Ebselen (2-phenyl-1,2-benzisoselinazol-3[2H]-one) is an organoselenium compound that has been shown to mediate the reduction of hydroperoxides by thiol compounds such as glutathione, thereby modeling the enzymatic activity of the selenoenzyme, glutathione peroxidase [50]. Many researchers have carried out a number of chemical, biochemical, mechanistic, and pharmacological studies on ebselen and its chemical analogs, and a commentary providing historical perspective on the early research and development of ebselen has recently been published [51]. Over the years, a key rationale underlying much of this interest in ebselen has been the possibility that selenium redox cycling in ebselen or its derivatives might supplement natural cellular defenses against oxidizing agents. However, it has now become quite clear that the redox cycle of ebselen consists of multiple reaction pathways with several intermediates and by-products [52–55]. Moreover, several investigations have shown that ebselen is an inefficient catalyst due to deactivating pathways, where unreactive intermediates hinder the regeneration of the original compound [54, 56–58]. In contrast, as noted above, stopped-flow kinetic and ESI-MS/MS results in this author's laboratory have demonstrated that the redox cycling of phenylaminoethyl selenides is a rapid and efficient two-step process, with initial formation of a thioselenurane intermediate followed by complete regeneration of the original selenide (Fig. 4).

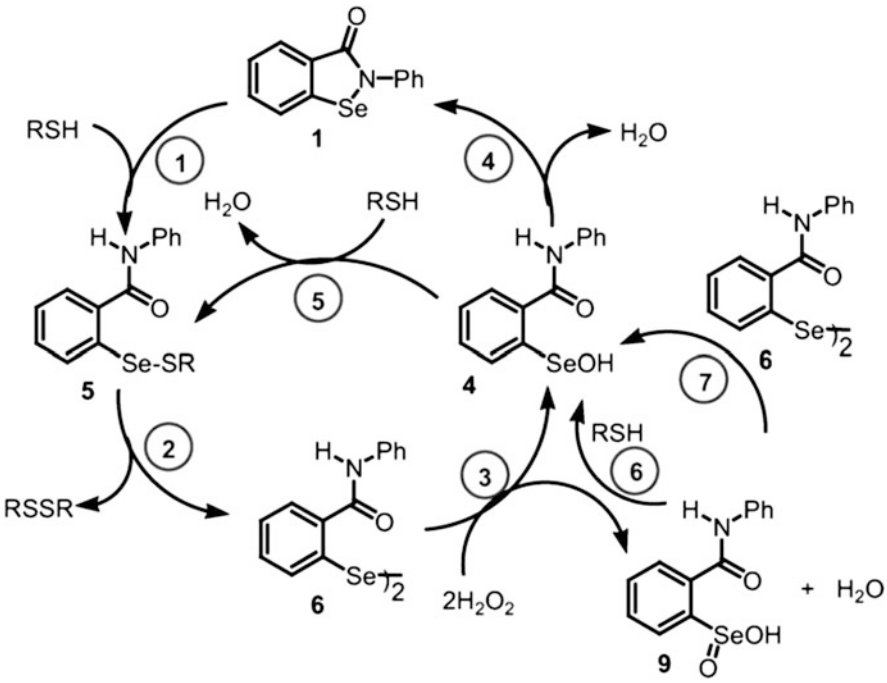
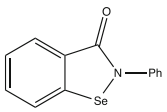


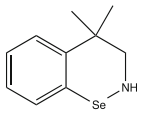
Fig. 4 Mechanism for the GPx activity of ebselen as proposed by Sarma and Mugesh [54]

Several years ago, a clinical trial was undertaken in Japan to assess whether ebselen is capable of protecting against the oxidative tissue damage which occurs following acute ischemic stroke [59]. The results of this trial showed that the most significant improvement was achieved in patients who started a 14-day course of ebselen treatment within 24 h of stroke onset; however, these benefits were statistically significant when evaluated after 1 month but not after 3 months. Subsequently, recruitment was announced for a phase III trial in Japan, sponsored by the Daiichi Pharmaceutical Company, to test the efficacy of ebselen in patients with acute cerebral infarction (cortical infarction). According to the Web site http://www.strokecenter.org/trials/TrialDetail.asp?trialName=list/trialPage298.htm, 394 patients were enrolled in this placebo-controlled, double-blind, randomized, multicenter trial, with enrollment to be completed by November 2002. Unfortunately, results from this trial have never been posted on this Web site, and this author could not find any publications in the open scientific literature reporting results from this trial. In their recent commentary on the early research and development of ebselen [51], Parnham and Sies state that the results of the trial were submitted for registration to the Japanese regulatory authority (MHLW), but the reviewers considered the drug efficacy to be insufficient for approval. According to media reports (see, e.g., http://www.bbsrc.ac.uk/news/health/2013/130114-n-new-drug-bipolar-disorder.aspx), researchers in Oxford have begun a small study in healthy volunteers to test whether ebselen has lithium-like effects on brain function in humans; if successful, these researchers reportedly plan to move on to a small phase II trial in people with bipolar disorder.

Ebselen has been investigated in animal models in a number of laboratories for other possible pharmacological activities. For example, Kono et al. [60] reported that twice-daily treatment of rats with 50 mg/kg ebselen significantly reduces early alcohol-induced liver injury, as measured by liver enzyme assays, inflammation, and liver necrosis. Another example is the report of Moussaoui et al. [61] that ebselen, when administered before, during, and after injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), prevents both neuronal loss and clinical symptoms in a primate MPTP model of Parkinson's disease. More recently, Chew et al. [62] studied the antiatherosclerotic and renoprotective effects of ebselen in diabetic apolipoprotein E/GPx1-double knockout mice that had been injected with streptozotocin. They found that that ebselen reduced atherosclerosis and that it also prevented the changes in renal structure and function and the inflammatory responses associated with nephropathy in the diabetic mice. These findings are reminiscent of the earlier work of Chander et al. [63], who reported that ebselen improved renal outcomes in the Zucker diabetic fat rat. Ebselen been reported to protect neuronal cells in the ventroposterior nucleus of stroke-prone renovascular hypertensive rats against damage resulting from a cerebral cortical infarction [64]. Along these lines, Yamagata and coworkers [65] have reported that ebselen exhibits protective effects against neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rats.



Ebselen



BXT-51072

BXT-51072 [4,4-dimethyl-2,3-dihydro-1,2-benzoselenazine], an organoselenium compound that is structurally related to ebselen, is severalfold more reactive than ebselen in catalyzing hydroperoxide reduction by glutathione [66]. A US patent [67] describes the development of BXT-51072 and analogs as potential treatments for inflammatory bowel disease (IBD) and for possible use in treating asthma, chronic obstructive pulmonary disease, and stroke. A successful phase I trial, carried out by Oxis International [68], was followed by an early phase II trial that apparently showed a promising improvement in patients with mild to moderate ulcerative colitis. However, in a subsequent form 10QSB filing with the US Securities and Exchange Commission [69], Oxis made the following statement:

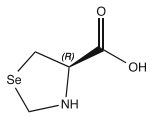
> "We have granted a licensee exclusive worldwide rights, in certain defined areas of cardiovascular indications, to develop, manufacture and market BXT-51072 and related compounds from our library of such antioxidant compounds. The licensee is responsible for worldwide product development programs with respect to licensed compounds. Due to the lack of financial resources, we ceased further testing of BXT-51072 but continue to review the possibility of further developing applications for BXT-51072 and related compounds outside of the areas defined in the license. However, further development and commercialization of antioxidant therapeutic technologies, oxidative stress assays or currently unidentified opportunities may require additional capital."..."No assurances can be given that we will be able to raise such funds in the future on terms favorable to us, or at all."

In January 2004, Axonyx Inc. acquired approximately 53% of the outstanding voting stock of Oxis International.

4 Selenazolidine Prodrugs

Roberts and coworkers [70, 71] have made the argument that inorganic forms of selenium have a relatively narrow therapeutic index, and since their metabolic conversion to H_2Se depletes glutathione, they may exhibit unacceptable toxicity for long-term or high-dose administration in humans. Moreover, these investigators pointed out that while the organoselenium compound, selenomethionine (often present in nutritional supplements as a component of "selenized" yeast), is able to provide selenium to correct nutritional deficiency, one of its major metabolic fates is nonspecific incorporation into proteins in place of methionine, which thereby decreases the therapeutic availability of the selenium by sequestering it inappropriately in protein. The metabolism of selenocysteine, on the other hand, does give rise to an increase in the selenium pool leading to selenoproteins as well as to formation of methylated metabolites; both of these effects can contribute to selenium-based anticancer activity. However, selenocysteine is chemically unstable since it is easily oxidized, and it is therefore relatively difficult to handle.

Based on these considerations, Roberts and coworkers [70–72] pioneered the development of selenazolidine-4(*R*)-carboxylic acids as prodrug forms of selenocysteine for potential use in therapeutic applications.



selenazolidine-4(R)-carboxylic acid

These prodrugs are condensation products of selenocysteine and carbonyl compounds, and contain selenazolidine rings with various substituents; they are designed to release selenocysteine either enzymatically or through spontaneous hydrolysis. The selenazolidines were found to be much less toxic than sodium selenite in cultured lung fibroblast cells; this is as expected since organoselenium compounds have generally been perceived as being less toxic than inorganic selenium species. Treatment of these cells with selenazolidines increased cellular glutathione peroxidase activity, and a clear stereochemical preference was observed, with the prodrugs of L-selenocysteine being more active than the D-enantiomers in increasing glutathione peroxidase activity. Overall, the selenazolidines exhibited comparable chemopreventive activity to selenocysteine, with decreased cytotoxicity and greater biological selenium availability than sodium selenite or selenomethionine.

As discussed below, many cancers are known to overexpress thioredoxin reductase, and knockdown of thioredoxin reductase can enhance the sensitivity of cancer cells to anticancer agents. Accordingly, Poerschke and Moos [73] extended the earlier work of Roberts by utilizing a lentiviral microRNA delivery system to knockdown thioredoxin reductase expression in human lung adenocarcinoma cells and then examining the cytotoxic activities of the selenazolidine prodrugs, 2-butylselenazolidine-4(*R*)-carboxylic acid and 2-cyclohexylselenazolidine-4-(*R*)-carboxylic acid. They found that thioredoxin reductase knockdown increased the cytotoxicity of selenazolidines in these adenocarcinoma cells via a mechanism involving mitochondrial dysfunction and caspase-independent activation of the apoptosis-inducing factor.

5 Selenium-Carrier Conjugates

In a series of US patents [74–76], Spallholz and Reid described the methodology for preparing selenium-carrier conjugates which could then be specifically targeted for delivery to tumors or to sites of pathogenic infection. The underlying rationale of this methodology is the idea of utilizing specific organoselenium compounds to

generate superoxide and other free radicals in a localized environment. Selenium Ltd. (http://www.selenbio.com), a company established in 2004 based on the work of these investigators, claims to have developed the necessary technology for covalently attaching selenium-containing molecules to antibodies, peptides, polymers, and drugs. Thus, for example, selenium-modified humanized antibodies and proteins have been used to effectively kill human prostate, colorectal, and lymphatic cancer cells in tissue culture, with the operative mechanism being the generation of superoxide by the antibody-linked organoselenium compound at the target site which induces apoptosis and destroys the tumor cell. Similarly, an organoselenium-labeled CD-4 peptide directed against the HIV-1 virus destroyed the virus before it was able to infect T cells, with 95% of a virulent isolate of HIV being inactivated in 2 h.

Another potential use for selenium-carrier conjugates is in the treatment of infections caused by bacteria, especially in a biofilm. Tran et al. [77] examined the ability of an organoselenium–methacrylate polymer (Se-MAP) to block biofilm formation by both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These bacteria were chosen since they cause a major share of wound infections and because drug-resistant forms of these bacteria have become a serious problem in the treatment and management of such infections. The results showed that 0.2% (wt/wt) Se in Se-MAP, covalently attached to cellulose disks, completely inhibited *P. aeruginosa* and *S. aureus* biofilm formation. The authors surmise that inhibition of biofilm formation is due to damage to bacterial cell walls and DNA by superoxide, which is generated via selenium-mediated thiol oxidation. The authors also found that the Se-MAP coating is stable and not cytotoxic to mammalian cells. Thus, they suggest that the application of Se-MAP-coated gauze to the debrided tissues of burn wounds may prevent the development of biofilms and facilitate wound healing. This approach might also be used for dental applications [78] and to combat infections in hospitals caused by catheters and other medical implants.

Kunstelj et al. [79] have patented the idea of producing a water-soluble polymer possessing a reactive selenium group (e.g., PEG coupled to selenocysteine or selenocystamine). The polymer is then reacted with a pharmaceutically active agent such as granulocyte colony-stimulating factor (G-CSF) to form a conjugate through linkage of the polymer's Se moiety to a Cys of the pharmaceutically active agent via an –Se–S– bond. The inventors visualize the use of this type of conjugate for the treatment of diseases such as neutropenia. Miki et al. [80] have patented an approach for inhibiting tumor cell growth that entails the administration of a selenium-containing prodrug while also administrating directly to the tumor an expression system for an enzyme for which the prodrug is a substrate. Thus, for example, SeMet is administered together with a vector for expression of methionine lyase, such that the vector preferentially replicates in rapidly proliferating cells or is under control of a promoter that is operable selectively in tumor tissue, thus conferring specificity for tumor cells. The lyase cleaves the prodrug at the C–Se bond, thus liberating a toxic form of selenium (presumably RSe^-) at the site of the tumor in an amount sufficient to inhibit tumor cell growth.

6 Targeting the Thioredoxin–Thioredoxin Reductase System

A number of investigators have been pursuing the development of compounds that target the mammalian selenoenzyme, thioredoxin reductase (TrxR), which catalyzes the NADPH-dependent reduction of the redox protein, thioredoxin (Trx). The Trx/TrxR system functions as a donor of reducing equivalents for enzymes such as ribonucleotide reductase, which is essential for DNA synthesis, and protein disulfide reductase, which is critically involved in thiol/disulfide redox regulation (Fig. 5). Therefore, the inhibition of TrxR would be expected to be highly detrimental to the growth of tumor cells, especially since the levels of both TrxR and Trx have been found to be elevated in tumor cell lines, and expression profiles of thioredoxin family proteins correlate with cell proliferation, survival, and tumor grade [81–84]. Moreover, knockdown of thioredoxin reductase in murine lung carcinoma cells reversed the malignant phenotype and decreased tumor growth and metastasis [85].

As mentioned above, Poerschke and Moos [73] have reported that knockdown of thioredoxin reductase in human lung adenocarcinoma cells using a lentiviral microRNA delivery system increased the cytotoxicity of selenazolidines in these adenocarcinoma cells. In earlier work, Powis and coworkers [86] found that certain organoselenium and organotellurium compounds, primarily of the diaryl chalcogenide type, were inhibitors of TrxR and also growth inhibitors of cultured tumor cells; these compounds, with one exception, did not inhibit glutathione reductase. Subsequently, these investigators reported [87] that water-soluble organotellurium

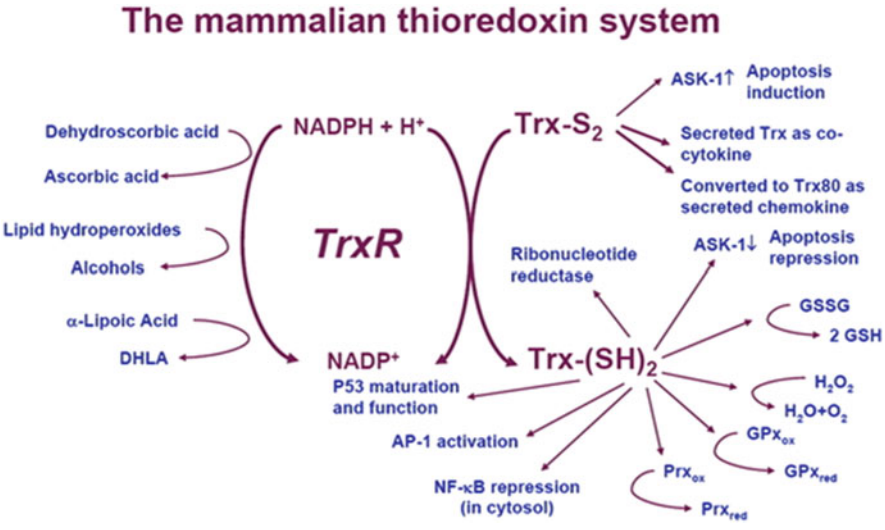


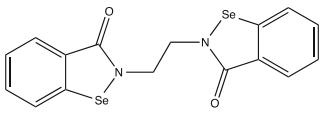
Fig. 5 The mammalian thioredoxin–thioredoxin reductase (Trx/TrxR) system is linked to many cellular processes (from http://biochem.mbb.ki.se/Arner/research/img/Trx_TrxR.jpg)

compounds of the diaryl telluride, alkyl aryl telluride, and dialkyl telluride types are very potent inhibitors of TrxR, and these tellurides inhibited the growth of MCF-7 and HT-29 human cancer cells in culture at the 5–10 micromolar level; however, their hydrophilicity did seem to restrict cellular uptake.

In 2002, Holmgren and coworkers reported [88] that ebselen is an excellent substrate for human TrxR. They showed that TrxR, in the presence of thioredoxin, catalyzes a very rapid reduction of ebselen to ebselen selenol, with NADPH serving as the electron donor. The reduced ebselen can, in turn, react rapidly with hydrogen peroxide (peroxidase activity), thus setting up a catalytic cycle whereby hydrogen peroxide and lipid peroxides can be eliminated. Since TrxR and Trx are present in all cells throughout the body, the authors proposed that this peroxidase activity provides a mechanistic explanation for the antioxidant and anti-inflammatory effects of ebselen and, in particular, for its activity in protecting cells against ischemic tissue damage. They further stated that their results "demonstrate that the mechanism of action of ebselen may be predominantly via the thioredoxin system rather than via glutathione." Recently, Lu and Holmgren [89] have pointed out that the absence of a glutathione-based antioxidant system in pathogenic bacteria such as *Helicobacter pylori*, *Mycobacterium tuberculosis*, and *S. aureus* makes the bacterial thioredoxin-based antioxidant system essential for the survival of such organisms under conditions of oxidative stress. They therefore conclude that the inhibition of TrxR accounts for ebselen's antibacterial activity in such organisms.

This issue of the relationship between the thioredoxin-based and glutathione-based antioxidant systems, and the possibility of cross talk between these systems, is very relevant to the findings of Casagrande et al. [90], who showed that Trx undergoes glutathionylation in human T cell blasts that had been exposed to oxidative stress. Glutathionylation, which was shown by MALDI-TOF mass spectrometry to occur on rhTrx's Cys72 residue, abolished the ability of Trx to act as a disulfide reductase in the presence of TrxR and NADPH. The authors conclude that their finding of Trx regulation by glutathionylation indicates that cross talk exists between the thioredoxin and the glutathione systems.

With the recognition that many tumor cells exhibit a high level of expression of Trx and TrxR, which renders such cells more resistant to apoptosis and chemotherapy, there is obviously considerable interest in the clinical evaluation of TrxR inhibitors as potential anticancer agents. Ethaselen (1,2-[bis(1,2-benzisoselenazolone-3(2H)-ketone)]ethane), a novel organoselenium compound developed by Zeng and coworkers [91], is the first selenium-containing inhibitor of mammalian TrxR1.



Ethaselen

Ethaselen specifically targets the catalytically essential cysteine–selenocysteine (Cys–Sec) redox pair at the C-terminal active site of mammalian TrxR thereby

inhibiting the enzyme, and this leads to an accumulation of oxidized Trx and increased levels of reactive oxygen species. Ethaselen exhibits anticancer activity with low toxicity in tumor cells and in animal models, and inhibition of cancer cell growth by ethaselen correlates with TrxR1 inactivation in several tumor cell lines. As of June 2014, recruitment was underway in China for a phase 1c single-arm study of ethaselen for the treatment of non-small cell lung cancer in patients who had previously received more than two lines of standard treatment (clinicaltrials.gov identifier: NCT02166242). Reportedly, ethaselen has now entered phase II clinical trials – presumably in China – targeting gastric, lung, and colon cancers (see Li et al. [92]).

It should be noted that a number of non-selenium-based anticancer drugs undergoing clinical evaluation are thought to target the Trx/TrxR system. Among these are PX-12 (1-methylpropyl 2-imidazolyl disulfide) for patients with advanced gastrointestinal cancers (phase Ib) [93] and motexafin gadolinium, in combination with doxorubicin (phase I) for patients with advanced solid tumors [94] and in combination with pemetrexed (phase II) for second-line treatment of patients with non-small cell lung cancer [95]. In addition, a number of well-established chemotherapeutic agents are known inhibitors of the Trx system (see Mahmood et al. [96] and references therein).

7 Selenium-Containing Metabolites

Several years ago, Combs [97, 98] pointed out that a number of selenium compounds had been found to be antitumorigenic in a variety of animal models at intakes that are substantially greater than those associated with maximal expression of the known selenocysteine-containing enzymes. This supports the view that the antitumorigenic effects seen in selenium supplementation studies arise at least in part from enhanced production of specific Se-containing metabolites, not just from maximal expression of selenoenzymes. Combs proposed a two-stage model in which selenium supplementation of individuals with low, nutritionally deficient, natural intakes of this element enhances the activities of protective selenoenzymes, whereas selenium supplementation of individuals who are not selenium deficient results in the beneficial buildup of antitumorigenic selenium metabolites. At about the same time, Ganther [99] reemphasized that alterations in selenoenzyme activity had failed to explain the anticancer effects of selenium, and therefore mechanisms involving low-molecular-weight selenium metabolites must also be involved. In subsequent years, a number of low-molecular-weight selenium-containing compounds known – or, quite often, simply postulated – to be formed in the course of selenium metabolism have been studied for possible therapeutic potential (see [100, 101] for recent reviews).

In this author's view, it stands to reason that the specific metabolic state of a particular cell, tissue, or organ should sensitively affect the bioactivity of a given selenium supplement or metabolite. Thus, for example, as recently mentioned by

Zeng and coworkers [91], the well-studied chemopreventive selenium compounds selenite, selenodiglutathione, and methylseleninate are all substrates of mammalian TrxR1, and the product selenium metabolites formed from these compounds play important roles in selenium-induced cytotoxicity and apoptosis in cancer cells. Therefore, the degree to which TrxR1 activity is upregulated or downregulated in a given metabolic situation or disease state would be expected to have a very significant effect on the steady-state levels of these bioactive product metabolites. In this regard, Weekley and Harris recently published [101] an excellent comprehensive review of the current evidence for the various metabolic pathways of common dietary selenium compounds, such as selenite, selenomethionine, methylselenocysteine, and selenocysteine. They conclude that dietary selenium compounds should be considered prodrugs, whose biological activity depends on the activity of the various metabolic pathways in, and the redox status of, cells and tissues. Obviously, such factors will need to be considered carefully when selecting selenium compounds for future trials of disease prevention and treatment by selenium supplementation.

