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New Approaches to Natural Anticancer Drugs

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Preface

During past decades, anticancer drug discovery has grown quickly and hundreds of novel active compounds of natural origin have been examined to find a way toward clinical trials, and then to the world pharmaceutical markets. Now is the time to take a look behind to see what we have gained from natural resources, challenges and developments, then to the future, new windows to modern developing approaches in order to find the best candidates for more investigations for sure.

Undoubtedly, high throughput screenings based on reverse pharmacology and pharmacognosy are able to result in further novel and lead compounds in this regard. On the other side, biotechnology and nanotechnology are strong tools to reach the effective compounds via targeted approaches rapidly toward both animal studies and clinical trials. In fact, the main objective of this book is to provide an up-to-date review on the recently identified natural anticancer/anti-tumor compounds from various natural origins including plants, animals, fungi, mushrooms, as well as micro- and marine organisms, and also novel candidates for clinical trials and new approaches used. Although there have been some books on natural anticancer drugs, no book has been published that deals with quick and useful information of different varieties of natural anticancer compounds all together in one book.

The present book can be used by researchers in the field of pharmaceutical sciences, students and residents in pharmacy and medicine schools regarding their courses and thesis, academic scientists in the mentioned fields, as well as those who work on the research areas of phytochemistry and natural products.

This book has six chapters explaining the challenges and development in anticancer drug discovery approaches, traditional remedies for prevention and treatment of cancer, marine-derived anticancer compounds, and antibiotics used as anticancer agents, as well as different classes of terpenoids and carbohydrates, which have attracted the scientists' attention in this field as the efficient anticancer candidates.

I would like to thank the contributors of the chapters, who are my great colleagues, for their kind endeavors in creating this text. Moreover, I would like

to acknowledge the support of the Springer staff, especially Ms. Manika Power. Eventually, any comments and feedback from the experts in the field of this book are welcome to consider for future editions.

Tehran, Iran Soodabeh Saeidnia

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Chapter 1
Anticancer Drug Discovery Approaches; Challenges and Development

Contents

Abstract Despite costly and time-consuming medical and pharmaceutical research and some promising developments the cancer storm is still blowing and threatening people's life around the world. There remain numerous challenges with medical diagnosis and treatment approaches, and pharmaceutical faults in finding effective candidates, preparing them as suitable formulations for targeted drug delivery, higher effectiveness and lower adverse reactions, presenting New Drug Applications (NDA) in the cancer field, and developing economical methods for sufficient production of these new drugs. In this chapter, we aim to review the practical solutions like integration of traditional pharmaceutical approaches (pharmacognosy) and modern ones (reverse pharmacognosy), high throughput screening, biotechnology and nanotechnology that may accelerate discovery of new anticancer drugs from natural sources.

Keywords Anticancer drugs • Reverse pharmacognosy • Biotechnology • Nanotechnology • High throughput screening • Traditional medicine

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1.1 Introduction

The remaining documents, originating from the ancient civilizations of Asia and Middle East, demonstrate that herbal medicines were used for a long time to maintain human health (Wang et al. 2010). Egyptian phytomedicines (like myrrh, cassia, thyme, henna, juniper, castor, caraway, marjoram and spearmint), Chinese herbal medicines and Ayurveda remedies have been proved to apply in different human illnesses and disorders (Patwardhan and Mashelkar 2009). Currently used anticancer drugs are mainly originated from natural origin or the semi-synthetic derivatives from natural leads. Literature revealed that 69 % of the approved anticancer agents since 1980 to 2002 are originated or developed from natural sources (Newman and Cragg 2007). Although plants are considered as the main source of anticancer drugs, only 5–15 % of the approximately 250,000 species of higher plants have been studied for the presence of bioactive compounds and this is why there is a huge potential to exploit nature for new anticancer compounds (Saeidnia and Abdollahi 2014).

Cancer is one of the most predominant diseases causing death in the world. Since various side effects are frequently reported from the present chemotherapeutic and chemo-preventive antitumor drugs, there is a growing trend towards new drug discovery, especially from natural origins. In order to facilitate anticancer drug discovery and development, new strategies should be considered to obtain more effective or new lead compounds from natural sources. On the other hand, not only traditional screening of medicinal plants, marine algae or other natural sources seems necessary to find effective compounds with lower toxicity and higher activity, but also new approaches in this area regarding new aspects of "reverse pharmacognosy" should be coupled with high throughput screening, virtual screening and in-silico databases to promote anticancer drug discovery. In fact, if chemistry knowledge and high-throughput screening are combined, identification of numerous selective active compounds would be possible (Saeidnia and Abdollahi 2014). Here in this chapter, we aim to explain how the new approaches like biotechnology, nanotechnology and high throughput screening methods have opened new windows to anticancer drug discovery from natural sources.

1.2 Linking Between Traditional Medicine Knowledge and High Throughput Screening

Historically, old screening methods and drug development plans were limited in scale and evaluated the anticancer activity of small numbers and/or particular types of anticancer agents. At first, in the USA, Harvard and then National Cancer Institute (NCI), established a screening plan for evaluation and isolation of bacterial polysaccharides using mice bearing sarcoma 37 for necrosis and hemorrhage, which was quickly extended to plant extracts. In the early 1950s, the mentioned

system had tested several hundreds of plant extracts. Two of these materials reached clinical trials stage.Larger-scale screenings emanated in 1955, when chemical agents like nitrogen mustard and folic acid antagonists were found to be able to produce remissions of malignant lymphomas. Gradually, discovery and development systems were placed at pharmaceutical companies, research institutions, and various universities around the world and requirement of high throughput screening (HTS) methods were felt (Burger and Fiebig 2004).

However, designing and performing an appropriate HTS is not a simple process for drug discovery programs and involves numerous challenges. In the natural product area, when a screening bioassay is in place, compounds can be examined for their biological activity, while screening of extracts is always problematic. Although new techniques like pre-fractionation of extracts can resolve some of these problems, it seems that the challenges in HTS have still been an important issue in drug discovery from medicinal plants (Balunas and Kinghorn 2005). However, if novel targets are considered responsible for driving cancer progression, the possibility of finding mechanism-based agents will increase. These agents are expected to be more efficient and less toxic than the usual cytotoxic drugs previously discovered. Numerous candidates found as tyrosine kinase inhibitors, p53-based modulators, mitotic inhibitors and Ras pathway inhibitors are just some examples (Aherne et al. 2002).

Traditional treatments using natural medicines have been successful in many populations worldwide, representing various aspects of knowledge that are sometimes neglected in modern medicine due to differences in the concepts of disease. Creating a link between traditional data and rapid screening systems can be a successful way to discover novel anticancer agents. As an example, a cell-based high throughput screening method was reported to be applied as a modern bio-sensor that selectively detected apoptosis on the basis of the fluorescence resonance energy transfer (FRET) technique (Tian et al. 2007). The mentioned cell-based HTS method was suggested to be employed in finding anticancer compounds, since numerous anticancer drugs are able to induce apoptosis that can be detected by this sensitive technique. Moreover, this method can detect apoptosis (not necrosis), and therefore it is suggested to be used instead of MTT in anticancer screening. The authors concluded that they were successful in detecting the active isolated compounds from two medicinal plants used in Chinese medicine.

1.3 Reverse Pharmacognosy

By developing the screening systems toward high throughput evaluations, scientists in the field of pharmacognosy were faced with a huge amount of data and information that has been produced and accumulated over years. The question was “How can scientists and researchers cope with thousands of data obtained?” Actually, “reverse pharmacognosy” was proposed with this purpose, in which the direction of movement proceeds from bioactive natural compounds to

active organisms employing biological assays. Novel computational techniques were required, such as silicon technology to reach that aim. Surprisingly, not only the results of this approach provide evidence for the medicinal properties of traditionally used herbal drugs, but also it can be applied to find other plants or organisms that consist of similar compounds in order to suggest for the same application. On the other side, special groups of secondary metabolites are bio-synthesized by particular organisms, and thus if a natural compound shows a considerable activity, probably more active and/or less toxic derivatives would be expected to be found in similar organisms containing the achievement. Moreover, reverse pharmacognosy (from diverse active molecules to plants) is a complementary to pharmacognosy(from bio-diverse plants to active molecules) and is capable to apply new techniques including virtual screening and a knowledge database containing the traditional uses of plants. The specialists in this area believe that integrating pharmacognosy and reverse pharmacognosy may provide an efficient and rapid tool for natural drug discovery, especially when the pharmaceutical industries need new drug candidates in clinical trials, and are faced with drug repositioning, since reverse pharmacognosy can contribute to addressing such issues by eliciting the required information from pharmacognosy knowledge (Blondeau et al. 2010; Do and Bernard 2004; Saeidnia and Abdollahi 2014).

For instance, to reveal the efficacy of this concept, a study on eviniferin, an active ingredient for cosmetic development has been reported, which is weakly defined in terms of biological properties. Actually, inverse docking computer software (named SELNERGY) was employed to find supposed binding biological targets for eviniferin amongst 400 proteins, of which two targets were preserved. Interestingly, cyclic nucleotide phosphodiesterase 4 was the most interesting candidate, while other PDE subtypes (1–6) were not maintained, exhibiting a selective action for subtype-4.Additionally, the laboratory binding examination demonstrated a significant selectivity of eviniferin for the subtype-4. Furthermore, the authors reported this selectivity evaluation of eviniferin on the secretion of TNF-α and Interleukin-8and concluded that eviniferin showed anti-inflammatory effects via suppression of subtype-4.Regarding such reports, finding new applications for previously known compounds is another advantage of reverse pharmacognosy (Do et al. 2005). Figure 1.1 shows the correlations between such new approaches to promote anticancer drug discovery and development.

1.4 Nanotechnology

Finding new targets is the main goal in drug discovery of cancer. Moreover, nanoparticles show a great promise in the treatment of a wide range of diseases due to their flexibility in structure, composition and properties.Nanoparticle-targeting anticancer drugs have been further considered recently because of biodegradability, biocompatibility, surface modification, stability, excellent pharmacokinetic control

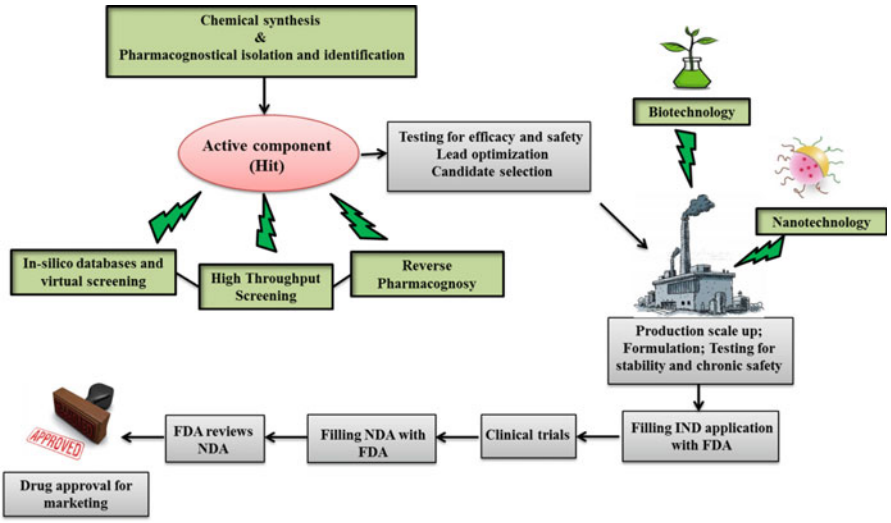

Fig. 1.1 Various novel approaches in promoting anticancer drug discovery from natural origin

and suitability for entrapping a wide range of therapeutic agents but concerns on the possible toxicity of all nano-compounds still remain (Saeidnia and Abdollahi 2014). On the other side, since anticancer chemotherapeutic agents are mainly toxic and hydrophobic compounds, they need to be adequately diluted for slow infusion into the body. To improve and cope with the limitations and drawbacks of conventional chemotherapy, nanotechnology and biocompatible polymeric nanoparticles have been developed as a significant output of modern science. A literature review reveals a variety of formulations on the basis of nanoparticles that are used in anticancer drug discovery and developments. Some of the most important nano-carriers are listed below that are frequently reported for evaluation against different cancer cell lines. Here we focused only on their applications in breast cancer research.

1.4.1 Dendrimers

Dendrimers are synthetic biocompatible materials used as the suitable biological nano-carriers of cytotoxic drugs due to their inception and nano-size (1–100 nm) property with long half-life,and the multifunctional central region. These polymers are usually branched with terminal surface active groups (Lee et al. 2005). For instance, different generations of these polymers have been reported in cancer research, of which a biocompatible sixth generation (G6) anionic lysine dendrimer has been recently exhibited with modified surface by glutamate and coupling with trastuzumab mAb and fluorescent label. More recently, this conjugated formulation was tested in both HER2 (human epidermal growth factor receptor) positive and

negative breast cancer cell lines to assess the targeting efficiency and cellular internalization compared to the free antibody application. The authors concluded that high binding affinity, lower cytotoxicity and lysosomal trafficking were observed in HER2 positive cells in a dose dependent manner in comparison to the HER2 negative cells (Miyano et al. 2010).

1.4.2 Micelles

Micelles are known as the colloidal dispersion system forming by the spontaneous aggregation of amphiphilic molecules or surface active agents in an aqueous phase or dispersion medium. Actually, these are the sphere-shaped nanoparticles (size range from 10 to 100 nm) concluding two distinctive sections: a central portion and a polar or hydrophilic head (Rangel-Yagui et al. 2005). As a quick example for application of these nanoparticles in cancer research, Lee et al. (2009) reported the evaluation of an anti-HER2 antibody (Herceptin) coupled with a cationic micellar formulation that resulted in considerable loads of Taxol and enhancement of anticancer effects against both HER2 positive and negative breast cancer cell lines in comparison with the commercial lipid based protein (BioPorter).

1.4.3 Liposomes

Liposomes are valid and suitable biocompatible carrier with multipurpose applications in both medicine and biological science. In fact, these are amphiphilic unilamellar or multilamellar membrane of nontoxic phospholipids and steroids (similar to cholesterol) forming lipid bilayers with size range from 80 to 100 nm (Silva et al. 2011). For example, Alexis et al. (2008) prepared a Paclitaxel loaded HER2 specific affibody fabricated nanoparticles (affisomes) to measure the toxicity contrary to HER2 expressed cancer cell lines like SKBR-3 (cell viability: 70 ± 5 % and $p < 0.01$) and SKOV-3 (cell viability: 59 ± 5 %, $p < 0.05$). Actually, anti-HER2affibody ligands are known as the small size and highly specific mimic monoclonal antibodies exhibiting an interesting feature to target HER2 receptor. A main concern about using liposomes is limitation of liposomal formulation regarding their stability that researchers are faced with the issue about the release of drugs from the nano-system especially after *iv* administration.

1.4.4 Poly (Lactic-co-Glycolic-Acid) or PLGA

Today, PLGA is a widely used polymer under investigation in order to reach an effective cancer drug delivery. With no doubt, PLGA is significantly a successful

nano-carrier composing biodegradable copolymers that easily undergo to break down into two metabolites, lactic acid and glycolic acid upon hydrolysis of ester linkage within the human body. Surprisingly, PLGA is approved by the main regulatory agencies like US FDA and European Medicine Agency (EMA) to employ in a variety of drug delivery systems in human. The reason is behind its biocompatibility, easy preparation techniques, mechanical strength, and lower systemic toxicity compared to other nano-carriers (Irache et al. 2011). Doxorubicin loaded magnetic PLGA multi-modal nanoparticles (Yang et al. 2007), Docetaxel loaded PLGA-PEG/PLGA nanoparticles with Herceptin (Liu et al. 2010), trastuzumab decorated paclitaxel loaded PLGA/montmorillonite (PLGA-MMT)nanoparticles (Sun et al. 2008), and monoclonal antibody-functionalized fluorescein loaded PLGA nanoparticles (Kocbek et al. 2007) are just some examples of recent studies, in which PLGA has been employed to target particular receptors in breast cancer cells.

1.5 Biotechnology

The human efforts for treating cancer and the importance of using natural plant-derived compounds as anticancer agents have been clear for centuries, when Egyptians mentioned some anticancer natural products in the Ebers papyrus in 1550 BC. Modern screening of plants to find anticancer compounds was extensively considered by National Cancer Institute (NCI) in the United States of America from 1950s. Although most of screened compounds failed in different phases of clinical trials, the value of the rest was undeniable in treating different types of cancer. In this regard, vincristine, vinblastine, podophyllotoxin and paclitaxel are some examples of natural anticancer compounds commercially marketed in different countries and saved the patient's life around the world (Misawa and Goodbody 1996; Cragg and Newman 2004).

Low concentration of anticancer phytochemicals in plant extracts is an important limiting factor, which compels producers toward large scale extraction of wild or cultivated plants. This procedure is mostly difficult with low efficiency. Moreover, commercial scale production with chemical synthesis is lengthy or almost impossible and not cost-effective due to structural and stereo-chemical complexity and numerous chiral centers in the molecular backbone of phytochemicals with anticancer activity (Misawa and Goodbody 1996; Wink et al. 2005).

Cancer outbreak, ever increasing demand for natural anticancer agents and high market price of plant derived anticancer drugs have attracted researchers to investigate simpler or more efficient methods for the economical production of secondary metabolites with anticancer activity since decades ago. Different biotechnological approaches such as plant cell, tissue, organ or hairy root culture, micropropagation and biotransformation techniques have been tried to get desired compounds and to improve the yield of production. Independence of geographical, seasonal and environmental variations, qualitatively and quantitatively uniform supply of products, rapidity of production, stereo- and regio-specificity and random

production of novel anticancer agents during biotechnological processes are some inspiring points and advantages of these approaches in comparison with conventional methods like synthesis,semi-synthesis or natural product isolation and purification (Ramachandra Rao and Ravishankar 2002; Wink et al. 2005).

Although it seems that limiting parameters such as slow growth and instability of cell lines, the low yield of production and difficulties in scale up processes can decrease efficiency of biotechnological approaches, different strategies like screening of efficient cell lines, optimizing the culture environment, using elicitors and cell immobilization and permeabilization can partially address these limitations. Moreover, improving the knowledge of biosynthetic pathways of phytochemicals production in plant cells and application of genetic engineering techniques will be helpful in coming years (Smetanska 2008; Roja and Rao 2000; Fuss 2003; Kuhlmann et al. 2002; Petersen and Alfermann 2001).

In spite of all that, there are still a few examples of successful, economical and commercialized applications of biotechnological approaches in anticancer pharmaceutical industry, which indicate that the research and development window will probably be open for years in this matter (Ionkova 2011; Saeidnia and Abdollahi 2014; Malik et al. 2014; Farkya et al. 2004). Some of these successful examples are presented here:

1.5.1 Pclitaxel and Other Taxanes

Paclitaxel is a diterpenoid alkaloid obtained from the stem bark and leaves of different *Taxus* species especially *T. brevifolia* Nutt. It has been widely used to treat different types of cancer since 1992 after its first FDA approval for the treatment of ovarian cancer with Taxol® trademark. As *Taxus* species grow slowly and produce trace amounts of paclitaxel with high cost (US$5 million/kg in 2005), *in vitro* production methods specifically biotechnology techniques should be considered as replacements. Several research projects were approved for yield improvement and scale up of callus and suspension cultures of *Taxus* from 1990s, which finally resulted in sustainable biotechnological production of paclitaxel by Phyton Biotech Inc. (Germany) in 2002 to supply Bristol-Myers Squibb Co. (USA) demand. Phyton Biotech Inc. have recently succeeded in biotransforming of taxanes to docetaxel using plant cell cultures. Moreover, genetic modification of paclitaxel producing fungi isolated from *Taxus* species can be considered as future research strategies (Wink et al. 2005; Majumder and Jha 2009; Zhou and Wu 2006; Phyton Biotech 2014).

1.5.2 Podophyllotoxin (PTOX) and Related Lignans

Podophyllotoxin is a lignan produced in several plant families, including Cupressaceae, Berberidaeae, Polygalaceae, Lamiaceae and Linaceae. PTOX itself

is very toxic for therapeutic purposes and it's often used as a precursor for the production of more potent and less toxic anticancer agents like etoposide, etopophos and tenoposide. PTOX can commercially be exploited from the roots and rhizomes of a few *Podophyllum* species especially *P. hexandrum* and *P. peltatum*. These species, as the only viable options to PTOX isolation, are critically endangered due to over-exploitation, lack of organized cultivation and long juvenile phase. These problems beside total chemical synthesis deficiencies caused investigation of other useful methods for PTOX production. Despite the vast examination of *Podophyllum* species for biotechnological approaches like callus or cell suspension culture from 1980s, low yield and slow growth of cultures and their tendency to browning resulted in replacement of *Podophyllum* species with *Linum* species especially *L. album* from 2000 onwards. Several *Linum* species have been shown suitable yield (PTOX % dry weight range from 0.06 to 2.26) in cell suspension, callus and hairy roots cultures. In spite of these improvements, the successful scale up attempts were limited. Recent isolation of two endophytic fungi, *Trametes hirsute* from *P. hexandrum* and *Fusarium oxysporum* from *Juniperus revurva*, which can biosynthesize PTOX, is a promising aspect of future researches because of higher growth rate and easier scale up processes of fungi cultures in comparison with plant cell ones (Wink et al. 2005; Majumder and Jha 2009; Yousefzadi et al. 2010).

1.6 Conclusion

Cancer remains one of the most prevalent and predominant diseases causing death. Unfortunately, more than 10 million people are annually diagnosed with cancer diseases worldwide. Although surgery, radiation therapy, hormone therapy, immunotherapy and chemotherapy are currently well established as general effective therapeutic approaches to treat different types of cancer, traditional cancer treatments are faced with some drawbacks and limitations. Traditional treatments by using natural medicines have been successful in many populations worldwide, representing various aspects of knowledge that are sometimes neglected in modern medicine due to differences in the concepts of disease. Therefore, creating a link between traditional data and rapid screening systems can be a successful way to discover novel anticancer agents particularly from natural sources. On the other side, reverse pharmacognosy as a complementary to pharmacognosy is able to use various modern techniques including virtual screening and a knowledge database containing the traditional uses of plants, and may promote an efficient and rapid tool for natural drug discovery, especially when the pharmaceutical industries need new drug candidates in clinical trials.

Nowadays nanotechnology has provided a modern opportunity for specific drug delivery with higher efficacy and lower adverse events. Developing the effective and reliable systems in therapy and diagnosis of cancer can be precisely available with the main approach of nanotechnology.Bio-conjugation strategies on chemotherapeutic drugs loaded nanoparticles with monoclonal antibodies have provided

the smart intensive approaches in both *in vitro* and *in vivo* studies. Alongside nanoscience practice, biotechnology can offer an alternative method for further production of high quantitative natural metabolites, where there are restrictions on production of secondary metabolites. Plant biotechnology comprises several *in vitro* techniques, in which manipulating the parameters makes it possible to influence the growth and metabolism of cultured tissues. Taken together, all the above explained aspects of modern approaches in cancer drug discovery and development can help to promote finding further candidates, more efficient drugs, better targeting and delivery, as well as new diagnosis and therapy approaches in the field of cancer research.

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Chapter 2
An Evidence Based Approach to Traditional Herbal Remedies for the Management of Cancer

Contents

Abstract Traditional herbal remedies are one of the frequently used categories of complementary and alternative medicines (CAM) among cancer patients. The aim of this chapter is to review popular medicinal plants used in traditional medicine of different areas for the management of cancer, and additionally to gather evidence for their effectiveness and pharmacological mechanisms in scientific literatures. Traditionally used medicinal plants have been shown to induce apoptosis, and suppress proliferation, invasion, angiogenesis and metastasis in tumor cells. Various mechanisms have been suggested to explain the antitumor activity of them including antioxidant, anti-inflammatory activities by inhibition of nuclear

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receptors, transcription factors, inflammatory cytokines, anti-apoptotic proteins, cell survival pathways, and cell cycle–related- proteins as well. Since these herbal medicines are prevalently consumed by cancer patients, interactions between traditionally used medicinal plants and conventional anti-neoplastic agents should be considered. Because most of evidence about antitumor activity of these plants is obtained from *in vitro* or animal studies, designing human studies and clinical trials is required to conclude the results thereof.

Keywords Traditional medicine • Drug interaction • Herbal medicine • Medicinal plants • Complementary and alternative medicine • Traditional medicine

2.1 Introduction

Cancer is a major problem worldwide, and is the most common or second most common cause of death in many countries. Complementary and alternative medicine (CAM) is reported to be used by about 40 % of all cancer patients. Patients generally do not suppose these approaches to cure their disease but mainly use them to alleviate pain, boost their immune system, or to control treatment-related side effects (Cramer et al. 2013). The results from a systematic review demonstrated that advanced cancer patients, who are female and more educated, younger, have longer duration of disease, and also who have previously consumed CAM are more likely to use that during this stage of illness (Truant et al. 2013). Complementary oncological therapies are classified as alternative medical systems (*e.g.*, homeopathy and traditional Chinese medicine), biologically based medicines (*e.g.*, herbs, vitamins and food), manipulative practices (*e.g.*, massage), energy medicine (*e.g.*, reiki), and mind-body practices (*e.g.*, yoga and meditation) (Cramer et al. 2013). Traditional medicinal herbs as a subcategory of CAM modalities for cancer seems to be beneficial in cancer through several ways: delaying or prevention of disease onset by blocking some of the molecular alterations in the process of cancer formation, preventing the development or metastasis of cancer, improving quality of life, and reducing adverse effects due to conventional cancer treatments including chemotherapeutic agents and radiotherapy (Cassileth and Deng 2004; de Jong et al. 2005). The aim of this chapter is to review popular medicinal plants frequently used in traditional medicine of different areas for the management of cancer, and additionally to gather evidence for their effectiveness and pharmacological mechanisms in scientific literatures.

2.2 Medicinal Plants Traditionally Used for Cancer

In this section, different medicinal plants, used in traditional medicines of different areas, are reported especially those are employed in traditional Chinese medicine (TCM), traditional Iranian medicine (TIM) and ayurvedic medicine for treatment of cancer (Fig. 2.1).

Fig. 2.1 Some pictures derived from traditional Iranian medicine literatures that show the importance of medicinal plants in the management of ailments and their administration by Iranian physicians (Adapted from "Old hand written literatures and resources in Library of Faculty of Traditional Medicine, Tehran, Iran")

2.2.1 Allium sativum *L. (Garlic)*

Garlic is a widely used traditional herbal remedy which considered by National Cancer Institute (NCI) as a vegetable with high potency in cancer prevention (Steinmetz and Potter 1996). Garlic has been demonstrated cytotoxicity against various cancer cells such as colon cancer, glioblastoma, and hepatocarcinoma cells via apoptosis and autophagy. Experimental studies have provided evidence that garlic supplements might protect against colorectal cancer (Chu et al. 2013). However, a meta-analysis of prospective studies demonstrated that the usage of garlic supplements was significantly correlated with an increased risk of colorectal cancer demonstrating a controversy on effectiveness of garlic (Zhu et al. 2014).

The anti-carcinogenic effect of garlic is mainly related to its organosulfur compounds. S-allylmercaptocysteine, a water-soluble organosulfur derivative in garlic, is able to induce apoptosis in many types of cancer cells through the mitogen-activated protein kinase (MAPK) and tumor growth factor-beta (TGF-β) signaling pathways (Tong et al. 2014). Diallyl disulfide showed anticancer properties through immunomodulation (Bauer et al. 2014). It induced cell death through induction of apoptosis in mice leukemia cells and promoted immune responses in leukemic and normal mice *in vivo* (Hung et al. 2014). Diallyl trisulfide inhibited estrogen receptor-α activity in human breast cancer cells and suppressed proliferation of human pancreatic cancer cells by inducing apoptosis (Hahm and Singh

2014; Ma et al. 2014). Moreover, S-allylcysteine suppressed proliferation and induced apoptosis in ovarian cancer cells (Xu et al. 2014).

2.2.2 Boswellia *species*

The oleogum resin, secreted by *Boswellia* trees and commonly known as Frankincense, has been used for the prevention and treatment of chronic inflammatory diseases especially cancer in many countries from ancient times (Hamidpour et al. 2013). Its cytotoxic properties have been frequently reported (Ni et al. 2012; Winking et al. 2000; Frank et al. 2009). *Boswellia* extract induced apoptosis in cervical cancer cells by inducing endoplasmic reticulum stress and generation of reactive oxygen species (Kim et al. 2008; Bhushan et al. 2009).

Triterpenic acids seem to be the major compounds in *Boswellia* responsible for its anti- tumor activities. Boswellic acid caused apoptosis in glioma and leukemia cell lines via p21 expression (Winking et al. 2000; Bhushan et al. 2007). A pentacyclic triterpenediol (TPD) which exists in nature as an isomeric mixture of 3α, 24-dihydroxyurs-12-ene and 3α, 24-dihydroxyolean-12-ene from *Boswellia serrata* showed significant cytotoxic and apoptotic activity in various human cancer cell lines. It has also exhibited *in vivo* antitumor activity in Sarcoma-180 solid tumor bearing mice (Bhushan et al. 2013). Another study found that acetyl-11-keto-beta-boswellic acid suppressed signal transducer and activator of transcription 3 (STAT3), which linked with survival, proliferation, chemo-resistance, and angiogenesis of tumor cells (Kunnumakkara et al. 2007). Furthermore, this compound suppressed human prostate tumor growth via inhibition of angiogenesis induced by vascular endothelial growth factor (VEGF) receptor 2 signaling pathways (Pang et al. 2009).

Boswellia resin has shown anti-edema and antitumor activity in brain tumors. Ethanolic extract of the gum resin of *B. serrata* reduced peritumoral brain edema in patients with brain tumor. A study showed that administration of 3,600 mg/day of *Boswellia* extract to patients with malignant glioma, for 7 days prior to surgery, caused decrease in both the fluid around the tumor and the signs of brain damage. Another study in patients with malignant cerebral tumors, who received *Boswellia* extract as an adjuvant to radiotherapy, showed decrease in cerebral edema and also the ratio of tumor over volume (Hamidpour et al. 2013).

2.2.3 Commiphora mukul *Engl. (Guggul)*

Guggul oleogum resin is one of the ancient drugs, having been first described in Atharva Veda (2000 B.C). According to Ayurvedic literatures, guggul is useful medicine for treatment of various ailments like liver dysfunction, sudden paralytic seizures, internal tumors, malignant sores and ulcers, and intestinal worms (Shishodia et al. 2008).

There are many studies on anti-carcinogenic effects of guggulsterone, the main constituent of guggul gum. Guggulsterone has been found to modulate various stages of cancer. It suppresses proliferation of various tumor cells including those of human leukaemia, head and neck carcinoma, multiple myeloma, breast carcinoma, melanoma, lung carcinoma, and ovarian cancer cell lines; while normal human fibroblasts were found to be resistant to its anti-proliferative effect (Shah et al. 2012; Shishodia et al. 2007). Alongside its anti-proliferative action, guggulsterone induced apoptosis in a wide variety of cells by both mitochondria-dependent and mitochondria-independent mechanisms (Singh et al. 2007; Shishodia et al. 2007). Guggulsterone also suppresses the activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which cause down-regulation of anti-apoptotic genes, and thereby enhance apoptotic activity (Shishodia and Aggarwal 2004). In contrast to apoptotic activity of guggulsterone in cancer cells, this activity was not observed in normal epithelial cells (Singh et al. 2007; Jiang et al. 2013). Guggulsterone was found to down-regulate matrix metalloproteinase-9 (MMP-9) expression, and therefore inhibit tumor cell invasion (Noh et al. 2013). Guggulsterone inhibited invasion of cells in head and neck squamous cell carcinoma via inhibition of STAT-3 (Leeman-Neill et al. 2009). It can also cause inhibition of metastasis by down-regulation of NF-κB regulated gene products (Shishodia and Aggarwal 2004). Moreover than guggulsterone, some other steroids have been isolated from guggul gum with significant anti-proliferative activity against human cancer cell lines (Shen et al. 2012).

2.2.4 Coriandrum sativum *L. (Coriander)*

Aqueous extract of Coriander aerial parts has employed in TIM for treatment of cancer (Emami et al. 2012). Anti-proliferative activity of different extracts from various parts of coriander including roots, leaves and stems were investigated in human breast cancer MCF-7 cell line. The ethyl acetate extract of root with highest phenolic content showed the uppermost anti-proliferative and antioxidant activity. The caspase cascade signaling system as an important component in the process of apoptosis was also activated by this extract. Moreover, *C. sativum* root inhibited DNA damage and prevented MCF-7 cell migration induced by H_2O_2 suggesting its role in cancer prevention and suppression of metastasis (Tang et al. 2013). Linalool, the main constituent of coriander essential oil, seems to be responsible for antitumor activity of *C. sativum* (Jana et al. 2014).

2.2.5 Descureania Sophia *L. (Flixweed)*

The seed of *Descureania Sophia* has been claimed to be effective in the treatment of cancer in TIM (Aghili 2009). Ethanol extract of *D. s*ophia seeds exhibited a

potent cytotoxic effect on human lung cancer cells through biphasic regulatory mechanism involving activation of metabolism-related pathways and signaling-related pathways (Kim et al. 2013). Among different compounds isolated from *D. Sophia* seeds, a cardenolide glycoside showed potent cytotoxicity against human cancer cell lines related to different organs including liver, prostate, colon, ovary, stomach, skin and lung (Lee et al. 2013). Glucosinolates, other major compounds in flixweed seed, are precursors of isothiocyanates which are among the most extensively studied chemoprotective agents. several mechanisms involved in their chemopreventive activity including induction of cytoprotective proteins, inhibition of proinflammatory responses, induction of cell cycle arrest and apoptosis, effects on heat shock proteins, and inhibition of angiogenesis and metastasis (Dinkova-Kostova2013).

2.2.6 Iris germanica *L. (Iris)*

The rhizomes of *Iris* species have been used in the treatment of cancer, inflammation and infections from the ancient time (Ibrahim et al. 2012). Two isoflavones, iriskashmirianin A and germanaism H, isolated from the rhizomes of *I. germanica* demonstrated anti-proliferative effects on Ehrlich's ascites carcinoma cancer cell line (Xie et al. 2013). However, evaluation of six other isoflavones isolated from the rhizomes including irisolidone, irisolidone 7-O-alpha-D-glucoside, irigenin, irilone, iriflogenin, and iriskashmirianin showed no significant cytotoxic activity (Wollenweber et al. 2003).

2.2.7 Linum usitatissimum *L. (Flax)*

Flaxseed has been used in TIM for treatment of cervical cancer (Arzani 2005). Flaxseed inhibits the growth and metastasis of human breast cancer (Chen et al. 2002; Lee and Cho 2012), prostate cancer (Lin et al. 2002), and melanoma (Yan et al. 1998) cells both *in vitro* and *in vivo*. Also, dietary flaxseed has a chemopreventive activity in developing intestinal and colon tumors by increasing omega −3 fatty acid levels and lignans, and decreasing cyclooxygenase (COX)-1 and COX-2 levels (Bommareddy et al. 2009). Flaxseed also decreased tumor biomarkers in men with prostate cancer (Demark-Wahnefried et al. 2008) and in women with breast cancer (Thompson et al. 2005). Its inhibitory role in human breast cancer growth and metastasis is due in part to down-regulation of insulin-like growth factor I and epidermal growth factor receptor expression (Chen et al. 2002). It can induce apoptosis by up-regulating p53 mRNA in breast cancer cell lines (Lee and Cho 2012). Moreover than its chemopreventive role, flaxseed has also reduced radiation therapy-induced lung damage and improved survival (Christofidou-Solomidou et al. 2011).

2.2.8 Matricaria recutita *L. (Chamomile)*

M. recutita has been used in TIM for treatment of cervical cancer (Arzani 2005). The first report about cytotoxic activity of chamomile flowers was in 2007 by Srivastava and Gupta. A significant decrease in cell viability in various human cancer cell lines was reported by aqueous and methanolic extracts of chamomile (Srivastava and Gupta 2007). Infusion from chamomile flower exhibited selective cytotoxicity against some human malignant cell lines including cervix adenocarcinoma, melanoma, breast adenocarcinoma, colon carcinoma and chronic myelogenous leukemia cells (Matić et al. 2013). Weak cytotoxicity and no differential apoptosis in normal cells show selectivity of chamomile in its antitumor action. Phenolic compounds was suggested to be responsible for this selective cytotoxic activity (Matić et al. 2013; Srivastava and Gupta 2007). Apigenin, as a dominant phenolic compound, exhibited anti-proliferative and apoptotic actions in various types of human cancer cell lines (Shukla and Gupta 2010; Srivastava and Gupta 2007). Actually, aglycon form is more active than its glycoside (Srivastava and Gupta 2007). Beside phenolic compounds, essential oil of chamomile has recently been reported to be cytotoxic against three human tumor cell lines including prostate carcinoma, lung carcinoma and breast cancer cells (Zu et al. 2010).

2.2.9 Plantago *species*

Plantago cordata, *P. coronopus*, *P. griesebachii*, *P. lagopus*, *P. lanceolata*, *P. macrostachys*, *P. major*, *P. media*, *P. mexicana*, *P. minor*, *P. ovata*, *P. psyllium*, *P. rocae*, *P. rugelii*, *P. sericea*, *P. tomentosa*, and *P. ureades* have been reported as plants used by humans against cancer (Hartwell 1982). A review on plants ethnomedicinally used against cancer has reported the consumption of *P. asiatica* on Easter Island, *P. hirtella* in Mexico, and *P. oparalias* in Argentina for anticancer purposes (Graham et al. 2000). Furthermore, a review on traditional uses of *P. major* reported its use in Canary Islands, Chile, Venezuela and Panama for treatment of tumors (Samuelsen 2000). Flavonoids are the major constituents in *Plantago* species and among them luteolin-7-*O*-glucoside is the major one in most of *Plantago* species (Kawashty et al. 1994). Methanolic extracts from leaves of seven *Plantago* species (*P. psyllium*, *P. bellardii*, *P. coronopus*, *P. lagopus*, *P. lanceolata*, *P. major* and *P. serraria*) as well as their major component, luteolin-7-*O*-glucoside, revealed cytotoxicity against three human cell lines including renal and breast adenocarcinoma as well as melanoma via topoisomerase-mediated DNA damage (Gálvez et al. 2003). A comparative ecological study of Spanish provinces showed an inverse trend between the consumption of *P. ovata* and colorectal cancer mortality (López et al. 2009). Hot water extract of *P. major* and *P. asiatica* suppressed proliferation of various cancer cell lines via immunomodulatory activity (Chiang et al. 2003). Methanolic extract of

P. major significantly suppressed neoplastic cell transformation by inhibiting the kinase activity of the epidermal growth factor receptor (Choi et al. 2012). Investigation of antitumor activity of *P. major* extract in Ehrlich ascites tumor bearing mice showed inhibitory effect in a dose dependent manner (Ozaslan et al. 2007).

2.2.10 Portulaca oleracea *L. (Purslane)*

Portulaca oleracea has been used as a folk medicine to treat different ailments like cancer (Mirabzadeh et al. 2013). Aqueous and ethanolic extracts from *P. oleracea* leaf showed significant inhibitory effect on murine mammary adenocarcinoma but human Rhabdomyosarcoma cells showed less response toward both extracts. The normal cell line was resistant to cytotoxicity of both extracts (Zakaria and Hazha 2013).

A polysaccharide isolated from *P. oleracea* aerial parts showed anti-proliferative activity on human cervical cancer cells. In addition, it significantly inhibited tumor growth in cervical carcinoma cells-bearing mice. Sub-G1 phase cell cycle arrest, triggering DNA damage and inducing apoptosis seem to be the major mechanisms involved in its cytotoxic activity (Zhao et al. 2013). Three homoisoflavonoids from aerial parts of *P. oleracea* showed *in vitro* cytotoxic activities towards four human cancer cell lines (Yan et al. 2012). Additionally, *P. oleracea* has shown preventive role in adverse events induced by chemotherapy. Pre-treatment with *P. oleracea* extract was found to provide significant protection against cisplatin-induced hepatotoxicity (Sudhakar et al. 2010).

2.2.11 Solanum nigrum *L. (Black Nightshade)*

S. nigrum was among the five most frequently used traditional Chinese medicinal plants for the treatment of cancer, and has also been used as a major ingredient of folk prescriptions for anticancer treatment in China (Meng et al. 2013; Ding et al. 2012). Different studies showed the *in vitro* cytotoxic activity of *S. nigrum* against different human tumor cell lines. Treatment of human colon carcinoma cells with *S. nigrum* significantly inhibited proliferation, adhesion, migration and invasion in these cells (Hu et al. 2013). The aqueous extract of S. nigrum induced autophagy in human colorectal and endometrial cancer cells. It also enhanced cytotoxicity of chemotherapeutic agents in these tumor cells (Tai et al. 2012, 2013). A significant cytotoxic effect of S. nigrum leaf extract on breast cancer cells was mediated via autophagy and apoptosis (Huang et al. 2010). The polyphenolic extract derived from ripe berries of *S.* nigrum caused cell cycle arrest and apoptosis in various human prostate cancer cells without affecting normal prostate epithelial cells (Nawab et al. 2012). This extract showed similar effects in hepatocellular carcinoma cells (Wang et al. 2011). An animal study has demonstrated the potential of *S. nigrum* for treating metastatic melanoma (Wang et al. 2010).

Solamargine, a major steroidal alkaloid glycoside isolated from *S. nigrum*, has been documented to inhibit the growth and induce apoptosis in various cancer cells. Solamargine inhibited proliferation and induced apoptosis in lung cancer cells through suppression of phosphorylation of p38 MAPK and protein expression of STAT3 (Zhou et al. 2014). It also significantly inhibited the growth of hepatoma cells and induced cell apoptosis through the activation of caspase-3 and regulation of cell cycle progression at the G2/M phase (Ding et al. 2012). Solamargine and three other steroidal glycoalkaloids isolated from S. nigrum (solasonine, β1-solasonine and solanigroside P) exhibited cytotoxicity against human gastric cancer cells by induction of apoptosis (Ding et al. 2013). Polysaccharides from *S. nigrum* exhibited certain anti-tumor effect, which is related to the cellular immune function. These polysaccharides significantly inhibited the growth of mouse solid tumors and improved the survival time of tumor-bearing mice via immunomodulatory activity (Chen and Qi 2013). The significant growth inhibition of these polysaccharides on cervical cancer and protective effect on thymus tissue of tumor-bearing mice was also demonstrated (Li et al. 2010).

2.2.12 Urtica dioica *L. (Stinging Nettle)*

Stinging nettle is the most commonly used medicinal plant among cancer patients in Turkey (Kucukoner et al. 2012; Karalı et al. 2012). Avicenna, a great Iranian scientist (11th century), in his famous book, The Canon of Medicine, has claimed that stinging nettle seed is topically effective for treatment of cancer (Avicenna 2008).

Stinging nettle has shown beneficial effect in prostate cancer via inhibition of adenosine deaminase activity in prostate tissue (Durak et al. 2004). U. dioica agglutinin (UDA), an N-acetylglucosamine- specific lectin from the rhizomes of nettle, seems to be the major anti-prostatic compound inhibiting the binding of epidermal growth factor (EGF) to its receptor (EGF-R) (Wagner et al. 1995). The aqueous extract of nettle showed significant anti-proliferative activity against human breast cancer cells by induction of apoptosis (Fattahi et al. 2013).

In addition, nettle can ameliorate toxicity of chemotherapeutic agent. A study showed the hepatoprotective, nephroprotective, and antioxidant activity of nettle methanolic extract against cisplatin toxicity in Erhlich ascites tumor-bearing mice (Özkol et al. 2012). Also, it is used as one of the most common CAM for reducing radiation toxicity (Aksu et al. 2008).

2.2.13 Viola odorata *L. (Sweet Violet)*

Viola odorata is documented in TIM literatures as a first choice for management of cancer (Arzani 2005). Cycloviolacin O2 (CyO2), a cyclotide isolated from

V. odorata, demonstrated to have antitumor effects (Lindholm et al. 2002). Disruption of cell membranes seems to play a crucial role in the cytotoxic effect of CyO2 (Svangård et al. 2007). The cytotoxic activity of CyO2 was evaluated in the breast cancer cell line MCF-7, and its drug resistant sub-line MCF-7/ADR in the presence and absence of doxorubicin. Increased cellular internalization of doxorubicin in drug resistant cells was observed when co-exposed to CyO2. Interestingly, CyO2 did not produce significant membrane disruption in normal cells suggesting its specificity toward tumor cells (Gerlach et al. 2010).

2.2.14 Zingiber officinale *Roscoe (Ginger)*

The rhizome of ginger has widely been used as a spice and condiment in different societies. Additionally, ginger has a long history of medicinal use in various cultures for treatment of common colds, rheumatic disorders, gastrointestinal complications and cancer. Preclinical studies have demonstrated chemopreventive and antineoplastic properties of ginger (Pereira et al. 2011). Since, inhibitors of COX and thus prostaglandin E2 (PGE2) are promising colorectal cancer preventives, ginger has been shown to inhibit COX and decrease PGE2 concentrations in subjects at normal risk for colorectal cancer. However, oral administration of 2 g per day of ginger for 28 days was not able to decrease eicosanoid levels in subjects at increased risk for colorectal cancer (Zick et al. 2014).

The most important chemical constituents with anticancer activity in ginger are gingerols and shogaols. 6-gingerol was effective in the suppression of the transformation and hyperproliferation of cells as well as inflammation that initiate and promote carcinogenesis, angiogenesis and metastasis. 6-gingerol suppressed growth of human colon cancer cells implanted in nude mice. 10-gingerol exerted a potent inhibitory effect on DNA synthesis and caused apoptosis in human promyelocytic leukaemia cells. It induced the formation of Ca^{+2}, which is cytotoxic to the colorectal cancer cell (Poltronieri et al. 2014). 6-shogaol is known to exhibit anti-proliferative, anti-metastatic, and pro-apoptotic activities through suppression of STAT3 expression-regulated gene products in tumor tissues (Kim et al. 2014). It effectively reduced survival and induced apoptosis in human and mouse prostate cancer cells via inhibition of STAT3 and NF-κB activity in these cells. It was more effective than two other compounds found in ginger, 6-gingerol and 6-paradol (Saha et al. 2014). 6-shogaol inhibited the growth of human pancreatic tumors and sensitized them to gemcitabine by suppressing inflammatory pathways linked to tumorigenesis (Zhou et al. 2014). Cysteine-conjugated metabolites of shogaols can be presumed as novel dietary colon cancer preventive agents (Fu et al. 2014). Ginger has also been found to be effective in reducing symptoms of chemotherapy-induced nausea and vomiting (Marx et al. 2013). Furthermore, preclinical studies revealed that ginger and its constituents, dehydrozingerone and zingerone, have protective effects against radiation-induced sickness and mortality via several mechanisms including

antioxidant, anti-inflammatory and anti-clastogenic activity. Additionally, zingerone selectively protects the normal tissues against the tumoricidal effects of radiation (Baliga et al. 2012).

2.3 Interaction Between Traditional Herbs and Anti-neoplastic Agents

Since traditional medicinal plants are prevalently consumed by cancer patients, understanding interaction between them and conventional antineoplastics is highly important. Extensive use of herbs by people suffering from cancer has been reported across the Middle East: in Turkey (32 %), Jordan (35 %), Arab patients in Israel (34 %) and the Palestinian authority (61 %). Patients' motives for herbal use include relief of cancer-related symptoms, lessening of side effects from chemotherapeutic agents, slowing down disease progression, and cure of disease (Almog et al. 2014). A survey in Turkey showed that 66 % of cancer patients used herbal medicine stated that their usage of herbal medicines was based on the media and the Internet as a source, and 64 % stated that they received information about herbal medicines from relatives and friends. Only 24 % of herbal medicines users had consulted or discussed their use with a physician (Tuna et al. 2013). A list of medicinal plants that demonstrated potential interactions with conventional anti-neoplastic agents have been shown in Table 2.1. Some other medicinal plants are also proposed to have interactions with novel anti-neoplastic agents but human studies have demonstrated no interactions yet. For example, reduction in cytotoxicity of cisplatin by *Cimicifuga racemosa* has demonstrated in animal model without enough clinical evidence (Rockwell et al. 2005). Another example is interaction of garlic with docetaxel, which expected because of cytochrome P450 inhibitory effect of garlic and metabolization of docetaxel by these isoenzymes, although no effect of garlic on pharmacokinetic profile of docetaxel has been observed (Cox et al. 2006).