As stated at the outset, a full discussion of the numerous studies on nutritional and epidemiological aspects of selenium metabolites and supplements is beyond the scope of this review. Nevertheless, a few illustrative examples will be mentioned here (Fig. 6).

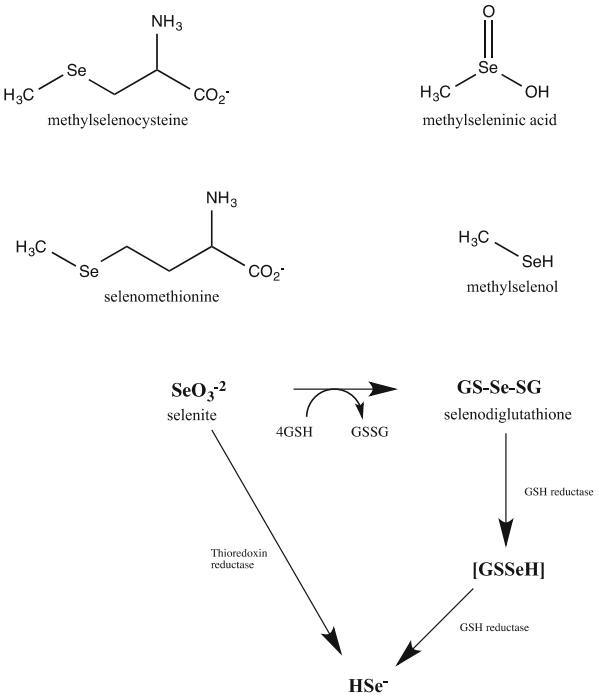


Fig. 6 Structures of some selenium metabolites

The initial step in metabolism of inorganic selenite (SeO_3^{2-}) is its reaction with glutathione to form selenodiglutathione (GS-Se-SG), which is subsequently reduced enzymatically to hydrogen selenide (HSe^-). Selenodiglutathione has been extensively studied by many investigators and shown to be a potent carcinostatic agent in animals and an inhibitor of cell growth and inducer of apoptosis in tumor cells [102–104]. Spyrou et al. [105] have shown that both selenodiglutathione and selenite inhibit the binding of the transcription factor AP-1 to DNA nuclear extracts of 3B6 lymphocytes. Selenodiglutathione is also a facile substrate for the thioredoxin reductase system and glutathione reductase [106] and was found to inhibit human thioredoxin by oxidation of structural thiol groups [107]. It should be noted that there are numerous published studies reporting anticancer effects of inorganic selenite itself, and it is possible that some chemoresistant malignant cells may exhibit enhanced sensitivity to selenite [108]. One promising study in *human patients* was reported by Asfour et al. [109], who found that administering high doses of sodium selenite along with standard chemotherapy increased apoptosis of lymphoma cells in adult patients with non-Hodgkin's lymphoma.

The methylated selenium metabolites methylselenocysteine and methylseleninic acid inhibit the progression and metastasis of cancer and increase survival in the transgenic adenocarcinoma of prostate mouse model [110]. Methylseleninic acid reduces the spontaneous metastasis of Lewis lung carcinoma in mice [111] and also increases the apoptosis potencies of the topoisomerase I inhibitor 7-ethyl-10-hydroxycamptothecin, the topoisomerase II inhibitor etoposide, or the microtubule inhibitor paclitaxel/taxol, in cell lines of advanced human metastatic prostate cancer against which standard chemotherapeutic treatments have limited efficacy [112]. Methylselenocysteine, which is found in foods such as garlic, onions, and broccoli, has been shown in combination with tamoxifen to inhibit MCF-7 breast cancer xenografts in nude mice through elevated apoptosis and reduced angiogenesis [113]. Methylselenocysteine and methylseleninic acid are cleaved metabolically to produce methylselenol ($MeSe^-$); Ganther and coworkers [114] have shown that the production of this monomethylated selenium metabolite is a key step in cancer chemoprevention by compounds such as methylselenocysteine and methylseleninic acid. Methylselenocysteine has recently been called "a promising antiangiogenic agent for overcoming drug delivery barriers in solid malignancies for therapeutic synergy with anticancer drugs" [115].

Selenomethionine is the form of selenium used in the National Cancer Institute's SELECT trial and in a number of other selenium supplementation studies. Selenomethionine is capable of binding to methionine t-RNA and becoming incorporated into proteins in place of methionine [116]. Selenomethionine at micromolar concentration was found to inhibit growth of three human tumor cell lines, whereas growth inhibition of normal diploid fibroblasts required millimolar concentrations [117]. Thus, at least in these cell lines, cancer cells apparently exhibit greatly enhanced sensitivity to the growth inhibitory effects of selenomethionine. Mice treated orally or topically with selenomethionine were found to have significantly lowered UV irradiation-induced skin damage and reduced incidence of skin cancer

[118]. Frenkel and Caffrey [119] have reported that two selenium compounds, selenite and selenomethionine, are able to reduce the induction of resistance by ovarian cancer cells or xenografts to the chemotherapeutic agents melphalan or cisplatin. Similarly, selenomethionine and selenite were found to prevent the induction of resistance to the antitumor agent cisplatin in mice bearing tumors derived from human ovarian cells [120]. These are interesting findings, since the development of drug resistance is considered to be a major cause for the failure of chemotherapy in several types of cancer.

A clever approach to selenium-based gene-directed enzyme prodrug therapy for cancer was devised by Miki et al. [121]. Their strategy exploits the toxic prooxidant property of methylselenol, which is released from selenomethionine by cancer cells that had been transduced with the adenoviral-delivered methionine α,γ-lyase gene. In these cells, the cytotoxicity of the selenomethionine prodrug was increased up to 1,000-fold compared with nontransduced cells. A strong bystander effect occurred due to methylselenol release from the transduced cells and uptake by surrounding tumor cells. Methylselenol damaged the mitochondria via oxidative stress and caused cytochrome c release into the cytosol, thereby activating the caspase cascade and apoptosis. These investigators also reported that treatment using their prodrug approach inhibited tumor growth in rodents and significantly prolonged their survival.

8 Diaryl Diselenides

In a series of nutritional and behavioral studies, diphenyl diselenide compounds were found to improve memory in a rodent model of Alzheimer's-type sporadic dementia, thereby ameliorating learning performance in these animals [122]. In subsequent studies, these investigators investigated the molecular mechanism of neuroprotection by *p*,*p*′-methoxyl-diphenyl diselenide in cortical neurons exposed to amyloid-β (Aβ) peptide as well as in Aβ-infused mice [123]. The diselenide compound was found to prevent Aβ-induced cell death associated with the inhibition of caspase-3 and caspase-9 activities, poly(ADP-ribose) polymerase cleavage, and JNK activation. In addition, oral administration of the diselenide at 5 mg/kg for 5 days rescued memory impairment in mice that had been exposed to Aβ fragment via intracerebroventricular infusion. Taken together, these findings are both timely and noteworthy, given the intense current interest in developing new approaches for the treatment of Alzheimer's disease.

Diaryl diselenides have also been investigated as potential anti-inflammatory agents. Shin et al. [124] reported that bis-(3-hydroxyphenyl) diselenide inhibits LPS-stimulated iNOS and COX-2 expression in RAW 264.7 macrophages. The diselenide compound also significantly reduced the release of TNF-α, interleukin (IL)-1β, and IL-6, and the authors provided evidence that these activities are due to the downregulation of NF-κB binding activity. In addition, disubstituted diaryl diselenides have been found to reduce carrageenan-induced paw edema in rats

[100], and there is a report [125] that diphenyl diselenide produces a significant peripheral antinociceptive (i.e., analgesic) effect via a mechanism that is unlike the activation of opioid, dopaminergic D2, or muscarinic cholinergic receptors.

It should be noted that in a recent review article, Nogueira and Rocha [126] refer to diphenyl diselenides as "Janus-faced" molecules, since the interaction of diphenyl diselenide with thiols can give rise to either toxic or beneficial effects. In truth, this appellation could apply as well to a number of other organoselenium compounds (see below).

9 Selenium Versus Sulfur in Drug Design

Although selenium and sulfur are members of the same periodic table group, there are important differences between the chemical properties of these two elements (see Iwaoka and Arai [127] and references therein for a detailed comparison). Selenium has a lower electronegativity and larger atomic radius than sulfur. Bond dissociation energies for C–Se, Se–H, and Se–Se covalent bonds are 58, 67, and 44 kcal/mol, respectively, whereas the corresponding values for sulfur are 65, 88, and 63 kcal/mol. Thus, organoselenium compounds should be more reactive than organosulfur compounds; selenols are more reactive than thiols and Se–Se bonds can be more easily cleaved than S–S bonds. Importantly, the ability of selenoxides to act as mild oxidizing agents has been recognized for decades [128].

The differences between selenium and sulfur can be very significant from the perspective of drug design. One illustrative example is provided by work carried out in this author's laboratory on the markedly differing effects of selenide versus sulfide compounds on norepinephrine production in adrenal chromaffin granules [26–29]. Both phenylaminoethyl selenide and its sulfur cognate, phenylaminoethyl sulfide, are readily taken up into chromaffin granules, where they undergo facile DBM-catalyzed heteroatom oxygenation to produce the corresponding selenide or sulfoxide product, respectively. However, only the selenoxide product has the ability to propagate a turnover-dependent redox cycle that results in local depletion of reduced ascorbate within the chromaffin vesicle. In contrast, the sulfoxide product formed enzymatically from phenylaminoalkyl sulfide is not capable of propagating such a redox cycle, and depletion of reduced ascorbate in the chromaffin granule consequently does not occur with the sulfide compound. We demonstrated [34] on the basis of potentiometric titrations that these findings are fully consistent with the redox potentials of the phenylaminoethyl selenoxides, which range from +410 to +480 mV (depending on the ring substituents) at the pH of the chromaffin granule milieu. Cyclic voltammetry experiments confirmed that the peak potentials for the reductive waves, E*pc*, for sulfoxides are ca. 500 mV lower than the E*pc* for the corresponding phenylaminoethyl selenoxides.

More than two decades ago, Ip and Ganther [104] compared the cancer chemopreventive efficacy in a rat mammary tumor model of three pairs of selenium and sulfur analogs; the compounds tested were selenocystamine/cysteamine,

Se-methylselenocysteine/S-methylcysteine, and selenobetaine/sulfobetaine. In all cases, the selenium compounds were found to be far more active in chemoprevention than their structurally similar sulfur cognates, and the authors concluded that selenium compounds may both prevent cellular transformation and delay or inhibit the expression of malignancy after carcinogen exposure. More recently, Xiao and Parkin [129] investigated the effects of 27 selenium compounds and 16 structurally related organosulfur compounds on phase II enzyme activity induction (a chemopreventive mechanism) in murine hepatoma cells, and identified nine highly active species, all of which were organoselenium compounds (Fig. 7).

The issue of the relative activities of organoselenium versus organosulfur compounds as antioxidants has been discussed by a number of investigators. For example, Steinmann et al. [130] found that the reactions of selenocysteine and selenoglutathione with tyrosyl radicals in *N*-Ac-Tyr-NH_2 and in insulin proceed orders of magnitude more rapidly than the reactions of the corresponding sulfur analogs. These authors point out that the effective concentration of a selenocysteine residue in the microenvironment of a selenoprotein is much higher than its concentration in the aqueous compartments of the cell, so a protective function that entails repair of radical damage to neighboring residues can be envisioned for selenocysteine. Similarly, phenylaminoethyl selenides have been found to be much more effective in protecting pUC19 DNA against peroxynitrite-induced damage than the corresponding sulfur analogs [34]. Iwaoka and Arai [127] have expressed the view that "progress in selenium biology [*has*] allowed researchers in biological chemistry to expand their research arena from sulfur to selenium." While this statement is quite evidently true, there are likely major differences between the mechanisms that underlie the biological activities of organoselenium versus organosulfur compounds, and this must certainly be kept in mind when extrapolating to selenium from the large body of information that has accumulated over the

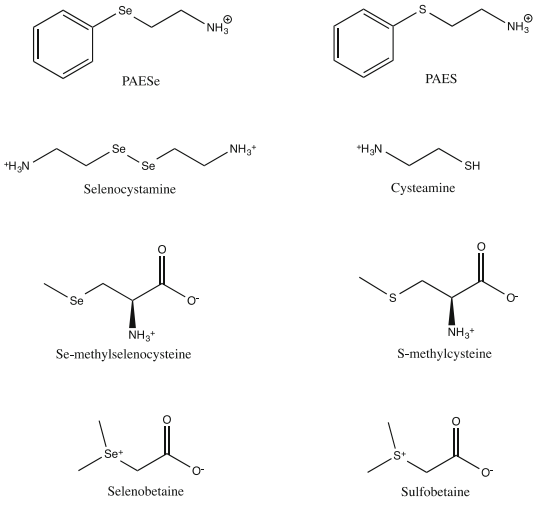


Fig. 7 Selenium compounds and their sulfur analogs

years about the biology of sulfur compounds. It is reasonable to expect that relevant mechanistic details will continue to be further elucidated in future investigations.

10 Conclusions and Perspectives

Selenium has a long history of association with human health and disease. We now recognize that this element is an essential nutrient that is critical to key cellular processes and to the activities of a number of important enzymes. Great strides have been made over the past few years in our understanding of the biochemistry of selenium metabolism, and these efforts have been facilitated by structural determinations of low-concentration metabolites using advanced separation and mass spectral techniques. With regard to selenoproteins, we now know that the selenoproteins constituting the human selenoproteome are encoded by 25 genes in the human genome [13], and very significant progress is being made in characterizing selenoproteins and their physiological functions. As discussed by Labunskyy et al. in their excellent and very recent review [131], selenoproteins not only function as antioxidant enzymes but also in diverse cellular processes such as thyroid hormone function, selenophosphate synthesis, protein folding, and protein degradation. However, the biological functions of approximately half of the human selenoproteins still remain unknown [84]. Over the coming years, we can anticipate that research efforts will be directed at answering important questions about selenoprotein function and the mechanisms by which these functions are linked to the health effects of dietary selenium and selenium metabolites in normal and disease states.

The idea that selenium-containing dietary supplements could be effective in preventing disease has gone through both optimistic and pessimistic phases. It has been recognized for decades that a deficiency of dietary selenium in certain populations is linked to cancer, cardiovascular disease, and various other disorders. Approximately 20 years ago, the Associated Press, Reuters, the New York Times, newspapers and media in many cities, and numerous Internet sites all enthusiastically reported the results of large epidemiological studies indicating that higher selenium levels are associated with a reduced risk of advanced prostate cancer. The subsequent failure of the very large and expensive SELECT trial a decade later greatly dampened the initial enthusiasm for the notion that simple dietary selenium supplementation could be an effective approach for cancer prevention. Some subsequent "postmortem" analyses of perceived flaws in the SELECT protocols did mitigate this disappointment to some extent, but it seems unlikely that government funding would be provided in the foreseeable future for any large-scale selenium supplementation trials similar to SELECT. As recently noted by Weekley and Harris [101], "with regards to the connection between selenium supplementation and disease prevention and treatment, there is an astounding gap between the

efficacy observed in laboratory studies and the mixed results of clinical trials. . .choice of selenium supplement speciation [*sic*] in laboratory and clinical studies may explain some of this efficacy gap." Another concern that also needs to be addressed in any future long-term supplementation protocol is the possibility of long-term dietary selenium toxicity. As well stated by Nogueira and Rocha [100], a balance must be struck in supplementation studies between nutritional requirements and the potential toxicological consequences of over- or under-selenium intake. Clearly, careful attention needs to be paid to the proper formulation and dosage of selenium dietary supplements.

Dietary supplementation on the one hand and the development of new therapeutic entities on the other are quite different matters. Glutathione peroxidase (GPx) was the first mammalian enzyme whose activity was clearly shown to be dependent on selenium. Since this selenoenzyme plays such an essential role in cellular defense against detrimental oxidants, a very strong impetus emerged for the development of selenium-containing small-molecule "GPx mimics" as potential therapeutic agents against disease states known to be associated with oxidative stress. In this regard, Roberts and coworkers [70] state as follows: "GPx activity has been previously observed to plateau with increased selenium administration, even as chemoprevention increases. . .this is one reason that it is no longer thought to play an important role in selenium-mediated chemoprevention." In this author's opinion, it is very unlikely that this statement holds true for all classes of selenium-based compounds. As detailed in this review, many current efforts continue to focus on compounds that modulate the glutathione-based – as well as the thioredoxin-based – antioxidant systems. Nevertheless, a general concern regarding the disappointing clinical outcomes to date of therapy with *natural* antioxidants in general must be kept in mind. As stated by Murphy [132], "a large number of well-conducted clinical trials have been carried out using several different antioxidants on a range of pathologies with little improvement in clinical outcome for the patients." Clearly, as Murphy himself concludes, more sophisticated drug design efforts are needed in the development of new chemical entities as antioxidants. It should be clear from the work described in this review that a number of such efforts with selenium-based compounds are underway.

In conclusion, it is quite evident that a number of selenium-based molecules with therapeutic potential are under development. While most of the efforts that entail *designed* organoselenium compounds – as opposed to inorganic selenium metabolites – are still at the preclinical stage, evidence is emerging that selenium-based compounds can operate by several biochemical and pharmacological mechanisms. Since our understanding of the biology, biochemistry, and pharmacology of selenium and selenoproteins is rapidly expanding, we can anticipate that the coming years will bring further development of new selenium-based pharmaceutical agents with therapeutic potential against human diseases.

References

1. Olcott HS, Brown WD, Van Derveen J (1961) Selenomethionine as an antioxidant. Nature 191:1201–1202
2. Walter R, Schwartz IL, Roy J (1972) Can selenoamino acids act as reversible biological antioxidants? Ann N Y Acad Sci 192:175–180
3. Beck MA, Shi Q, Morris VC, Levander OA (1995) Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nat Med 1(5):433–436
4. Levander OA, Beck MA (1997) Interacting nutritional and infectious etiologies of Keshan disease. Insights from coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E. Biol Trace Elem Res 56(1):5–21. doi:10.1007/BF02778980
5. May SW (1999) Selenium-based drug design: rationale and therapeutic potential. Expert Opin Investig Drugs 8(7):1017–1030. doi:10.1517/13543784.8.7.1017
6. Shamberger RJ, Frost DV (1969) Possible protective effect of selenium against human cancer. Can Med Assoc J 100(14):682
7. Schrauzer GN, Rhead WJ (1971) Interpretation of the methylene blue reduction test of human plasma and the possible cancer protecting effect of selenium. Experientia 27(9):1069–1071
8. Clark LC, Cantor KP, Allaway WH (1991) Selenium in forage crops and cancer mortality in U.S. counties. Arch Environ Health 46(1):37–42. doi:10.1080/00039896.1991.9937427
9. Mugesh G, du Mont WW, Sies H (2001) Chemistry of biologically important synthetic organoselenium compounds. Chem Rev 101(7):2125–2179
10. Nogueira CW, Zeni G, Rocha JB (2004) Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev 104(12):6255–6285. doi:10.1021/cr0406559
11. May SW (2002) Selenium-based pharmacological agents: an update. Expert Opin Investig Drugs 11(9):1261–1269. doi:10.1517/13543784.11.9.1261
12. May SW, Pollock SH (1998) Selenium-based antihypertensives. Rationale and potential. Drugs 56(6):959–964
13. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN (2003) Characterization of mammalian selenoproteomes. Science 300(5624):1439–1443. doi:10.1126/science.1083516
14. Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB, Giovannucci E (1998) Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J Natl Cancer Inst 90(16):1219–1224
15. van den Brandt PA, Zeegers MP, Bode P, Goldbohm RA (2003) Toenail selenium levels and the subsequent risk of prostate cancer: a prospective cohort study. Cancer Epidemiol Biomarkers Prev 12(9):866–871
16. Beck MA, Handy J, Levander OA (2004) Host nutritional status: the neglected virulence factor. Trends Microbiol 12(9):417–423. doi:10.1016/j.tim.2004.07.007
17. Beck MA, Levander OA, Handy J (2003) Selenium deficiency and viral infection. J Nutr 133 (5 Suppl 1):1463S–1467S
18. Duntas LH (2009) Selenium and inflammation: underlying anti-inflammatory mechanisms. Horm Metab Res 41(6):443–447. doi:10.1055/s-0029-1220724
19. Beckett GJ, Arthur JR (2005) Selenium and endocrine systems. J Endocrinol 184 (3):455–465. doi:10.1677/joe.1.05971
20. Council NR (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC
21. Parnham M, Sies H (2000) Ebselen: prospective therapy for cerebral ischaemia. Expert Opin Investig Drugs 9(3):607–619. doi:10.1517/13543784.9.3.607
22. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park HK, Sanders BB Jr, Smith CL, Taylor JR (1996) Effects of selenium supplementation for cancer prevention in patients with