2.4 Conclusion

Traditional medicinal plants from different countries could be considered as a valuable source for discovering new drugs. Cancer is proposed as a chronic ailment, in which frequent use of traditional herbal remedies has been reported. The considerable properties of traditionally used medicinal plants differentiate them from conventional anti-neoplastic agents especially regarding their specificity to cancer cells without anti-proliferative activity on normal cells. Most of the studies have investigated *in vitro* cytotoxicity on various human cancer cell lines or *in vivo* antitumor activity in tumor bearing animals, although human studies and clinical

Table 2.1 Important herbs that are capable of showing interactions with conventional anti-neoplastic agents

Herb (scientific name)	Common name	Antineoplastic agent	Result(s)	Reference(s)
Angelica sinensis	Chinese angelica	Tamoxifen	↓Efficacy of tamoxifen → stimulation of breast cancer growth	Boyle (1997)
Aronia melanocarpa	Black chokeberry	Trabectedin	↓CYP3A4 → ↑BA of trabectedin → rhabdomyolysis	Strippoli et al. (2013)
Cannabis sativa	Hemp, Marijuana	Cisplatin	↑Risk of stroke by cisplatin	Russmann et al. (2002)
Citrus paradisi	Grapefruit	Etoposide	↓BA of etoposide	Reif et al. (2002)
Hypericum perforatum	St. John's wort	Irinotecan	↑CYP3A4 → ↓ irinotecan → ↓toxicity and efficacy	Rahimi and Abdollahi (2012)
		Imatinib	↓BA of imatinib	
Panax ginseng	Ginseng	Imatinib	↓CYP3A4 → ↑BA of imatinib → hepatotoxicity	Bilgi et al. (2010)
		Tamoxifen	↓Efficacy of tamoxifen → stimulation of breast cancer growth	Cui et al. (2006)

↑ means increase and ↓ means decrease, *BA* bioavailability, *CYP* cytochrome P

trials on the efficacy of these plants are rare. A clinical pilot study on ginger shows its preventive role in subjects with normal risk for colorectal cancer, while no effect was observed in subjects with increased risk for colorectal cancer (Zick et al. 2014). Another example is a comparative ecological study indicating an inverse trend between the consumption of *P. ovata* and colorectal cancer mortality (López et al. 2009). Also, human studies have revealed the decreased tumor biomarkers in men with prostate cancer (Demark-Wahnefried et al. 2008) and in women with breast cancer (Thompson et al. 2005) following consumption of flaxseed. It seems that the most human studies are on *Boswellia* demonstrating its anti-edema and anti-tumor effects in subjects with brain tumor. For this reason, *Boswellia* and its major compounds, boswellic acids, are considered as promising complementary drugs for this type of cancer (Hamidpour et al. 2013). Some of the investigated traditional herbal remedies have been demonstrated to be effective in different stages of cancer (proliferation, invasion, angiogenesis and metastasis of tumor cells) including *Boswellia* resin and its triterpenic acids, oleogum resin of *Commiphora* species, *S. nigrum* and its alkaloid solamargine, as well as ginger and its constituents, gingerols and shogaols.

Furthermore, some of traditional herbal remedies could manage treatment-related side effects. Reduction of radiation therapy-induced lung damage by flaxseed (Christofidou-Solomidou et al. 2011), protection against cisplatin-induced

hepatotoxicity by *P. oleracea* extract (Sudhakar et al. 2010), reduction of cisplatin- and radiation- induced toxicity by stinging nettle (Özkol et al. 2012; Aksu et al. 2008), and reducing symptoms of chemotherapy-induced nausea and vomiting together with radiation-induced sickness and mortality by ginger (Marx et al. 2013; Baliga et al. 2012) are some examples.

Due to high prevalence of medicinal plants consumption among cancer patients, interactions between these plants and conventional anti-neoplastic agents should be considered. Interactions may lead to increase drug metabolism and consequently decrease the bioavailability and efficacy of such drugs. On the other hand, medicinal plants may decrease drug metabolism and subsequently increase adverse events and toxicity of anti-neoplastic agents. Beside pharmacokinetic interactions, pharmacodynamic mechanisms may also involve such as interaction between *Angelica sinensis* and tamoxifen, which is mediated by the effects of *A. sinensis* on estrogen receptors, and antagonizing the effects of tamoxifen on these receptors. Since most of evidence about anti-tumor activity of these plants is from *in vitro* or animal studies, designing human studies to obtain more conclusive results is suggested.

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Chapter 3
Marine-Derived Anticancer Compounds

Contents

Abstract Huge number of taxonomically diverse creatures live in oceans and produce structurally various types of metabolites. Because of such an ample resource and successful experiences in delivering potent therapeutics to the market, the scope of marine pharmaceuticals is now very wide. Particularly, great effort has been put into the isolation and clinical development of compounds that can overcome cancer, the leading cause of death over the world. Beside compounds like trabectedin and eribulin mesylate, which have succeeded in reaching the marketplace, a large number of marine compounds or modified derivatives thereof are currently in preclinical and clinical trial stages. Therefore, an increased number of approved agents against cancer would be anticipated in the near future. The scope of marine natural products through delivering safer and potent anticancer medicines into the market give some hope of finding successful treatments for cancer. This chapter gives useful information about the anticancer marine compounds, which have recently entered into the marketplace or currently are in the stages of preclinical and clinical development.

Keywords Algae • Marine organisms • Clinical trial • Mechanism of action • Sponge • Tunicate

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3.1 Introduction

During the past few decades, there has been an increasing worldwide trend in exploring new natural compounds from the marine environment. Covering of about 95 % of the biosphere's surface and harboring numerous marine creatures that started their lives from around 3.5 billion years ago and have gradually evolved, marine environment offers a vast and ample resource for discovery of natural products (Sithranga Boopathy and Kathiresan 2010). Most of the marine floras like tunicates, sponges, bryozoans, mollusks, and even marine microorganisms are known to be toxic or produce metabolites with toxic effects to the other marine creatures or human being. They generate structurally complex and diverse poisonous compounds enabling them to defend and compete with others in intensely competitive and hostile habitats of the marine ecosystem and remain alive (Arumugam et al. 2013). There is a famous dictum that "every drug is a poison and every poison may be a drug". Thus, it is not strange that many of the isolated marine compounds have shown significant biological activities particularly against cancer. In recent years, enormous efforts of science and industry researchers led to the development of a number of marine-derived compounds or synthetic analogues thereof into anticancer therapeutics. Although a few compounds have gained approval for treatment of cancerous diseases, there are lots of compounds in preclinical and clinical trial stages warranting promising and encouraging perspective on the scope of marine natural product researches (Gerwick and Moore 2012; Mayer et al. 2010).

The intention of this chapter is to offer useful information about source, chemical structure, clinical status and mechanisms of action for the most important marine compounds currently ongoing trials due to their potential anticancer properties. Moreover, it provides recent, brief and helpful information for experienced researchers and clinicians. Meanwhile, compounds here have been classified on the basis of their sources.

3.2 Tunicates

Tunicates, also known as "sea squirts", are marine animals with a bag like body covered by an outer protective (tunic). Adult tunicates are mostly sessile and attach to hard surfaces such as rocks and corals (Gupta et al. 2013). Tunicates are recently considered as a rich source of potential anticancer agents. A few tunicate-derived compounds such as trabectedin and plitidepsin demonstrated significant therapeutic impact and promising results in clinical trials in the USA and Europe (Cooper and Yao 2012).

Trabectedin (ET-743, Ecteinascidin, Yondelis®) is an isoquinoline-type alkaloid originally derived from the Caribbean tunicate *Ecteinascida turbinata*. It is currently produced by a semi-synthetic process from the antibiotic cynosafracin B, which is obtained from bacterial fermentation. Chemical structure

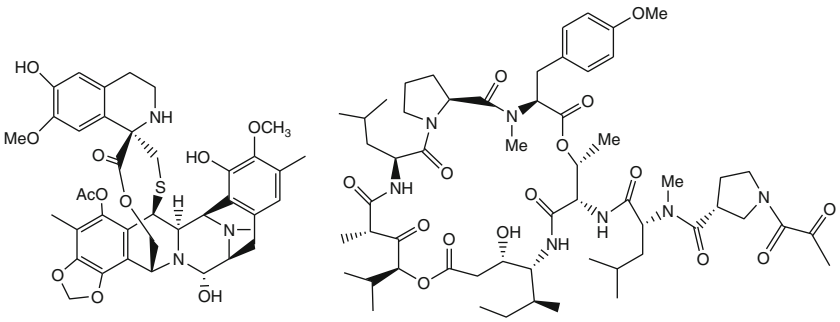


Fig. 3.1 Chemical structures of two anticancer compounds from tunicates

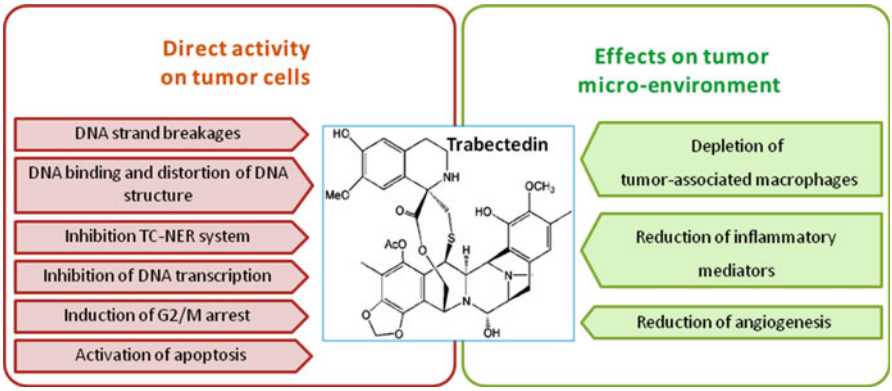


Fig. 3.2 Mechanisms of action for trabectedin. Trabectedin directly acts against tumor cells and also modulates tumor microenvironment

of trabectedin contains a pentacyclic skeleton made up of two fused tetrahydroisoquinoline rings (subunits A and B). It is connected to a third tetrahydroisoquinoline ring (subunit C) *via* a 10-membered sulfide-containing lactone (Fig. 3.1). Two fused rings (subunits A and B) covalently interact with the N2 amino group guanine in the DNA minor groove leading to DNA denaturation, while traditional alkylating drugs interact with guanine at the N7 or O6 position. Subunit C does not involve in interaction with DNA, but binds to nuclear proteins such as transcription factors or DNA repair proteins (D'Incalci and Galmarini 2010; Christinat and Leyvraz 2009). Although trabectedin is often defined as a DNA binding agent, this is only a part of its several mechanisms of action underlying its anti-tumor and anti-metastatic activities (Fig. 3.2). It also disrupts transcription-coupled nucleotide excision repair (TC-NER) system and causes lethal DNA strand breakages *via* interacting DNA-NER proteins. TC-NER is a system that recognizes DNA damage and exploits various factors to repair them in order to provide continuity of transcription and cell survival. Consequently, trabectedin blocks cell cycle in G2/M phases and

activates p53-independent process of apoptosis pathways leading to cell death (D'Incalci and Galmarini 2010; D'Incalci et al. 2014). In addition to the unique actions of this agent on neoplastic cells, its ability to modulate tumor microenvironment attracts interest. Trabectedin suppresses production of chemokines and cytokines such as CCL2 (chemokine (C-C motif) ligand 2), CXCL8 (chemokine (C-X-C motif) ligand 8), IL-6 (Interleukin 6) and VEGF (vascular endothelial growth factor) by monocytes, macrophages, and tumor-associated macrophages. These factors play important roles in tumor progression *via* activities such as chemotaxis for monocytes and other cells at the tumor site, growth stimulation for several neoplastic cell types and induction of angiogenesis. *In vivo* animal and clinical studies revealed that trabectedin selectively reduces mononuclear phagocytes, including tumor-associated macrophages (TAM) through inducing rapid apoptosis. TCMs are found abundantly in tumor masses and involve in inflammatory reactions that promote cancer progression and resistance to therapies, so they are currently considered as an interesting therapeutic target in cancer (D'Incalci and Galmarini 2010; Germano et al. 2013).

In vitro studies on neoplastic cell lines and *in vivo* animal studies on xenografted tumors showed that trabectedin significantly acts against sarcomas particularly in liposarcomas and leiomyosarcomas (Campo et al. 2013). After passing all phases of clinical testing, "European Commission" approved trabectedin for the treatment of patients with soft tissue sarcoma in patients who have prior failed chemotherapy regimens containing anthracyclines and ifosfamide. The combination of trabectedin with pegylated liposomal doxorubicin for the treatment of patients with platinum-sensitive recurrent ovarian cancer was another approval that the "European Commission" has granted (Poveda et al. 2014). Obtaining promising results from preclinical combinations of trabectedin with other anticancer agents resulted in several current studies for clinical trials of trabectedin combinations (D'Incalci and Galmarini 2010). Trabectedin is shown to be a well-tolerated drug, lacking cumulative organ-specific toxicity. Its common adverse effects are neutropenia, thrombocytopenia, and transient increase of hepatic tests. Pretreatment with dexamethasone decreases the risk of its hepatoxicity and myelosuppression (Christinat and Leyvraz 2009).

Plitidepsin (dehydrodidemnin B, Aplidin®) is a cyclic depsipeptide initially extracted from the Mediterranean tunicate *Aplidium albicans*, while it is currently produced by chemical synthesis (Fig. 3.1). Like trabectedin, plitidepsin has different molecular targets on both cancer cells and their molecular environment. Strong apoptotic activity, reduction of intracellular glutathione, JNK (c-Jun NH(2)-terminal protein Kinases) pathway activation and reactive oxygen species induction as well as anti-angiogenic activity *via* inhibition of VEGF secretion and down-regulation of VEGFR-1 (VEGF receptor 1) are some of its mechanisms (Danu et al. 2013). This agent is now being tested in a phase III study for the treatment of refractory/relapsed multiple myeloma patients. It also has shown clinical activity in phase II clinical trials for treatment of T cell lymphoma (Galmarini et al. 2014; Mayer et al. 2010). Similar to trabectedin, plitidepsin is a well-tolerated drug and the most frequently occurring adverse events after its consumption are nausea, fatigue and myalgia (Galmarini et al. 2014).

3.3 Sponges

Sponges are multi-cellular primitive organisms. They are sessile animals, which have no body and tissues and their asymmetric body are full of pores and channels allowing water flow through them continuously. The water brings in nourishment and oxygen, and it also removes waste products. Sponges produce molecular responses to defend against predators, and control symbiotic bacteria or compete with other sessile species. Accordingly, they are a rich source of bioactive molecules, a number of these compounds and analogues thereof, such as eribulin mesylate and α-galactosylceramide have shown a potential therapeutic effect against various cancers (Monaco and Quinlan 2014).

Halichondrin B is a sponge-derived large polyether macrolide. It was originally isolated from a marine sponge, *Halichondria okadai* (Fig. 3.3). Initial investigations showed that this agent displays potent anticancer activity *in vitro* and *in vivo* (Menis and Twelves 2011; Preston and Trivedi 2012). Further studies indicated that

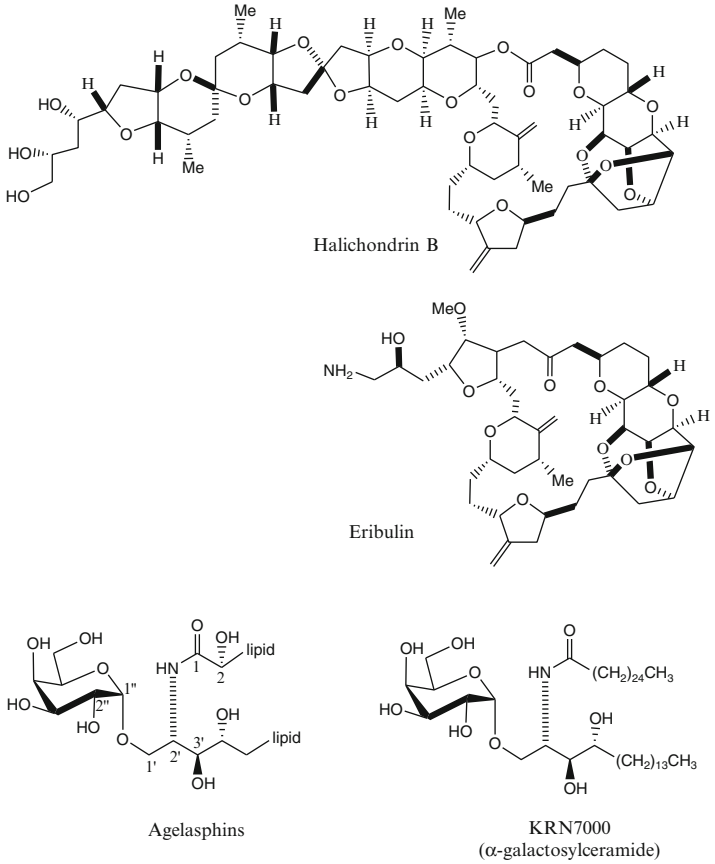


Fig. 3.3 Chemical structures of some anticancer compounds from sponges

halichondrin B arrests mitosis *via* depolimerizing tubulins, but its mechanisms of interaction with tubulin is novel and differs from those of other antitubulin drugs such as taxanes, vinca alkaloids and epothilones (Huyck et al. 2011). However, scarcity of natural source for production of halichondrin B prevented its development as a therapeutic agent. To overcome this barrier, the development of a simple synthetic route for production of halichondrin B and its analogues was considered as the next step. Eribulin (E7389; Halaven®), as one of the structurally simplified synthetic analogue of halichondrin B, showed anticancer activity comparable to that of the parent compound (Fig. 3.3). It is available as a mesylate salt and showed considerable anticancer activity in preclinical and clinical studies (Cortes et al. 2012; Towle et al. 2011). A large number of preclinical studies demonstrated that eribulin possesses potent anticancer activities, including promising activity against taxan-resistant cancer cells (Pean et al. 2012). In 2010, US food and drug administration (FDA) approved eribulin for the treatment of patients with metastatic breast cancer, who have at least two prior failed chemotherapy regimens containing an anthracycline and a taxane. One year later, "European Commission" granted similar approval throughout the European Union for eribulin (Pean et al. 2012; European Medicines Agency 2011; Administration UFaD 2010). Due to exhibiting high anticancer efficacy, low incidence of hypersensitivity, and being useful even in taxane resistant cases, eribulin was subjected for further clinical trials to be assessed for a variety of other indications such as lung, ovarian, pancreatic, bladder and soft tissue tumors (Shetty and Gupta 2014). The most common side effects with eribulin are weakness and fatigue, neutropenia, alopecia, nausea, peripheral neuropathy, constipation and leukopenia (Pean et al. 2012).

Agelasphins are sponge-derived anticancer glycolipids isolated from *Agelas mauritanius*. Their chemical structures comprise saccharides that link in an α or β-configuration to a long-chain amino alcohol, a phytosphingosine-based ceramide (Fig. 3.3). Structure-activity relationship studies (SAR) of agelasphins were followed by the synthesis of a promising analog, KRN7000 (commonly known as α-galactosylceramide). It consists of D-erythro-phytosphingosine, which is N-acylated with a 26-carbon fatty acid and a galactosyl moiety α-linked to it (Fig. 3.3) (Anderson et al. 2013; Franck 2012).

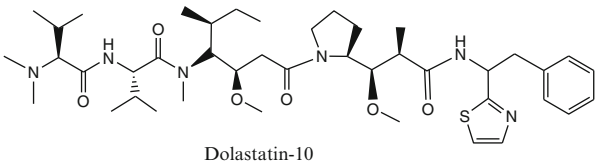
The α-galactosylceramide acts as a ligand for the activation of CD1d-mediated natural killer T (NKT) cells. NKT cells are a T lymphocyte subpopulation expressing both T cell and natural killer (NK) receptors (Wu et al. 2008; Motohashi et al. 2009). They involve in regulation of the immune system *via* stimulation of cytokine release. Activation of NKT cells leads to activation of other immune cells, such as NK cells, dendritic cells (DC), macrophages, B lymphocytes, and conventional T cells. Consequently, other cytokines are secreted by these cells and extensive responses of the immune system are triggered (Wu et al. 2008). Ligand-binding site of the CD1d receptor contains two hydrophobic channels, which show affinity for hydrophobic long chains (eg. fatty acyl and sphingosine tails). In addition, hydroxyls of galactose and 3′ hydroxyl of sphingosine form hydrogen bonding with protein residues of the receptor. After forming complexes, secretion of regulatory cytokines including IL-12, IL-4 and IFNγ (Interferon

gamma) are stimulated (Wu et al. 2008; Franck 2012). In animal models, intravenous administration of α-galactosylceramide showed its efficacy in the treatment of various tumors such as melanoma, sarcoma, colon carcinoma and lymphoma. Lately, Tatsumi et al. (2011) showed the α-galactosylceramide activity against liver tumors in mice and investigated its detailed mechanism of action in activating liver DCs and immune cells. Kim et al. (2010) used α-galactosylceramide as an adjuvant to potentiate a DNA vaccine that induces the generation of cytotoxic T lymphocytes. This analogue also has shown efficacy in the treatment of several diseases such as malaria, microbial infections and hepatitis B as well as autoimmune disorders (Wu et al. 2008).

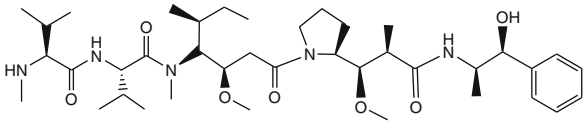
3.4 Cyanobacteria

Cyanobacteria are a diverse group of prokaryotic photosynthetic organisms. They have raised an enormous interest due to nitrogen fixing ability and producing novel and active nitrogen-containing metabolites. Culturability is another characteristic of these microorganism that lets them to be more useful as a source of natural products. A growing number of anticancer compounds are isolated from cyanobacteria that target tubulin in eukaryotic cells (Tan 2007; Burja et al. 2001).

Dolastatins are a class of these compounds. They are natural cytotoxic peptides initially isolated from the mollusk, *Dolabella auricularia*. However, further studies revealed their presence in marine cyanobacteria consumed by the mollusk (Villa and Gerwick 2010). Dolastatin-10 with linear pentapetide structure was known as the most promising cytotoxic agent of this class (Fig. 3.4) (Suarez-Jimenez et al. 2012). It exhibited potent activity against several cancerous cell lines via microtubule assembly inhibition and apoptotic activation. Dolastatin-10 has been evaluated in various phase I and II clinical trials. However, observed toxicities like moderate peripheral neuropathy, and minimal activity in patients with advanced breast cancer or recurrent platinum-sensitive ovarian carcinoma lead



Dolastatin-10



MMAE

Fig. 3.4 Chemical structures of dolastatin-10, an anticancer compound from cyanobacteria and its synthetic analogue, MMAE (monomethyl auristatin E)

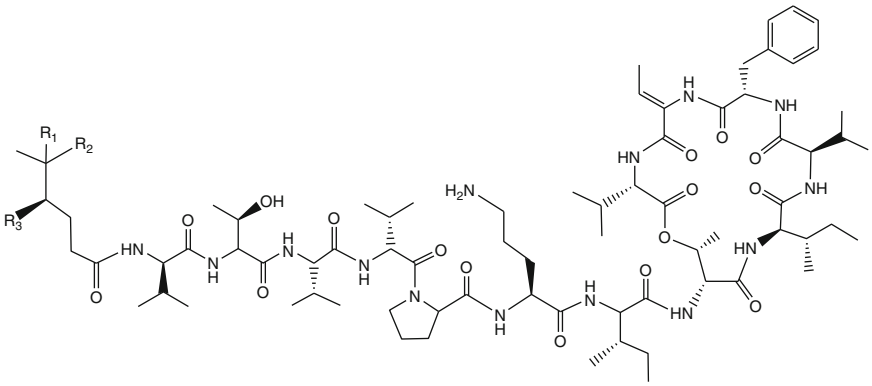
to synthesis and development of less toxic and more potent analog compounds such as monomethyl auristatin E (MMAE) (Fig. 3.4) (Perez et al. 2005; Villa and Gerwick 2010). MMAE is the component of two antibody-drug-conjugates (ADC), brentuximab vedotin (cAC10-vcMMAE or SGN-35) and glembatumumab vedotin (CR011-vcMMAE or CDX-011). Brentuximab vedotin consists of MMAE, cAC10 (a CD30-targeted monoclonal antibody), and a lysosomal enzyme-cleavable linker that attaches MMAE to cAC10. After binding this drug to CD30 and internalization, the complex of brentuximab vedotin and CD30 transferred to the lysosome, where lysosomal enzymes cleave the linker. Then released MMAE leads to apoptosis and inhibition of microtubule assembly (Pro and Perini 2012).

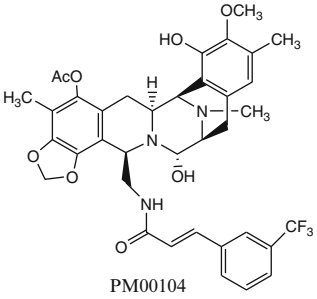
On August 2011, FDA granted approval to brentuximab vedotin (Adcetris®) for the treatment of Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or at least two multiagent chemotherapy regimens, and for systemic anaplastic large-cell lymphoma after failure of at least one multiagent chemotherapy regimen (Newland et al. 2013). Glembatumumab vedotin comprises a different monoclonal antibody (CR011) directed against glycoprotein NMB (GPNMB). Showing significant efficacy in the treatment of melanoma and breast cancer, Glembatumumab vedotin is currently undergoing early clinical studies (Vaklavas and Forero 2014).

3.5 Mollusks

Mollusks are a large phylum of invertebrate animals. Although some types of mollusks are shelled, they all have soft and vulnerable bodies. Mollusks produce a wide range of bioactive compounds, which circulate in their haemolymph, or secret in their body surface mucus in order to protect them against predators and pathogens. They also produce metabolites to form chemical communication systems. An increasing number of bioactive metabolites have been isolated from mollusks, and a few of them as well as their synthetic analogues are reached to different stages of clinical trial testing for treatment of cancerous tumors (Benkendorff 2010).

Kahalalides is a class of depsipeptides isolated from the sacoglossan mollusk *Elysia rufescens* for the first time, and then in much lower concentration separated from its algal diet *Bryopsis pennata*. Kahalalide F is a large cyclic tridecapeptide that comprises several unusual amino-acid residues (Fig. 3.5). Mechanisms of anticancer action of kahalalide F have not yet been fully established. However, induction of vacuolization *via* disturbances in lysosomal function, induction of necrosis-like cell death *via* suppression of ERBB3 protein, and inhibition of the PI3K–AKT signaling pathway, as well as increasing cell-membrane permeability are involved in its activity. Kahalalide F is currently under phase II clinical trials for non-small-lung cancer, melanoma and hepatocellular carcinoma (Serova et al. 2013; Molinski et al. 2009).





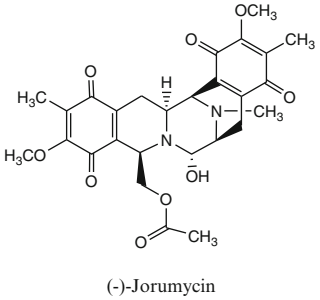


Fig. 3.5 Chemical structures of some molluscan-derived anticancer compounds

Elisidepsin (PM02734, Irvalec®) is a synthetic analogue of kahalalide F with significant antineoplastic results against human solid tumors and acceptable preclinical toxicology profile (Fig. 3.5). It is currently in early-stage of clinical development for squamous non-small cell lung cancer and advanced or metastatic esophageal, gastric or gastroesophageal junction cancer (Coronado et al. 2011).

(−)-Jorumycin was isolated from the enclosing epidermis and mucus of a mollusk, *Jorunna funebris*. This compound structurally belongs to the isoquinoline marine alkaloids and its chemical structure is very similar to two fused isoquinoline rings (subunits A and B) of trabectedin (Fig. 3.5). However, it lacks 10-membered lactone bridge and the third tetrahydroisoquinoline moiety (see trabectedin structure in Fig. 3.1). *In vitro* studies on various human tumor cell lines indicated its potent anti-proliferative activity at nanomolar concentrations (Chen et al. 2013). PM00104 (Zalypsis®) is one of the promising synthetic analogues of (−)-jorumycin with the same structural bases of dimeric isoquinoline rings (Fig. 3.5). Therefore, it can bind to the minor groove of DNA (similar to trabectedin) resulting in DNA double strand breakages, cell cycle blockade in S-phase and apoptosis (Fig. 3.5) (Capdevila et al. 2013; Jones et al. 2014; Romano et al. 2013). However,

due to chemical differences in subunit C structure, which involves in disruption of TC-NER, PM00104 does not show TC-NER-dependency of trabectedin in cellular targeting. In other words, DNA damage induced by PM00104 does not depend on the activity of NER complex. Thus, it differs from trabectedin and platinum derivatives, which selectively affect TC-NER-proficient cells (Guirouilh-Barbat et al. 2009). Currently, PM00104 is in phase II clinical investigations for several cancer types including multiple myeloma, cervical and endometrial cancer, and Ewing's sarcoma (Moneo et al. 2014). Guirouilh-Barbat et al. suggested that histone γ-H2AX can be considered as a pharmacodynamic biomarker of this compound (2009). Moreover, Moneo et al. indicated that activation of tyrosine kinase receptor can be used as a determinant parameter for resistance to PM00104 (2014).

3.6 Algae

Algae is a diverse group of photosynthetic organisms. They are grouped into two microalgae (unicellular microscopic organisms) and macroalgae (multicellular and large organisms). They have been recently interested as functional foods by the food and pharmaceutical industries owing to their bioactive metabolites. Many studies demonstrated the immunomodulating and anti-tumor activities of algae (Ibañez and Cifuentes 2013). Fucoxanthin and fucoidan are two important algae-derived compounds presented here.

Fucoxanthin is a common algal pigment found in various classes of microalgae, as well as brown macroalgae and diatoms. It plays an essential role in the photosynthetic process of these marine organisms *via* light harvesting function. Fucoxanthin is a xanthophyll that its chemical structure includes a polyenic chain along with two unusual moieties, an allenic bond and an epoxy group (Fig. 3.6) (Kumar et al. 2013; Rengarajan et al. 2013). In addition, hydroxyl, carbonyl and carboxyl moieties are other functional groups of this structure contributing in its medicinal activities (Peng et al. 2011). Some edible brown seaweed such as *Undaria pinnatifida, Hijikia fusiformis*, *Laminaria japonica* and *Sargassum fulvellum* are rich sources of fucoxanthin. Extract forms of these macroalgae are commercially available as food supplements (Rengarajan et al. 2013; D'Orazio et al. 2012). Fucoxanthin possesses a variety of medicinal properties including antioxidant, anti-inflammatory, anticancer, anti-obese, anti-diabetic, anti-angiogenic, antimalarial and anti-photoaging activities. Dietary fucoxantin was shown to convert to two active metabolites contributing in the mentioned medicinal activities; fucoxanthinol is directly derived from fucoxantin through deacetylation by digestive enzymes in the gastrointestinal tract and further, converted to amarouciaxanthin A in the liver (Fig. 3.6) (D'Orazio et al. 2012; Peng et al. 2011).

Many studies have revealed that fucoxanthin has remarkable potential as a chemotherapeutic or chemopreventive agent. Anti-proliferation, activation of apoptosis pathway, blocking cell cycle progression, and anti-angiogenesis have

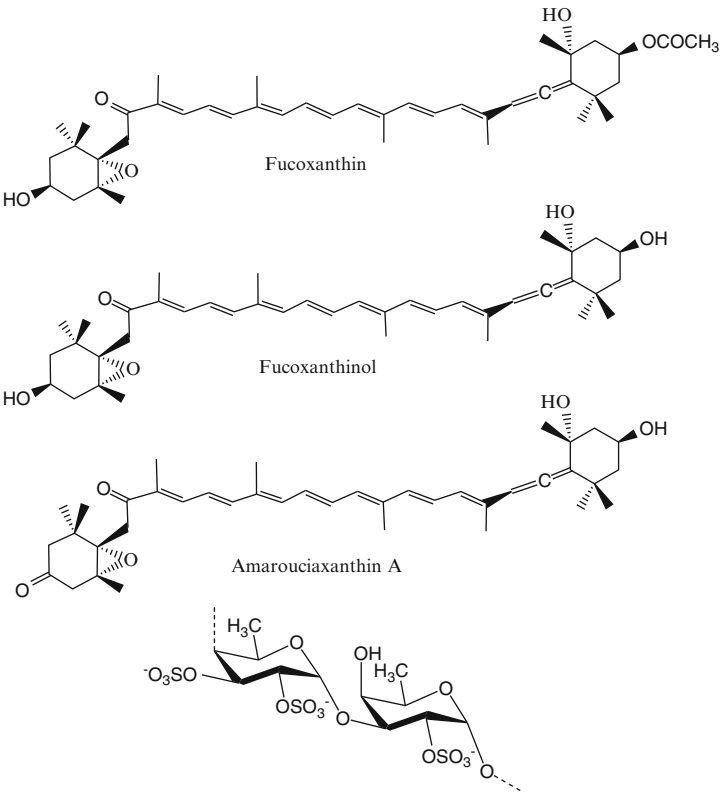


Fig. 3.6 Chemical structures of fucoxanthin, its two metabolites, fucoxanthinol and amarouciaxanthin A, and a common structural unit of fucoidans

been found as the main anticancer mechanisms of this compound (Rengarajan et al. 2013). Fucoxanthin, as an anticancer and chemopreventive agent, diminishes incidence and growth of tumors and exhibits cytotoxicity against cancer cells. Impressively, several studies exhibited that fucoxanthin exerts selective cytotoxicity to cancerous cells rather than normal cells (Kumar et al. 2013). Moreover, Chung et al. reported that fucoxanthin has a considerable inhibitory effect on the cancer metastasis and is able to apply for preventing cancer metastasis (2013). It also shows synergistic activity in combination with established cytotoxic drugs. However, there has been a lack of clinical studies on this marine natural compound so far and more clinical studies should be conducted to develop fucoxanthin as a new drug (Rengarajan et al. 2013; Kumar et al. 2013).

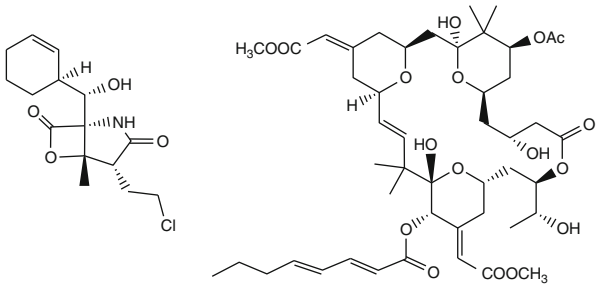
Fucoidan is a sulfated polysaccharide found mainly in the cell-wall matrix of various brown algae species such as *Laminaria* and *Fucus*. It fundamentally contains fucose (a hexose deoxy sugar) unit and sulfate ester groups (Fig. 3.6). It also composes small amounts of galactose, mannose, xylose, glucose and/or glucuronic

acid. Fucoidan structure varies depending on the extraction methodology and the seaweed species used as a source of isolation. Like edible sources of fucoxanthin, some fucoidan-containing brown algae are available as dietary supplements (Wijesinghe and Jeon 2012; Senthilkumar et al. 2013; Kwak 2014). Fucoidans have been reported to display anti-coagulant, anti-thrombotic, immunomodulatory, anti-inflammatory, anticancer, cardioprotective, antivirus, gastric mucosal protective, neuroprotective antioxidant and anti-lipidemic activities. Particularly, recent studies have shown its effectiveness in fighting cancer. Fucoidan shows growth inhibitory effects through induction of cell cycle arrest, induction of apoptosis, modulation of growth signaling molecules and anti-metastatic and anti-angiogenetic activities. Beside these fucoidan direct actions on tumor cells, it also fights cancer cells and their metastasis through stimulation of the immune system in several ways (Senthilkumar et al. 2013; Wijesinghe and Jeon 2012). Anticancer activities of fucoidans were observed to vary depending on their structures, SAR studies of these compounds revealed that their biological activities are potentiated by an increase in degree of sulfation. In addition, the positions of the sulfated groups and the backbone's macromolecular structure affect their functional properties (Wijesinghe and Jeon 2012). A recent study revealed that fucoidan could potentiate the anticancer activity of established chemotherapeutic agents such as cisplatin, tamoxifen or paclitaxel against breast cancer cells (Zhang et al. 2013). Another study demonstrated that fucoidan as an immunostimulatory agent could display cytoprotective effects against 5-FU-induced cellular damage on dendritic cells (Jeong et al. 2012). Although many studies demonstrate therapeutic effects of fucoidan for the prevention and treatment of cancer, more complete preclinical and clinical trial studies are needed to develop this compound as a therapeutic agent.

3.7 Other Marine Sources of Anticancers

Generally, important sources of marine-derived anticancer agents and their metabolites, which have received considerable attention in the literature, were introduced and discussed in previous sections. However, there are some other marine creatures as bryozoans, marine bacteria and marine fungi and so on, which have remained unmentioned.

Bryostatins are cyclic polyketides, which are originally isolated from the marine invertebrate Bryozoan, *Bugula neritina*. They all similarly comprise macrolactone core with three tetrahydropyran rings. Among them, bryostatin 1 has been extensively studied preclinically and clinically (Fig. 3.7) (Trindade-Silva et al. 2010). Studies demonstrated that bryostatin 1 activate protein kinase C (PKC). This agent also has been reported to have immunomodulatory, radioprotective and cell differentiative activities. Moreover, frequent studies demonstrate its synergistic therapeutic effects in combination with other anti-tumors such as doxorubicin, paclitaxel, vincristine, dolastatin, *etc*. (Molinski et al. 2009). On the basis of the promising results of preclinical studies, over thirty phase I and II clinical trials



Salinosporamide A Bryostatin 1

Fig. 3.7 Chemical structures of a bryozoans-derived compound, bryostatin 1 and a marine bacterial compound, salinosporamide A

have studied efficacy of bryostatin 1, either as a single agent or in combination with other chemotherapeutic agents on various cancers. However, none of them has yet progressed to phase III clinical tests. Currently, bryostatin 1 entered early- stages of clinical development for Alzheimer's disease (Trindade-Silva et al. 2010).

Salinosporamide A (NPI-0052, marizomib) is a potent proteasome inhibitor, which was first isolated from the marine actinomycetes bacterium, *Salinispora tropica*. Its chemical structure is characterized by γ-lactam-β-lactone pharmacophore (Fig. 3.7) (Gerwick and Moore 2012). Salinosporamide A potently and irreversibly inhibits all three proteolytic activities of 20S proteasome. Adverse events such as peripheral neuropathy, neutropenia and thrombocytopenia commonly induced by bortezomib (a proteasome inhibitor previously approved), have not been reported frequently after salinosporamide A administration. Moreover, this agent showed anti-tumor activity in bortezomib-resistant tumors. Accordingly, salinosporamide A has advanced from preclinical to clinical development (Millward et al. 2012). Recently, a series of phase I dose-escalation studies evaluated this compound as a single agent in patients with multiple myeloma, solid tumors, and lymphomas. Also, a phase I study indicated its high synergistic anti-tumor activity and safety in combination with the histone deacetylase inhibitor, vorinostat (Millward et al. 2012).

3.8 Conclusion

The area of drug development from marine sources has been increasingly active and productive in the past few decades. Figure 3.8 shows the current clinical status of major marine drugs with a schematic view. There are many other compounds, especially in preclinical evaluation and early stages of clinical trials, not mentioned here. Although a few compounds have gained approval for cancer treatment so far, it can be surmised that such a rich pipeline will deliver more drugs into the market in the near future. Based on the examples illustrated above, novelty in chemical structure and mechanisms of action of marine compounds as well as multi-target

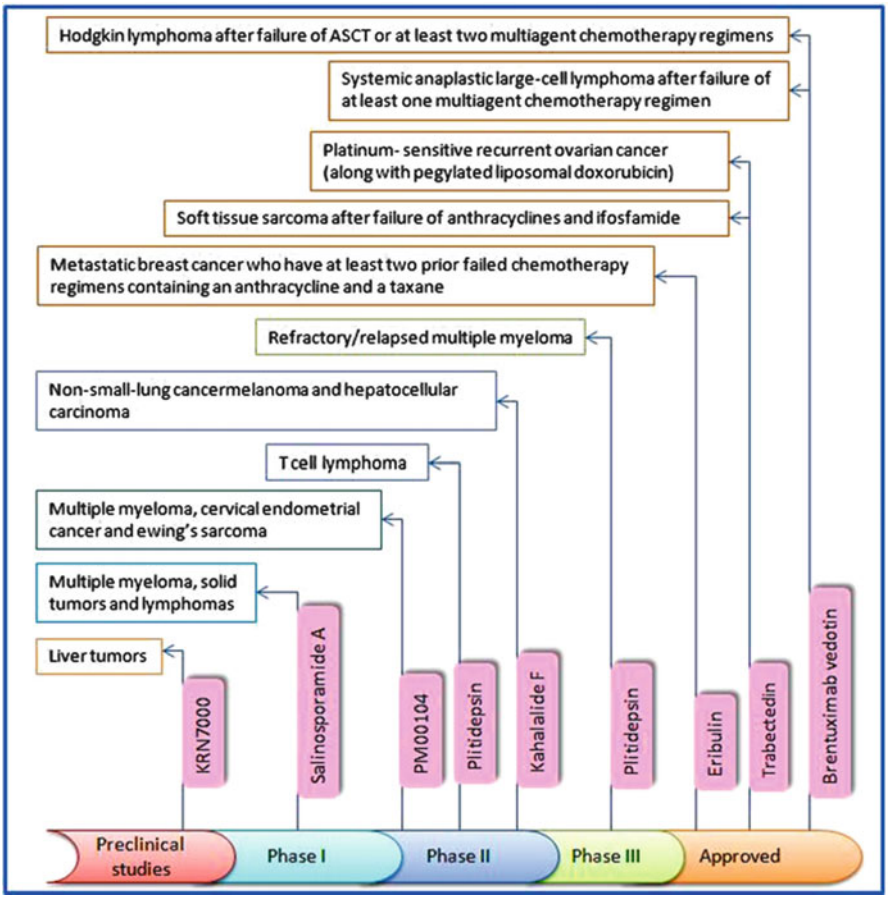


Fig. 3.8 A schematic view that shows applications and clinical statuses of important marine-derived anticancer agents in mid-2014

action for some of them in the treatment of cancer may be the reasons underpinning interest of researchers in this area. Besides, high therapeutic impact and overcoming chemoresistance occurring to clinically established chemotherapeutics are additional values of some marine-derived compounds. However, providing a sufficient supply for chemical substances has been the common limitation for development and production of these compounds. Contributions of sciences like medicinal chemistry and biotechnology have been remarkable in transcending such limitations. Medicinal chemists not only attempt to design commercially efficient synthetic routes for the production of marine natural compounds, but also they modify lead structures to obtain novel analogues with enhanced pharmaceutical properties compared with parents thereof. Altogether, constant collaboration between industry and academia, as well as collaboration between basic and clinical sciences are essential for more success and progress in this area of science.

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Chapter 4
Anticancer Antibiotics

Contents

Abstract Cancer chemotherapy is indeed indebted to microorganisms and their products, antibiotics. Since the discovery of the first antibiotic with anticancer properties, a lot of research has been focused on isolation, modification, partial or total synthesis, as well as uncovering the mechanism of action, increasing the efficacy, and meanwhile reducing the toxicity of these potential metabolites. Different classes of antibiotics such as aromatic polyketides (anthracyclines), glycopeptides (bleomycins), indolocarbazoles, *etc*. have presented effective medications to fight against cancer. The effort is continuing and a number of promising drugs from a variety of classes are under clinical investigations for future establishment in the cancer chemotherapy regimen.

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SpringerBriefs in Pharmaceutical Science & Drug Development,
DOI 10.1007/978-3-319-14027-8_4

Keywords Antibiotics • Anticancer drugs • Actinomycetes • Anti-tumor • Microorganisms • Toxicity

4.1 Introduction

The word "antibiotic" used to refer to compounds of natural origin, while synthetic antibiotics have confirmed their role in today's scientific investigations; though these compounds have the support of nature as their background (Pelaez 2006). Actinomycetes, non-filamentous bacteria and filamentous fungi have taken their role in offering antibiotics by more than 50 %, 10–15 % and 20 %, respectively. Moreover, their semi-synthetic derivatives are also provided through chemical modification or bioconversion (Demain 2014).

Since the presentation of penicillin to the world of medicine in 1940s, much research has been focused on introducing, developing and derivatization of new antibiotics. Evaluating antibiotics for anticancer properties goes back to the World War II, when actinomycin D was found to possess remarkable anti-tumor effects and applied in pediatric malignancies (DeVita Jr and Chu 2008). This was the starting point for the considerable endeavors, which have consequently led to the introduction of a number of commonly used anti-tumor antibiotics.

4.2 Antibiotics as Anticancer Agents

The majority of cancer chemotherapeutic agents have been introduced by microorganisms. Antibiotics such as actinomycin D, anthracyclines, and the anthracenones have been approved as anticancer agents (Demain 2014). Waksman and Woodruff discovered and introduced the first anticancer antibiotic "actinomycin D" from *Actinomyces antibioticus* in 1940, which was highly toxic to animals (Waksman and Woodruff 1941), while it was proved to be efficient in some childhood tumors afterwards (Nobili et al. 2009). This was the onset of using antibiotics in cancer chemotherapy and from this time various antibiotics either natural or synthetic have been employed in the treatment of cancer.

4.3 Classification of Anticancer Antibiotics

Anticancer antibiotics comprise different categories, which are not much similar in structure. A summarized classification is represented in Table 4.1.

Table 4.1 Anti-tumor compounds

Group	Example(s)
Aromatic polyketides (anthracyclines)	Daunorubicin, doxorubicin (adriamycin), epirubicin, pirarubicin, idarubicin, valrubicin, amrubicin
Glycopeptides (bleomycins)	Bleomycin, phleomycin
Non-ribosomal peptides	Actinomycin D (dactinomycin)
Mitosanes	Mitomycin C
Enediynes	Calicheamicin
Indolocarbazoles	Rebeccamycin
Epothilones	Ixabepilone
Other agents	Mithramycin

Adapted from Demain (2014) with some modifications

4.3.1 Aromatic Polyketides (Anthracyclines)

Bacteria, fungi and some plants are able to produce aromatic polyketides. These compounds possess a unique polycyclic aromatic structure, which distinguishes them from other polyketides (Shen 2000). Soil actinomycetes, in particular *Streptomyces* have been the center of many research programs due to their ability to produce a wide range of bioactive components (Metsä-Ketelä et al. 2002). Actinomycetes are capable of synthetizing aromatic polyketides by using polyketide synthases enzymes (Das and Khosla 2009). These enzymes catalyze sequential decarboxylative condensation between the starter and extender units, and provide a β-ketone intermediate, which bears regiospecific reduction, aromatization or cyclization thereafter, leading to the polycyclic aromatic structures, which undergo other enzymatic changes afterwards resulting in biologically active components (Shen 2000). Aromatic polyketides are also known as anthracyclines due to the presence of a tetracyclic bearing an anthraquinone chromophore in their aglycone moiety (Arcamone 2005). Antracyclines are effective inhibitors of topoisomerase II and they are also able to attach to DNA. Important anticancer agents such as daunorubicin and its derivative doxorubicin (adriamycin) are members of this class of antibiotics.