carcinoma of the skin. A randomized controlled trial .Nutritional Prevention of Cancer Study Group. JAMA 276(24):1957–1963
23. Lippman SM, Goodman PJ, Klein EA, Parnes HL, Thompson IM Jr, Kristal AR, Santella RM, Probstfield JL, Moinpour CM, Albanes D, Taylor PR, Minasian LM, Hoque A, Thomas SM, Crowley JJ, Gaziano JM, Stanford JL, Cook ED, Fleshner NE, Lieber MM, Walther PJ, Khuri FR, Karp DD, Schwartz GG, Ford LG, Coltman CA Jr (2005) Designing the selenium and vitamin E cancer prevention trial (SELECT). J Natl Cancer Inst 97(2):94–102. doi:10.1093/jnci/dji009
24. Ledesma MC, Jung-Hynes B, Schmit TL, Kumar R, Mukhtar H, Ahmad N (2011) Selenium and vitamin E for prostate cancer: post-SELECT (selenium and vitamin E cancer prevention trial) status. Mol Med 17(1–2):134–143. doi:10.2119/molmed.2010.00136
25. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD 3rd, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr, Baker LH, Coltman CA Jr (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 301(1):39–51. doi:10.1001/jama.2008.864
26. May SW, Herman HH, Roberts SF, Ciccarello MC (1987) Ascorbate depletion as a consequence of product recycling during dopamine beta-monooxygenase catalyzed selenoxidation. Biochemistry 26(6):1626–1633
27. May SW, Wimalasena K, Herman HH, Fowler LC, Ciccarello MC, Pollock SH (1988) Novel antihypertensives targeted at dopamine beta-monooxygenase: turnover-dependent cofactor depletion by phenyl aminoethyl selenide. J Med Chem 31(6):1066–1068
28. Herman HH, Wimalasena K, Fowler LC, Beard CA, May SW (1988) Demonstration of the ascorbate dependence of membrane-bound dopamine beta-monooxygenase in adrenal chromaffin granule ghosts. J Biol Chem 263(2):666–672
29. Wimalasena K, Herman HH, May SW (1989) Effects of dopamine beta-monooxygenase substrate analogs on ascorbate levels and norepinephrine synthesis in adrenal chromaffin granule ghosts. J Biol Chem 264(1):124–130
30. Pollock SH, Herman HH, Fowler LC, Edwards AS, Evans CO, May SW (1988) Demonstration of the antihypertensive activity of phenyl-2-aminoethyl selenide. J Pharmacol Exp Ther 246(1):227–234
31. Herman HH, Pollock SH, Fowler LC, May SW (1988) Demonstration of the potent antihypertensive activity of phenyl-2-aminoethyl sulfides. J Cardiovasc Pharmacol 11(5):501–510
32. May SW, Wang L, Gill-Woznichak MM, Browner RF, Ogonowski AA, Smith JB, Pollock SH (1997) An orally active selenium-based antihypertensive agent with restricted CNS permeability. J Pharmacol Exp Ther 283(2):470–477
33. Overcast JD, Ensley AE, Buccafusco CJ, Cundy C, Broadnax RA, He S, Yoganathan AP, Pollock SH, Hartley CJ, May SW (2001) Evaluation of cardiovascular parameters of a selenium-based antihypertensive using pulsed Doppler ultrasound. J Cardiovasc Pharmacol 38(3):337–346
34. De Silva V, Woznichak MM, Burns KL, Grant KB, May SW (2004) Selenium redox cycling in the protective effects of organoselenides against oxidant-induced DNA damage. J Am Chem Soc 126(8):2409–2413. doi:10.1021/ja037294j
35. Woznichak MM, Overcast JD, Robertson K, Neumann HM, May SW (2000) Reaction of phenylaminoethyl selenides with peroxynitrite and hydrogen peroxide. Arch Biochem Biophys 379(2):314–320. doi:10.1006/abbi.2000.1893
36. Cowan EA, Oldham CD, May SW (2011) Identification of a thioselenurane intermediate in the reaction between phenylaminoalkyl selenoxides and glutathione. Arch Biochem Biophys 506(2):201–207. doi:10.1016/j.abb.2010.11.007
37. Cowan EA, Taylor JL, Oldham CD, Dasari M, Doyle D, Murthy N, May SW (2013) Cellular antioxidant activity of phenylaminoethyl selenides as monitored by chemiluminescence of

peroxalate nanoparticles and by reduction of lipopolysaccharide-induced oxidative stress. Enzyme Microb Technol 53(6–7):373–377. doi:10.1016/j.enzmictec.2013.08.002
38. Kumar S, Singh HB, Wolmershauser G (2006) Protection against peroxynitrite-mediated nitration reaction by intramolecularly coordinated diorganoselenides. Organometallics 25:382–393
39. Reich HJ, Jasperse CP (1987) Organoselenium chemistry. Redox chemistry of selenocysteine model systems. J Am Chem Soc 109(18):5549–5551
40. Back TG, Moussa Z, Parvez M (2004) The exceptional glutathione peroxidase-like activity of di(3-hydroxypropyl) selenide and the unexpected role of a novel spirodioxaselenanonane intermediate in the catalytic cycle. Angew Chem Int Ed 43:1268–1270
41. Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 279(6):L1005–L1028
42. Sarsour EH, Kumar MG, Chaudhuri L, Kalen AL, Goswami PC (2009) Redox control of the cell cycle in health and disease. Antioxid Redox Signal 11(12):2985–3011. doi:10.1089/ARS.2009.2513
43. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49(11):1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006
44. Hande KR (1998) Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta 1400(1–3):173–184
45. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC (2002) Antioxidant nutrients and adriamycin toxicity. Toxicology 180(1):79–95
46. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56(2):185–229. doi:10.1124/pr.56.2.6
47. Pearlman M, Jendiroba D, Pagliaro L, Keyhani A, Liu B, Freireich EJ (2003) Dexrazoxane in combination with anthracyclines lead to a synergistic cytotoxic response in acute myelogenous leukemia cell lines. Leuk Res 27(7):617–626
48. Tebbi CK, London WB, Friedman D, Villaluna D, De Alarcon PA, Constine LS, Mendenhall NP, Sposto R, Chauvenet A, Schwartz CL (2007) Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J Clin Oncol 25(5):493–500. doi:10.1200/JCO.2005.02.3879
49. Kang JY, Costyn LJ, Nagy T, Cowan EA, Oldham CD, May SW, Arnold RD (2011) The antioxidant phenylaminoethyl selenide reduces doxorubicin-induced cardiotoxicity in a xenograft model of human prostate cancer. Arch Biochem Biophys 515(1–2):112–119. doi:10.1016/j.abb.2011.08.008
50. Parnham M (1996) The pharmaceutical potential of selenium. Pharmaceut News 3:7–10
51. Parnham MJ, Sies H (2013) The early research and development of ebselen. Biochem Pharmacol 86(9):1248–1253. doi:10.1016/j.bcp.2013.08.028
52. Back TG, Moussa Z, Parvez M (2004) The exceptional glutathione peroxidase-like activity of di(3-hydroxypropyl) selenide and the unexpected role of a novel spirodioxaselenanonane intermediate in the catalytic cycle. Angew Chem Int Ed 43(10):1268–1270. doi:10.1002/anie.200353128
53. Kunwar A, Mishra B, Barik A, Kumbhare LB, Pandey R, Jain VK, Priyadarsini KI (2007) 3,3′-diselenodipropionic acid, an efficient peroxyl radical scavenger and a GPx mimic, protects erythrocytes (RBCs) from AAPH-induced hemolysis. Chem Res Toxicol 20 (10):1482–1487. doi:10.1021/tx700137a
54. Sarma BK, Mugesh G (2008) Antioxidant activity of the anti-inflammatory compound ebselen: a reversible cyclization pathway via selenenic and seleninic acid intermediates. Chemistry 14(34):10603–10614. doi:10.1002/chem.200801258
55. Back TG, Moussa Z (2003) Diselenides and allyl selenides as glutathione peroxidase mimetics. Remarkable activity of cyclic seleninates produced in situ by the oxidation of

allyl omega-hydroxyalkyl selenides. J Am Chem Soc 125(44):13455–13460. doi:10.1021/ja0357588
56. Sarma BK, Mugesh G (2005) Glutathione peroxidase (GPx)-like antioxidant activity of the organoselenium drug ebselen: unexpected complications with thiol exchange reactions. J Am Chem Soc 127(32):11477–11485. doi:10.1021/ja052794t
57. Davies R, Bartholomeusz DA, Andrade J (2003) Personal sensors for the diagnosis and management of metabolic disorders. IEEE Eng Med Biol Mag 22(1):32–42
58. Balkrishna SJ, Kumar S, Azad GK, Bhakuni BS, Panini P, Ahalawat N, Tomar RS, Detty MR, Kumar S (2014) An ebselen like catalyst with enhanced GPx activity via a selenol intermediate. Org Biomol Chem 12(8):1215–1219. doi:10.1039/c4ob00027g
59. Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y, Asano T, Yasuhara H (1998) Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. Stroke 29(1):12–17
60. Kono H, Arteel GE, Rusyn I, Sies H, Thurman RG (2001) Ebselen prevents early alcohol-induced liver injury in rats. Free Radic Biol Med 30(4):403–411
61. Moussaoui S, Obinu MC, Daniel N, Reibaud M, Blanchard V, Imperato A (2000) The antioxidant ebselen prevents neurotoxicity and clinical symptoms in a primate model of Parkinson's disease. Exp Neurol 166(2):235–245. doi:10.1006/exnr.2000.7516
62. Chew P, Yuen DY, Stefanovic N, Pete J, Coughlan MT, Jandeleit-Dahm KA, Thomas MC, Rosenfeldt F, Cooper ME, de Haan JB (2010) Antiatherosclerotic and renoprotective effects of ebselen in the diabetic apolipoprotein E/GPx1-double knockout mouse. Diabetes 59 (12):3198–3207. doi:10.2337/db10-0195
63. Chander PN, Gealekman O, Brodsky SV, Elitok S, Tojo A, Crabtree M, Gross SS, Goligorsky MS (2004) Nephropathy in Zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. J Am Soc Nephrol 15(9):2391–2403. doi:10.1097/01.ASN.0000135971.88164.2C
64. He M, Xing S, Yang B, Zhao L, Hua H, Liang Z, Zhou W, Zeng J, Pei Z (2007) Ebselen attenuates oxidative DNA damage and enhances its repair activity in the thalamus after focal cortical infarction in hypertensive rats. Brain Res 1181:83–92. doi:10.1016/j.brainres.2007.08.072
65. Yamagata K, Ichinose S, Miyashita A, Tagami M (2008) Protective effects of ebselen, a seleno-organic antioxidant on neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rat. Neuroscience 153(2):428–435. doi:10.1016/j.neuroscience.2008.02.028
66. Moutet M, d'Alessio P, Malette P, Devaux V, Chaudiere J (1998) Glutathione peroxidase mimics prevent TNFalpha- and neutrophil-induced endothelial alterations. Free Radic Biol Med 25(3):270–281
67. Erdelmeier I, Chaudiere J, Moutet M, Yadan J-C (1999) Compounds having a benzisoselenazoline and -azine structure, method for preparing same and therapeutic uses thereof. United States Patent 5,968,920
68. Lange RW (2000) BXT-51072 OXIS international. Curr Opin Investig Drugs 2(4):338–341
69. Oxis International, Inc. (2005) Form 10-QSB quarterly report pursuant to Section 13 or 15 (d) of the Securities Exchange Act of 1934 for the quarterly period ended March 31, 2005
70. Short MD, Xie Y, Li L, Cassidy PB, Roberts JC (2003) Characteristics of selenazolidine prodrugs of selenocysteine: toxicity and glutathione peroxidase induction in V79 cells. J Med Chem 46(15):3308–3313. doi:10.1021/jm020496q
71. Li L, Xie Y, El-Sayed WM, Szakacs JG, Roberts JC (2004) Characteristics of selenazolidine prodrugs of selenocysteine: toxicity, selenium levels, and glutathione peroxidase induction in A/J mice. Life Sci 75(4):447–459. doi:10.1016/j.lfs.2003.12.018
72. Franklin MR, Moos PJ, El-Sayed WM, Aboul-Fadl T, Roberts JC (2007) Pre- and post-initiation chemoprevention activity of 2-alkyl/aryl selenazolidine-4(R)-carboxylic acids against tobacco-derived nitrosamine (NNK)-induced lung tumors in the A/J mouse. Chem Biol Interact 168(3):211–220. doi:10.1016/j.cbi.2007.04.012

73. Poerschke RL, Moos PJ (2011) Thioredoxin reductase 1 knockdown enhances selenazolidine cytotoxicity in human lung cancer cells via mitochondrial dysfunction. Biochem Pharmacol 81(2):211–221. doi:10.1016/j.bcp.2010.09.024
74. Spallholz JE, Reid TW (1998) Method for the preparation of free radical pharmaceuticals using selenium conjugates. United States Patent 5,783,454
75. Spallholz JE, Reid TW (2000) Method for the preparation of free radical pharmaceuticals diagnostics and devices using selenium conjugates United States Patent 6,077,714
76. Spallholz JE, Reid TW (1999) Selenium carrier conjugates. United States Patent 5,994,151
77. Tran PL, Hammond AA, Mosley T, Cortez J, Gray T, Colmer-Hamood JA, Shashtri M, Spallholz JE, Hamood AN, Reid TW (2009) Organoselenium coating on cellulose inhibits the formation of biofilms by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Appl Environ Microbiol 75(11):3586–3592. doi:10.1128/AEM. 02683-08
78. Tran P, Hamood A, Mosley T, Gray T, Jarvis C, Webster D, Amaechi B, Enos T, Reid T (2013) Organo-selenium-containing dental sealant inhibits bacterial biofilm. J Dent Res 92 (5):461–466. doi:10.1177/0022034513482141
79. Kunstelj M, Menart V, Ambrozic G, Gaberc-Porekar V (2013) Selenium containing modifying agents and conjugates. United States Patent 8,389,695
80. Miki K, Xu M, Tan Y (2005) Selenium-containing pro-drugs for cancer therapy. United States Patent 6,916,793
81. Powis G, Oblong JE, Gasdaska PY, Berggren M, Hill SR, Kirkpatrick DL (1994) The thioredoxin/thioredoxin reductase redox system and control of cell growth. Oncol Res 6 (10–11):539–544
82. Biguet C, Wakasugi N, Mishal Z, Holmgren A, Chouaib S, Tursz T, Wakasugi H (1994) Thioredoxin increases the proliferation of human B-cell lines through a protein kinase C-dependent mechanism. J Biol Chem 269(46):28865–28870
83. Fernandes AP, Capitanio A, Selenius M, Brodin O, Rundlof AK, Bjornstedt M (2009) Expression profiles of thioredoxin family proteins in human lung cancer tissue: correlation with proliferation and differentiation. Histopathology 55(3):313–320. doi:10.1111/j.1365-2559.2009.03381.x
84. Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN (2014) Selenium and selenocysteine: roles in cancer, health, and development. Trends Biochem Sci 39(3):112–120. doi:10.1016/j.tibs.2013.12.007
85. Yoo MH, Xu XM, Carlson BA, Gladyshev VN, Hatfield DL (2006) Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. J Biol Chem 281(19):13005–13008. doi:10.1074/jbc.C600012200
86. Engman L, Cotgreave I, Angulo M, Taylor CW, Paine-Murrieta GD, Powis G (1997) Diaryl chalcogenides as selective inhibitors of thioredoxin reductase and potential antitumor agents. Anticancer Res 17(6D):4599–4605
87. Engman L, Kandra T, Gallegos A, Williams R, Powis G (2000) Water-soluble organotellurium compounds inhibit thioredoxin reductase and the growth of human cancer cells. Anticancer Drug Des 15(5):323–330
88. Zhao R, Masayasu H, Holmgren A (2002) Ebselen: a substrate for human thioredoxin reductase strongly stimulating its hydroperoxide reductase activity and a superfast thioredoxin oxidant. Proc Natl Acad Sci U S A 99(13):8579–8584. doi:10.1073/pnas.122061399
89. Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66:75–87. doi:10.1016/j.freeradbiomed.2013.07.036
90. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, Salmona M, Chang G, Holmgren A, Ghezzi P (2002) Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. Proc Natl Acad Sci U S A 99 (15):9745–9749. doi:10.1073/pnas.152168599
91. Wang L, Yang Z, Fu J, Yin H, Xiong K, Tan Q, Jin H, Li J, Wang T, Tang W, Yin J, Cai G, Liu M, Kehr S, Becker K, Zeng H (2012) Ethaselen: a potent mammalian thioredoxin

reductase 1 inhibitor and novel organoselenium anticancer agent. Free Radic Biol Med 52 (5):898–908. doi:10.1016/j.freeradbiomed.2011.11.034
92. Li DD, He J, Zeng HH (2012) Biological evaluation of novel selenazole-based compounds as potential thioredoxin reductase inhibitors. Appl Organomet Chem 26:619–624. doi:10.1002/aoc.2910
93. Baker AF, Adab KN, Raghunand N, Chow HH, Stratton SP, Squire SW, Boice M, Pestano LA, Kirkpatrick DL, Dragovich T (2013) A phase IB trial of 24-hour intravenous PX-12, a thioredoxin-1 inhibitor, in patients with advanced gastrointestinal cancers. Invest New Drugs 31(3):631–641. doi:10.1007/s10637-012-9846-2
94. Traynor AM, Thomas JP, Ramanathan RK, Mody TD, Alberti D, Wilding G, Bailey HH (2011) Phase I trial of motexafin gadolinium and doxorubicin in the treatment of advanced malignancies. Invest New Drugs 29(2):316–322. doi:10.1007/s10637-009-9364-z
95. Edelman MJ, Otterson G, Leach J, Malpass T, Salgia R, Jones D, Mody TD, Govindan R (2011) Multicenter phase II trial of Motexafin gadolinium and pemetrexed for second-line treatment in patients with non-small cell lung cancer. J Thorac Oncol 6(4):786–789. doi:10.1097/JTO.0b013e31820a443f
96. Mahmood DF, Abderrazak A, El Hadri K, Simmet T, Rouis M (2013) The thioredoxin system as a therapeutic target in human health and disease. Antioxid Redox Signal 19 (11):1266–1303. doi:10.1089/ars.2012.4757
97. Combs GF Jr, Gray WP (1998) Chemopreventive agents: selenium. Pharmacol Ther 79 (3):179–192
98. Combs GF Jr (1999) Chemopreventive mechanisms of selenium. Med Klin 94(Suppl 3):18–24
99. Ganther HE (1999) Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis 20(9):1657–1666
100. Nogueira CW, Rocha JB (2011) Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch Toxicol 85(11):1313–1359. doi:10.1007/s00204-011-0720-3
101. Weekley CM, Harris HH (2013) Which form is that? The importance of selenium speciation and metabolism in the prevention and treatment of disease. Chem Soc Rev 42 (23):8870–8894. doi:10.1039/c3cs60272a
102. Lanfear J, Fleming J, Wu L, Webster G, Harrison PR (1994) The selenium metabolite selenodiglutathione induces p53 and apoptosis: relevance to the chemopreventive effects of selenium? Carcinogenesis 15(7):1387–1392
103. Cho DY, Jung U, Chung AS (1999) Induction of apoptosis by selenite and selenodiglutathione in HL-60 cells: correlation with cytotoxicity. Biochem Mol Biol Int 47(5):781–793
104. Ip C, Ganther HE (1992) Comparison of selenium and sulfur analogs in cancer prevention. Carcinogenesis 13(7):1167–1170
105. Spyrou G, Bjornstedt M, Kumar S, Holmgren A (1995) AP-1 DNA-binding activity is inhibited by selenite and selenodiglutathione. FEBS Lett 368(1):59–63
106. Ren X, Bjornstedt M, Shen B, Ericson ML, Holmgren A (1993) Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. Biochemistry 32(37):9701–9708
107. Bjornstedt M, Kumar S, Holmgren A (1995) Selenite and selenodiglutathione: reactions with thioredoxin systems. Methods Enzymol 252:209–219
108. Nilsonne G, Olm E, Szulkin A, Mundt F, Stein A, Kocic B, Rundlof AK, Fernandes AP, Bjornstedt M, Dobra K (2009) Phenotype-dependent apoptosis signalling in mesothelioma cells after selenite exposure. J Exp Clin Cancer Res 28:92. doi:10.1186/1756-9966-28-92
109. Asfour IA, El-Tehewi MM, Ahmed MH, Abdel-Sattar MA, Moustafa NN, Hegab HM, Fathey OM (2009) High-dose sodium selenite can induce apoptosis of lymphoma cells in adult patients with non-Hodgkin's lymphoma. Biol Trace Elem Res 127(3):200–210. doi:10.1007/s12011-008-8240-6

110. Wang L, Bonorden MJ, Li GX, Lee HJ, Hu H, Zhang Y, Liao JD, Cleary MP, Lu J (2009) Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit. Cancer Prev Res 2(5):484–495. doi:10.1158/1940-6207.CAPR-08-0173
111. Yan L, DeMars LC (2012) Dietary supplementation with methylseleninic acid, but not selenomethionine, reduces spontaneous metastasis of Lewis lung carcinoma in mice. Int J Cancer 131(6):1260–1266. doi:10.1002/ijc.27355
112. Hu H, Jiang C, Ip C, Rustum YM, Lu J (2005) Methylseleninic acid potentiates apoptosis induced by chemotherapeutic drugs in androgen-independent prostate cancer cells. Clin Cancer Res 11(6):2379–2388. doi:10.1158/1078-0432.CCR-04-2084
113. Li Z, Carrier L, Belame A, Thiyagarajah A, Salvo VA, Burow ME, Rowan BG (2009) Combination of methylselenocysteine with tamoxifen inhibits MCF-7 breast cancer xenografts in nude mice through elevated apoptosis and reduced angiogenesis. Breast Cancer Res Treat 118(1):33–43. doi:10.1007/s10549-008-0216-x
114. Ip C, Thompson HJ, Zhu Z, Ganther HE (2000) In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Res 60(11):2882–2886
115. Bhattacharya A (2011) Methylselenocysteine: a promising antiangiogenic agent for overcoming drug delivery barriers in solid malignancies for therapeutic synergy with anticancer drugs. Expert Opin Drug Deliv 8(6):749–763. doi:10.1517/17425247.2011.571672
116. Hoffman JL, McConnell KP, Carpenter DR (1970) Aminoacylation of Escherichia coli methionine tRNA by selenomethionine. Biochim Biophys Acta 199(2):531–534
117. Redman C, Scott JA, Baines AT, Basye JL, Clark LC, Calley C, Roe D, Payne CM, Nelson MA (1998) Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett 125(1–2):103–110
118. Burke KE, Combs GF Jr, Gross EG, Bhuyan KC, Abu-Libdeh H (1992) The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. Nutr Cancer 17(2):123–137. doi:10.1080/01635589209514180
119. Frenkel GD, Caffrey PB (2001) A prevention strategy for circumventing drug resistance in cancer chemotherapy. Curr Pharm Des 7(16):1595–1614
120. Caffrey PB, Frenkel GD (2000) Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo. Cancer Chemother Pharmacol 46(1):74–78. doi:10.1007/s002800000127
121. Miki K, Xu M, Gupta A, Ba Y, Tan Y, Al-Refaie W, Bouvet M, Makuuchi M, Moossa AR, Hoffman RM (2001) Methioninase cancer gene therapy with selenomethionine as suicide prodrug substrate. Cancer Res 61(18):6805–6810
122. Pinton S, Bruning CA, Sartori Oliveira CE, Prigol M, Nogueira CW (2013) Therapeutic effect of organoselenium dietary supplementation in a sporadic dementia of Alzheimer's type model in rats. J Nutr Biochem 24(1):311–317. doi:10.1016/j.jnutbio.2012.06.012
123. Pinton S, Souza AC, Sari MH, Ramalho RM, Rodrigues CM, Nogueira CW (2013) p, p′-Methoxyl-diphenyl diselenide protects against amyloid-beta induced cytotoxicity in vitro and improves memory deficits in vivo. Behav Brain Res 247:241–247. doi:10.1016/j.bbr.2013.03.034
124. Shin KM, Shen L, Park SJ, Jeong JH, Lee KT (2009) Bis-(3-hydroxyphenyl) diselenide inhibits LPS-stimulated iNOS and COX-2 expression in RAW 264.7 macrophage cells through the NF-kappaB inactivation. J Pharm Pharmacol 61(4):479–486. doi:10.1211/jpp/61.04.0010
125. Zasso FB, Goncales CE, Jung EA, Araldi D, Zeni G, Rocha JB, Nogueira CW (2005) On the mechanisms involved in antinociception induced by diphenyl diselenide. Environ Toxicol Pharmacol 19(2):283–289. doi:10.1016/j.etap.2004.08.003
126. Nogueira CW, Rocha JBT (2010) Diphenyl diselenide a Janus-faced molecule. J Braz Chem Soc 21(11):2055–2071. doi:10.1016/j.bbr.2013.03.034