Daunorubicin (**1**) and doxorubicin (**2**) are used to overcome breast cancer, acute non-lymphocytic leukemia, Hodgkin and non-Hodgkin lymphomas, and sarcomas. The specific therapeutic use of daunorubicin is for treatment of acute leukemia (Kingston and Newman 2008; Trevor et al. 2008). Epirubicin (**3**), a semisynthetic derivative of doxorubicin with less cardiotoxic activity, is used in gastric and breast cancer. Idarubicin (**4**), an analogue of daunorubicin with higher efficacy, has been applied in acute myelogenous leukemia, while valrubicin (**5**) is beneficent in treatment of early bladder cancer (Cortés-Funes and Coronado 2007; Trevor et al. 2008). Pirarubicin (**6**) is another doxorubicin derivative, which is indicated in breast cancer. Furthermore, amrubicin (**7**), a synthetic antracycline related to doxorubicin, has recently been introduced for treatment of lung cancer in Japan

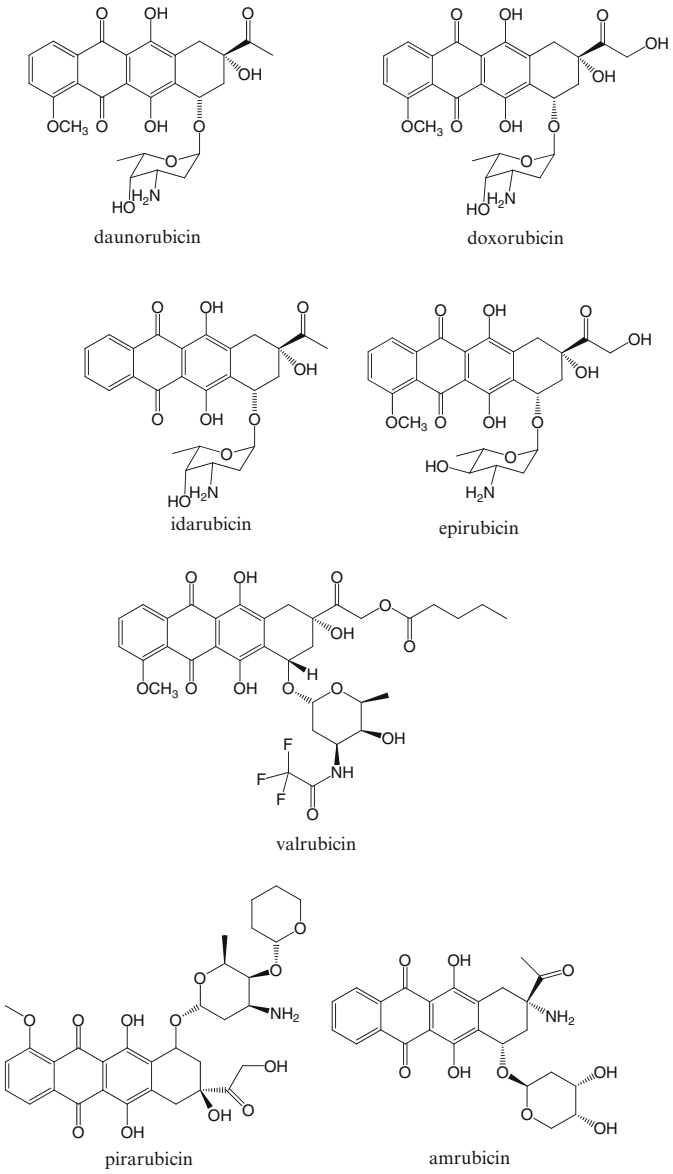


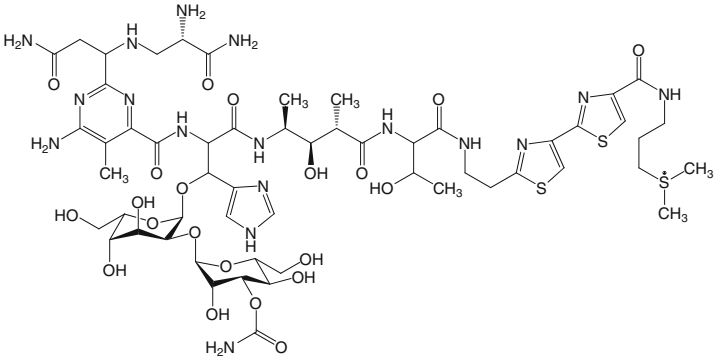
Fig. 4.1 Structures of daunorubicin (**1**), doxorubicin (**2**), epirubicin (**3**), idarubicin (**4**), valrubicin (**5**) pirarubicin (**6**) and amrubicin (**7**)

(Asai et al. 2014). The most considerable toxicity of aromatic polyketides has been found to be their cardiotoxic effect, whilst myelosuppression, thrombocytopenia and anemia have been reported too (Cortés-Funes and Coronado 2007) (Fig. 4.1).

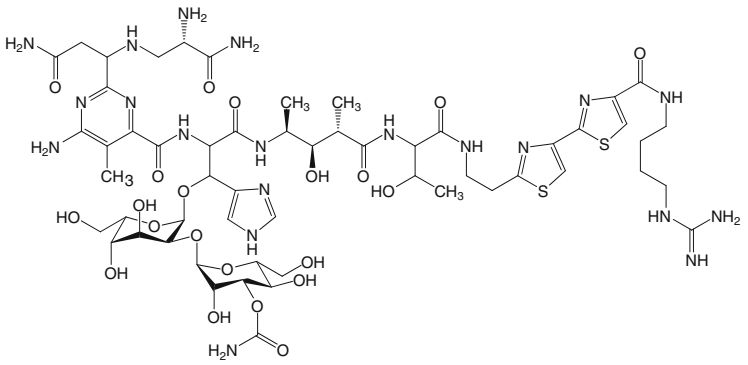
4.3.2 Glycopeptides (Bleomycins)

This class of antibiotics, which was initially recognized in some species of *Streptomyces,* consists of glycosylated oligopeptide derivatives that possess uncommon amino acids in their structures. Bleomycins (*e.g.* bleomycin A_2 (**8**)) may bind to DNA and RNA, and consequently cause breakage in their structures. They are also capable of inhibiting t-RNA, and it is found that their oxidative function needs a metal ion (Galm et al. 2005; Hecht 2005).

Bleomycin is a part of the drug mixture applied in lymphomas, testicular cancer and squamous cell carcinoma; it is also used to treat brain tumors (Trevor et al. 2008; Linnert and Gehl 2009). Although phleomycins (*e.g.* Phleomycin D (**9**)) were discovered prior to bleomycins, they did not come to the same fame due to their toxicity and possible instability (Hecht 2005). Hypersensitivity, mucocutaneous reactions and pulmonary disorder have been associated with using bleomycins in cancer chemotherapy (Ramotar and Wang 2003; Trevor et al. 2008) (Fig. 4.2).



bleomycin A2



phleomycin D

Fig. 4.2 Structures of bleomycin A_2 (**8**) and phleomycin D (**9**)

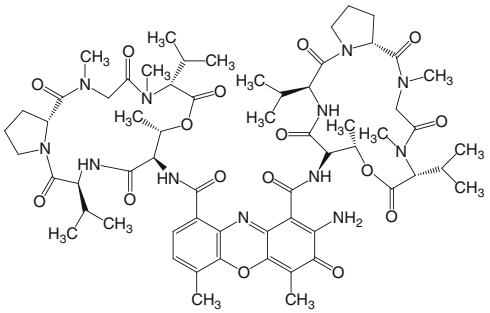


Fig. 4.3 Structure of actinomycin D (**10**)

4.3.3 Non-ribosomal Peptides

Non-ribosomal peptide synthases are the key enzymes in the biosynthesis of non-ribosomal peptides. These peptides have more diversity compared to the ribosomal peptides (Finking and Marahiel 2004). The non-ribosomal peptides, actinomycins, comprise series of chromopeptide antibiotics, which are structurally related. They enfold various amino acids in their structures. Actinomycin D (**10**) (dactinomycin), an inhibitor of RNA polymerase, owns the reputation of being the member of these primal anti-tumor antibiotics discovered in 1940s from *Actinomyces antibioticus*. It binds to DNA, intercalates between the base pairs, and finally hinders RNA synthesis. Similar to other actinomycins, it is capable of restraining both topoisomerase I and topoisomerase II (Baguley 2002; Mauger and Lackner 2005; Nobili et al. 2009). Actinomycin D has been used in the treatment of sarcomas, germ cell and trophoblastic tumors, melanoma and Wilms' tumor (da Rocha et al. 2001; Trevor et al. 2008). Actinomycin D also induces bone marrow suppression, and skin reactions, as well as gastrointestinal upsets (Trevor et al. 2008) (Fig. 4.3).

4.3.4 Mitosanes

Mitosanes were discovered in the late 1950s from *Streptotnyces caespitosus* and bear a distinct four-ringed structure, which includes an aziridine ring, saturation at carbons 9 and 9a, and eventually the quinone oxidation state (Lown and Weir 1978; Remers 2005). After turning into their active forms, this group of anticancer antibiotics attach to DNA similar to alkylating agents. (Galm et al. 2005; Remers 2005). Mitomycin C (**11**) is undoubtedly an outstanding member of this class of anti-tumor that stops mitosis and protein synthesis (Van Bergen et al. 2014). Mitomycin is used as a part of anticancer therapy regimen in treatment of adenocarcinomas of the cervix, stomach, pancreas and lung (Trevor et al. 2008). Toxicity to the heart, liver, lung, kidney and the bone marrow has been associated with the use of mitomycin (Trevor et al. 2008; Kingston and Newman 2008).

4.3.5 Enediynes

Enediynes contain an unsaturated core with two acetylenic groups conjugated to a double bond, in a nine- or ten-membered ring. They are originated from polyphenols, which turn into the final structure through a series of reactions that makes the final enediynes capable of intercalating to DNA leading to cleavage. Enediynes were discovered in 1980s (Van Lanen and Shen 2008; Hertweck 2009). They are provided by diverse natural sources (Joshi and Rawat 2012). Some enediynes have been isolated from a variety of microorganisms such as *Streptomyces* and *Actinomycetes* (Galm et al. 2005). Calicheamicin (Mylotarg) and a polymer derivative of neocarzinostatin (SMANCS) are used in cancer chemotherapy (Chen 2010).

Calicheamicin (Mylotarg) (**12**), an antibody-targeted agent, has been approved in treatment of acute myelogenous leukemia. Moreover, SMANCS is used to treat hepatocellular carcinoma in Japan (Grediak and Jeri 2007; Kingston and Newman 2008). In June 2010, FDA recommended that Mylotarg would not be available to new patients due to the safety issues arisen from a clinical trial. Myelosuppression, hyperbilirubinemia and elevated hepatic transaminase levels have been reported regarding the use of Mylotarg (Sievers et al. 2001) (Fig. 4.4).

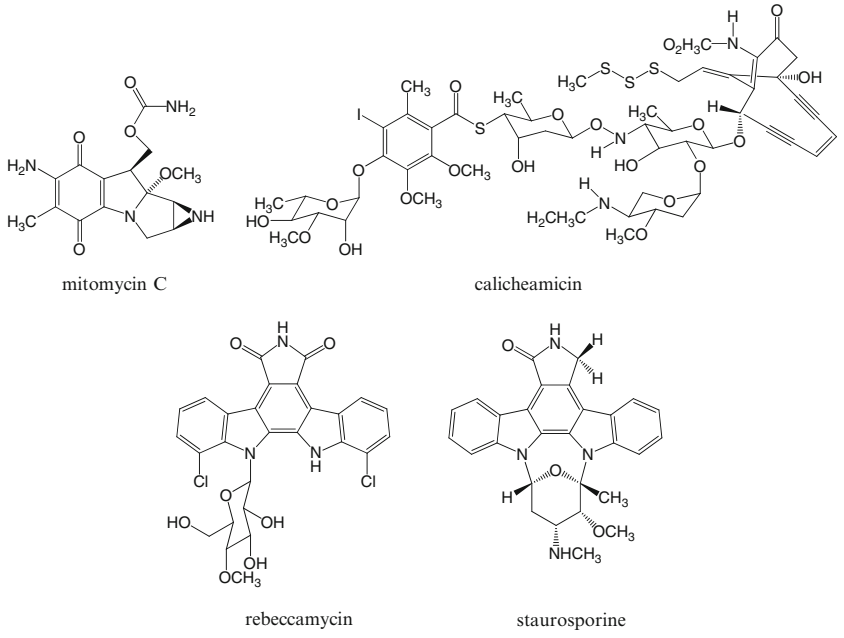


Fig. 4.4 Structures of mitomycin C (**11**), calicheamicin (**12**), rebeccamycin (**13**) and staurosporine (**14**)

4.3.6 Indolocarbazoles

An indole unit, fused to one of the benzenoid rings of a carbazole, constructs the core of indocarbazole anticancer antibiotics (Janosik et al. 2008). Considering the variety in the structure of these group of anti-neoplastic agents, different complex mechanisms have been attributed to their anti-tumor activity including Topoisomerase I (TOPO I) poisoning, Protein Kinase C (PKC), Protein Kinase A (PKA), CDK1/cyclin B, and CDK5/p25 inhibition (Li et al. 2009). A critical point is that the anticancer property is dependent to the sugar moiety (Animati et al. 2012). It has been suggested that biological targeting and selectivity could be effectively improved in indolocarbazoles by little modification in different parts of their chemical structures. The indolocarbazole rebeccamycin (**13**) isolated from *Saccharothrix aerocolonigenes* in 1987, executes its anti-tumor activity by inhibiting topoisomerase I and stabilizing the enzyme-DNA interaction; however it has shown no activity against PKC and PKA, while staurosporine (**14**), another indolocarbazole, has demonstrated no inhibitory activity towards topoisomerases; in contrast, it is a non-selective kinase inhibitor (Prudhomme 2005; Deslandes et al. 2009). Some indocarbazole anticancer agents are currently under clinical trial investigations.

4.3.7 Epothilones

The discovery of epothilones goes back to 1987, when some strains of myxobacterium such as *Sorangium cellulosum* were found to produce these polyketide macrolide lactones, which possessed antifungal activity (Cheng et al. 2008; Demain and Vaishnav 2011; Parajuli et al. 2014). Epothilones seem to demonstrate similarities with taxanes in their mechanism of anti-tumor activity as well as their side effects (Fornier 2007). These 16-membered ring macrolides are microtubule-stabilizing agents. They attach to β-tubulin, which results in stabilizing the microtubule thus inhibit its depolymerization. This mechanism finally induces apoptosis due to interrupting the microtubules and interfering with the cell cycle or other cellular procedures (Smaglo and Pishvaian 2014; Zhang et al. 2014). Epothilones B and D have served as interesting lead structures in cancer research due to their considerable effects, and also simplicity of their chemical structures (Taylor and Chen 2001). In 2007 ixabepilone (**15**), a semi synthetic analog of epothilone B, was approved by FDA (Parajuli et al. 2014). Furthermore, ixabepilone (ixempra) has been approved for treatment of metastatic and locally advanced breast cancer (Zhang et al. 2014). Unfortunately, neurologic toxicity has been associated with the use of ixabepilone (Cheng et al. 2008) (Fig. 4.5).

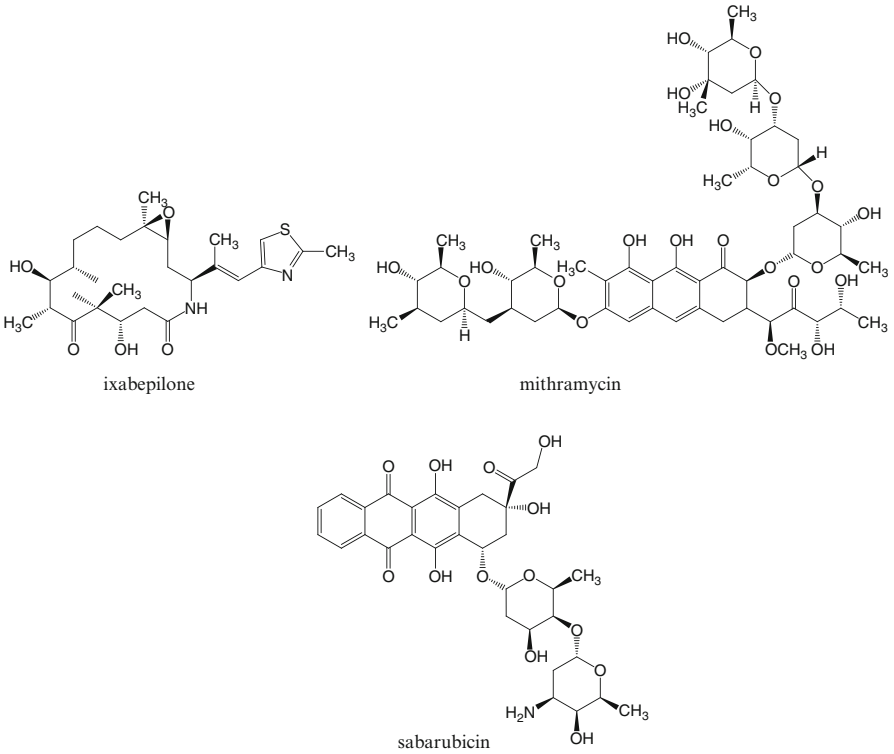


Fig. 4.5 Structures of ixabepilone (**15**), mithramycin (**16**) and sabarubicin (**17**)

4.3.8 Other Agents

Mithramycin (**16**), also known as plicamycin, is a glycosylated anthracenone provided by different species of *Streptomyces* such as *S. argillaceus and S. plicatus*. It might also be categorized in aureolic acid group of anti-neoplastic agents. Upon binding to DNA, mitramaycin obstructs RNA synthesis, which leads to its anti-tumor activity (Lombó et al. 2006). Mithramycin has been approved for the treatment of leukemia and testicular cancer. It has also been found beneficent in hypercalcemia related to cancer (Lombó et al. 2006; Kingston and Newman 2008; Osada et al. 2013). However, mithramycin is toxic to the liver, and might cause nausea or vomiting. It could also increase the serum creatinine (Leask 2012).

4.4 Drugs for the Future

Looking for more potent drugs with less cytotoxic effects in cancer chemotherapy has persuaded scientists to concentrate their efforts on finding new natural and/or whole or partially synthetic medications for treatment of cancer. Following, some

rather new agents that are in different steps of clinical trials have been discussed. Some information about these drugs has been published but they could also be pursuit from National Institute of Health (NIH) through the website address: http://clinicaltrials.gov/ specified as ClinicalTrials.gov Identifier, which consists of the letters NCT followed by an eight digit number as in NCT00397072. The drugs will be discussed here in accordance with the categories that have been discussed earlier in this chapter. Some other promising categories will also be considered later on.

4.4.1 Aromatic Polyketides (Anthracyclines)

The synthetic sabarubicin (**17**) is structurally related to anthracyclines, and has completed phase II clinical trial for treatment of prostate cancer (NCT00027781) (Fiedler et al. 2006) and phase I of two studies about treating solid tumors (NCT00003028, NCT00003982).

4.4.2 Indolocarbazoles

UCN-01 (7-hydroxystaurosporine) (**18**) is structurally related to staurosporine. The phase II evaluation in lymphoma of UCN-01 has been completed (NCT00082017). It has also been assessed along with carboplatin in treatment of solid tumors (NCT00036777); phase I of this study has been completed. Another completed study, which included a phase I clinical trial study incorporating UCN-01 in combination with irinotecan hydrochloride, has been designed to treat patients with metastatic solid tumors or triple negative breast cancer (NCT00031681). Also, phase II clinical trial of becatecarin (**19**), a synthetic analog of rebeccamycin, has been finished for treatment of breast cancer (NCT00005817). Some other finished studies of becatecarin include clinical trials comprising phase I investigations for leukemia (NCT00087204), phase II for lung cancer (NCT00006017), phase II for pediatric neuroblastoma (NCT00003737) and phase II for pediatric tumors (NCT00006102) (Langevin et al. 2008) (Fig. 4.6).

4.4.3 Epothilones

Among this interesting class of anticancer antibiotics, which present their antineoplastic effects similar to paclitaxel, epothilone D (**20**) has completed phase II of some clinical trials. It has been studied as a second line therapy for colorectal cancer (NCT00077259), in combination with trastuzumab in treatment of breast cancer (NCT00337649), for renal cell carcinoma (NCT00030992) and to treat non-small

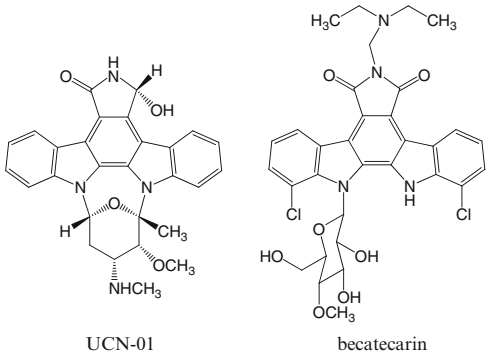


Fig. 4.6 Structures of UCN-01 (**18**) and becatecarin (**19**)

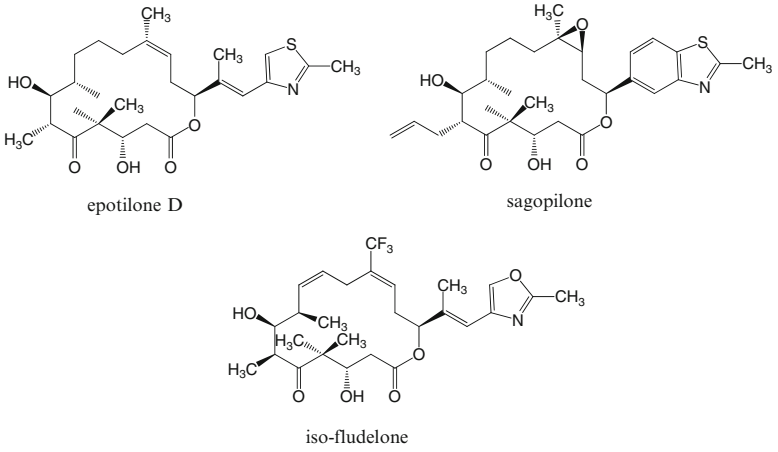


Fig. 4.7 Structures of epotilone D (**20**), sagopilone (**21**) and iso-fludelone (**22**)

cell lung cancer (NCT00081107), as well as some other malignancies. Sagopilone (**21**, ZK-EPO) is a synthetic epothilone, which has completed phase II of clinical studies for melanoma (NCT00598507), ovarian neoplasms in combination with carboplatin (NCT00325351) (McMeekin et al. 2012), prostate cancer along with prednisone (NCT00350051), breast cancer (NCT00313248) (Morrow et al. 2010), non-small cell lung carcinoma (NCT00160069) (Heigener et al. 2013) and glioblastoma (NCT00397072) (Silvani et al. 2009). Moreover, iso-fludelone (**22**), a synthetic analog of epothilones, has been recently enrolled in phase I clinical trial of solid tumors (NCT01379287) (Fig. 4.7).

4.4.4 Salinosporamides

Marine actinobacteria did not used to be the focus of much research in advance but they have attracted much attention currently for the reported clinical advantages of

their secondary metabolites (Goo et al. 2014). In 1980s, the scientists' interests were drawn toward such metabolites with anti-tumor activity. Since then, much effort has been focused on the isolation and biological investigations of these natural products (Newman and Cragg 2005). An actinomycete, known as *Salinispora,* is found in tropical and subtropical marine sediments (Jensen and Mafnas 2006). Some species (like *S. tropica*) produce salinosporamides, which might be able to demonstrate proteasome inhibitor properties (Williams et al. 2005). Salinosporamides share a γ-lactam-β-lactone bicyclic structure. Salinosporamide A (**23**), a member of this class of anticancer agents causes its cytotoxicity by inhibiting the 20S proteasome (Feling et al. 2003). Regarding its potent reported anticancer effects, analogs of salinosporamide A have been synthesized by medicinal chemists (Blasdel et al. 2013). Salinosporamide A (marizomib) in combination with vorinostat has finished phase I clinical studies for non-small cell lung cancer, pancreatic cancer, melanoma and lymphoma (NCT00667082).

4.4.5 Benzoquinoid Ansamycins

The structure of ansamycin antibiotics consist of an aliphatic bridge linking two non-adjacent positions of an aromatic moiety (Rinehart and Sheild 1976). Geldanamycin and its derivatives herbimycin A and macbecin I, belong to this group of antibiotics (Snader 2005).

4.4.5.1 Geldanamycins

Geldanamycin (**24**) was the first discovered benzoquinoid ansamycins found in 1970 from the cultures of *Streptomyces hygroscopicus.* Geldanamycin reveals its anti-neoplastic ability through inhibiting the expression of oncogenic proteins, blocking the cell cycle and anti-angiogenesis properties, and also by binding to the protein chaperone Hsp-90. Jurczyszyn and his colleagues have evaluated geldanamycin and its derivatives on myeloma cell lines and CD138+ cells derived from the bone marrow of patients with multiple myeloma to find that these compounds could inhibit the growth of myeloma cells (Ochel et al. 2001; Jurczyszyn et al. 2014). 17- allylamino-17-desmethoxygeldanamycin (17-AAG) or tanespimycin (**25**) has entered clinical trials due to its potency, less toxicity along with its special procedure of anti-neoplastic activity (Kingston and Newman 2008). Tanespimycin has been evaluated for various neoplasms in different phases of clinical trials. The ClinicalTrials.gov Identifiers such as NCT00118248, NCT00098423, and NCT00047047 contain the data about the phase II studies of thyroid cancer, phase I clinical trial of leukemia and phase I investigations for solid

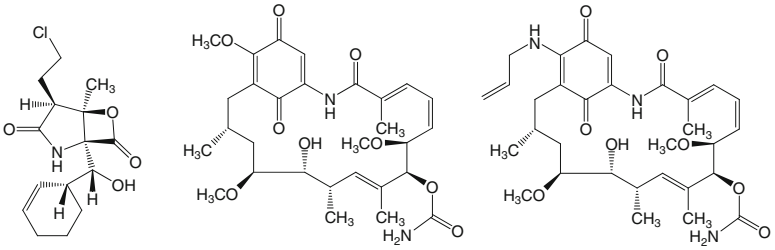


Fig. 4.8 Structures of salinosporamide A (**23**), geldanamycin (**24**) and tanespimycin (**25**)

tumors, respectively. Also there are a number of other studies in the course of different phases of clinical trials for anticancer studies (Fig. 4.8).

4.5 Conclusion

Antibiotics comprise several chemical structures and act by different mechanisms to reveal their anti-neoplastic properties. They have contributed enormously to cancer chemotherapy either in their natural forms or as synthetic or semisynthetic derivatives. A number of these anticancer agents have been considered in this short survey and some future expectations have been presented. Antibiotics have introduced some very effective anticancer agents both in clinical use and as lead structures for further anti-tumor developments. Much endeavor has been dedicated to the anticancer drug discovery in the field of antibiotics and the perspective is still promising. The most confining steps in this process have been the toxic side effect, poor solubility and decreased efficacy due to derivatization. Sometimes synthesis of a new analog is so complex and time consuming that draws back the effort. Mechanistic approach to the anticancer properties of the antibiotics could bestow a dynamic groundwork for developing new reasonable analogs. And last, but not least biotechnology and directing friendly microorganisms to provide our designed structures could be a successful method towards production of more potent, less toxic and cost effective antibiotics.

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Chapter 5
Anticancer Terpenoids

Contents

Abstract Terpenoids are a class of secondary metabolites with immense variety of structures and biological activity. They are divided into some groups including monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids and tetraterpenoids. Among them, there are some compounds with interesting

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anticancer activity capable for use in clinical or pre-clinical administration like D-limonene, perilyll alcohol and salvicine, as well as drugs derived from parthenolide, thapsigargin and artemisinin. Off-target effects, low bioavailability and adverse effects of some terpenoids like parthenolide derivatives make them unsuitable for clinical application. However, they can play a role as lead compounds for the development of semi-synthetic molecules, in which specificity and pharmacokinetic parameters would be improved. In addition, some of these compounds such as salvicine, triterpenoids and carotenoids have been able to overcome multi drug resistance by inhibition of p-glycoprotein overexpression in cancer cells demonstrating them as the proper candidate for co-administration with chemotherapy agents. Moreover, combination of these compounds with anticancer drugs may enhance the efficacy and decrease adverse effects of both drug and terpenoids.

Keywords Chemopreventive • Preclinical studies • Monoterpenes • Sesquiterpene lactones • Diterpenes • Triterpenes • Tetraterpenes

5.1 Introduction

"Terpens" are a class of secondary metabolites produced not only by plants but also by other organisms like bacteria and possess hydrocarbon skeleton, while "terpenoids" refer to the modified terpenes with addition of oxygen (as an example) in their structures. This class of secondary metabolites is used in pharmaceutical and food industries for their medicinal and flavoring properties.

Although there has been much scientific progression in the past decades, investigation of anticancer drugs is faced with some problems like limitation in chemical scaffold. Chemical diversity in natural products extensively attracts scientific attention to discover potential therapeutic agents like anticancer drugs from natural sources. For instance, over 50 % of the approved drugs in the last two decades have natural origins. A large number of applicable drugs with anticancer activity like vincristine, paclitaxel and etoposide represents diverse structures (Newman et al. 2003; Huang et al. 2012). Since terpenoids have immense structural diversity and display several biological activities, biosynthesis and classification of them along with preclinical or clinical trials of some molecules in this class together with their possible mechanisms have been discussed in this part.

5.2 Biosynthesis and Classification of Terpenoids

Structures of terpenoids biosynthetically change by loss or addition of carbons atoms. Although terpenoids are classified based on the number of isoprene units regardless to carbon loss or addiction, isoprene is not a precursor for terpenoid biosynthesis.

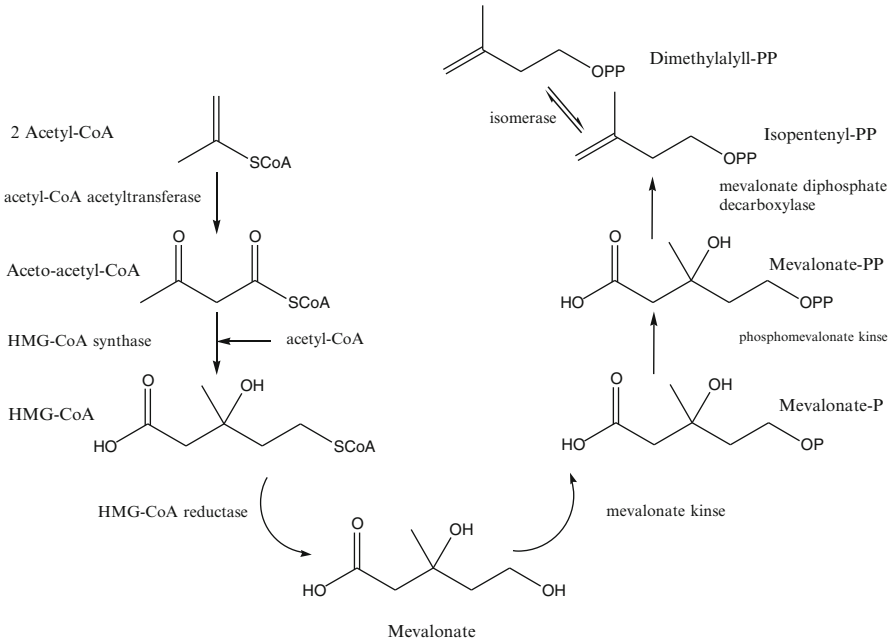

Fig. 5.1 Mevalonate pathway

In reality, mevalonic acid is converted to isopentenyl diphosphate (Fig. 5.1), which is involved in terpenoids biosynthesis. Mevalonate pathway (Fig. 5.1) is common in eucaryotes, archaebacteria, and cytosols of higher plants, which is vital for diverse cellular functions. Another pathway, non-mevalonate, has been discovered in many green algae, eubacteria, and chloroplasts of higher plants to produce the mentioned precursors (Fonseca et al. 2006; Kuzuyama 2002; Kuzuyama and Seto 2012). The non-mevalonate pathway is widely called 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway at present, because MEP is the first biosynthetic intermediate of this pathway (Kuzuyama and Seto 2012; Fonseca et al. 2006). Terpenoids are classified into some groups based on the number of isoprene units in their structures including monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), sesterterpenoids (C25), triterpenoids (C30), and tetraterpenoids (C40). Additionally, meroterpenoids with mixed biogenesis are identified to have terpenoid and non-terpenoid fragments in their structures like paclitaxel.

5.3 Monoterpenoids

5.3.1 Perilyll Alcohol

Perilyll alcohol, *p*-metha,1,7-diene-6-ol or 4-isopropenyl-cyclohexenecarbinol, is a menthane type monoterpene (Fonseca et al. 2006). The compound has been isolated

from essential oil of different plants like lavender, spearmint, peppermint, cherries, sage, celery seeds, cranberries, perilla, wild bergamot, ginger grass, savin, caraway, lemongrass and several other plants. Since this compound is in low amount in the oil of the plants, thus synthetic source of it is desirable. Preliminary studies revealed that incorporation of radiolabeled isoprenes into small G proteins inhibited by D-limonene and perilyll alcohol in carcinoma cell lines. There are some preclinical studies demonstrating perilyll alcohol activity against mammary, pancreatic, colon, prostate, liver and glioma tumors (Fonseca et al. 2006). Preclinical studies showed that perilyll alcohol is involved in both the chemopreventive and chemotherapeutic process (Fonseca et al. 2008).

Glioblastoma multiforme (GBM) is the most aggressive and most common type of malignant primary brain tumor in human glial cells. Standard treatments of GBM are rarely curative including surgical resection, radiation and/or chemotherapy. In GBM, over-expression of platelet-derived growth factor (PDGF) and its receptor, epidermal growth factor receptor (EGFR) as well as the mutant form of EGFR, epidermal growth factor receptor variant III (EGFRvIII) are observed (Fonseca et al. 2006, 2008). In EGFRvIII, 267 amino acids are deleted in the extracellular domain causing disability of the receptor to bind ligands (Gana et al. 2009). Since EGFRvIII has not been discovered in normal tissues at significant level, thus targeting these specific molecules probably has not affected the normal tissues. Over-expression of EGFR and EGFRvIII resulted in signaling through phosphatidylinositol-3-kinase (PI3K/Akt) and Ras mitogen activated protein kinase (Ras-MAPK) pathways (Fonseca et al. 2006). The function of Ras-MAPK pathway is to transfer signals from extracellular area to the cell nucleus. It is also involved in cell cycle regulation, division and differentiation (Molina and Adjei 2006). PI3K/Akt signaling is related to both cell proliferation and survival by phosphorylating a variety of substrates. Therefore, it is not only associated with tumor growth but also with cancer treatment (Osaki et al. 2004). Perilyll alcohol may decrease Ras transcription and translation or its degradation (Fig. 5.2) (Fonseca et al. 2006). Moreover, the molecule may regulate gene through over-expression of mannose 6-phosphate/insulin-like growth factor-II (M6P/IGF-II) and transforming growth factor beta (TGF-β type II) receptor genes. The TGF-β is a type of cytokine that controls proliferation, cellular differentiation and play a role in some diseases like cancer, diabetes, heart disease, bronchial asthma and Parkinson's disease, which may affected by perilyll alcohol. Perillyl alcohol also induces radio sensitization causing apoptosis in glioma cell lines. Recent studies revealed that this molecule induces apoptosis in human GBM lines along with inhibition of migration and angiogenesis of murine GBM cell lines. Perilyll alcohol affected tumor cells without any adverse effect on normal cells, and also change tumor cells to a differentiate state (Fonseca et al. 2006, 2008).

Generally, the activities of perillyl alcohol include different mechanisms such as G1 arrest, induction of apoptosis, gene regulation, reduction in the levels of isoprenylated Ras and Ras-related proteins. The maximum tolerated dose for perillyl alcohol was evaluated as 8.4 g/m^2 per day delivered orally in four divided doses. However, adverse effects such as early satiety, nausea, unpleasant taste,

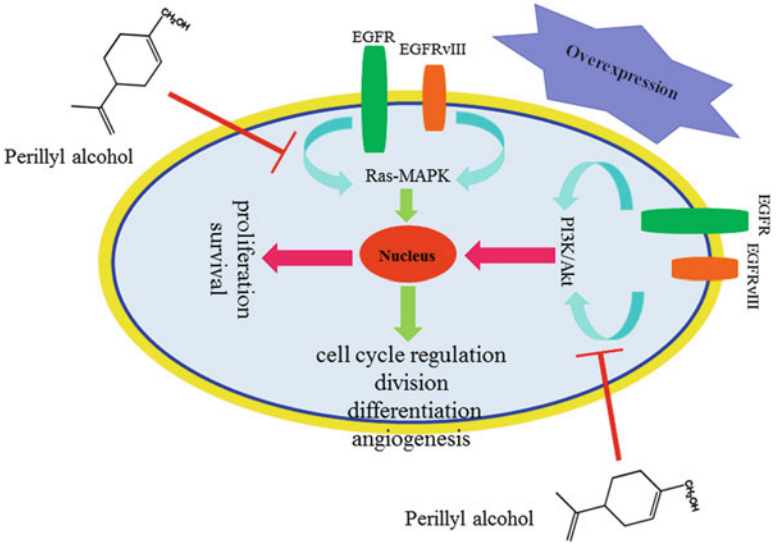

Fig. 5.2 Overexpression of EGFR and EGFRvIII occur in tumor cells resulting in signaling trough Ras-MAPK and PI3K/Akt pathways. Schematic pathway of therapeutic effect of perillyl alcohol through inhibition of Ras-MAPK and PI3K/Akt pathways suppressing division, differentiation, angiogenesis, proliferation, and survival of cancer cells

eructation, and fatigue suggest that oral administration of the drug is not a tolerable and compatible way for the patient (Fonseca et al. 2008). Olfactory epithelium plays a role as a gateway for molecules like small polar molecules, peptides and even the large proteins and polysaccharides like vaccines or DNA plasmids exploited for DNA vaccines that enter the central nervous systems (CNS) (Fonseca et al. 2006). Because the adverse effects reported in clinical trials phase I and II during oral administration of the drug and also efficacy of inhalation method, therefore inhalation of perillyl alcohol were applied in another clinical trials phase I and II (Fig. 5.3). The results of a study showed that intranasal perillyl alcohol has no toxicity and is well tolerated in the patient suggesting intranasal therapy could be a valuable therapeutic strategy for a patient with malignant gliomas (Fonseca et al. 2008).

5.3.2 D-Limonene

D-Limonene, a monocyclic hydrocarbon monoterpene, is widely distributed in plants essential oils mainly citrus oils including orange, mandarin, lemon, lime and grapefruit. In the Code of Federal Regulations it is recognized as a safe flavoring agent using in fruit juices, baked goods, soft drinks, pudding, and ice cream. Since D-limonene is an excellent solvent for cholesterol, therefore, it has been applied for

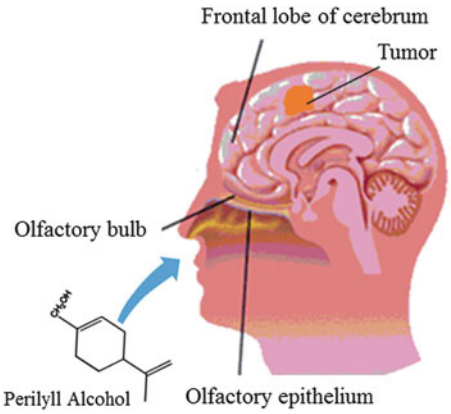

Fig. 5.3 Schematic pathway of olfactory bulb for administration of perilyll alcohol to the tumors in the central nervous system

treatment of gallstone containing cholesterol. Moreover, D-limonene neutralized gastric acid and supported normal peristalsis, and could be used in heartburn. The molecule showed pronounced chemopreventive and chemotherapeutic activity in animal tumor models with notably low toxicity. Dietary administration of D-limonene retarded rat mammary tumorigenesis during both initiation and promotion/progression stages. Tumor regression with D-limonene diet (10 %) was measured about 80 % and most of the regressions are complete with low toxicity. Post-translational isoprenylation of $p21^{ras}$, a small GTP-binding protein, was selectively inhibited by D-limonene. The $p21^{ras}$ regulates signal transduction and cell growth. The activity of Ras is related to the addition of farnesyl isoprenoid moiety to the C-terminal tetrapeptide catalyzed by farnesyl transferase, which may be inhibited by D-limonene. The inhibition of the enzyme may lead to suppression of signal transduction and gene expression leading to cytostasis, apoptosis, cellular re-differentiation and tumor regression. D-limonene prevents interaction of chemical carcinogens with DNA through activation of cytochrome p450 enzymes, which convert carcinogens to less toxic compounds. Toxicity of D-limonene in patients with solid tumor was mainly gastrointestinal side effects including nausea, diarrhea and vomiting. Partial beneficial activity in the patients with breast cancer was observed at dose of 8 g/m^2/day, for instance supraclavicular lymphadenopathy and bone pain were reduced in the patients as well (Sun 2007).

5.4 Sesquiterpenoids

5.4.1 Sesquiterpene Lactones

Among sesquiterpenes (C15), a group with γ-lactone (cyclic ester) ring in their structure has been called sesquiterpene lactones. In many cases, they contain α-methylene group, hydroxyl, esterified hydroxyl and epoxide ring. They are

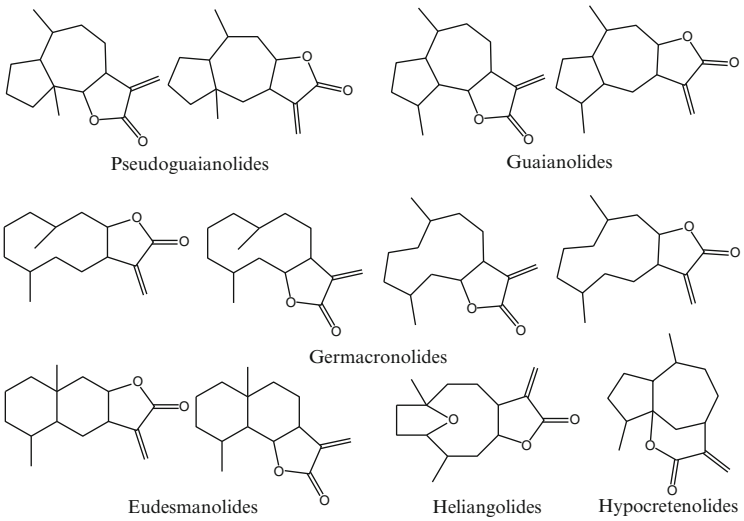

Fig. 5.4 Chemical structures of sesquiterpenoids lactones

isolated and identified from several plants family especially Asteraceae but may be found in other plant families with over 3,000 different structures (Ghantous et al. 2010; Chaturvedi 2011). They are primarily divided into smaller groups including pseudoguainolides, guaianalides, germanocranolides, eudesmanolides, heliangolides, hyptocretenolides, etc. (Fig. 5.4), based on their carboxylic skeletons. Usually, every individual plant species produces especial skeleton type of sesquiterpene lactones concentrated in flowers and leaves (Chaturvedi 2011). Extracts rich in sesquiterpene lactones have been used in treatment of human diseases like headache, inflammation and infections. Some drugs derived from parthenolide, thapsigargin and artemisinin are used in clinical trials for cancer treatments (Chaturvedi 2011). Recently it is found that they possess anticancer, anti-migraine, analgesic, sedative, antimalarial, antiviral, antibacterial and antifungal properties (Chadwick et al. 2013; Chaturvedi 2011).

Functionality of sesquiterpene lactones is generally related to γ-lactone and α-methylene groups. These groups react with nucleophiles, like cysteine sulfhydryl group in proteins, by fast Michael type reaction, which may be a possible mechanism in alkylation of growth regulatory macromolecules like enzymes that direct cells to apoptosis (Chaturvedi 2011; Chadwick et al. 2013). Sesquiterpene lactones may react with free intracellular glutathione (GSH) leading to disruption of GSH metabolism, which is vitally important in intracellular redox balance. Other properties like lipophilicity, molecular geometry, and chemical environment of the sulfhydryl groups may control the activity of sesquiterpene lactones. It is believed that exo-methylene group on lactone ring is essential for cytotoxicity of these molecules, since modification of this group resulted in the loss of cytotoxicity and tumor inhibition. It is also revealed that O = C-C = CH system is responsible

for the cytotoxicity of the compounds regardless of lactone group. Anti-tumor activity of the compounds *in vitro* strongly influenced by α-methylene γ-lactones and α, β-unsaturated cyclopentenone ring (or α-epoxycyclopentenone) (Chaturvedi 2011). Unsaturated acyl or hydroxyl groups near γ-lactone and α-methylene groups allow greater binding stability. Lipophilicity of the compound is another factor in affecting permeability of the compounds to the cell membrane and nuclei, where they exert their main effects (Chadwick et al. 2013).

Nuclear factor κB (NF-κB), a protein, mediated immune system in humans through controlling other effectors such as cytokines, cell adhesion and inflammatory molecules. It plays role in cancer by genetic regulation of apoptosis pathways and metastasis. The results of a comprehensive study demonstrated that two cysteine sulfhydryl groups (Cys 38 and Cys 120) in the subunit NF-κB (p65) are the targets of inhibition of NF-κB by sesquiterpene lactones. Inhibition of NF-κB expression reduced inflammation and cancer growth along with relaxation of smooth muscles, which has beneficial effect in cardiovascular diseases (Chadwick et al. 2013). Sesquiterpene lactones selectively target cancer cells while sparing normal cells, which makes them suitable for clinical trials. For instance, artemisinin-derived drugs are valuable candidates in laryngeal carcinomas, uveal melanomas and pituitary macroadenomas followed by phase I-II trials against lupus nephritis and breast, colorectal and non-small cell lung cancers. Additionally, clinical trials on phase I for thapsigargin-derived drugs are now undergoing for breast, kidney and prostate cancers. A parthenolide analog, dimethylamino-parthenolide, is orally bioavailable presenting in phase I against acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and other blood and lymph node cancers. It is believed that selectivity of parthenolide, thapsigargin and artemisinin toward cancer cells are related to their property to target particular proteases secreted by cancer cells including sarco/endoplasmic reticulum (ER) calcium ATPase (SERCA) pump, high iron content and cell surface transferrin receptors, NF-κB signaling, murine double minute 2 (MDM2) degradation and p53 activation, angiogenesis, metastasis and epigenetic mechanisms (Ghantous et al. 2010).

5.4.2 Parthenolide

Parthenolide has been isolated from aerial parts or mainly flowers and leaves of *Tanacetum parthenium* (feverfew). Since the molecule has not been totally synthesized, therefore it is commercially extracted from *Chrysanthemum parthenium* leaves. Parthenolide showed potent anti-tumor and anti-inflammatory activity attributed to its strong inhibition of NF-κB as well as targeting of epigenetic factors. Inhibitory effect of the compound related to the number of alkylating centers like methylene lactone and conjugated keto or aldehyde functional groups (Fig. 5.5). The results of another study highlighted the effect of lipophilicity and

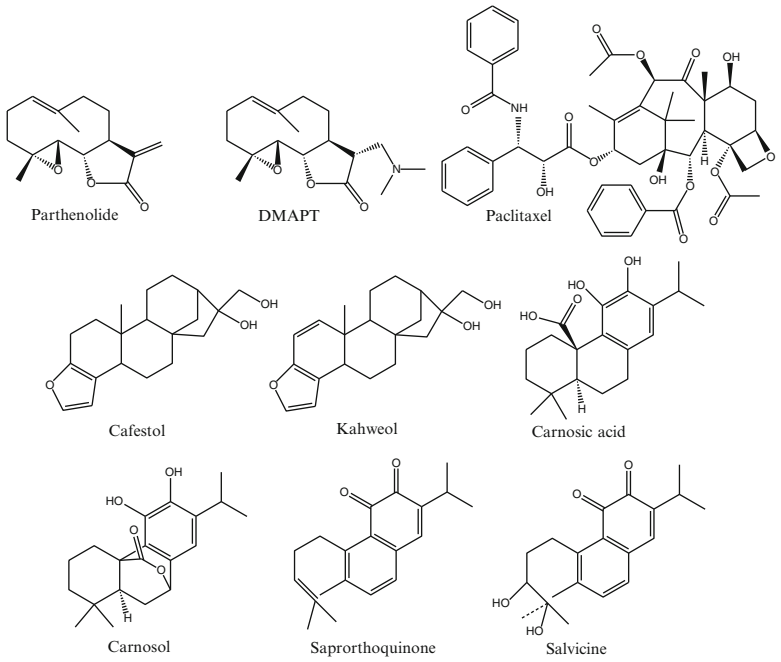

Fig. 5.5 Chemical structures of parthenolide, DMAPT, paclitaxel, cafestol, kahweol, canosic acid, carnosol, saprorthoquinone, and salvicine

polarity on potential of NF-κB inhibition. More polar derivatives of parthenolide more strongly inhibited NF-κB-driven transcription that may be related to their potential to cause hydrogen binding with amino acid residues adjacent to cysteine in NF-κB. Tubulin, major components of mitotic spindles during cell division, is detyrosinated by tubulin carboxypeptidase (TCP), therefore inhibition of TCP is a target in proliferative cells like tumors. Epoxide group and α, β-lactone are crucial for inhibition of TCP by parthenolide, hence the derivatives without these moieties showed no inhibitory activity (Fonrose et al. 2007). Parthenolide specifically inhibits DNA methyltransferase 1 (DNMT1) resulting in global DNA hypomethylation, while it has no effect on other DNMTs (Liu 2009). It is believed that underlying mechanism includes alkylating thiolate of cysteine-1226 in catalytic area of DNMT1. The nucleoside analogs decitabine and 5-azacytidine are common drugs with DNA methylation inhibitory effect, which makes them toxic. However, specific activity of parthenolide toward DNMT1 makes it suitable therapeutic drug with less toxicity (Ghantous et al. 2010). Redox homeostasis maintains by counteraction thioredoxin and glutathione systems, and parthenolide targets both systems. Few hours' treatment of parthenolide causes irreversible depletion in leukemic stem cells, which usually leads to unrepairable DNA damage (Ghantous et al. 2013).