127. Iwaoka M, Kenta A (2013) From sulfur to selenium. A new research arena in chemical biology and biological chemistry. Curr Chem Biol 7:2–14
128. Reich HJ (1978) Organoselenium oxidations. Oxidation in organic chemistry part C, pp 1–130
129. Xiao H, Parkin KL (2006) Induction of phase II enzyme activity by various selenium compounds. Nutr Cancer 55(2):210–223. doi:10.1207/s15327914nc5502_13
130. Steinmann D, Nauser T, Beld J, Tanner M, Gunther D, Bounds PL, Koppenol WH (2008) Kinetics of tyrosyl radical reduction by selenocysteine. Biochemistry 47(36):9602–9607. doi:10.1021/bi801029f
131. Labunskyy VM, Hatfield DL, Gladyshev VN (2014) Selenoproteins: molecular pathways and physiological roles. Physiol Rev 94(3):739–777. doi:10.1152/physrev.00039.2013
132. Murphy MP (2014) Antioxidants as therapies: can we improve on nature? Free Radic Biol Med 66:20–23. doi:10.1016/j.freeradbiomed.2013.04.010

Top Med Chem (2016) 17: 119–154
DOI: 10.1007/7355_2015_87

Published online: 27 June 2015

Selenium-Functionalized Molecules (SeFMs) as Potential Drugs and Nutritional Supplements

Rob Abdur and Zhen Huang

Abstract Selenium (Se) is an essential element to humans and animals. Since its diverse functions were discovered, its importance has been more and more revealed. After the discovery of selenium as a key component of many proteins, significant research efforts have been focused on its therapeutic and nutritional potentials and possible impacts on human and animal health. To date, 25 selenoprotein genes have been identified in humans and animals, where selenium exists in the form of an amino acid, selenocysteine (Sec). Sec incorporation into selenoproteins occurs through a unique process by recognizing UGA stop codon via the Sec-charged tRNA for delivering Sec into proteins. Most selenoproteins are antioxidant proteins and play critical roles in maintaining redox balance in living systems by removing reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). These reactive species are major threats to bio-macromolecules, such as DNAs, RNAs, proteins, lipids, and carbohydrates. They may cause molecular oxidation and DNA damage/mutation, alter gene expression patterns, weaken the immune system, and even lead to neurological disorders and other pathological conditions. As potential drugs and/or nutritional supplements, selenium-functionalized molecules (SeFMs) have shown benefits in preventing and/or treating many diseases, such as cancer, cardiovascular diseases, thyroid diseases, diabetes, AIDS, rheumatoid arthritis (RA), and neurodegenerative diseases. These promising results and therapeutic potentials of SeFMs have attracted tremendous attention from academia and pharmaceutical and biotech industries. However, further fundamental research and investigation are required to discover the ideal chemical, biochemical, and biological forms of selenium, appropriate dosages, and mechanisms of the selenium biological actions, since the tolerance window of selenium in humans may be limited.

R. Abdur and Z. Huang (✉)
Department of Chemistry, Georgia State University, P.O. Box 4098, Atlanta, GA 30303, USA

College of Life Sciences, Sichuan University, Chengdu, China
e-mail: huang@gsu.edu

Keywords DNA and RNA, Drug and supplement, Essential element, Nucleic acids, Nucleotides, Selenium functionalization and modification, Selenium-derivatized nucleosides

Contents

1 Introduction

Selenium is an essential trace element for humans and animals. It was discovered in 1817 by Jöns Jakob Berzelius (a Swedish physician and chemist), when he was trying to uncover the etiology of a mysterious disease among workers at a H_2SO_4 plant in Gripsholm (Sweden). The reason that selenium research has become an intense field of research is because this trace element with nutritional importance has unique sets of biological activities. During early years of the twentieth century, selenium was reported as a toxic element that was manifested by feeding livestock forage grown in seleniferous soil. The first evidence of selenium essentiality in living systems appeared in the middle of the twentieth century. Selenium was reported as a nutritional factor in the formation of formic dehydrogenase in *E. coli* and *Aerobacter aerogenes* bacteria [1] and in prevention of exudative diathesis in chicks [2]. Around the same time, Klaus Schwarz and Calvin M. Foltz reported that selenium could prevent liver necrosis in rats fed with torula yeast grown in a selenium-rich medium [3]. Soon thereafter, selenium deficiency was implicated as a factor for the development of several diseases, such as white muscle disease of calves, lambs and foals, and exudative diathesis of poultry. In the late 1960s, epidemiological surveys in different parts of the United States suggested an inverse correlation between the incidences of certain cancers (particularly breast

cancer) and the selenium content of the plants growing in different regions. It took about 15 years after the establishment of selenium essentiality in human health to determine one biological role of selenium. The real excitement appeared in 1973 when selenium was found as a natural part of a redox enzyme, glutathione peroxidase (GPx) [4]. Subsequently, two other selenium-containing redox enzymes, formate dehydrogenase [5] and glycine reductase of *Clostridium thermoaceticum* (belonging to the glutathione peroxidase family), were identified [6]. Analysis of the glycine-reductase complex labeled with ^{75}Se found an oxygen-labile chromophore, which implied the ionized -SeH group of the selenocysteine (Sec) residue [7]. Later, many other studies confirmed the existence of selenium in selenoproteins in the chemical form of selenocysteine [8, 9]. Sec is a cysteine (Cys) analog that differs from Cys by only one atom (selenium versus sulfur), and yet this switch dramatically influences the important aspects of the enzyme reactivity [10]. As Sec is invariably recognized by a codon and has its unique Sec-tRNA and other translation factors, Sec is referred as the 21st amino acid. Besides the glutathione peroxidase family, type 1 iodothyronine deiodinase (a Sec-containing enzyme), which catalyzes the hepatic conversion of thyroxine (T4) to triiodothyronine, was reported in the late 1980s [11, 12].

The puzzle of how selenium incorporates into the amino acid Sec and how Sec is stably co-translated into selenoprotein became clear in 1986. The mRNA sequences of glutathione peroxidase (*gpx*) of mouse [13] and formate dehydrogenase (*fdhF*) of *E. coli* [14] revealed an in-frame UGA codon (termination codon), which encodes for Sec. Simultaneously, another *cis*-acting element called Sec-insertion sequence (SECIS) element was identified at the 3′-untranslated region of mRNA, which forms the secondary structure and helps Sec-tRNA^{Sec} to get position at the UGA codon [15, 16]. To date, 25 selenoproteins (Table 1) have been identified and characterized with various functions, such as hormone metabolism, selenium transport, antioxidant/redox properties, anti-inflammatory properties, etc. In every selenoprotein, the Sec residue invariably occupies the core position of the active site and plays very diverse but crucial role in enzymatic catalysis. Besides Sec, another selenium-containing amino acid (selenomethionine, Se-Met) also presents in proteins of all genera. But the incorporation of Se-Met into proteins is not regulated as Sec. Se-Met is randomly incorporated into protein in place of Met, and its incorporation rate depends on the availability of Se-Met during protein synthesis. Se-Met replaces methionine (Met) by charging with the same methionine tRNA (tRNA^{Met}), which is efficiently recognized by all translational factors [54, 55]. The stably incorporated Se-Met does not significantly alter the protein structure, but may influence the activity of enzymes, if Se-Met replaces Met in the vicinity of the active site.

The natural occurrence of selenium is also reported in certain tRNAs from bacteria, archaea, and eukaryotes. The modified 5-methylaminomethyl-2-selenouridine (mnm5Se2U) has been reported to commonly occupy at the wobble position of the anticodons of tRNA^{Glu}, tRNA^{Gln}, and tRNA^{Lys} [56–59]. It is thought to play a critical role in fine-tuning of codon–anticodon base pairing by positioning the Se-modified nucleotide at the first position of the anticodon. The proposed

Table 1 Lists of 25 human selenoproteins identified to date

Entry	Selenoprotein	Short name	Main function	Subcellular or body localization
1	Deiodinase type 1	DIO1	To transform the prohormone thyroxine (T_4, 3,5,3′,5′-tetraiodothyronine) to the active hormone (T_3, 3,3′,5-triiodothyronine), convert T_4 to 3,3′,5′-triiodothyronine (rT_3), convert T_3 to 3,3-′-diiodothyronine (T_2), and convert rT_3 to T_2. It can deiodinate both rings	ER (endoplasmic reticulum) membrane, plasma membrane [17, 18]
2	Deiodinase type 2	DIO2	To transform T_4 to T_3 and convert rT_3 to T_2. It can only deiodinate the outer ring	Brain and other tissues [19]
3	Deiodinase type 3	DIO3	To transform T_4 to rT_3 and convert T_3 to T_2. It can only deiodinate the inner ring	Cell and endosome membrane [20]
4	Glutathione peroxidase 1	GPx1/cGPx	To provide antioxidant environment (detoxification of hydrogen peroxide)	Cytoplasm and nucleus [21]
5	Glutathione peroxidase 2	GPx2/GI (gastrointestinal)-GPx2	To provide antioxidant environment (detoxification of hydrogen peroxide)	Cytoplasm [22, 23]
6	Glutathione peroxidase 3	GPx3	Detoxification of hydrogen peroxide	Skeletal muscle other tissues [24]
7	Glutathione peroxidase 4	GPx4/PH (phospholipid hydroperoxide)-GPx	To provide antioxidant environment and protect against lipid peroxidation, redox signaling, and regulatory processes, such as inhibiting lipoxygenases and apoptosis	Cytoplasm, mitochondria, nucleus and cell membranes [25, 26]
8	Glutathione peroxidase 6	GPx6	Detoxification of hydrogen peroxide	Secreted, found only in olfactory epithelium and embryonic tissues [27, 28]
9	Thioredoxin reductase 1	TRxR1	Ubiquitous cytoplasmatic housekeeping enzyme, reduction of thioredoxin and other substrates	Cytoplasm, mitochondria, nucleus [29]
10	Thioredoxin reductase 2	TRxR2	Reduction of thioredoxin, disulfide bond isomerization, thioredoxin/glutaredoxin/glutathione reductase	Mitochondrial [30, 31]
11	Thioredoxin reductase 3	TRxR3	Reduction of thioredoxin, defense against oxidative stress	Mitochondrial, nucleus cytoplasm [32]

12	Selenoprotein H	SelH	Redox-sensing DNA-binding protein, antioxidant defense, and phase II detoxification	Overexpressed in brain, specific location unknown [33]
13	Selenoprotein I	SelI	Unknown	An integral membrane protein [34]
14	Selenoprotein K	SelK	Antioxidant defense, calcium regulation, ER-associated protein degradation	Membrane ER [35, 36]
15	Selenoprotein M	SelM	Thiol disulfide oxidoreductase, formation of disulfide bonds, calcium responses	ER, highly expressed in the brain [37, 38]
16	Selenoprotein N	SelN	Essential for muscle regeneration and satellite cell maintenance	ER membrane [39]
17	Selenoprotein O	SelO	Redox-dependent activity/specific function unknown	Unknown [40]
18	Selenoprotein P	SePP	Se transport and delivery, modulate metal ion-mediated Aβ aggregation, reactive oxygen species (ROS) production, and neurotoxicity	Secreted [41–43]
19	Selenoprotein S	SePS/SelS	Inflammatory response, regulation cytokine production, protection against ER-stress-induced apoptosis	Plasma and ER membrane protein [44, 45]
20	Selenoprotein T	SelT	Intracellular Ca^{2+} mobilization and neuroendocrine secretion	Plasma membrane/Golgi/ER [46]
21	Selenoprotein V	SelV	Redox-related function	Unknown/seminiferous tubules of the testes [34]
22	Selenoprotein W	SelW	Antioxidant, redox metabolism	Cytoplasm, cell membrane [47, 48]
23	Selenophosphate synthetase 2	SPS2	Conversion selenide to selenophosphate	Unknown [49, 50]
24	15 kDa	SeP15	Form complex with UDP-glucose:glycoprotein glucosyltransferase 1, prevent transport of improperly folded glycoproteins out of the ER	ER [51, 52]
25	Thioredoxin glutathione reductase	TGR	Testis-specific enzyme; it can reduce glutathione disulfide, while its specific function unknown	ER [53]

selenium function at the wobble position is supported by the recent research from Zhen Huang and co-workers [60–62]. In addition, the selenium introduction as a heavy atom via Se-Met into protein provides a magnificent tool to solve the phase problem in X-ray crystal structure determination of proteins. In the structure determination, selenium acts as a suitable resonance center for anomalous dispersion in MAD and SAD experiments, which require synchrotron radiation with precise beam wavelength control [63–68]. For nucleic acid structure studies, Zhen Huang and co-workers have developed selenium nucleic acids [61, 62, 68–72] to derivatize nucleic acids and protein–nucleic acid complexes for nucleic acid–protein X-ray crystallography [68].

The selenoproteins have a wide range of pleiotropic effects, ranging from antioxidant and anti-inflammatory properties to the production of active thyroid hormone. In the past 10 years, a large number of research projects have been dedicated to discover the disease-associated polymorphism in selenoprotein genes. Selenium is an essential microelement because of its participation in many important enzymes, and its deficiency hampers or reduces the activities of many enzymes essential for proper physiological functions. The two most notable classes of selenoproteins are antioxidants and deiodinases. The roles of antioxidant proteins are to scavenge or neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the body, where they are continuously generated during normal metabolic and respiratory processes. These ROS and RNS are the major oxidation threats to bio-macromolecules, and they or their by-products may also cause alkylation of DNA, RNA, proteins, lipids, and/or many other biomolecules, which are potentially linked with many diseases, such as cardiovascular diseases, cancers, AIDS, neurodegenerative diseases, thyroid diseases, rheumatoid arthritis, etc. Selenoproteins have been reported to prevent these diseases by removing ROS and RNS. Selenium supplementation may help to maintain a proper level of selenoproteins, thereby reducing the threat of ROS and/or RNS in plasma and preventing many deadly diseases. Selenoproteins have a number of biological functions in oxidoreductions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses. This review focuses on the recent advancement and a few pathological conditions and/or diseased states that may be prevented by or even cured with selenium-functionalized molecules (SeFM; Fig. 1).

2 Naturally Occurring Selenium Proteins

Twenty-five selenoproteins have been identified in humans with different functions in different subcellular locations (Table 1). Selenoproteins are mostly enzymes that constitute two major metabolic pathways (Fig. 2), namely, redox metabolism and thyroid hormone metabolism (discussed later). The selenoproteins with redox activities are ubiquitous and help to maintain redox balance along with other pathways in the body. The selenoprotein GPx operates with a small ubiquitous

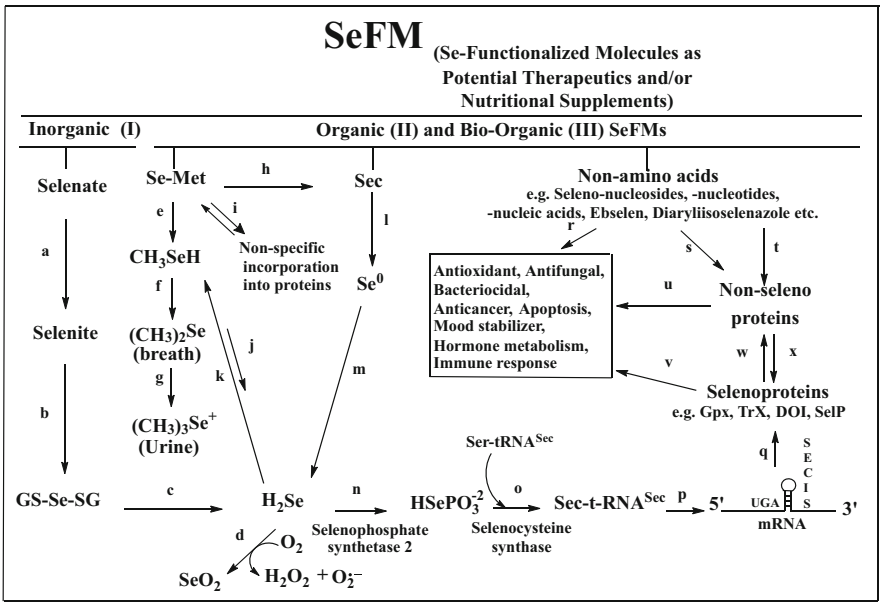


Fig. 1 Potential metabolic mechanisms of Se-functionalized molecules (SeFMs) in the human body. SeFM enters the body in inorganic (**I**), organic (**II**), and/or bio-organic (**III**) forms. Selenium inorganic forms (selenate and selenite taken as supplements) are converted into hydrogen selenide (less reactive) through several reductions (*a*–*c*). The hydrogen selenide is phosphorylated and converted into selenophosphate by selenophosphate synthetase enzyme in an ATP-driven process (*n*). The highly active selenium donor (selenophosphate) donates selenium functionality to the serine-charged $tRNA^{Sec}$, catalyzed by selenocysteine synthase, and produces Sec-$tRNA^{Sec}$. The Sec-$tRNA^{Sec}$ is positioned on an in-frame UGA codon of an mRNA containing SECIS secondary hairpin structure during translation process (*q*), generating selenoproteins. The most common forms of the organic seleno-compounds are Sec and Se-Met, which are derived from the hydrolysis of proteins ingested as diet. Se-Met can be readily incorporated into proteins nonspecifically (*i*). Sec, which can be formed from Se-Met by the *trans*-selenization process (*h*) and derived from hydrolysis of selenoproteins, undergoes several chemical transformations and forms hydrogen selenide via elemental selenium (*l* and *m*). Methylation of the hydrogen selenide could readily occur and produces methyl selenol (CH_3SeH) (*k*), which can also be formed from Se-Met (*e*). Further methylation of methyl selenol (CH_3SeH) produces dimethyl selenide [$(CH_3)_2Se$] and trimethyl selenonium ion [$(CH_3)_3Se^+$], which are excreted from the body through the breath and urine, respectively. The non-amino acid selenium compounds (including organic and bio-organic SeFMs) may also act as potential drugs and/or nutritional supplements [73, 74] in order to supply selenium for maintaining the proper level of selenoproteins and producing beneficial physiological effects [75, 76]

peptide called glutathione (GSH) and forms one of the major pathways to neutralize or reduce reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated continuously during metabolic and respiratory processes. In the GSH–GPx redox system, the sulfur-containing peptide glutathione (GSH) is a cofactor and acts as a reducing substrate in the peroxidase reaction. During the GPx catalytic cycle, the selenenic acid form (GPx–Se-OH) reacts with GSH and

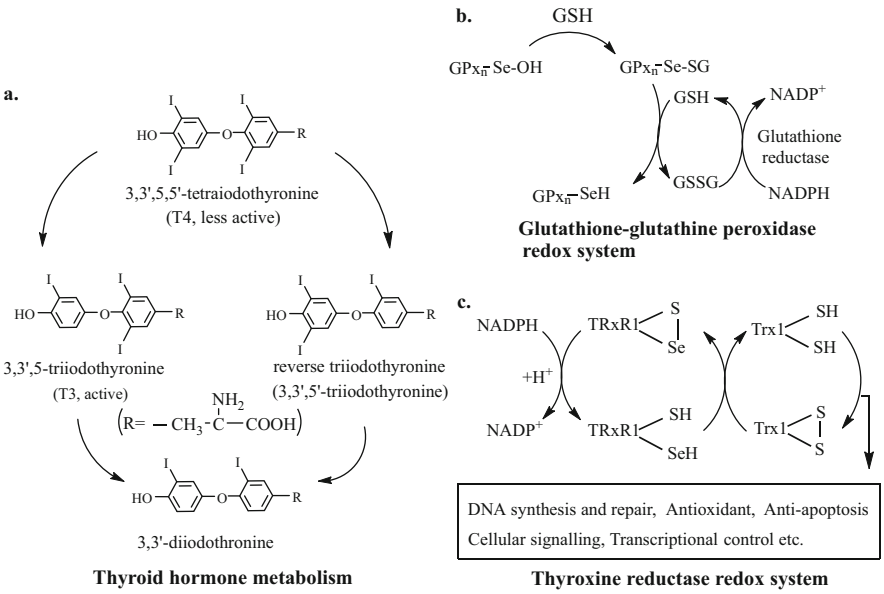


Fig. 2 General catalytic mechanisms of human selenoproteins. Selenoproteins constitute two major classes of enzymes: deiodinases (**a**) and antioxidants (**b** and **c**). Deiodinases are responsible for converting the less-active thyroid hormones T_4 into active form T_3. Among the several redox systems in humans, glutathione–glutathione peroxidase (**b**; $n = 1, 2, 3, 4$, or 6) and thioredoxin–thioredoxin reductase (**c**) redox systems are very important for detoxification of ROS in the body. In the glutathione–glutathione peroxidase redox system, glutathione is a cofactor and acts as a reducing substrate. During the catalytic cycle, oxidized di-glutathione is reduced by the enzyme, glutathione reductase. Thioredoxin–thioredoxin reductase (**c**) system can also detoxify ROS, but this system has broader substrate specificity

generates GPx–selenenyl-sulfide adduct (GPx–Se-SG). Subsequently, the adduct reacts with an additional GSH to regenerate the active selenol (GPx–SeH), which reduces a peroxide molecule (Fig. 2) [77, 78]. Another selenoprotein family, involved in redox defense system, consists of thioredoxin, thioredoxin reductase, and peroxiredoxin. This is also a major redox pathway and plays a diverse role in metabolism with broader substrate specificity. This powerful system is involved in many central intracellular and extracellular processes, including cell proliferation, the redox regulation of gene expression and signal transduction, protection against oxidative stress, anti-apoptotic functions, growth factor and co-cytokine effects, and regulation of the redox state of the extracellular environment [79, 80] (Fig. 2).

Selenium in proteins exists as amino acid selenocysteine (Sec) and selenomethionine (Se-Met). The presence of selenium atom in proteins confers many advantages in protein functionality. Selenium belongs to the same elemental family (VI A) as oxygen and sulfur in the periodic table. Although the selenium atom (atomic radius, 1.16 Å) is bigger than oxygen (atomic radius, 0.73 Å) and sulfur (atomic radius, 1.02 Å) atoms, their chemical and electronic properties are closely related. As a result of this functional similarity with oxygen and sulfur, but

bigger atomic size on the other hand, incorporation of selenium into biomolecules offers many unique and important advantages. Due to the larger electron density, one of the major advantages is that selenium can exist in multiple oxidation states, such as elemental selenium (0), selenide (−2), selenite (+4), and selenate (+6), which largely depend on the chemical properties of the environment. The reactivity of a selenoprotein arises from the selenol (-SeH) side chain of the Sec residue. This SeH functionality provides unique properties to selenoprotein over other residues in the protein. The pK_a value of the SeH group in Sec is lower (pK_a 5.2), compared to the SH group in Cys (pK_a 8.5). As a result of lower pK_a, the SeH side chain is more acidic and remains deprotonated at physiological pH, which makes the SeH a more powerful reductant and stronger nucleophile. Most of the selenoproteins are enzymes, and their Sec residues are conveniently located in or near an active site in the close proximity with Cys residue. The catalytic function of a selenoprotein enzyme is dependent on the interplay or shuttling of electrons among SeH, SH groups, and cofactors (if any).

On the other hand, selenium in Se-Met exists as –Se–CH_3 side chain, and proteins containing Se-Met do not completely depend on the Se function for their activity. Se-Met is an essential amino acid, and it cannot be synthesized de novo in humans. It must be ingested in the form of Se-Met or protein containing Se-Met. Plants and microorganisms can introduce selenium in place of sulfur during Met biosynthesis. In eukaryotes, if Se-Met is available, it can be charged onto $tRNA^{Met}$, which is recognized by all other translational components and stably incorporated into proteins in place of Met. Se-Met incorporation into protein is random, and it depends on the availability of Se-Met [81–83]. Se-Met does not significantly alter the protein structure, but may influence the activity of enzymes, if Se-Met replaces Met in the vicinity of the active site. In contrast, Sec may remain in the free form and cannot be introduced into protein readily. It is incorporated into protein via a specific translational control process, which is unique in relation to the standard protein translation [84]. The Sec incorporation into selenoproteins is discovered in all three domains of life: bacteria, archaea, and eukarya [85]. The pathway for selenocysteine incorporation into protein in archaea and eukarya is very similar to *E. coli*. Selenoprotein synthesis in *E. coli* begins with the acylation of the 3′-end of a special $tRNA^{Sec}$ (encoded by *selC* gene) with L-serine by seryl-tRNA ligase, thus producing seryl-$tRNA^{Sec}$ (Ser-$tRNA^{Sec}$) [86]. The $tRNA^{Sec}$ has many unique and unusual features, such as formation of *O*-phosphoseryl-tRNA [87], its anticodon (UCA) suppressing UGA termination codon [88], longer in size (90 nucleotides in eukaryotes), relatively fewer base modifications [89, 90], and the unique cloverleaf model with a long extra arm [91]. The serine residue on the Ser-$tRNA^{Sec}$ is converted into Sec and forms Sec-$tRNA^{Sec}$ in two-step reactions by the pyridoxal 5′-phosphate-dependent enzyme, selenocysteine synthase (product of *selA gene*, EC 2.9.1.1). In the first step, a Schiff base is formed between α-amino group of serine and carbonyl of pyridoxal 5′-phosphate, followed by 2,3-elimination of a water molecule to yield enzyme-bound dehydroalanyl-$tRNA^{Sec}$ intermediate. In the second step, nucleophilic addition of monoselenophosphate to the double bond of the dehydroalanyl residue produces selenocystyl-$tRNA^{Sec}$ [92–95]. The highly

active Se donor (monoselenophosphate) is formed by transferring γ-phosphate of ATP to hydrogen selenide, which is catalyzed by selenophosphate synthetase (product of *selD*) [96]. Once Sec-tRNA^{Sec} is formed, the Sec-tRNA^{Sec} is recognized by a *cis*-acting element, Sec-insertion sequence (SECIS), a secondary structure on the selenoproteins mRNA that helps in docking Sec-tRNA^{Sec} at UGA codon and directs selenocysteine incorporation into protein [97–99]. A Sec-specific elongation factor (product of *selB* gene) binds to Sec-tRNA^{Sec} and SECIS and guides the incorporation of Sec onto nascent selenoprotein [100–102]. In archaea and eukarya, *O*-phosphoseryl-tRNA^{Sec} kinase phosphorylates the hydroxyl group of serine on seryl-tRNA^{Sec} [103, 104], and the resulting *O*-phosphoseryl-tRNA^{Sec} is subsequently converted to selenocysteinyl-tRNA^{Sec} by Sec synthetase.

3 Naturally Occurring Selenium Nucleic Acids

The natural occurrence of selenium in certain tRNAs was also reported in bacteria [59, 105], archaea [58, 106], mammals [107], and plants [108]. The discovery of the presence of naturally occurring selenium in tRNAs further provides the evidence at the molecular level for the nutritional role of selenium in life. It is not clear whether selenium can be directly incorporated into nucleosides naturally. Nonetheless, the presence of seleno-modified uridine residue (5-methylaminomethyl-2-selenouridine, mnm^{5}Se^{2}U) in tRNAs was reported, and mnm^{5}Se^{2}U occupies the wobble (first) position of the anticodon of the tRNAs for glutamate (tRNA^{Glu}), glycine (tRNA^{Gln}), and lysine (tRNA^{Lys}) in bacteria [56, 59, 109]. This modified nucleoside is thought to play a critical role in the fine-tuning of codon–anticodon base pairing by positioning the Se modification at the first position of the anticodon. In the anaerobic microorganism, *Clostridium sticklandii*, the most prominent selenium-containing tRNA was identified as the major glutamate-accepting species in the bulk tRNA preparation. It has been proposed that the presence of selenium in this tRNA^{Glu} is essential for its aminoacylation activity [58, 106, 110].

4 Artificially Seleno-Modified Nucleosides and Nucleotides and Selenium Nucleic Acids (SeNA)

The presence of the Se modification in natural tRNA has encouraged Huang and co-workers to pioneer and incorporate the selenium functionalities at various selected positions of RNA and DNA, namely, selenium-derivatized nucleic acids (SeNA) [68–71, 111–115]. Their experimental data have demonstrated that nucleic acid oxygen in various positions can be stably replaced with selenium for structure and function studies, especially the facilitation of phase solution, crystallization, and high-resolution structure determination in X-ray crystallography. Their studies

imply that selenium functionalities on nucleobases might improve the accuracy and efficiency of translation, RNA transcription, and even DNA replication by enhancing base-pair interaction, selectivity, and fidelity. For instance, the biochemical study of RNase H with the Se-modified DNA/RNA duplexes showed that the Se-modified nucleobase functionality can enhance the enzyme turnover rate by manyfold [68]. The supporting evidence from X-ray crystallographic structures has revealed that as a larger atom, the selenium functionality causes local unwinding of DNA/RNA substrate duplex. The Se-functionalized DNA acts as a guiding sequence shifting the scissile phosphate of the RNA substrate closer to the active site, therefore further activating the attacking nucleophilic water molecule [68]. Previously, it has been reported that the selenium-modified nucleic acids can resist nuclease digestion [116–118]. Thus, this Se-atom-specific mutagenesis (SAM) has opened a new research area to further explore and design nucleic acid-based novel therapeutics as well as nutritional supplements.

Therapeutic nucleosides, nucleotides, and nucleic acids can be used to block or interfere with specific pathological processes or pathways. The most common targets of these therapeutics are cancers and viruses. The potential oligonucleotide therapeutics, such as small interference RNAs (siRNA), antisense DNAs, or CRISPR-RNA (crRNA) [119, 120], are based on sequence complementarity to the target RNAs (such as mRNAs) or DNAs (such as genomic DNA). This is an intensely advanced area, because the short oligonucleotides can target a specific RNA very precisely and hydrolyze the RNA by Dicer–Argonaute system [121–125] or target a specific DNA by crRNA and Cas system [119, 120, 126]. Potential application of the Se functionalities in short oligonucleotides could offer additional benefits to the oligonucleotides by improving the base-pair stability, selectivity, and fidelity, by enhancing the catalytic rate, and by conferring resistance to nuclease digestion. However, extensive in vivo research is needed in order to take full advantage of all Se modifications, especially the Se-nucleobase functionalities [61, 68, 72, 127].

As we know, nucleoside analogs are a major class of chemotherapeutic agents that disrupt DNA and/or RNA synthesis (or repair) in targeting malignant cancer cells or viruses. Because the proliferative status of a malignant cell is higher than that of a normal cell, the malignant cell always maintains an elevated concentration of nucleosides. Thus, the malignant cells are generally more sensitive to modified nucleoside agents disrupting nucleic acid synthesis. However, research on the Se-modified therapeutic nucleosides is limited; few Se-modified nucleosides have been investigated to target cancers and viruses. Recently, after pioneering and developing selenium nucleic acids (SeNA) for the function and X-ray crystal structure studies [68–71, 111–115], Huang and co-workers have also investigated several Se-modified nucleosides for anticancer activity against various tumor cell lines. They reported that the MeSe nucleosides have relatively fine solubility in aqueous solution, have anticancer effects, and can generally inhibit cancer cell growth (Fig. 3). Among them, 5′-Se-thymidine (**3** in Fig. 3a) is more active than the other Se nucleosides against these prostate cancer cell lines [128]. Recently, Kim and co-workers [129] reported the synthesis of two Se-modified nucleosides:

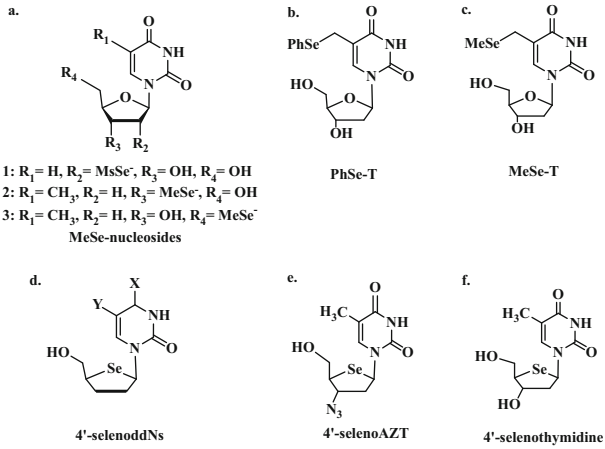


Fig. 3 Chemical structures of selenium-modified nucleosides used as anticancer and antivirus therapy. (**a**) Among these three MeSe nucleosides, compound 3 was more effective as cytotoxic agent. (**b**) and (**c**) PhSe-T and MeSe-T mediate apoptosis induced by p38 pathway. (**d**) 4′-SelenoddN, (**e**) 4′-seleno-AZT, and (**f**) 4′-selenothymidine; they are inhibitors of reverse transcriptase

5-phenylselenyl-methyl-2′-deoxyuridine (5-PhSe-T) and 5-methylselenyl-methyl-2′-deoxyuridine (5-MeSe-T). They have been evaluated for the cytotoxic effect on human cancer cells (Fig. 3b and c). Their caspase activity study suggested that these nucleoside derivatives induced apoptosis by interfering with caspase-2 and caspase-3 pathways and, to a lesser extent, caspase-8 pathway. Another study on HL-60 cells by the same group suggested that the apoptosis induced by 5-PhSe-T and 5-MeSe-T is attributed to p38 pathway. This p38 pathway served as a link between ROS generation and DNA damage/caspase activation [129]. Moreover, several other Se-modified nucleosides have been investigated against HIV as potent inhibitors of reverse transcriptase (Fig. 3d–f) [130].

5 Selenium Deficiency and Diseases

5.1 Thyroid Health

The role of selenium in thyroid function has been established for a long time. In thyroid gland, the amount of selenium per gram of the tissue is the highest among all organs in human body [131, 132]. As an essential micronutrient, selenium is as important as iodine for the production of thyroid hormone thyroxine (or triiodothyronine, T_3) and the maintenance of thyroid hormone homeostasis [133]. Thyroid peroxidase (TPO) is the major enzyme produced by the thyroid gland [134, 135], and it is a heme-containing oxidoreductase that catalyzes stepwise

iodination of the tyrosine residue on thyroglobulin to produce thyroxine or 3,3′,5,5′-tetraiodothyronine (T4). The selenoenzyme iodothyronine deiodinase converts inactive thyroxine (T4) to its more active 3,5,3′-triiodothyronine form (T3) by removing one iodine atom [136, 137]. Three isozymes of the selenoproteins, iodothyronine deiodinase, types 1, 2, and 3 (DIO1, DIO2, and DIO3), have been characterized and cloned [20, 138, 139]. The expression and function of these isozymes are tightly regulated in a tissue-specific manner and represent a new family of eukaryotic selenoproteins. Two other seleno-enzymes, the antioxidant enzyme (such as glutathione peroxidase) and the redox enzyme (such as thioredoxin reductase), are present in thyroid glands in order to maintain proper environment for thyroid activity [140]. The role of glutathione peroxidase in the gland is to protect the thyroid cells from oxidative damage by catalyzing the reduction of H_2O_2 with the help of thiol co-substrates, such as glutathione (GSH). The human TRxR also plays important roles in detoxifying ROS in the thyroid gland [132, 141, 142]. Therefore, adequate supply of both iodine and selenium is necessary for thyroid homeostasis and thyroid health. One of the most common thyroid diseases is hyperthyroidism, also called Graves' disease, often referred to as an overactive thyroid. Hyperthyroidism is a condition where the thyroid gland produces and secretes excessive amounts of thyroid hormones that circulate in the blood [143]. A number of studies have demonstrated an increase in oxidative stress and generation of ROS in Graves' disease and also reported an increased production of malondialdehyde found in urine [144].

In Graves' disease, the balance between intracellular and extracellular oxidants and antioxidants appears to be disturbed, and overall GPx activity has been reported to be diminished [145]. In treating hyperthyroidism, methimazole (MMI), propylthiouracil (PTU), and methyl thiouracil (MTU) are the commonly prescribed drugs for adult and pediatric patients. Recently, the FDA has issued a warning that use of PTU is associated with a higher risk for clinically serious or fatal liver injury compared to MMI in both adult and pediatric patients. There are also clinical study reports on the resistance of MMI in patients with hyperthyroidism in spite of good compliance [146]. In recent years there have been renewed interest is grown in developing selenium analogs of antithyroid drugs, such as, MMI (MSeI), PTU (PSeU), and MTU (MSeU) (Fig. 6). Besides toxicity and drug resistance, there are several other initial assumption was made for the selection of the selenium analog over thiol containing compounds. Selenium is a better nucleophile than sulfur; therefore, the seleno-compound may exhibit higher inhibitory effect toward ID-1, compared to the sulfur drug. As these compounds are anticipated to react with the selenenyl iodide intermediate of ID-1, the formation of Se–Se bond may occur more readily than the formation of Se–S bond [147]. In addition, the seleno-compounds may have a significant effect in neutralizing H_2O_2. There are a few other low-molecular-weight seleno-compounds listed in Fig. 4 that were designed and synthesized by several different groups to mimic Gpx activity as ebselen. These compounds are generated either by modifying the basic structure of ebselen or by incorporating some structural features of the native enzyme. The synthetic GPX mimics reported in the literature include benzoselenazolinones, selenenamide,

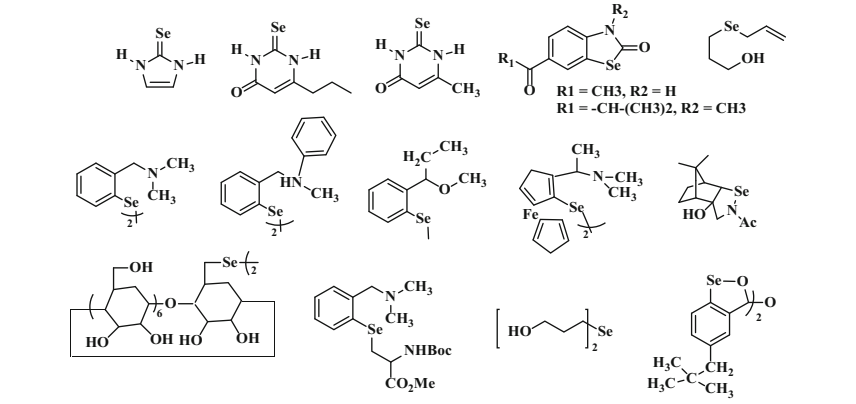


Fig. 4 Chemical structures of SeFMs for the treatment of thyroid diseases

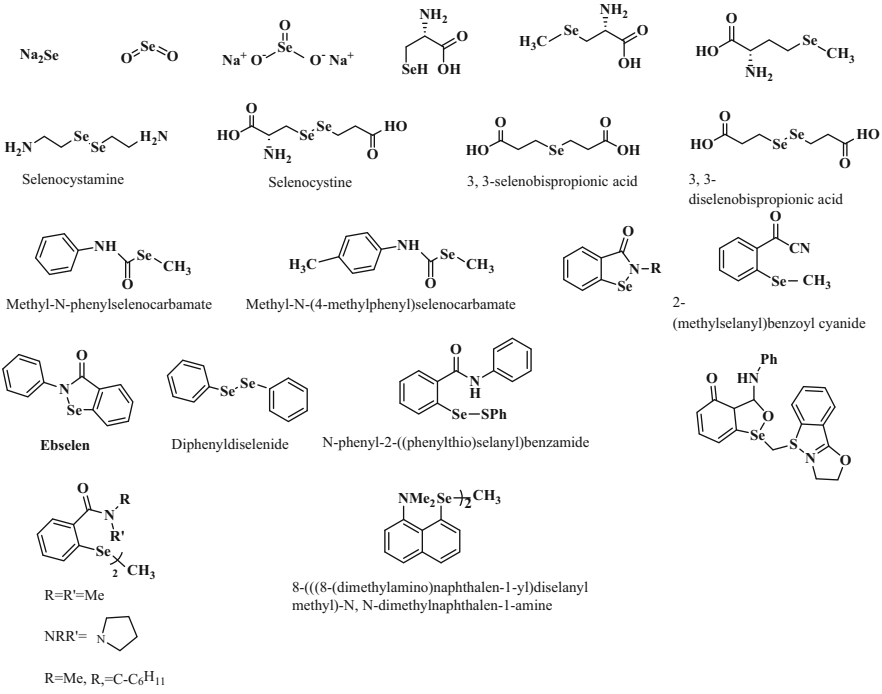


Fig. 5 SeFMs investigated for antioxidant activity, including ROS scavenging for the treatment of cardiovascular disease

diaryl selenide, various diselenides, hydroxyalkyl selenides, a selenocysteine derivative, and a selenenate ester (Fig. 4).

Selenium deficiency is implicated in several other thyroid diseases. Chronic lymphocytic thyroiditis is the most common thyroid autoimmune disease where iodine supply is insufficient. Administration of selenium in the form of selenomethionine or sodium selenite along with levothyroxine substitution therapy improves the pathogenic conditions [148, 149]. The thyroid disease myxoedematous cretinism is also found to associate with selenium deficiency. Myxoedematous cretinism is characterized by the persistence of hypothyroidism despite of supplement with iodine. It has been reported that supplementation with iodine alone has no effect, but when together with selenium, it improves the pathogenic condition of myxoedematous cretinism [150].

5.2 Cardiovascular Disease

Cardiovascular disease (also called heart disease) is the number one disease causing death and disability worldwide [151, 152]. There are diverse causes for cardiovascular disease, but the main causes are arteriosclerosis and hypertension [153]. The manifestation and progression of arteriosclerosis involve the triad: hyperlipidemia, oxidative stress, and inflammation [154]. Hyperlipidemia is the condition of elevated level of low-density lipoprotein (LDL) in the blood, which is eventually deposited in adipose tissues. Lipids are the primary targets for oxidative modification (oxidative stress consequence) enzymatically or by free radicals, because they are the main repository of oxidizable olefinic or double bond [155]. Subsequently, these oxidized lipids activate an NFκB-like transcription factor and induce the expression of genes containing NFκB binding sites, which initiate the production of various cytokines [156, 157] involved in inflammation. The inflammatory response leads to the development of the fatty streak and atherosclerotic plaque. Many studies have clearly demonstrated that selenium can greatly reduce cardiovascular risks (such as arteriosclerosis, myocardial hypertrophy, etc.) and help to maintain cardiac health by enhancing antioxidant role of selenoproteins, especially glutathione peroxidases. These cardioprotective effects are thought to confer through the antioxidant actions of selenoprotein enzymes, which directly limit the levels of ROS (such as hydrogen peroxide, H_2O_2) and reverse the oxidative damages to lipids and proteins [158–160]. It is generally accepted that the GPx level is decreased by the instability of GPx mRNA at low selenium intakes [161, 162].

In the glutathione–GPx redox system, the sulfur-containing peptide glutathione (GSH) is a cofactor and acts as a reducing substrate in the reduction of peroxidases. During the GPx catalytic cycle, the selenenic acid of GPx reacts with GSH to generate GPx–selenenyl-sulfide adduct (GPx–Se-SG). Subsequently, the adduct reacts with an additional GSH to generate the active selenol (GPx–SeH), which in turn reduces peroxide [77, 78]. Another selenoprotein family, involved in redox

defense system, consists of thioredoxin, thioredoxin reductase (TRxR), and peroxiredoxin, which play a pivotal role in both hematopoiesis and heart function. Among three types of TRxR in humans, the cytosolic TRxR1 and mitochondrial TRxR2 are required for protecting heart from ROS or free radical-mediated damages [163, 164]. The primary function of the TRxR system in normal cell is to keep thioredoxin (Trx) in reduced state, serve as a redox regulator for cell signaling, and contribute to the antioxidant defense to cells [165, 166].