Off-target effects of parthenolide, particularly at high doses, along with its hydrophobicity limited the drug bioavailability and restricted its clinical usage. Pharmacokinetic parameters of parthenolide like oral bioavailability and plasma concentration were improved by producing more hydrophilic derivatives of parthenolide. National Cancer Institute (NCI) disqualified parthenolide in 1970 because of its highly reactive groups interacted with all thiol moieties resulting in undesirable cytotoxicity (Ghantous et al. 2013). Some changes in the structure of parthenolide like reduction of epoxidation of the endocyclic alkene, α-methylene, or oxidation of the allylic methyl groups reduced its activity. On the other hand, similar potency with better hydrophilicity was achieved by conjugate addition of aromatics to the α-methylene compounds particularly aromatics with a tyramine moiety or aliphatic amines (Nasim and Crooks 2008; Neelakantan et al. 2009; Peese 2010). Among those parthenolide derivatives, dimethylamino-parthenolide (DMAPT, an aliphatic acyclic derivative), secondary amine with a dimethyl amino group, are more potent than primary amines against AML cells. Higher anti-leukemic activity was observed by compounds with at least one N-methyl group rather than those lacking it. Moreover, acyclic amines have higher anti-leukemic activity than cyclic amines (Neelakantan et al. 2009). Recently, fluorinated derivatives of parthenolide were developed, among which 13-(3-trifluoromethylphenyl)-PTL displays higher cytotoxicity toward multiple myeloma, while 13-(4-chlorophenyl)-PTL demonstrated opposite trend. Fluorinated compounds are potentially able to use as metabolic and imaging probes in positron emission tomography (PET) (Gunn et al. 2011). The analog of DMAPT, more water-soluble and orally bioavailable analog of parthenolide, exhibits 70 % oral bioavailability in rat, with the major metabolite of mono N-demethylation. Aminoparthenolides undergo retro-Michael additions to regenerate the parent parthenolide, therefore negligible parthenolide are detected in rat plasma. In cell culture, DMAPT shows <3 % degradation to parthenolide after 24 h (Cheng et al. 2005; Peese 2010).

It is important to note that administration of parthenolide orally and DMAPT by injection does not eradicate tumor. Although both drugs were applied in low concentration in the previous examinations, the *in vivo* inefficacy could be related to their high plasma protein binding levels exceeding 75 % (Cheng et al. 2005). Therefore, in some studies the drugs have been used into or near the tumor or *ex vivo* pretreatment to increase drug potency. Parthenolid and DMAPT, preferentially target the cancer stem cells, which constitute small fraction of tumor volume. Both drugs inhibit metastasis and engraftment of tumors, in which cancer stem cells are crucial. This is a highly desirable property of drugs to use in anticancer therapy but requires combination with other drugs that target the tumor (Ghantous et al. 2013). Tumor cells are heterogeneous, in which cancer stem cells are tumor initiating cells with potential to self-renew, invade and engraft into new tissues. These cells have slower proliferation rates compared to more differentiated cancer cells, and also they are often more rare but believed to constitute the root or seed of the tumor. They might be the main reason of ineffective standard chemotherapy, cancer eradication, resistance and tumor relapse (Valent et al. 2012; Guzman and Jordan 2005).

5.5 Diterpenoids

5.5.1 Novel Taxanes

Taxanes are diterpenoids with nitrogen in their structures, which stabilize microtubules and prevent depolymerization arresting mitosis. These compounds are produced by the plants of *Taxus* genus specially bark of slow growing Pacific yew tree, *Taxus brevifolia*. Administration of taxanes is associated with toxicity like arthralgia and myalgia, myelo-suppression, neuropathy, and fatigue. They cause severe but rarely life threatening hypersensitivity reaction and pulmonary toxicity in a dose-dependent manner. Newer taxanes are in development to reduce toxicity, increase efficacy of the molecules to inhibit microtubules, induce mitotic arrest, overcome resistance to paclitaxel (Fig. 5.5) and docetaxel, and formulate oral taxanes to ease their administration (Spigel and Greco 2008).

One of the more developed taxanes in oncology is albumin-bound paclitaxel. This formulation consists of albumin bound to paclitaxel with ratio of 9:1 with no solvent, and is developed to reduce hypersensitivity to previous formulation like Cremophor®-based paclitaxel. Cremophor (polyoxyethylated castor oil) is a vehicle associated with hypersensitivity reactions, when used with other chemotherapies. In addition, this formulation needs non-di (2-ethylhexyl) phthalate tubing and in-line filters for administration. On the other hand, the new formulation of paclitaxel have the opportunity to use more standard intravenous tubing (polyvinylchloride: PVC) sets over a shorter period without steroid or antihistamine premedication (Ibrahim et al. 2002; Spigel and Greco 2008). Albumin-bind paclitaxel has been approved for the treatment of advanced breast cancer due to improvement of its overall response rate, and greater time to progression compared with paclitaxel. It is associated with more grade 3 neuropathy but less neutropenia and no hypersensitivity reactions.

Paclitaxel polyglumex is another new formulation of taxanes, in which paclitaxel is linked with a biodegradable water soluble amino acid polymer. Infusion time for this formulation is brief as 10–20 min with no Cremophor®. High solubility of this new formulation increases therapeutic index of paclitaxel, tumor/drug exposure, and limiting exposure to normal cells. Neutropenia and grade 3/4 neuropathy were the prominent toxicity of the drug in 18 % of patients. In a study, paclitaxel polyglumex/carboplatin was compared with standard paclitaxel/carboplatin, and the results indicated that paclitaxel polyglumex was associated with significantly less alopecia, arthralgia/myalgia, and cardiac events. Importantly, neuropathy was delayed in the paclitaxel polyglumex administration. However in other studies, paclitaxel polyglumex was compared with gemcitabine, vinorelbine, and docetaxel, and showed no survival difference (Spigel and Greco 2008).

Docosahexaenoic acid (DHA) paclitaxel is a novel covalent conjugate of paclitaxel with the natural fatty acid docosahexaenoic acid. This formulation plays role as pro-drug, since released paclitaxel specifically into tumor cells. However, Cremophor® is used in this formulation but in lower amount than

standard paclitaxel, therefore premedication and non-PVC infusion set are still required. It is less toxic than paclitaxel with 15-fold longer half-life and higher delivery dose to the tumor. Dose-related grade 3/4 neutropenia occurred in 76 % and no severe neuropathy was seen (Harries et al. 2004).

BMS-184476 is another taxane analogue more soluble than paclitaxel with greater therapeutic index. Cremophor® is required in lesser amounts for dissolution of this new analogue. Furthermore, this analogue was designed to overcome paclitaxel elimination via p-glycoprotein efflux pump, and improve multidrug resistance as a result of tubulin mutation. Severe neutropenia and neuropathy were observed in 20 and 9 % of the patients, respectively. Other novel taxanes are in development for cancer treatment including oral formulation like DJ-927 with toxicity including neutropenia (47 %) and anemia (19 %). Paclitaxel-loaded polymeric micelle is another Cremophor®-free novel taxanes that allows high doses of paclitaxel to be delivered (Spigel and Greco 2008).

5.5.2 Cafestol and Kahweol

Epidemiological studies showed an inverse relation between coffee consumption and risk of some cancers like colorectal cancer. Furthermore, animal studies confirmed this chemopreventive effect. It is also revealed that coffee or coffee components cause protection against carcinogenic compounds like nitrosamines or 1,2-dimethylhydrazine. Green or roasted coffee (Fig. 5.5) showed protection in 7,12-dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis in different tissue of animal models.

Some components of coffee are responsible for the chemoprotective effect of coffee including caffeine and polyphenols like chlorogenic acids. Lipid fraction of coffee potentially has chemoprotective activity, and the main part of this fraction was identified as diterpenoids, cafestol and kahweol (Fig. 5.5). These constituents are highly unstable and hardly purified, therefore biological activity of their mixture was examined. A mixture of cafestol and kahweol prevents formation of DNA adducts as a result of several genotoxic carcinogens like aflatoxin B1. They also prevent DNA binding induced by procarcinogene heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which is a pyrolysis product in cooked meat and fish. PhIP induces breast, prostate, lymphatic system and colon cancers. The key mechanism of chemoprevention of cafestol and kahweol were identified in several carcinogen models including induction of phase II enzymes involved in carcinogen detoxification, reduction in the expression of phase I enzyme responsible for carcinogen activation, specific inhibition of the enzymatic activity of phase I enzyme responsible for carcinogen activation, and stimulation of intracellular antioxidant defense mechanisms (Cavin et al. 2002). These findings in animal models can explain beneficial effects of coffee ant its constituents in human.

5.5.3 *Carnosol*

Carnosol is a diterpenoid with broad anticancer properties in several cell models such as prostate, breast, leukemia, skin, and colon cancers followed by other activities like antioxidant and anti-inflammatory effects. Mediterranean herbs like sage and rosemary contain variety of polyphenols like carnosol, rosmarinic acid and carnosic acid (Chang et al. 2008; Johnson 2011). Carnosol has abietane carbon skeleton with ortho hydroxyl groups and a lactone moiety across ring B. It is a product of oxidative degradation of carnosic acid (Fig. 5.5). Carnosol may be purified from rosemary with expensive process or produced semi-synthetic using pisiferic acid extracted from *Chamaecyparis pisifera* (Tada et al. 2010).

Carnosol has a potential to modulate cell cycle related proteins, PI3K/AKT, and apoptotic related proteins (Khan et al. 2007). Antibody array in prostate cancer cells demonstrated that protein subunit of 5-AMP-activated protein kinase was up-regulated, which plays role in growth and survival of cancer cells (Johnson 2011). Furthermore, carnosol has a unique property that can disrupt both androgen (AR-α) and estrogen (ER-α) receptors. Carnosol may provide a lead structure for development of a dual modulator for androgen and estrogen receptors in order to use in the chemoprevention and/or chemotherapy of hormone-responsive cancers (Johnson et al. 2010). The results of a study showed that carnosol suppressed tumor growth in treated mice. Both carnosol and carnosic acid developed cytotoxicity toward MCF-7 (breast cancer cell line). Furthermore, carnosol inhibited tumorigenesis induced by DMBA may be due to the activation of glutathione s-transferase (GST) and quinone reductase enzymes. Carnosol inhibited migration and invasion of B16/F10 mouse melanoma cells. The activity of NF-κB was also arrested by carnosol. The mitochondrial membrane potential is disrupted in leukemia cell lines of SEM, RS4:11, and MV4:11 resulting in apoptosis. It is suggested that carnosol targets anti-apoptotic members of the B-cell lymphoma 2 (Bcl-2).

It is found that carnosol has significant antioxidant and anti-mutagenic activity using Ames *Salmonella* tester strain TA102 similar to ascorbic acid. The results of several studies showed that carnosol is a protective agent against environmental toxins may be trough stimulation of phase II detoxification enzymes. The bioavailability and metabolism of carnosol in animals or in humans have not been clarified. However, bioavailability of carnosic acid with similar structure to carnosol was evaluated as 65.09 %, when it was administered intragastrically. Some pre-clinical studies showed that carnosol selectively targets tumor cells and it is well tolerated in animals (Johnson 2011).

5.5.4 *Salvicine*

Salvicine is a diterpenoid quinone (Fig. 5.5) obtained from structure modification of a natural lead, saprorthoquinone that isolated from *Salvia prionitis* (Lu et al. 2011).

The compound exhibited potent anticancer activity toward a wide spectrum of cancer cell lines *in vitro* and in mice bearing human tumor xenograft by profound cytotoxicity toward multi-drug resistant (MDR) cell. Furthermore, the compound significantly decreased the lung metastatic foci of MDA-MB-435 orthotopic xenograft but no inhibition on tumor growth was observed. Therefore, the compound is a promising candidate for anticancer drugs as it is in phase II of clinical trial. Salvicin inhibits Topo II dependent to reactive oxygen specious (ROS) generation along with GSH depletion-driven via H_2O_2 generation. It acts as Topo II poison through effect on Topo II-mediated DNA double-strand breaks without any interaction with DNA (Cai et al. 2008; Meng and Ding 2007). Results of molecular modeling revealed that salvicine binds to the ATP pocket in ATPase domain and superimposes on the phosphate and ribose group. It is believed that salvicine acts as an ATP competitor, therefore, the compound is the first non-intercalative eukaryotic Topo II poison that works in the mentioned way (Hu et al. 2006). Inhibition of Topo II activity is abrogated by GSH, a ROS scavenger, indicating that salvicine inhibits Topo II activity may be through ROS generation (Meng and Ding 2007). Topo II poisons exhibit anti-proliferative activity by induction of DNA double strand beaks, triggering DNA damage response cascade and ultimately apoptosis. Salvicine stimulates intracellular production of ROS, which was already inhibited by N-acetyl cysteine as an antioxidant (Lu et al. 2005). Most of anticancer drugs with quinone moiety stimulate ROS as a part of their anti-tumor and cytotoxic effects (Wang et al. 2001). Furthermore, salvicine down-regulates the activity of telomerase via induction of telomere erosion (Liu et al. 2002, 2004). The activity of the compound is attributed to the ability of quinone carbonyl to accept one or two electrons to create the corresponding radical onion or dianion species. Moreover, changes in side chain have directly influenced on the cytotoxic activity of the salvicine analogues (Lu et al. 2011).

One of the most important impediments in cancer monotherapy is MDR. Thus, overcoming MDR by inhibition of p-glycoprotein overexpression is a valuable effect of salvicine. The mean resistance factor for salvicine (1.42) is much lower than the standard drugs like vincristine (344.35), doxorubicin (233.19) and etoposide (71.23) (Miao et al. 2003). The compound possesses anti-metastatic activity beside its anti-proliferative effect. It is revealed that salvicine obviously down-regulates genes related to cell adhesion and motility including fibronectin, integrin alpha 3, integrin beta 3, integrin beta 5, Federasie van Afrikaanse Kultuurvereniginge (FAK), paxillin, and RhoC (Lang et al. 2005). Salvicine disrupts formation of focal adhesions and actin stress fibers, thus inhibits cell adhesion to fibronectin and collagen leading to rounding up the cells. The compound down-regulates β1 integrin affinity, clustering, and signaling via FAK and paxillin followed by activation of ERK and p38 MAPK via triggering ROS generation (Meng and Ding 2007).

5.6 Triterpenoids

5.6.1 Anticancer Activity of Pentacyclic Triterpenoids

Among all the triterpenoids, biogenic pentacyclic triterpenoids are the most potent natural products with a wide range of activity like anticancer and anti-inflammatory followed by other biological and pharmacological effects like bactericidal, fungicidal, antiviral, cytotoxic, analgesic, spermicidal, cardiovascular, anti-allergic and so on. These compounds are commonly found in plant kingdom (Shanmugam et al. 2012). Regarding Fig. 5.6, these compounds structurally consists of 30 carbon skeleton with five six-membered rings (ursane, oleanane and friedelanes) or four six-membered rings plus one five-membered ring (lupane) (Fig. 5.6). Results of previous studies suggested that betulin, betulinic acid, oleanolic acid, ursolic acid and lupeol are multi target agents. In several assays, these compounds revealed differences in their efficacy, and consequently combination of them could be a way to improve their potencies. Furthermore, they may apply in combination with other chemotherapeutic agents to decrease both the dosage used and adverse effects by increasing the efficacy of treatment through synergism with standard drugs. However, these compounds are insoluble in water, and thus their bioavailability is not optimal and requires improvement by optimizing the galenic form or the application route (Laszczyk 2009).

5.6.2 Betulin and Betulinic Acid

Betulin, belonging to lupane class, is the most abundant triterpenoid in the nature, which is the precursor of betulinic acid found in plant species of the Betulaceae family. For instance, the bark of hazel (*Corylus avellana*), hornbeam (*C. betulus*)

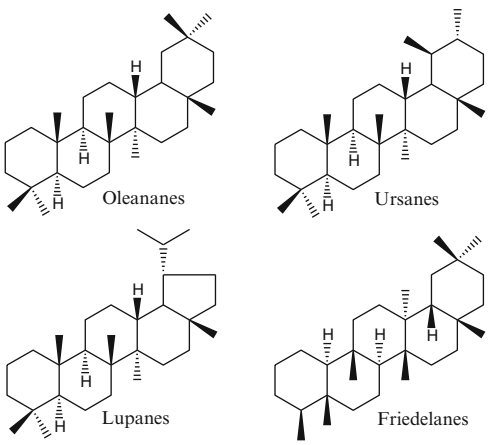

Fig. 5.6 Chemical structures of pentacyclic triterpenoids

and a number of *Alnus* species are the main source of the compound. The compound is found mainly in the outer part of birch bark, whereas white color of the plant bark is mainly related to betulin. Betulin has been extracted from the birch bark by sublimation or extraction with organic solvents. The compound is used in cosmetic products and its derivatives are applied in production of plastic materials. Betulinic acid exerted cytotoxic activity toward neuroblastoma cells, glioblastoma and melanoma cell lines. The compound selectively induces apoptosis through induction of mitochondrial pathway and activation of MAPK kinase pathway in cancer cell lines without affecting normal cells (Shanmugam et al. 2012). The activity of betulinic acid increased, when pH decreased *in vitro*. In euthymic mice carrying human melanoma xenograft, betulinic acid was administered by *intraperitoneal* injection (500 mg/kg) and concentration of the compound in the tumor tissue with lower pH was observed higher than other tissues like liver, kidney and lung. This finding could explain accumulation and specific cytotoxicity of the triterpenoid acids, although more investigations are needed with more details in future studies (Laszczyk 2009). Betulinic acid inhibits nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBα) kinase activation, phosphorylation of IkBα and p65 translocation to the nucleus, which cause down regulation of different proliferative, anti-apoptotic, metastatic genes products like cyclooxygenase 2 (COX-2), cyclin D1, Bcl-2, B-cell lymphoma-extra-large (Bcl-xL) and matrix metalloproteinases (MMPs) (Shanmugam et al. 2012). Moreover, betulinic acid inhibits activation of signal transducer and activator of transcription 3 (STAT3), Src kinase, Janus kinase 1 (JAK1) and JAK2, and also induces the expression of the protein tyrosine phosphatase, SHP-1. Betulinic acid significantly down regulated Bcl-xL, Bcl-2, cyclin D1 and surviving (Pandey et al. 2010). It is demonstrated that betulinic acid causes release of mitochondrial outer membrane cytochrome-c in a Bcl-2 or Bcl-xL-dependent manner, but independently of caspase in a cell-free system (Fulda and Kroemer 2009; Fulda and Debatin 2000). Anticancer activity of betulinic acid was markedly enhanced in combination with conventional chemotherapy, ionizing radiation or cytokine. Oral administration of betulinic acid causes prevention of photo-carcinogenesis induced by ultraviolet B (Shanmugam et al. 2012).

5.6.3 Oleanolic Acid

Oleanolic acid is an oleanane type triterpenoid, which abundantly found in dietary and medicinal plants. In the mouse skin carcinogenesis model, oleanolic acid inhibits both initiation and progression of tumor. Apoptosis induced by oleanolic acid through activation of caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavage in HL-60 leukemia cell line (Shanmugam et al. 2012; Zhang et al. 2007). Masilinic acid, 2-α-OH derivative of oleanolic acid, induced apoptosis and inhibited COX-2 in low concentrations in HT-29 cells. Other derivatives of oleanolic acid like epi-oleanolic acid isolated from Korean mistletoe and

amooranin isolated from the stem bark of *Amoora rohituka* are able to induce apoptosis in several cancer cell lines such as MCF-7 and MDA-MB-468 cells through activation of caspase pathway (Bishayee et al. 2011; Jung et al. 2004). Synthetic oleanolic acid derivatives modulated multiple signaling pathways and intracellular signaling molecules including NF-κB, AKT, STAT3, mTOR, caspases-3, -8, and -9, intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), and PARP in a variety of cancer cells. The results of some studies showed that both oleanolic acid and its synthetic derivatives inhibited angiogenesis trough inhibition of vascular endothelial growth factor-induced ERK1/2 pathway (Shanmugam et al. 2012). Oleanolic acid increases ROS in astrocytoma cell lines in a dose dependent manner (Martin et al. 2007).

5.6.4 Ursolic Acid

Ursolic acid is an ursane type triterpenoid derived from leave, flower, and fruit of medicinal plants with anti-inflammatory and anticancer activity. Several studies reported that the compound inhibited proliferation and promotion of tumor cells, as well as metastasis and angiogenesis in animal model of cancer. Ursolic acid induces apoptosis in a variety of human cell lines like HL-60 (leukemia cancer cell lines), K562, HEC108 and SCG-II (endometrial cancer cells), M4Beu (melanoma cells), A549 (non-small cell lung cancer cells), PC-3 and LNCaP (prostate cancer cells), Ha-CaT (keratinocyte cells), and eventually Burkitt's lymphoma Daudi cells in a dose- and time-dependent manner. The compound induces apoptosis through several pathways including inhibition of DNA replication, activation of caspases, inactivation of protein tyrosine kinases, and induction of Ca^{2+} release (Shanmugam et al. 2012). Furthermore, ursolic acid inhibited NF-κB, which is induced in the presence of several carcinogens like TNF-α, phorbol ester, okadaic acid, H_2O_2 and cigarette smoke (Shishodia et al. 2003). The compound reversely activates NF-κB, STAT3 and AKT, and thus inhibited the inflammatory networks that consist of these agents. In addition, ursolic acid suppresses COX-2 and nitric oxide activity. Thus, the compound is a promising anti-inflammatory agent for the future triterpenoid-based anticancer candidates (Shanmugam et al. 2012).

5.6.5 Lupeol

Lupeol, a pentacyclic triterpenoid, is abundantly found in medicinal plants as well as edible vegetables and fruits like mangoes, olive, fig, cabbage, pepper, cucumber, tomato, carrot, pea, bitter root, soy bean, ivy gourd, black tea, strawberries, red grapes, mulberries, date palm and guava. Lupeol is also found in medicinal plants such as Shea butter plant, licorice, *Tamarindus indica*, *Celastrus paniculatus*, *Zanthoxylum riedelianum*, *Allanblackia monticola*, *Himatanthus sucuuba*,

Leptadenia hastata, *Crataeva nurvala*, *Bombax ceiba*, *Sebastiania adenophora*, *Aegle marmelos* and *Emblica officinalis* (Siddique and Saleem 2011). Lupeol exhibits several pharmacological activities against several diseases including inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer. The compound showed low toxicity in a dose of 2 g/kg with no adverse effects in mice and rat (Siddique and Saleem 2011). The compound is also reported to suppress proliferation, metastasis, and invasion in different cancers (Shanmugam et al. 2012). Lupeol is able to inhibit several pro-inflammatory mediators and oncogenic kinase pathways like PI3K/AKT dependent pathway along with pro-inflammatory molecules like prostaglandin E2, TNF-α, and interleukin (IL-1b) (Prasad et al. 2009). Tumor promoters activate NF-κB resulting in translocation of activated NF-κB to the nucleus, where it acts as a transcriptional factor. NF-κB activates several target genes requiring for the tumor growth, while lupeol significantly inhibited the NF-κB translocation and its DNA binding activity in a mouse model of skin tumorigenesis (Saleem et al. 2004). It showed strong anti-mutagenic effect in both *in vitro* and *in vivo* tests along with chemopreventive and chemotherapeutic activity against a variety of cancers. Lupeol suppresses the growth of different type of cancers by modulating key molecules in pathways of proliferation, apoptosis, and survival. Interestingly, the compound has not toxic effect toward normal cells in its therapeutic dose (Siddique and Saleem 2011).