SeFMs as potential therapeutics and nutritional supplements have been reported to prevent cardiovascular risks and/or to treat cardiovascular diseases. Some SeFMs (Fig. 5) have been studied for preventing or treating cardiovascular diseases by many research laboratories. Though the exact mechanisms of actions for these compounds are not clear, it has been proposed that the cardioprotective effects may be attributed to the antioxidant defense [167]. One particular organo-seleno-compound, diphenyl diselenide (DPDS), has been reported to inhibit human LDL oxidation, and this phenomenon was related to the thiol-peroxidase activity [168]. Another SeFM, ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one), has been investigated for a long time and is a nontoxic organo-seleno-compound that has anti-inflammatory, anti-atherosclerotic, and cytoprotective properties. Ebselen acts by mimicking the active site of GPx, and it is also an excellent scavenger of peroxynitrite. Moreover, it inhibits activities of cyclooxygenase and lipoxygenases at the micromolar concentrations [169, 170].

5.3 SeFMs for Diabetes Treatment

Diabetes mellitus (DM) affects over 170 million people worldwide, with more than 90% of the patients suffering from type 2 diabetes (T2D) [171]. T2D is a heterogeneous metabolic disorder characterized by increased blood sugar level resulting from the resistance of liver, skeletal muscle, and fat tissue to insulin. These conditions cause dyslipidemia, hyperglycemia, and an increase in insulin secretion by pancreatic beta cells for compensation of the poor insulin response by major target tissues [172–175]. T2D is a major risk factor for the incidence of cardiovascular disease characterized by arteriosclerosis and hypertension [153, 176, 177]. In the United States, 12.9% of the adults older than 20 have T2D, of which 39.8% remain undiagnosed [178, 179]. The relationship between glucose metabolism and selenium status in the body is conflicting [180]. In spite of the antioxidant effect of many selenoproteins in maintaining redox balance in the cellular system, some studies indicate a deleterious effect of selenium in T2D. A cross-sectional study on almost 9,000 American adults, along with other analysis, showed a positive link between high selenium levels and T2D [181, 182]. A similar study performed on 7,182 women from Northern Italy has indicated that the increased dietary selenium intake is associated with an increased risk of T2DM [183]. It has also been suggested that selenium impairs hepatic insulin sensitivity through opposite regulation of ROS [184].

In the insulin signaling pathway, binding of insulin to its receptors initiates a signaling cascade with a mild oxidative burst, where H_2O_2 acts as secondary messenger [180]. In turn, H_2O_2 oxidizes redox-regulated Cys residues, therefore leading to the deactivation of tyrosine phosphatase 1B (PTP-1-B) and tensin homolog protein (PTEN). PTP-1B deactivates insulin receptor substrate (IRS), whereas PTEN inhibits phosphatidylinositol 3-kinase (PI3K). This results in overall stimulation of the signaling pathway for glucose uptake [185–187]. The selenoproteins (such as glutathione peroxidase 1) reduce H_2O_2; therefore, the presence of antioxidant proteins produces an inhibitory action on the signaling cascade [188, 189]. However, it has been observed that insulin combined with selenium significantly decreased blood glucose levels and maintained the expression of insulin receptor substrate (IRS-1), phosphatidylinositol-3 kinase (PI3K), and glucose transporter 4 (GLUT4) in skeletal muscles of diabetic rats [190]. This experimental result indicates that the relation between insulin and selenium still remains a challenging mystery, which requires more investigation.

5.4 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune condition with unknown etiology. It is characterized by chronic inflammatory polyarthritis with progressive erosion of tissues within and surrounding the joints. It affects 1% of the population and is associated with significant morbidity and increased mortality [191]. Recent studies indicate a possible role of selenium on the etiology and disease progression of RA. Clinical data shows that patients with RA have a lower level of selenium in serum, compared to healthy controls [192, 193]. The exact mechanism is unknown, but it is assumed that selenium deficiency affects the signaling pathway of nuclear factor kappa-B (NF-κB), which is correlated with interleukin-6 and TNF-α production [194, 195]. Recently, diaryl isoselenazole compounds (4,5-diaryl isoselenazoles) have been reported (Fig. 6) as multiple target nonsteroidal anti-inflammatory drugs (MTNSAIDs) for the inhibition of inflammatory processes [196]. This SeFM could be a safe alternative as a nonsteroidal anti-inflammatory drug (NSAID), which is associated with serious side effects, such as hypertension, gastrointestinal hemorrhage, ulceration, and kidney failure [197]. The mechanism of NSAIDs is to inhibit cyclooxygenase enzymes (COX-1 and COX-2), which catalyze the biotransformation of arachidonic acid to the prostaglandins [198, 199]. But the new class MTNSAIDs (4,5-diaryl isoselenazoles) have been

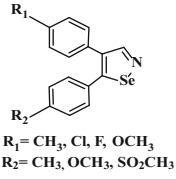


Fig. 6 Diaryl isoselenazole, a new class of SeFMs with dual COX/LO inhibition property

described as dual COX/LO inhibitors with potential radical scavenging by the selenium moiety. This compound is related to the well-known SeFM ebselen with a variety of beneficial properties, such as antioxidant function, radical inactivation, and lipid peroxidase inhibition.

5.5 SeFMs for Cancer Prevention and Treatment

Cancer is becoming an increasingly deadly disease, and there are seven million deaths occurring around the globe each year. According to World Cancer Research Fund and American Institute for Cancer Research, 40% of all cancer can be prevented by the combination of appropriate diets and physical activities. Therefore, cancer management becomes an ideal approach for reduction of cancer mortality by preventing, delaying, or even reversing cancer incidence. Since the initial discovery of selenium as an essential element in humans, the nutritional aspect of selenium has become an intense field of research for connecting the possible role of selenium in cancer prevention. Extensive epidemiological and specific studies have started after the identification of selenoproteins decades ago. Since then, many selenol compounds have been examined, and some have demonstrated potential clinical and molecular role in cancer prevention. A few selenol compounds have been found very effective against many types of cancers, such as lung cancer [200], bladder cancer [201], colorectal cancer [202], liver cancer [203], esophageal cancer [204], gastric–cardiac cancer [205], thyroid cancer [206], and prostate cancers [202, 207–210]. The pivotal role of selenium in cancer prevention is the antioxidant defense, which protects the cells against ROS. Both inorganic forms (such as sodium selenite: Na_2SeO_3) and organic forms of selenium (such as selenocysteine or selenomethionine) have shown effectiveness in antioxidant defense [76]. Several mechanisms have been proposed for the selenium-mediated anticancer effects. The major mechanisms are (1) the reduction of DNA damage [211, 212], oxidative stress [213–218], and inflammation [219], (2) induction of phase II conjugating enzymes that detoxify carcinogens [220, 221], (3) Se incorporation into selenoproteins, (4) alteration in DNA methylation status of tumor suppressor genes [222–224], and (5) induction of apoptosis and kinase modulation [225–227].

In addition to chemopreventive and anticarcinogenic properties, several selenocompounds have been reported to halt progression and metastasis of various types of cancers [228–230]. Among them, prostate cancer has been studied most extensively with SeFMs. Administration of oral capsules of sodium selenate to patients with prostate cancer significantly boosts the activity of the protein PP2A phosphatase and impedes tumor neovascularization and angiogenesis [231]. Selenium effect has also been examined in ovine pulmonary adenocarcinoma (OPA), as an animal model for studying lung cancer, by treating animals with sodium selenate [232]. Selenium at higher concentration suppresses expression of TrxR in tissues with lung cancer and causes rapid cell death [233]. The possible mechanism is due

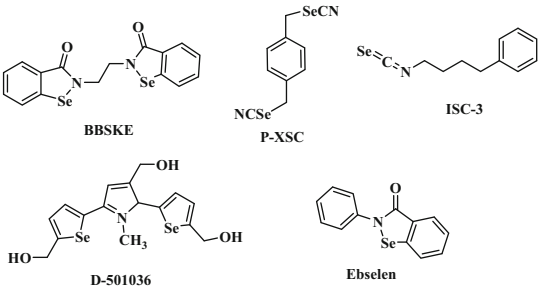


Fig. 7 Examples of seleno-compounds with cytotoxic effect on cancer cells. The Se nucleoside analogs for cancer chemotherapy are in Fig. 3

to an impaired function of SBP2 (SECIS binding protein 2), therefore reducing TrxR production and activity [234]. Methylselenol, a selenium metabolite, has been reported to induce cell cycle arrest in G1 phase and mediate apoptosis via inhibiting extracellular-regulated kinase 1/2 (ERK1/2) pathway and c-Myc expression [235].

More SeFMs are listed in Fig. 7, and they have been examined as cytotoxic agents with anticancer properties. A clinical trial report revealed that the administration of 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) to 40 patients with end-stage cancers has increased predicted survival time in 76% of these cases [236]. The cytotoxic effect of this compound is thought to function by modulating TrxR activity through mitochondrial dysfunction [237]. Another selenol derivative cytotoxic compound acting by Akt3 signaling modulation is isoselenocyanate-4 (ISC-4, similar to ISC-3 in Fig. 7) with a four-carbon alkyl chain. Topical application of ISC-4 on xenografted melanoma mice resulted in reducing tumor cell expansion and development by 80% and increasing apoptosis rates by threefold [238]. Another heterocyclic selenium compound, 1,2-[bis(1,2-benzisoselenazolone-3-(2*H*)-ketone)]ethane (BBSKE), has been reported to induce apoptosis of the tongue cancer Tca8113 cells by activating caspase-3 pathway. BBSKE is structurally related to ebselen, a thioredoxin reductase inhibitor (Fig. 7) [239].

5.6 SeFMs for HIV Treatment

HIV belongs to the retrovirus family. It carries a single copy of genomic RNA and RNA genes required for reproduction and pathogenesis. The HIV infection process begins with attachment of viral protein gp120 to the specific type of CD4 receptor and a co-receptor on the surface of the CD4 cell. Upon its entry into cells, the viral RNA genome is reversely transcribed into DNA (cDNA) by reverse transcriptase (viral protein), followed by integration of the double-stranded DNA into the host genome. The picture of the HIV-infected population is dire. According to the recent estimates from UNAIDS, there were 35.3 million people living with HIV in 2012, which was up from 29.4 million in 2001. HIV is a leading cause of death worldwide, and the number one cause of death by disease in Africa. Sub-Saharan Africa is most heavily affected by HIV and AIDS than any other regions in the world. An

estimated 22.9 million people live with HIV in this region, which is approximately two-thirds of the total number. In 2012, 1.6 million deaths were caused by AIDS worldwide. Though enormous research has been performed on many aspects of HIV/AIDS, critical research on nutritional aspect (especially micronutrient) on HIV epidemic has not been done thoroughly. There are growing concerns on why HIV infection, disease progression, and mortality rates are much higher in the underdeveloped areas in the world. Recently, a randomized clinical trial on the effect of micronutrient supplementation on HIV-infected ART (antiviral treatment) has been reported. It has been found that the combined supplementation with selenium and vitamins B, C and E largely improved the CD4 cell counts of the HIV-infected patients. A supplement containing multivitamins and selenium was found to be safe. The supplement significantly reduced the risk of immune decline and morbidity, if the supplement treatment was started in the early stages of HIV infection [240].

The HIV-infected individuals are immunocompromised. The mechanisms on why vitamins and selenium exert immune stimulatory effects are not fully elucidated, but the role of each micronutrient has been documented. The role of selenium in HIV infection, activation, and disease progression has been investigated in human and animal models extensively [241]. Like many other pathological conditions, oxidative stress is also a potent inducer of HIV activation and DNA damage to the infected cells [242]. The antioxidant properties of selenoproteins are assumed to play an important regulatory role in immune response [243, 244]. Since HIV infection causes the inflammatory response, many inflammatory cytokines are elevated during HIV infection [245], which include tumor necrosis factor-alpha (TNFα), interferon-α, and various interleukins (IL-1, IL-4, IL-6, IL-10) [246]. Elevation of TNFα was related with viral activation, a high level of free radicals, and DNA damage [242].

Oxidative stress is also reported to upregulate the activation of viral replication through the actions of nuclear factor kappa-B (NFκB) [247, 248]. All of these free radical productions lead to body-wide inflammation and depletion of primary antioxidants that cells use to protect themselves against free radicals. In addition, various free radicals generated from inflammatory response cause hypermutation of the viral genome, which lead to the generation of HIV with new pathogenesis. Several identified selenoproteins can decrease the viral replication by mitigating the oxidative stress. It has been found that the levels of selenoproteins that are expressed in T cells, including thioredoxin reductase, glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidases, are increased in the presence of selenium supplements [249–253]. Unlike some Se-containing compounds used as potential antioxidant supplements, several Se-modified nucleosides have been reported as potential antiviral agents. The 2′,3′-dideoxy-4′-selenonucleosides [254] and 4′-seleno-AZT [255], which are bioisosteric analogs of 2′,3′-dideoxynucleosides (ddNs) and AZT, have been reported as potential inhibitors of HIV reverse transcriptase (Fig. 3).

5.7 SeFMs for Tropical Disease Treatment

Tropical diseases refer to diseases that occur solely or principally in the tropics, such as tuberculosis, malaria, leishmaniasis, schistosomiasis, onchocerciasis, lymphatic filariasis, Chagas disease, African trypanosomiasis, and dengue [256, 257]. As a result of selenium functionality being a natural component of antioxidants and redox proteins in mammals, many experiments have been designed to observe the effect of selenium on tropical diseases as a preventive agent and cure. Among the different diseases that constitute tropical disease, only tuberculosis (TB) and leishmaniasis are discussed here in relation to potential selenium drugs.

Leishmaniasis is a tropical disease, prevalent in the Southeast Asia and East Africa. It occurs all over the world, but the form of leishmaniasis is different from one region to another [258, 259]. Leishmaniasis is caused by several species of flagellated protozoa belonging to the genus *Leishmania* in the *Trypanosomatidae* family, whose members are characterized by the presence of the kinetoplast, a unique form of mitochondrial DNA [260]. Leishmaniasis can be caused by over 20 *Leishmania* species, and it is transmitted to humans through the bite of infected female phlebotomine sand flies [261, 262]. The World Health Organization (WHO) estimates that leishmaniasis results in two million new cases a year and threatens 350 million people in 88 countries. There are 12 million people currently infected worldwide [261, 263]. Recently, the trace element selenium has been identified as a protective agent against oxidative damage caused by *Leishmania* infection [264, 265]. The biochemical pathways that constitute the cellular targets for selenium are still under investigation.

A class of compounds, which were originally developed as anticancer drugs, has been found very effective as antiprotozoal agents (Fig. 8) [265–268]. These selenocompounds were synthesized and reported as potential antileishmanial drugs by Carmen Sanmartín's laboratory. It has also been reported that between the selenocyanide (Fig. 8a) and diselenide (Fig. 8b) derivatives, the diselenide compound with aryl ring substitution (especially with 4-aminophenyl) showed better cytotoxic effects, compared to selenocyanide [265]. Another class of imidothiocarbamate and imidoselenocarbamate compounds (Fig. 8c) has been synthesized and tested against leishmaniasis. Among various substitutions, the imidoselenocarbamate compound (especially with X = Se, Y = N, R = methyl, and R′ = 2Cl groups) was very effective as an antileishmaniasis agent [264]. In 2013, the same laboratory has synthesized and tested a new class of compounds for the treatment of leishmaniasis. The new compound was designed based on the 4,4′-diselanediyldianiline (Fig. 8d) with sulfonamide moieties bound to different rings (Fig. 8e) [269]. Their data suggest that compounds with R group, such as 4-methylphenyl, 4-flurophynyl, or 8-quinolinyl, exhibit high leishmanicidal activity and low cytotoxicity, which are required for antileishmanial drugs.

Tuberculosis (TB) is a highly infectious disease. The etiological agent of TB is *Mycobacterium tuberculosis*. The notoriety of this highly infectious disease is rested on the fact that the *Mycobacterium tuberculosis* could develop multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains within

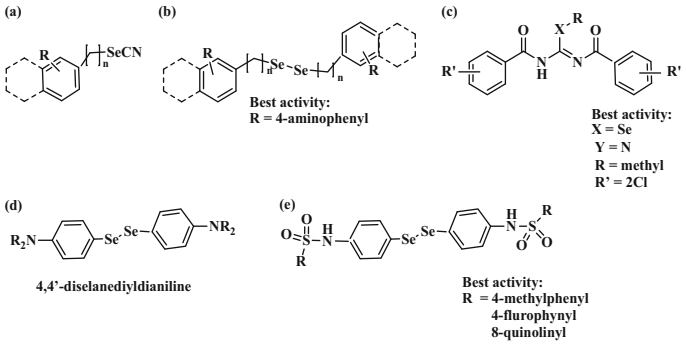


Fig. 8 Selenium containing antiprotozoal agents. (a) Selenocyanide, (b) Diselenide derivatives (best cytotoxic effect with aryl ring substitution with 4-aminophenyl), (c) Imidothiocarbamate and imidoselenocarbamate compounds (among various substitutions, X = Se, Y = N, R = methyl, R' = 2Cl was shown very effective), (d) 4,4′-diselanediyldianiline (e) 4,4′-diselanediyldianiline with sulfonamide moieties bound to different rings

the long treatment regimen [270]. The bacteria have evolved drug resistance mechanisms through a persistent phenotype and mutations in drug target genes [271]. Recently, a new target, the antigen 85 (Ag85) protein family consisting of Ag85-A, Ag85-B, and Ag85-C, has been identified. Ag85 has mycoloyl transferase activity, which produces an envelope lipid called trehalose dimycolate (TDM) and cell wall with arabinogalactan-linked mycolic acids, and it is required for cell wall synthesis [272–274]. The organoselenium compound ebselen has been reported to inactivate the Ag85 activity by covalently linking with a cysteine residue located near the mycoloyl transferase active site. Mutational and crystal structure studies indicate that linking ebselen to the cysteine residue disrupts the hydrogen bonding network within the active site that is essential for enzymatic activity [275]. Tuberculosis often coincides with nutritional deficiencies. The effect of selenium on tuberculosis has also been explored in the nutritional aspect. It has been reported that a 2-month intervention with vitamin E and selenium supplementation reduced oxidative stress and improved the outcome in patients undergoing TB chemotherapy [276, 277].

5.8 SeFMs for Neurodegenerative Disease Treatment

Free radicals or, more generally, reactive oxygen species are generated continuously during the process of normal oxidative metabolism. There is growing evidence that free radicals play an important role in onset and progression of several neurodegenerative diseases. Those diseases include cerebral ischemia, Parkinson's disease, Huntington's disease, epilepsy, amyotrophic lateral sclerosis, Down's syndrome, and Alzheimer's disease (AD) [278–281]. Among these neurodegenerative diseases, Alzheimer's disease (AD) is among the most pressing health

concerns. The pathology of AD is characterized by the formation of large insoluble plaques made of misfolded amyloid beta (Aβ) protein. Amyloid beta (Aβ) protein is a short peptide that is a proteolytic by-product of the transmembrane protein amyloid precursor protein (APP), whose function is unclear, but thought to be involved in neuronal development [282, 283]. The experimental data suggests that beta-amyloid aggregation and toxicity are probably caused by the cellular oxidative stress, which plays a key role in the conversion of soluble to insoluble beta-amyloid, suggesting that the oxidative stress is primary to the beta-amyloid cascade [284, 285].

The effect of selenium on neurodegenerative diseases has been investigated. It has been found that the brain is enriched with selenium and its level declines with age, which may be linked to cognitive impairment and AD development. Administration of selenium in a rat model of acute dementia is found to reduce tau phosphorylation and improve memory loss, which implies the neuroprotective role of selenium [37, 286]. It has also been reported that selenium could attenuate Aβ-induced neuronal death by reducing amyloidogenic β- and γ-secretase activities [286]. Gene knockdown experiments have revealed a potent effect of glutathione peroxidase 4, which protects cells against lipid hydroperoxide damage, the characteristic feature of neurodegenerative disease and AD [287]. A number of studies suggest that GPx4 is an antioxidant enzyme possessing broader substrate specificity than other glutathione peroxidases. It accepts many reductant substrates in addition to glutathione and reacts with a wide array of organic and inorganic peroxides [25, 288, 289]. The neuroprotective role of SelP protein has also been investigated. SelP protects neuronal cells from Aβ-induced toxicity, suggesting a neuroprotective role for SelP in preventing neurodegenerative disorders in AD [290, 291]. Consistently, several studies have demonstrated the significantly lower Se levels (micronutrient status) in plasma, erythrocytes, and nails in AD patients [292]. The therapeutic effect of *p*,*p*′-methoxyl-diphenyl diselenide [$(MeOPhSe)_2$], a SeFM, against streptozotocin (STZ)-induced sporadic dementia of Alzheimer's type (SDAT) in rats has been evaluated [293]. Several interesting observations from $(MeOPhSe)_2$ supplementation on SDAT rats have been reported. The results indicate that dietary supplementation with $(MeOPhSe)_2$ reverts STZ-induced memory impairment in rats and also reverts oxidative stress in the STZ group by decreasing reactive species and tyrosine nitration levels and enhancing nonprotein thiol levels [294]. The dietary supplementation with $(MeOPhSe)_2$ has also shown to normalize acetylcholinesterase (AChE) activity, which was reported to enhance by STZ injection, but did not revert the deficit in cerebral energy metabolism caused by STZ [293, 295].

Huntington's disease (HD) is also a fatal neurodegenerative disorder caused by CAG-repeat expansion encoding a polyglutamine tract in the huntingtin (Htt) protein characterized by motor and psychiatric disturbances and cognitive decline leading to dementia [296, 297]. In HD neurodegeneration, an increase in intracellular reactive oxygen species (ROS) and caspase-3 activity has been reported. Oxidative stress from ROS is primarily implicated to the impaired activity of the major endogenous glutathione redox system. This impaired system causes oxidative damage and formation of 8-hydroxydeoxyguanosine (8-OH-dG) [298, 299] as

a DNA oxidative damage marker, malondialdehyde (MDA) [300] as a lipid peroxidation marker, and protein oxidation [301]. The role of antioxidant defense by glutathione peroxidases in HD has been demonstrated via genome-wide screening, two suppressors of Htt encode glutathione peroxidases, and their overexpression could ameliorate the toxicity of a mutant Htt fragment in yeast [302]. Moreover, studies indicated that mGPx1 overexpression and ebselen treatment mimicing GPx activity can lower the ROS production and significantly reduce caspase-3/caspase-7 activation.

Selenium supplementation and therapeutics have also been examined in treating bipolar disease. Recently, ebselen has been examined as a potential drug for bipolar disease. They reported ebselen as an effective drug candidate inhibiting inositol monophosphatase (IMPase) activity and acting as a lithium mimetic in mouse models of bipolar disorder [303]. Lithium has been used as the most effective mood stabilizer for the treatment of bipolar disorder, but it is toxic at only twice the therapeutic dosage and has many undesirable side effects [304, 305]. The selenium moiety of ebselen irreversibly inhibits IMPase by forming a selenylsulfide (–Se–S–) bond with the cysteine residue, which is conserved in both human and mouse isoforms. It has also been demonstrated that ebselen permeates blood–brain barrier and inhibits endogenous inositol monophosphatase in mouse brain with no selenium toxicity at pharmacological dosage.

Epilepsy, which affects about 2–3% of the general population, is a common chronic neurological disorder with various etiological factors [306]. Several causes have been suggested to be responsible for idiopathic epilepsy, such as imbalances in trace elements [307] and genetic reasons, including mutations in ion and non-ion channel genes [308, 309] and mutations in genes involved in the antioxidant system [310]. The impaired antioxidant system, which generates oxidative stress by producing free radicals, has been implicated as a leading cause to seizures or the risk of their recurrence in idiopathic epilepsy [311, 312]. A control case study suggests that the Se and Zn levels are significantly decreased in patients with epilepsy and there are no significant differences in gender or age, which implies the possible cause for impaired antioxidant system in epilepsy patients [313].

6 SeFMs in Clinical Trials

After discovery of several selenocysteine-containing key enzymes in humans and vertebrates, tremendous attention has been paid to evaluate synthetic Se compounds for pharmaceutical applications. Among many Se compounds that have been studied, ebselen is studied and tested most extensively for its therapeutic value in many pathological conditions. Ebselen [2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one] was first synthesized in 1924. In 1984, it was first reported that ebselen has the properties of thiol peroxidase and antioxidant and acts by mimicking glutathione peroxidase (GPx; Fig. 9) [315, 316]. Since then, a large number of research articles have been published: approximately 1000 ebselen-related publications may be found in the NCBI database. Due to its properties as antioxidant [317, 318] and

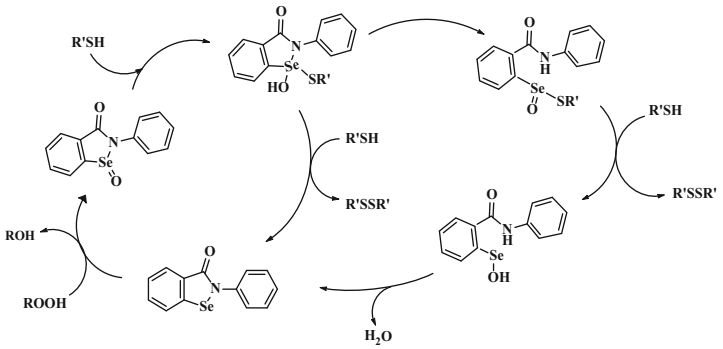


Fig. 9 Mechanism of glutathione peroxidase-like activity of ebselen. R = polyunsaturated phospholipids, cholesterol esters, cholesterol, or any other organic hydroperoxide or hydrogen. R′ = glutathione, dihydrolipoate, *N*-acetylcysteine, or thiol protein residue [314]

anti-inflammatory [319, 320] and its low toxicity in vivo [321], ebselen was first clinically tested for using in the stroke treatment by Daiichi Pharmaceuticals, Japan, in 1997 [322]. Unfortunately, it was not authorized (as harmokisane) for registration by MHLW, the Japanese regulatory authority in therapeutics [323]. Ebselen has a broad range of biological activities due to the GPx-like activity, which catalyzes the reduction of hydrogen peroxide and other hydroperoxides by utilizing glutathione and a variety of other thiols [324]. Ebselen can also reduce the peroxynitrite ($ONOO^-$) to nitrite, thereby diminishing harmful effect of peroxynitrite to cellular constituents in host, when the concentration of peroxynitrite is too high [325]. Ebselen is in the National Institutes of Health Clinical Collection, a chemical library of bioavailable drugs considered clinically safe but without proven use.

Ebselen was studied clinically for treating the late stages of development of stroke. The pathological condition of stroke involves a variety of destructive mechanisms, which include oxygen radical generation [326], lipid peroxidation [326], calcium overload [327, 328], and the generation of inflammatory mediators [329–331]. In vitro and animal studies showed that Ebselen can mimic glutathione peroxidase, react with peroxynitrite, and inhibit acute ischemic stroke-related enzymes, such as cyclooxygenase [332], lipoxygenases [169], NO synthases [333, 334], NADPH oxidase [335–337], and H(+)/K(+)-ATPase [317].

Ebselen is the most characterized for its high bioavailability. It can readily cross the blood–brain barrier and also produce promising results in clinical trials for noise-induced hearing loss (NIHL) along with stroke. NIHL is manifested by the acute exposure to loud noise, which affects several structural elements in auditory hair cells, including cell membrane and intracellular biochemical pathways [338]. Loud noise can reduce GSH and increase the level of oxidized glutathione in the inner ear, therefore leaving it prone to mediated cell damages, which also overwhelm resident detoxification and other antioxidant mechanisms [339, 340]. Intriguingly, GPx activity, which regenerates reduced GSH, has been

reported to decrease following noise exposure. Therefore, administration of ebselen improves NIHL conditions by mimicking GPx activity [341–343].

7 Conclusion and Perspective

Because it is a natural part of proteins and tRNAs, selenium is an essential trace element for living systems. Many selenoproteins (such as GPx, TRxR, and Sel proteins) modulate the redox status and systems. Therefore, selenium deficiency will lead to insufficient and/or nonfunctional selenoprotein production, which disrupts the balance of cellular redox systems, thereby resulting in pathological conditions, such as cardiovascular disease, thyroid disease, cancer, neurological disease, depression, diabetes, pancreatitis, and tropical diseases. Furthermore, the deiodinase selenoprotein family is responsible for maintaining a circulating level of thyroid hormone. As an atypical element, selenium has been successfully incorporated into numerous potential therapeutic agents. Approximately 30,000 research articles on selenium have been published and deposited into the NCBI database, indicating the importance of selenium in human health. However, the tolerance range of elemental selenium in humans is narrow: from 55 μg/day (the adequate intake level) to 400 μg/day (the tolerable upper intake level). Epidemiological and pharmacological studies and randomized clinical trials should be conducted to further investigate the link between Se supplement intake and health benefits.

Clearly, selenium function in living systems and its effects on human health are essential. As selenium naturally occurs in protein and tRNA via replacement of oxygen or sulfur atom(s), we predict that the selenium functionality may also present in genomic DNA to assist genome functions, including DNA condensation and accuracy and efficiency of DNA replication and RNA transcription. Thorough research is required to discover and fully understand the selenium functionalities in living organisms. Moreover, selenium-atom-specific modification (SAM) will transform the design of potential oligonucleotide therapeutics via antisense DNA, siRNA, miRNA, and crRNA strategies, by improving the molecular specificity and substrate cleavage efficiency [68].

References

1. Pinsent J (1954) Biochem J 57:10
2. Schwarz K, Bieri JG, Briggs GM, Scott ML (1957) Proc Soc Exp Biol Med 95:621
3. Schwarz K, Foltz CM (1957) J Am Chem Soc 79:3292
4. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Science 179:588
5. Andreesen JR, Ljungdahl LG (1973) J Bacteriol 116:867
6. Turner DC, Stadtman TC (1973) Arch Biochem Biophys 154:366
7. Cone JE, Del Rio RM, Davis JN, Stadtman TC (1976) Proc Natl Acad Sci U S A 73:2659

8. Jones JB, Stadtman TC (1981) J Biol Chem 256:656
9. Motsenbocker MA, Tappel AL (1982) Biochim Biophys Acta 704:253
10. Hondal RJ, Marino SM, Gladyshev VN (2013) Antioxid Redox Signal 18:1675
11. Beckett GJ, Beddows SE, Morrice PC, Nicol F, Arthur JR (1987) Biochem J 248:443
12. Behne D, Kyriakopoulos A, Meinhold H, Kohrle J (1990) Biochem Biophys Res Commun 173:1143
13. Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR (1986) EMBO J 5:1221
14. Zinoni F, Birkmann A, Stadtman TC, Bock A (1986) Proc Natl Acad Sci U S A 83:4650
15. Zinoni F, Heider J, Bock A (1990) Proc Natl Acad Sci U S A 87:4660
16. Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD et al (1991) Nature 353:273
17. Leonard JL, Rosenberg IN (1978) Endocrinology 103:274
18. Maciel RM, Ozawa Y, Chopra IJ (1979) Endocrinology 104:365
19. Baqui MM, Gereben B, Harney JW, Larsen PR, Bianco AC (2000) Endocrinology 141:4309
20. Baqui M, Botero D, Gereben B, Curcio C, Harney JW et al (2003) J Biol Chem 278:1206
21. Bera S, Weinberg F, Ekoue DN, Ansenberger-Fricano K, Mao M et al (2014) Cancer Res 74:5118
22. Florian S, Wingler K, Schmehl K, Jacobasch G, Kreuzer OJ et al (2001) Free Radic Res 35:655
23. Kipp A, Banning A, Brigelius-Flohe R (2007) Biol Chem 388:1027
24. Baez-Duarte BG, Mendoza-Carrera F, Garcia-Zapien A, Flores-Martinez SE, Sanchez-Corona J et al (2014) Arch Med Res 45:375
25. Imai H, Nakagawa Y (2003) Free Radic Biol Med 34:145
26. Januel C, El Hentati FZ, Carreras M, Arthur JR, Calzada C et al (2006) Biochim Biophys Acta 1761:1228
27. Kryukov GV, Kryukov VM, Gladyshev VN (1999) J Biol Chem 274:33888
28. Choi J, Liu RM, Kundu RK, Sangiorgi F, Wu W et al (2000) J Biol Chem 275:3693
29. Karimpour S, Lou J, Lin LL, Rene LM, Lagunas L et al (2002) Oncogene 21:6317
30. Miranda-Vizuete A, Damdimopoulos AE, Pedrajas JR, Gustafsson JA, Spyrou G (1999) Eur J Biochem 261:405
31. Prasad R, Chan LF, Hughes CR, Kaski JP, Kowalczyk JC et al (2014) J Clin Endocrinol Metab 99:E1556
32. Lee SR, Kim JR, Kwon KS, Yoon HW, Levine RL et al (1999) J Biol Chem 274:4722
33. Novoselov SV, Kryukov GV, Xu XM, Carlson BA, Hatfield DL, Gladyshev VN (2007) J Biol Chem 282:11960
34. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O et al (2003) Science 300:1439
35. Verma S, Hoffmann FW, Kumar M, Huang Z, Roe K et al (2011) J Immunol 186:2127
36. Shchedrina VA, Everley RA, Zhang Y, Gygi SP, Hatfield DL, Gladyshev VN (2011) J Biol Chem 286:42937
37. Reeves MA, Bellinger FP, Berry MJ (2010) Antioxid Redox Signal 12:809
38. Dikiy A, Novoselov SV, Fomenko DE, Sengupta A, Carlson BA et al (2007) Biochemistry 46:6871
39. Castets P, Bertrand AT, Beuvin M, Ferry A, Le Grand F et al (2011) Hum Mol Genet 20:694
40. Han SJ, Lee BC, Yim SH, Gladyshev VN, Lee SR (2014) PLoS One 9:e95518
41. Schomburg L, Schweizer U, Holtmann B, Flohe L, Sendtner M, Kohrle J (2003) Biochem J 370:397
42. Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF et al (2003) J Biol Chem 278:13640
43. Hill KE, Zhou J, McMahan WJ, Motley AK, Burk RF (2004) J Nutr 134:157
44. Liu J, Rozovsky S (2013) Biochemistry 52:5514
45. Olsson M, Olsson B, Jacobson P, Thelle DS, Bjorkegren J et al (2011) Metabolism 60:114
46. Steinbrenner H, Hotze AL, Speckmann B, Pinto A, Sies H et al (2013) J Mol Endocrinol 50:31

47. Whanger PD (2009) Biochim Biophys Acta 1790:1448
48. Vendeland SC, Beilstein MA, Yeh JY, Ream W, Whanger PD (1995) Proc Natl Acad Sci U S A 92:8749
49. Xu XM, Carlson BA, Irons R, Mix H, Zhong N et al (2007) Biochem J 404:115
50. Tamura T, Yamamoto S, Takahata M, Sakaguchi H, Tanaka H et al (2004) Proc Natl Acad Sci U S A 101:16162
51. Kasaikina MV, Fomenko DE, Labunskyy VM, Lachke SA, Qiu W et al (2011) J Biol Chem 286:33203
52. Veres Z, Kim IY, Scholz TD, Stadtman TC (1994) J Biol Chem 269:10597
53. Prast-Nielsen S, Huang HH, Williams DL (2011) Biochim Biophys Acta 1810:1262
54. Engler R, Lombart C, Jayle MF (1972) Biochem Biophys Res Commun 46:1483
55. Dosseto M, Rohner C, Pierres M, Goridis C (1981) J Immunol Methods 41:145
56. Ching WM, Alzner-DeWeerd B, Stadtman TC (1985) Proc Natl Acad Sci U S A 82:347
57. Ching WM, Tsai L, Wittwer AJ (1985) Curr Top Cell Regul 27:497
58. Ching WM, Wittwer AJ, Tsai L, Stadtman TC (1984) Proc Natl Acad Sci U S A 81:57
59. Wittwer AJ, Tsai L, Ching WM, Stadtman TC (1984) Biochemistry 23:4650
60. Sun H, Sheng J, Hassan AE, Jiang S, Gan J, Huang Z (2012) Nucleic Acids Res 40:5171
61. Sheng J, Gan J, Soares AS, Salon J, Huang Z (2013) Nucleic Acids Res 41:10476
62. Sun H, Jiang S, Caton-Williams J, Liu H, Huang Z (2013) RNA 19:1309
63. Hendrickson WA, Pahler A, Smith JL, Satow Y, Merritt EA, Phizackerley RP (1989) Proc Natl Acad Sci U S A 86:2190
64. Yang W, Hendrickson WA, Crouch RJ, Satow Y (1990) Science 249:1398
65. Gonzalez A (2007) J Synchrotron Radiat 14:43
66. Ouerdane L, Mester Z (2008) J Agric Food Chem 56:11792
67. Ferre-D'Amare AR, Zhou K, Doudna JA (1998) Nature 395:567
68. Abdur R, Gerlits OO, Gan J, Jiang J, Salon J et al (2014) Acta Crystallogr D Biol Crystallogr 70:354
69. Carrasco N, Ginsburg D, Du Q, Huang Z (2001) Nucleosides Nucleotides Nucleic Acids 20:1723
70. Lin L, Sheng J, Huang Z (2011) Chem Soc Rev 40:4591
71. Wen Z, Abdalla HE, Zhen H (2013) Sci China Chem 56:273
72. Sheng J, Zhang W, Hassan AE, Gan J, Soares AS et al (2012) Nucleic Acids Res 40:8111
73. Rahmanto AS, Davies MJ (2012) IUBMB Life 64:863
74. Fleet JC (1997) Nutr Rev 55:277
75. Fleming J, Ghose A, Harrison PR (2001) Nutr Cancer 40:42
76. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK et al (1996) JAMA 276:1957
77. Hayes JD, McLellan LI (1999) Free Radic Res 31:273
78. Yao JK, Leonard S, Reddy R (2006) Dis Markers 22:83
79. Shahpiri A, Svensson B, Finnie C (2008) Plant Physiol 146:789
80. Biaglow JE, Miller RA (2005) Cancer Biol Ther 4:6
81. Yang X, Tian Y, Ha P, Gu L (1997) Wei Sheng Yan Jiu 26:113
82. Schrauzer GN (2000) J Nutr 130:1653
83. Guo X, Wu L (1998) Ecotoxicol Environ Saf 39:207
84. Leinfelder W, Forchhammer K, Zinoni F, Sawers G, Mandrand-Berthelot MA, Bock A (1988) J Bacteriol 170:540
85. Bock A, Stadtman TC (1988) Biofactors 1:245
86. Leinfelder W, Zehelein E, Mandrand-Berthelot MA, Bock A (1988) Nature 331:723
87. Hatfield D, Portugal FH (1970) Proc Natl Acad Sci U S A 67:1200
88. Diamond A, Dudock B, Hatfield D (1981) Cell 25:497
89. Hatfield DL, Carlson BA, Xu XM, Mix H, Gladyshev VN (2006) Prog Nucleic Acid Res Mol Biol 81:97
90. Ishii TM, Kotlova N, Tapsoba F, Steinberg SV (2013) J Biol Chem 288:13337
91. Ohama T, Yang DC, Hatfield DL (1994) Arch Biochem Biophys 315:293

92. Tormay P, Wilting R, Lottspeich F, Mehta PK, Christen P, Bock A (1998) Eur J Biochem 254:655
93. Forchhammer K, Leinfelder W, Boesmiller K, Veprek B, Bock A (1991) J Biol Chem 266:6318
94. Commans S, Bock A (1999) FEMS Microbiol Rev 23:335
95. Atkinson GC, Hauryliuk V, Tenson T (2011) BMC Evol Biol 11:22
96. Leinfelder W, Forchhammer K, Veprek B, Zehelein E, Bock A (1990) Proc Natl Acad Sci U S A 87:543
97. Engelberg-Kulka H, Liu Z, Li C, Reches M (2001) Biofactors 14:61
98. Copeland PR (2005) Genome Biol 6:221
99. Fourmy D, Guittet E, Yoshizawa S (2002) J Mol Biol 324:137
100. Tormay P, Sawers A, Bock A (1996) Mol Microbiol 21:1253
101. Rother M, Wilting R, Commans S, Bock A (2000) J Mol Biol 299:351
102. Keeling PJ, Fast NM, McFadden GI (1998) J Mol Evol 47:649
103. Kaiser JT, Gromadski K, Rother M, Engelhardt H, Rodnina MV, Wahl MC (2005) Biochemistry 44:13315
104. Carlson BA, Xu XM, Kryukov GV, Rao M, Berry MJ et al (2004) Proc Natl Acad Sci U S A 101:12848
105. Wittwer AJ, Stadtman TC (1986) Arch Biochem Biophys 248:540
106. Politino M, Tsai L, Veres Z, Stadtman TC (1990) Proc Natl Acad Sci U S A 87:6345
107. Mizutani T, Watanabe T, Kanaya K, Nakagawa Y, Fujiwara T (1999) Mol Biol Rep 26:167
108. Huang KX, An YX, Chen ZX, Xu HB (2001) Biol Trace Elem Res 82:247
109. Chen CS, Stadtman TC (1980) Proc Natl Acad Sci U S A 77:1403
110. Ching WM, Stadtman TC (1982) Proc Natl Acad Sci U S A 79:374
111. Hassan AE, Sheng J, Zhang W, Huang Z (2010) J Am Chem Soc 132:2120
112. Zhang W, Huang Z (2011) Org Lett 13:2000
113. Sheng J, Huang Z (2010) Chem Biodivers 7:753
114. Salon J, Gan J, Abdur R, Liu H, Huang Z (2013) Org Lett 15:3934
115. Thompson A, Spring A, Sheng J, Huang Z, Germann MW (2015) J Biomol Struct Dyn 33:289
116. Brandt G, Carrasco N, Huang Z (2006) Biochemistry 45:8972
117. Carrasco N, Caton-Williams J, Brandt G, Wang S, Huang Z (2006) Angew Chem Int Ed Engl 45:94
118. Caton-Williams J, Huang Z (2008) Chem Biodivers 5:396
119. Haurwitz RE, Jinek M, Wiedenheft B, Zhou K, Doudna JA (2010) Science 329:1355
120. Li H (2015) Structure 23:13
121. Schmitter D, Filkowski J, Sewer A, Pillai RS, Oakeley EJ et al (2006) Nucleic Acids Res 34:4801
122. Castellano L, Stebbing J (2013) Nucleic Acids Res 41:3339
123. Song JJ, Smith SK, Hannon GJ, Joshua-Tor L (2004) Science 305:1434
124. Hutvagner G, Zamore PD (2002) Science 297:2056–2060
125. Macrae IJ, Zhou K, Li F, Repic A, Brooks AN et al (2006) Science 311:195
126. Liu T, Li Y, Wang X, Ye Q, Li H et al (2015) Nucleic Acids Res 43:1044
127. Salon J, Sheng J, Jiang J, Chen G, Caton-Williams J, Huang Z (2007) J Am Chem Soc 129:4862
128. Lin L, Sheng J, Momin RK, Du Q, Huang Z (2009) Nucleosides Nucleotides Nucleic Acids 28:56
129. Kim BM, Rode AB, Han EJ, Hong IS, Hong SH (2012) Apoptosis 17:200
130. Goudgaon NM, Schinazi RF (1991) J Med Chem 34:3305
131. Drutel A, Archambeaud F, Caron P (2013) Clin Endocrinol (Oxf) 78:155
132. Kohrle J (1999) Biochimie 81:527
133. Schomburg L, Kohrle J (2008) Mol Nutr Food Res 52:1235
134. Contempre B, Duale NL, Dumont JE, Ngo B, Diplock AT, Vanderpas J (1992) Clin Endocrinol (Oxf) 36:579

135. Duntas LH (2002) Thyroid 12:287
136. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Endocr Rev 23:38
137. Sellitti DF, Suzuki K (2014) Intrinsic regulation of thyroid function by thyroglobulin. Thyroid 24:625–638. doi:10.1089/thy.2013.0344
138. Chiu-Ugalde J, Wirth EK, Klein MO, Sapin R, Fradejas-Villar N et al (2012) Antioxid Redox Signal 17:902
139. Berry MJ, Banu L, Larsen PR (1991) Nature 349:438
140. Poncin S, Van Eeckoudt S, Humblet K, Colin IM, Gerard AC (2010) Am J Pathol 176:1355
141. Zagrodzki P, Nicol F, Arthur JR, Slowiaczek M (2001) Biofactors 14:223
142. Mitchell JH, Nicol F, Beckett GJ, Arthur JR (1996) J Mol Endocrinol 16:259
143. Pizzulli A, Ranjbar A (2000) Biol Trace Elem Res 77:199
144. Guerra LN, Rios de Molina Mdel C, Miler EA, Moiguer S, Karner M, Burdman JA (2005) Clin Chim Acta 352:115
145. Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F et al (2010) Biol Trace Elem Res 138:107
146. Li H, Okuda J, Akamizu T, Mori T (1995) Endocr J 42:697
147. Taurog A, Dorris ML, Guziec LJ, Guziec FS Jr (1994) Biochem Pharmacol 48:1447
148. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D (2010) Thyroid 20:1163
149. Wiersinga WM (2014) Nat Rev Endocrinol 10:164
150. Contempre B, Dumont JE, Denef JF, Many MC (1995) Eur J Endocrinol 133:99
151. Guilbert JJ (2003) Educ Health (Abingdon) 16:230
152. Mathers CD, Lopez AD, Murray CJL (2006) The burden of disease and mortality by condition: data, methods and results for 2001. In: Lopez AD, Mathers CD, Ezzati M, Murray CJL, Jamison DT (eds) Global burden of disease and risk factors. Oxford University Press, New York, pp 45–240
153. Clauser M, Altenberger J (2013) Herz 38:610
154. Alexander RW (1995) Hypertension 25:155
155. Lei C, Niu X, Wei J, Zhu J, Zhu Y (2009) Clin Chim Acta 399:102
156. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL et al (1995) Circulation 91:2488
157. Liao F, Andalibi A, deBeer FC, Fogelman AM, Lusis AJ (1993) J Clin Invest 91:2572
158. Hoffmann FW, Hashimoto AS, Lee BC, Rose AH, Shohet RV, Hoffmann PR (2011) Arch Biochem Biophys 512:38
159. Nogales F, Ojeda ML, Fenutria M, Murillo ML, Carreras O (2013) Reproduction 146:659
160. Reeves MA, Hoffmann PR (2009) Cell Mol Life Sci 66:2457
161. Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ et al (1995) Biochem J 311(Pt 2):425
162. Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ et al (1996) Biol Trace Elem Res 51:211
163. Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A et al (2004) Mol Cell Biol 24:9414
164. Stanley BA, Sivakumaran V, Shi S, McDonald I, Lloyd D et al (2011) J Biol Chem 286:33669
165. Rundlof AK, Arner ES (2004) Antioxid Redox Signal 6:41
166. Fujino G, Noguchi T, Takeda K, Ichijo H (2006) Semin Cancer Biol 16:427
167. Battin EE, Brumaghim JL (2009) Cell Biochem Biophys 55:1
168. de Bem AF, Farina M, Portella Rde L, Nogueira CW, Dinis TC et al (2008) Atherosclerosis 201:92
169. Schewe C, Schewe T, Wendel A (1994) Biochem Pharmacol 48:65
170. Baran H, Vass K, Lassmann H, Hornykiewicz O (1994) Brain Res 646:201
171. Rathmann W, Giani G (2004) Diabetes Care 27:2568, author reply 9
172. Pillay TS, Makgoba MW (1991) S Afr Med J 79:607
173. Olefsky JM (1993) Adv Exp Med Biol 334:129
174. Muoio DM, Newgard CB (2008) Nat Rev Mol Cell Biol 9:193
175. Olefsky JM, Nolan JJ (1995) Am J Clin Nutr 61:980S
176. Wilson PW (1998) Am J Kidney Dis 32:S89

177. Shara NM, Wang H, Valaitis E, Pehlivanova M, Carter EA et al (2011) Am J Cardiol 107:399
178. Song M (2010) J Cardiovasc Nurs 25:93
179. Kadulina AA, Kurash TP, Kuskova LM, Grinberg EM, Vorob'ev VI (1982) Probl Endokrinol (Mosk) 28:11
180. Steinbrenner H, Speckmann B, Pinto A, Sies H (2011) J Clin Biochem Nutr 48:40
181. Laclaustra M, Navas-Acien A, Stranges S, Ordovas JM, Guallar E (2009) Circ Cardiovasc Qual Outcomes 2:369
182. Wiernsperger N, Rapin J (2010) Diabetol Metab Syndr 2:70
183. Stranges S, Sieri S, Vinceti M, Grioni S, Guallar E et al (2010) BMC Public Health 10:564
184. Wang X, Zhang W, Chen H, Liao N, Wang Z et al (2014) Toxicol Lett 224:16
185. Miki H, Funato Y (2012) J Biochem 151:255
186. Tsou RC, Bence KK (2012) Front Neurosci 6:192
187. Tiganis T (2013) FEBS J 280:445
188. Loh K, Deng H, Fukushima A, Cai X, Boivin B et al (2009) Cell Metab 10:260
189. Wang XD, Vatamaniuk MZ, Wang SK, Roneker CA, Simmons RA, Lei XG (2008) Diabetologia 51:1515
190. Xu T, Liu Y, Meng J, Zeng J, Xu X, Mi M (2013) Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 29:1245
191. Kaplan MJ (2010) Rheum Dis Clin North Am 36:405
192. Peretz AM, Neve JD, Famaey JP (1991) Semin Arthritis Rheum 20:305
193. Peretz A, Neve J, Famaey JP, Molle L (1987) J Pharm Belg 42:395
194. Sehnert B, Burkhardt H, Wessels JT, Schroder A, May MJ et al (2013) Proc Natl Acad Sci U S A 110:16556
195. Prabhu KS, Zamamiri-Davis F, Stewart JB, Thompson JT, Sordillo LM, Reddy CC (2002) Biochem J 366:203
196. Scholz M, Ulbrich HK, Dannhardt G (2008) Eur J Med Chem 43:1152
197. McCabe CJ, Akehurst RL, Kirsch J, Whitfield M, Backhouse M et al (1998) Pharmacoeconomics 14:191
198. Dannhardt G, Kiefer W (2001) Eur J Med Chem 36:109
199. Laneuville O, Breuer DK, Dewitt DL, Hla T, Funk CD, Smith WL (1994) J Pharmacol Exp Ther 271:927
200. Zhuo H, Smith AH, Steinmaus C (2004) Cancer Epidemiol Biomarkers Prev 13:771
201. Amaral AF, Cantor KP, Silverman DT, Malats N (2010) Cancer Epidemiol Biomarkers Prev 19:2407
202. Peters U, Takata Y (2008) Mol Nutr Food Res 52:1261
203. Kim IW, Bae SM, Kim YW, Liu HB, Bae SH et al (2012) Biol Trace Elem Res 148:25
204. Wei WQ, Abnet CC, Qiao YL, Dawsey SM, Dong ZW et al (2004) Am J Clin Nutr 79:80
205. Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H et al (2000) J Natl Cancer Inst 92:1753
206. Glattre E, Thomassen Y, Thoresen SO, Haldorsen T, Lund-Larsen PG et al (1989) Int J Epidemiol 18:45
207. Rayman MP (2009) Biochim Biophys Acta 1790:1533
208. Etminan M, FitzGerald JM, Gleave M, Chambers K (2005) Cancer Causes Control 16:1125
209. Penney KL, Schumacher FR, Li H, Kraft P, Morris JS et al (2010) Cancer Prev Res (Phila) 3:604
210. Li H, Stampfer MJ, Giovannucci EL, Morris JS, Willett WC et al (2004) J Natl Cancer Inst 96:696
211. Battin EE, Zimmerman MT, Ramoutar RR, Quarles CE, Brumaghim JL (2011) Metallomics 3:503
212. Prabhu P, Bag PP, Singh BG, Hodage A, Jain VK et al (2011) Free Radic Res 45:461
213. Mohamed J, Wei WL, Husin NN, Alwahaibi NY, Budin SB (2011) Pak J Biol Sci 14:1055
214. Reszka E (2012) Clin Chim Acta 413:847
215. Brozmanova J (2011) Klin Onkol 24:171

216. Selenius M, Rundlof AK, Olm E, Fernandes AP, Bjornstedt M (2010) Antioxid Redox Signal 12:867
217. Reid ME, Duffield-Lillico AJ, Slate E, Natarajan N, Turnbull B et al (2008) Nutr Cancer 60:155
218. Duffield-Lillico AJ, Dalkin BL, Reid ME, Turnbull BW, Slate EH et al (2003) BJU Int 91:608
219. Krehl S, Loewinger M, Florian S, Kipp AP, Banning A et al (2012) Carcinogenesis 33:620
220. Ip C, Lisk DJ (1997) Nutr Cancer 28:184
221. Duntas LH (2006) Thyroid 16:455
222. Davis CD, Uthus EO (2004) Exp Biol Med (Maywood) 229:988
223. Davis CD, Uthus EO (2003) J Nutr 133:2907
224. Zeng H, Combs GF Jr (2008) J Nutr Biochem 19:1
225. Sanmartin C, Plano D, Sharma AK, Palop JA (2012) Int J Mol Sci 13:9649
226. Park SH, Kim JH, Chi GY, Kim GY, Chang YC et al (2012) Toxicol Lett 212:252
227. Sanmartin C, Plano D, Font M, Palop JA (2011) Curr Cancer Drug Targets 11:496
228. Alwahaibi N, Mohamed J, Alhamadani A (2010) J Trace Elem Med Biol 24:119
229. Rayman MP (2005) Proc Nutr Soc 64:527
230. Peters U, Chatterjee N, Church TR, Mayo C, Sturup S et al (2006) Cancer Epidemiol Biomarkers Prev 15:315
231. Corcoran NM, Hovens CM, Michael M, Rosenthal MA, Costello AJ (2010) Br J Cancer 103:462
232. Humann-Ziehank E, Wolf P, Renko K, Schomburg L, Ludwig Bruegmann M et al (2011) J Trace Elem Med Biol 25(Suppl 1):S30
233. Selenius M, Fernandes AP, Brodin O, Bjornstedt M, Rundlof AK (2008) Biochem Pharmacol 75:2092
234. Papp LV, Lu J, Striebel F, Kennedy D, Holmgren A, Khanna KK (2006) Mol Cell Biol 26:4895–4910
235. Zeng H, Wu M, Botnen JH (2009) J Nutr 139:1613
236. Hertz N, Lister RE (2009) J Int Med Res 37:1961
237. Poerschke RL, Moos PJ (2011) Biochem Pharmacol 81:211
238. Nguyen N, Sharma A, Sharma AK, Desai D, Huh SJ et al (2011) Cancer Prev Res (Phila) 4:248
239. Xing F, Li S, Ge X, Wang C, Zeng H et al (2008) Oral Oncol 44:963
240. Baum MK, Campa A, Lai S, Sales Martinez S, Tsalaile L et al (2013) JAMA 310:2154
241. Cirelli A, Ciardi M, de Simone C, Sorice F, Giordano R et al (1991) Clin Biochem 24:211
242. Baruchel S, Wainberg MA (1992) J Leukoc Biol 52:111
243. Stone CA, Kawai K, Kupka R, Fawzi WW (2010) Nutr Rev 68:671
244. Hoffmann PR, Berry MJ (2008) Mol Nutr Food Res 52:1273
245. Makropoulos V, Bruning T, Schulze-Osthoff K (1996) Arch Toxicol 70:277
246. Roux-Lombard P, Modoux C, Cruchaud A, Dayer JM (1989) Clin Immunol Immunopathol 50:374
247. Westendorp MO, Shatrov VA, Schulze-Osthoff K, Frank R, Kraft M et al (1995) EMBO J 14:546
248. Peterhans E (1997) J Nutr 127:962S
249. Taylor EW, Cox AG, Zhao L, Ruzicka JA, Bhat AA et al (2000) J Acquir Immune Defic Syndr 25(Suppl 1):S53
250. Ferencik M, Ebringer L (2003) Folia Microbiol (Praha) 48:417
251. Stambullian M, Feliu S, Slobodianik NH (2007) Br J Nutr 98(Suppl 1):S140
252. Lopez-Herce JA (1998) An Med Interna 15:623
253. Taylor EW, Nadimpalli RG, Ramanathan CS (1997) Biol Trace Elem Res 56:63
254. Jeong LS, Choi YN, Tosh DK, Choi WJ, Kim HO, Choi J (2008) Bioorg Med Chem 16:9891
255. Alexander V, Choi WJ, Chun J, Kim HO, Jeon JH et al (2010) Org Lett 12:2242
256. Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R et al (2014) PLoS Negl Trop Dis 8: e2865

257. Cattand P, Desjeux P, Guzmán MG, Jannin J, Kroeger A, Medici A, Musgrove P, Nathan MB, Shaw A, Schofield CJ (2006) Tropical diseases lacking adequate control measures: dengue: leishmaniasis, and African trypanosomiasis. In: Disease control priorities in developing countries. 2nd edn. World Bank, Washington DC
258. Postigo JA (2010) Int J Antimicrob Agents 36(Suppl 1):S62
259. Carvalho LP, Passos S, Schriefer A, Carvalho EM (2012) Front Immunol 3:301
260. da Silva MS, Monteiro JP, Nunes VS, Vasconcelos EJ, Perez AM et al (2013) PLoS One 8: e81397
261. Tiwananthagorn S, Bhutto AM, Baloch JH, Soomro FR, Kawamura Y et al (2012) Parasitol Res 111:125
262. Bates PA (2007) Int J Parasitol 37:1097
263. Kedzierski L, Sakthianandeswaren A, Curtis JM, Andrews PC, Junk PC, Kedzierska K (2009) Curr Med Chem 16:599
264. Moreno D, Plano D, Baquedano Y, Jimenez-Ruiz A, Palop JA, Sanmartin C (2011) Parasitol Res 108:233
265. Plano D, Baquedano Y, Moreno-Mateos D, Font M, Jimenez-Ruiz A et al (2011) Eur J Med Chem 46:3315
266. Fuertes MA, Nguewa PA, Castilla J, Alonso C, Perez JM (2008) Curr Med Chem 15:433
267. Sharma U, Singh S (2008) J Vector Borne Dis 45:255
268. Haouas N, Pesson B, Boudabous R, Dedet JP, Babba H, Ravel C (2007) Am J Trop Med Hyg 77:1054
269. Baquedano Y, Moreno E, Espuelas S, Nguewa P, Font M et al (2014) Eur J Med Chem 74:116
270. van den Boogaard J, Kibiki GS, Kisanga ER, Boeree MJ, Aarnoutse RE (2009) Antimicrob Agents Chemother 53:849
271. Connolly LE, Edelstein PH, Ramakrishnan L (2007) PLoS Med 4:e120
272. Kremer L, Maughan WN, Wilson RA, Dover LG, Besra GS (2002) Lett Appl Microbiol 34:233
273. Warrier T, Tropis M, Werngren J, Diehl A, Gengenbacher M et al (2012) Antimicrob Agents Chemother 56:1735
274. Elamin AA, Stehr M, Oehlmann W, Singh M (2009) J Microbiol Methods 79:358
275. Favrot L, Grzegorzewicz AE, Lajiness DH, Marvin RK, Boucau J et al (2013) Nat Commun 4:2748
276. Villamor E, Mugusi F, Urassa W, Bosch RJ, Saathoff E et al (2008) J Infect Dis 197:1499
277. Seyedrezazadeh E, Ostadrahimi A, Mahboob S, Assadi Y, Ghaemmagami J, Pourmogaddam M (2008) Respirology 13:294
278. Gutteridge JM (1994) Ann N Y Acad Sci 738:201
279. Tabet N, Mantle D, Orrell M (2000) Adverse Drug React Toxicol Rev 19:127
280. Atwood CS, Huang X, Moir RD, Tanzi RE, Bush A (1999) Met Ions Biol Syst 36:309
281. Bus AI, Huang X, Fairlie DP (1999) Neurobiol Aging 20:33, discussion 9–42
282. Forlenza OV, Diniz BS, Teixeira AL, Stella F, Gattaz W (2013) Rev Bras Psiquiatr 35:284
283. Maccioni RB, Lavados M, Maccioni CB, Mendoza-Naranjo A (2004) Curr Alzheimer Res 1:307
284. Riederer BM, Leuba G, Elhajj Z (2013) Exp Biol Med (Maywood) 238:519
285. Butterfield DA (2002) Free Radic Res 36:1307
286. Gwon AR, Park JS, Park JH, Baik SH, Jeong HY et al (2010) Neurosci Lett 469:391
287. Yoo MH, Gu X, Xu XM, Kim JY, Carlson BA et al (2010) Antioxid Redox Signal 12:819
288. Thomas JP, Geiger PG, Maiorino M, Ursini F, Girotti AW (1990) Biochim Biophys Acta 1045:252
289. Thomas JP, Maiorino M, Ursini F, Girotti AW (1990) J Biol Chem 265:454
290. Chen P, Wang C, Ma X, Zhang Y, Liu Q et al (2013) PLoS One 8:e66384
291. Takemoto AS, Berry MJ, Bellinger FP (2010) Ethn Dis 20:S1–S92
292. Cardoso BR, Ong TP, Jacob-Filho W, Jaluul O, Freitas MI, Cozzolino SM (2010) Br J Nutr 103:803

293. Pinton S, Bruning CA, Sartori Oliveira CE, Prigol M, Nogueira CW (2013) J Nutr Biochem 24:311
294. Pinton S, da Rocha JT, Gai BM, Prigol M, da Rosa LV, Nogueira CW (2011) Cell Biochem Funct 29:235
295. Pinton S, da Rocha JT, Zeni G, Nogueira CW (2010) Neurosci Lett 472:56
296. Barron LH, Rae A, Holloway S, Brock DJ, Warner JP (1994) Hum Mol Genet 3:173
297. Warner JP, Barron LH, Brock DJ (1993) Mol Cell Probes 7:235
298. Chen CM, Wu YR, Cheng ML, Liu JL, Lee YM et al (2007) Biochem Biophys Res Commun 359:335
299. Hersch SM, Gevorkian S, Marder K, Moskowitz C, Feigin A et al (2006) Neurology 66:250
300. Stoy N, Mackay GM, Forrest CM, Christofides J, Egerton M et al (2005) J Neurochem 93:611
301. Sorolla MA, Rodriguez-Colman MJ, Vall-llaura N, Tamarit J, Ros J, Cabiscol E (2012) Biofactors 38:173
302. Mason RP, Casu M, Butler N, Breda C, Campesan S et al (2013) Nat Genet 45:1249
303. Singh N, Halliday AC, Thomas JM, Kuznetsova OV, Baldwin R et al (2013) Nat Commun 4:1332
304. Okusa MD, Crystal LJ (1994) Am J Med 97:383
305. Groleau G, Barish R, Tso E, Whye D, Browne B (1987) Am J Emerg Med 5:527
306. Hauser WA, Annegers JF, Rocca WA (1996) Mayo Clin Proc 71:576
307. Hamed SA, Abdellah MM, El-Melegy N (2004) J Pharmacol Sci 96:465
308. Child ND, Benarroch EE (2014) Neurology 82:989
309. Armijo JA, Shushtarian M, Valdizan EM, Cuadrado A, de las Cuevas I, Adin J (2005) Curr Pharm Des 11:1975
310. Naziroglu M (2009) Neurochem Res 34:2181
311. Willmore LJ, Rubin JJ (1981) Neurology 31:63
312. Savaskan NE, Brauer AU, Kuhbacher M, Eyupoglu IY, Kyriakopoulos A et al (2003) FASEB J 17:112
313. Seven M, Basaran SY, Cengiz M, Unal S, Yuksel A (2013) Epilepsy Res 104:35
314. Haenen GR, De Rooij BM, Vermeulen NP, Bast A (1990) Mol Pharmacol 37:412
315. Muller A, Cadenas E, Graf P, Sies H (1984) Biochem Pharmacol 33:3235
316. Wendel A, Fausel M, Safayhi H, Tiegs G, Otter R (1984) Biochem Pharmacol 33:3241
317. Schewe T (1995) Gen Pharmacol 26:1153
318. Yoshikawa T, Naito Y, Kondo M (1993) J Nutr Sci Vitaminol (Tokyo) 39(Suppl):S35
319. Renson M, Dereu N (1990) J Pharm Belg 45:322
320. Brune B, Diewald B, Ullrich V (1991) Biochem Pharmacol 41:1805
321. Sies H (1993) Free Radic Biol Med 14:313–323
322. Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y et al (1998) Stroke 29:12
323. Parnham MJ, Sies H (2013) Biochem Pharmacol 86:1248
324. Cotgreave IA, Morgenstern R, Engman L, Ahokas J (1992) Chem Biol Interact 84:69
325. Masumoto H, Kissner R, Koppenol WH, Sies H (1996) FEBS Lett 398:179
326. Braughler JM, Hall ED (1989) Free Radic Biol Med 6:289
327. De Ley G, Weyne J, Demeester G, Stryckmans K, Goethals P, Leusen I (1989) Stroke 20:357
328. Nakase T, Sohl G, Theis M, Willecke K, Naus CC (2004) Am J Pathol 164:2067
329. Barone FC, Feuerstein GZ (1999) J Cereb Blood Flow Metab 19:819–834
330. Worthmann H, Tryc AB, Goldbecker A, Ma YT, Tountopoulou A et al (2010) Cerebrovasc Dis 30:85
331. Rosenzweig S, Carmichael ST (2013) Stroke 44:2579
332. Pratta MA, Ackerman NR, Arner EC (1998) Inflamm Res 47:115
333. Zembowicz A, Hatchett RJ, Radziszewski W, Gryglewski RJ (1993) J Pharmacol Exp Ther 267:1112

334. Kim HR, Kim JW, Park JY, Je HD, Lee SY et al (2001) J Auton Pharmacol 21:23
335. Smith SM, Min J, Ganesh T, Diebold B, Kawahara T et al (2012) Chem Biol 19:752
336. Yoshizumi M, Fujita Y, Izawa Y, Suzaki Y, Kyaw M et al (2004) Exp Cell Res 292:1
337. Tepperman BL, Chang Q, Soper BD (1999) Br J Pharmacol 128:1268–1274
338. Plontke S, Zenner HP (2004) GMS Curr Top Otorhinolaryngol Head Neck Surg 3:Doc06
339. Ohlemiller KK, McFadden SL, Ding DL, Lear PM, Ho YS (2000) J Assoc Res Otolaryngol 1:243
340. Henderson D, Bielefeld EC, Harris KC, Hu BH (2006) Ear Hear 27:1
341. Kil J, Pierce C, Tran H, Gu R, Lynch ED (2007) Hear Res 226:44
342. Lynch ED, Gu R, Pierce C, Kil J (2004) Laryngoscope 114:333
343. Pourbakht A, Yamasoba T (2003) Hear Res 181:100

Index