Lupeol inhibits the chemically-induced DNA damage under *in vitro* conditions (Sultana et al. 2003). Cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP) is a major anti-apoptotic protein and an important cytokine and chemotherapy resistance factor that suppresses cytokine- and chemotherapy induced apoptosis. Lupeol decreases the transcriptional activation of cFLIP gene as well as its translational levels in pancreatic cancer cells (Murtaza et al. 2009). Taken together, lupeol is a chemopreventive and chemotherapeutic agent with no toxicity.

5.6.6 Quassinoids

Quassinoids are a group of compounds with bitter taste presented in Simaroubaceae family. They are chemically degraded triterpenoids with five distinct groups including C-18, C-19, C-20, C-22 and C-25 types (Fig. 5.7). It is believed that quassinoids are biosynthesized through triterpenoids biogenetic pathway.

They have wide range of biological and pharmacological activity including anti-tumor, antimalarial, antiviral, anti-inflammatory, anti-feedant, insecticidal, amoebicidal, anti-ulcer and herbicidal effects. Among them, C-20 quassinoids have been extensively investigated for their biological activities especially anti-leukemic activity. Quassinoids display anti-tumor activity with different potencies. Among them, bruceantin, bruceantinol, glaucarubinone and simalikalacton D are the most potent molecules. They inhibited the protein synthesis by suppression

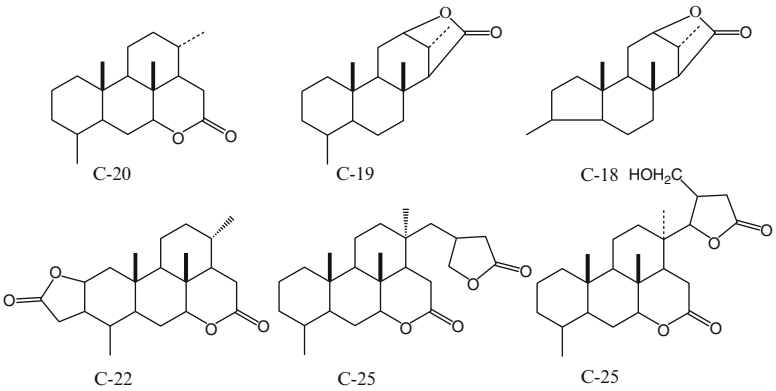

Fig. 5.7 Chemical structures of different types of quassinoids

of the ribosomal peptidyl transferase activity resulted in termination of chain elongation. Both, bruceantin and brusatol suppressed poly (U)-directed polyphenylalanine synthesis as a consequence of ribosomes runoff. The obtained data suggested that quassinoids inhibited the peptidyl transferase elongation reaction of protein synthesis, although they can do the same only after one round of protein synthesis completely. Another plausible mechanism for their anti-tumor activity is that the A-ring enone acts as Michael acceptor for biological nucleophiles. Free hydroxyl group in C-1 or C-3 enhances the biological effects of the compounds presumably through intramolecular hydrogen binding between the hydroxyl and the oxygen of the enone, and thus further activation may happen in the enone towards nucleophilic attacks. Moreover, substituting the "C-1 hydroxyl" with "C-1 methyl ether" can result in completely loss of cytotoxicity. The lactone ring was found to be essential for biological activity. Lipophilic side chain is important in both potency and spectrum of response may be through increasing cell membrane permeability. Although bruceantin once had been used in a clinical trial phase II, it has been withdrawn due to the lack of significant potency, while some other derivatives showed cytotoxicity *in vitro* (Guo et al. 2005). It seems that more observations in this class of compounds could be the subject of future studies.

5.6.7 Cucurbitacins

Cucurbitacins refer to a group of tetracyclic triterpenoids (Fig. 5.8). They were initially isolated and identified in Cucurbitaceae family, which are traditionally well known for antipyretic, analgesic, anti-inflammatory, antimicrobial, and anti-tumor activities. According to the side chain variations, they are divided into 12 groups of cucurbitacins. Hundreds of derivatives are derived from 17 main molecules from cucurbitacins A-T. It is important to note that all the cucurbitacins from the same category don't have essentially similar anticancer activity; for instance,

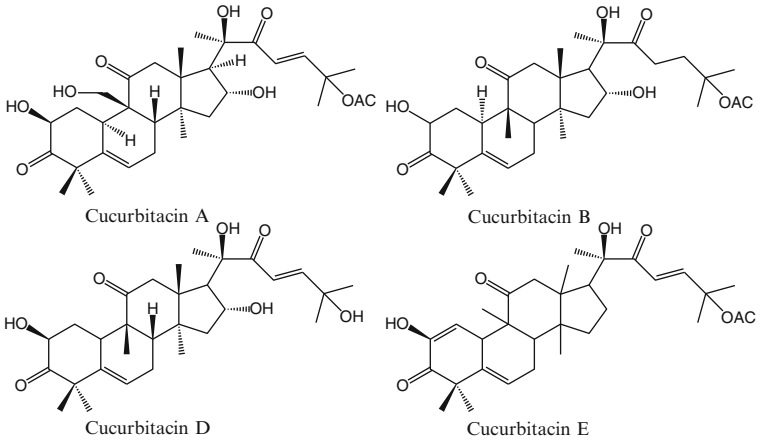

Fig. 5.8 Chemical structures of some cucurbitacins

cucurbitacin D possesses potent anticancer activity, while 2-O-glucoside derivative does not show such potency (Lee et al. 2010).

Cucurbitacins cause several morphological and physiological changes in cancer cells including rounding, pinocytic blebbing, swelling, sub-membranous inclusions, and blisters within a couple of hours. Dysregulation of cytoskeletons may be a reason for morphological changes occurred in the presence of cucurbitacins in cancer cell lines. It is reported that cucurbitacin E caused dramatic changes in F-actin to G-actin ratio, as well as abnormal reorganization of the vimentin network in prostate cancer cell lines. Besides, aggregation of F-actin by cucurbitacin B treatment occurred in several cancer cell lines. Another morphological change is multi-nucleation in cancer cells resulting in disruption of cell cycle, when they exposed to cucurbitacin for more than 24 h. This phenomenon implies that the compounds block cytokinesis but not karyokinesis. This fact is related to the observation that the compounds disrupted actin, which involves in cytokinesis, but have no effect on microtubules that involve in karyokinesis.

Cucurbitacins target several oncogenic signaling pathways including the JAK-STAT, the Akt-PKB, and the MAPK pathways. Activation of STAT3 and STAT5 in several cancer cell lines play important role in tumorigenesis. Inhibition of JAK-STAT pathway influenced several downstream targets playing role in pro-growth signaling (e.g., c-myc, cyclins and survivin) and apoptosis (e.g., p53, Bcl-xL and Bcl-2). Therefore, G2/M arrest and apoptosis may be related to JAK-STAT inhibition in the presence of cucurbitacin B in pancreatic cancer cell lines.

Clinical application of cucurbitacins has been restricted despite of the excellent anticancer activity of these molecules, since cucurbitacins have low therapeutic index with nonspecific toxicity. One way to overcome this problem is administration of cucurbitacins in combination with other chemotherapeutic agents to decrease toxicity and increase efficacy of the treatment. For instance, cucurbitacin E enhances accumulation of doxorubicin in tumor cell lines through facilitating influx and inhibition of efflux of drug to tumor cell lines (Chen et al. 2005).

5.7 Tetraterpenoids

5.7.1 Carotenoids

Carotenoids include the hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls), which may be synthesized through mevalonic acid pathway or non-mevalonic acid pathway (Bolhassani et al. 2014). These compounds display anticancer activity via several mechanisms like induction of apoptosis, suppression of oncogenes and angiogenesis along with cancer prevention effect (Garattini et al. 2007). Epidemiological study showed that high intake of fruits and vegetables rich in carotenoids can reduce the risk of cancers (Palozza et al. 2004). Some carotenoids showed high affinity to p-glycoprotein in mice lymphoma cell lines, while they did not show the same results in human cancer cells including colon and breast cancer cell lines. Carotenoids are substrates for p-glycoprotein and they decreased expression of MDR1-gene in Caco-2 cell lines. The results of a study suggested that fucoxanthin and canthaxanthin were more active toward p-glycoprotein than other carotenoids like β-carotene, retinoic acid and crocin (Fig. 5.9). However, all the tested carotenoids were more potent than verapamil in inhibition of p-glycoprotein. Therefore, they could be introduced as chemosensitizer. Co-administration of carotenoids with chemotherapeutic drugs

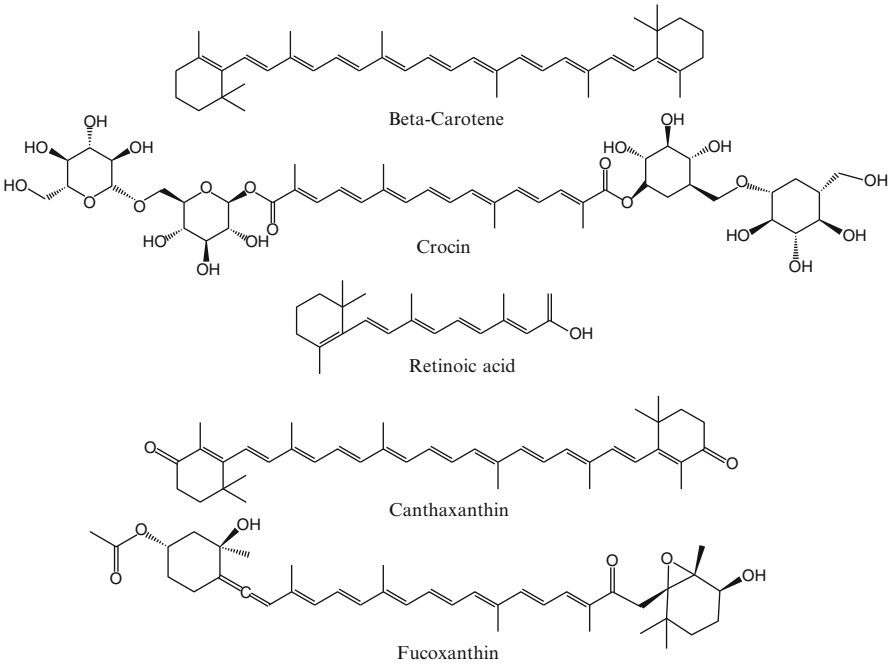

Fig. 5.9 Chemical structures of some carotenoids

enhances the cytotoxicity of standard drugs. Among them, fucoxanthin was the most effective reversal agent in co-administration with vinblastine, etoposide and 5-FU. It seems that more hydrophobic carotenoids like fucoxanthin and canthaxanthin are better substrates for p-glycoprotein in comparison with other polar carotenoids like crocin. In the structure of fucoxanthin, 5,6-monoepoxide and an allenic bond may bind to amino and SH- groups in proteins, so that it can alkylated the transporter proteins leading to irreversible inhibition of those proteins (Yehia Eid et al. 2012).

5.8 Conclusion

Terpenoids provide a promising source for discovering new drugs in several aspects of human disorders, since this class of secondary metabolites present considerable diversity in their chemical structures as well as biological activity. These compounds are synthesized through mevalonate and non mevalonate pathways that the former one is common in eucaryotes as well. Several studies properly prepare data for beneficial effects of this class of secondary metabolites in cancer. Some of these compounds like perilyll alcohol and D-limonene are well tolerated in patients with low toxicity introducing them as a competent drug for application in clinic in the near future. Off-target effects and low bioavailability of some of these compounds like parthenolide make them unsuitable for clinical application. However, it can play role as a lead compound for development of synthetic molecules, in which specificity and pharmacokinetic parameters improved.

Although taxanes are approved as anticancer drugs used in clinics there are newer taxanes are under development in order to reduce their toxicity, increase their efficacy to inhibit microtubules, and induce mitotic arrest, as well as overcome cell resistance (e.g. to paclitaxel). The biological activity of these compounds are interesting; for example carnosol provides a lead structure for development of a dual modulator of androgen and estrogen receptors for applying in the chemoprevention and/or chemotherapy of hormone-responsive cancers. In addition, carnosol showed selective activity toward cancer cell lines rather than normal cells. Some of these compounds (cafestol and kahweol) are in dietary plants like coffee beans that show protection effect against cancers, which is proved by epidemiological studies. Terpenoids and carotenoids are also rich in vegetables and fruits reducing risk of cancer. Another valuable characterization of these compounds is overcoming MDR by inhibition of p-glycoprotein overexpression in cancer cells. Overexpression of p-glycoproteins is one of the major impediments in chemotherapy of cancer. Salvicine, triterpenoids and carotenoids demonstrate inhibition of p-glycoproteins and/or interaction with active sites of the proteins suggesting them as proper candidates for co-administration with chemotherapy agents in clinic by considering all the aspects such as their pharmacological properties. Moreover, combination of these compounds with anticancer drugs may enhance the efficacy of treatment and decrease adverse effects of both drug and terpenoids. Generally, this class of

natural products offers valuable prospects in chemotherapy or chemoprevention of cancer providing new agents to modulate cancer cell lines.

Since mechanisms of action and the efficacy of these compounds in human cancers are not fully recognized, scientists in this field are faced with new challenges in their future experiments.

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Chapter 6
Carbohydrates Against Cancer

Contents

Abstract Using natural medicines containing carbohydrates has been valued from ancient times for therapeutic reasons including anticancer activity. In recent years, new natural carbohydrates (mostly polysaccharides and polysaccharide-protein complexes) from herbal, fungal, animal and algal sources have been found, some of which are taking the last steps toward being established as the anticancer agents to be used alone or in combination with conventional chemotherapeutics. Furthermore, some efforts have been devoted to develop new carbohydrate-base cancer vaccines and also glycoconjugates that may play an important role in prevention and treatment of cancer.

Keywords Carbohydrates • Polysaccharides • Medicinal mushrooms • Cancer vaccine • Sulfated polysaccharides

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6.1 Introduction

Besides being the first biological product formed in the life cycle as glucose, carbohydrates/saccharides are the most abundant and one of the most important classes of biomolecules on earth (Cui and Brummer 2005; Dewick 2009; Hudak and Bertozzi 2014) consisting of carbon, hydrogen and oxygen atoms with general formula of $C_x(H_2O)_y$.

Monosaccharides are usually considered as primary metabolites (Dewick 2009). On the other hand, their important biological functions including their roles in fertilization, preventing pathogenesis, blood homeostasis, immunity and development, and also pharmacologic activities (Maton 1997; Hudak and Bertozzi 2014; Chang 2007) make them important biomolecules for human beings. In cancer therapy, they are now considered as functional molecules, which can be used for targeting cancerous cells (discussed below).

Oligosaccharides are formed by O-glycosidic linkage between monosaccharides and often found in combination with proteins or lipids. They play important biological roles inside and outside of the cells especially in cell membranes (Witczak 2008). Therapeutic uses of oligosaccharides as anticancer agents in natural form or by some modification on larger polysaccharides include increasing the number of beneficial microflora in the intestinal column and using as anticancer agents (Macfarlane et al. 2008; Leclere et al. 2013).

Natural polysaccharides, found in all living organisms, are structurally diverse polymeric carbohydrates with a wide range of subunits (usually 200–2,500), and display two main roles in living organisms: reservation of energy (e. g. starch), and important building structures (e. g. cellulose). They are usually isolated from plants, animals, fungi and seaweeds (Dewick 2009; Zong et al. 2012; Delattre et al. 2011). Other biological functions of polysaccharides include cell-cell communication, fertilization, prevention of pathogenesis, blood homeostasis, *etc*. (Zong et al. 2012). Polysaccharides and peptide-bound polysaccharides exhibit different biological roles and therapeutic applications including anticancer and immunomodulation, and by some modifications, semi-synthetic forms of polysaccharides with advanced therapeutic properties are also available now (Chang 2007; Zong et al. 2012). Using alone or in combination with conventional treatment systems, some of them have shown clinical efficacy in a spectrum of cancers/tumors including gastric, lung, breast, colorectal, *etc*.

The immunosuppression, caused by tumor cells, is a fundamental key in approaching cancer. It is demonstrated that the mechanism of anticancer/antitumor action of most polysaccharides involves nonspecific immunomodulation that leads to activation of macrophages, production of cytokines (including interleukins and tumor necrosis factor) and improving host defense, which ends in eliminating tumor cells (Zhang et al. 2014; Chang 2007; Popa 2011; Lu et al. 2014). The other major mechanism is to counter the effects of mutated tumor suppressor gene p53. This mutation leads to reduction of p32 protein production and disturbance of cell division control, and exists in about 50 %

of cancerous statuses in human. Natural polysaccharides have also shown antioxidant activity. This is probably effective in preventing tumor cell formation by reducing oxidative stress and free radical damages to cells, which may lead to dysfunctions in cell genes and tumor initiation. Other involving mechanisms are induction of differentiation or apoptosis, prevention of metastasis and anti-angiogenic effects that would extend the bioactivity spectrum of these compounds as anticancer agents (Zhang et al. 2014).

A number of anticancer polysaccharides are now being evaluated for effectiveness and safety and many are still as a part of nutraceuticals with few studies concerning their health benefits. Regarding their beneficial effects in prevention and treatment of tumor/cancer, they are generally used as anticancer regimen adjuvants, in which they can ameliorate side-effects of other therapeutic agents or techniques used for treatment of cancer (i.e. chemotherapy or radiotherapy) as well as improving the well-being of cancer patients (Chang 2007).

Recent studies on structure-activity relationship of polysaccharides have been mainly on the β-glucans and pectins. Usually higher molecular weight is associated with greater immunomodulation activity. It has also been found that the process of isolation, presence of helixes, branching in the molecule and the composition of saccharide or non-saccharide portion (especially protein or sulfate groups) is effective in medicinal properties of these polymers (Giavasis 2014). Polysaccharides with anti-tumor activity have been shown to contain mainly specific monosaccharides (e.g. glucose, galactose, arabinose and ribose), and usually glucan structural units, of which β-1,6-glucans, β-1,3-D-glucans and α-1,3-glucans previously showed greater activity (Zhang et al. 2014; Liu et al. 2004; Chang 2007).

After genomics and proteomics, in glycomic era (Delattre et al. 2011), by means of efficient techniques of separation, purification and structural determination, and also standardization of carbohydrates, it is now possible to isolate and screen them for discovery of new effective anticancer agents that could be presented as medicine or adjuvant by well-designed clinical trials (Zhang et al. 2014; Chang 2007; Giavasis 2014).

6.2 Recent Findings in Plant Polysaccharides with Anti-tumor and Anticancer Activities

Plant polysaccharides have exhibited different pharmacological activities including immunomodulation, anti-tumor, antiviral, etc. (Zhang et al. 2014). They are usually extracted by hot water, which may be assisted by other novel techniques such as microwave and ultrasonic (Jin et al. 2013). Activation of monocyte/macrophages and dendritic cells (via Toll-like receptors), modulating the production of NO, Interleukins 1, 2, 4, 6, 8, 10, 12, TNF- α, Interferon-γ (through NK cells), activating spleen lymphocyte or p38 kinase and colony stimulating factor, cytotoxic effect, and also increasing apoptosis of cancerous cells are among major mechanisms, by

which these polysaccharides display their anti-tumor and anticancer activity (Zhang et al. 2014; Chang 2007).

In recent years, many plants have been tested for their anticancer effects, and the effectiveness of some plants has been found to be related to polysaccharide content. Following, are the most important polysaccharide containing plants, which have been revealed to show anticancer effect.

Acanthopanax senticosus, *Aconitum coreanum, Actinidia eriantha*, *Alchornea cordifolia*, *Aloe barbadensis*, *Anemone raddeana, Angelica sinensis*, *Asparagus officinalis, Astragalus membranaceus*, *Astragalus mongholicus*, *Auricularia auricula-judae*, *Brassica napus*, *Boschniakia rossica, Bupleurum smithii var. parvifolium*, *Camellia oleifera*, *Camellia sinensis, Carthamus tinctorius, Codonopsis pilosula, Curcuma zedoaria, Curcuma longa, Curcuma kwangsiensis, Cymbopogon citrates, Dahlia spp., Dendrobium huoshanense, Dendrobium moniliforme*, *Dendrobium nobile*, *Dendrobium officinale*, *Dendrobium thyrsiflorum*, *Dimocarpus longan*, *Elsholtzia ciliate*, *Ephedra sinica*, *Fagopyrum* esculentum, *Gastrodia elata*, *Ginkgo biloba*, *Glycyrrhiza uralensis*, *Gynostemma pentaphyllum*, *Hedysarum polybotrys*, *Inonotus obliquus*, *Ipomoea batatas*, *Juniperus scopulorum*, *Lycium barbarum*, *Melia toosendan*, *Ornithogalum caudatum*, *Orostachys japonicas*, *Opuntia ficus-indica*, *Passiflora edulis*, *Patrinia scabra*, *Phaseolus vulgaris*, *Prunella vulgaris*, *Pulsatilla chinensis*, *Punica granatum*, *Schisandra chinensis*, *Taxus yunnanensis*, *Thuja occidentalis*, and *Zizyphus jujuba*, *Opuntia polyacantha*, *Panax ginseng*, *Panax quinquefolius*, *Phellinus linteus*, *Platycodon grandiflorum*, *Pleurotus tuber-regium*, *Poria cocos*, *Prunella vulgaris*, *Salvia miltiorrhiza*, *Sanguisorba officinalis*, *Solanum nigrum*, *Sophora flavescens*, *Sophora subprosrate* and *Tamarindus indica* are among the most studied plants with bioactive polysaccharides against cancer (Zong et al. 2012; Zhang et al. 2014; Thangam et al. 2014; Joseph et al. 2014).

Furthermore, there are some examples of recently identified polysaccharides/polysaccharide fractions with antitumor activity, extracted from medicinal plants. For instance, *Lycium barbatum* polysaccharide has been found to be active against prostate, breast, bladder, liver, colon and stomach cancer cells by induction of apoptosis or cell cycle arrest, and suppression of angiogenesis (Jin et al. 2013).

Moreover, *Astragalus membranaceous* root contains a polysaccharide (APS) that is demonstrated for its anticancer activity through stimulating the immune system. It belongs to the branched α-1,4 or α-1,3-glucans, and possesses its activity via up-regulation of p53 gene expression. It has been effective in the treatment of leukemia and non-small-cell lung cancer. It has also been used in combination with cisplatin and vinorelbine, and has improved the quality of life for patients compared to using chemotherapeutic agents alone (Zong et al. 2012).

There are ample studies demonstrating the anti-tumor activity of ginseng (*Panax ginseng*) polysaccharides, which contains neutral and acidic polysaccharides including homogalacuronan-rich pectin. The anticancer effect of acidic fraction is mainly mediated through inhibition of tumor growth, induction of cell cycle arrest and supporting the immune system, while the neutral fraction has exhibited immunostimulating effect. It has also been used as an adjuvant with Fluorouracil, and protected against immune system toxicity of the drug as well as its synergistic

effect against cancerous cells. An injectable form of ginseng polysaccharide has been produced in china and used as an adjuvant for chemo- and irradiation therapy (Zong et al. 2012). A unique polysaccharide component (AC-PS), isolated from *Antrodia camphorate* and mainly composed of (1 → 3)-linked β-D-glucopyranosyl residues, has revealed anti-tumor activity in both *in vitro* and *in vivo* models that might be due to the activation of host Immune response mononuclear cells, increasing interleukins production, proliferation of spleen cells, and cytolytic activity of them (Liu et al. 2004). Purified polysaccharide/polysaccharide-peptides from the roots of *Angelica sinensis* (namely, APS-1d and three acidic polysaccharides including APS-2a, APS-3b and APS-3c) have been effective in tumor growth inhibition and life-span extending of tumorous mice. They had immune-stimulating and apoptotic effect on the host body and tumor cells, respectively. Another polysaccharide fraction, AP, has been reported to possess protective effect against gastrointestinal and hematologic side effects of cyclophosphamide in animal study (Zong et al. 2012).

Pectin is a branched, extremely complex and structurally diverse group of polysaccharides that mainly composed of D-galactose, L-arabinose and D-xylose, and widely distributed in a variety of fruits of higher-plants. It has been studied for its anticancer properties. Because of low solubility of pectin in water, its bioavailability is very low, where modified pectins have no problem in this regard. As a part of dietary fibers, natural pectins could protect the digestive track from mutagenic agents by detoxification/trapping. There are also different ways for modifying the natural pectins to change the solubility, and then the bioavailability of them including pH change, heat treatment or irradiation. For instance, modified *Citrus* pectin (MCP) is prepared by treating *Citrus* pectin with NaOH (pH = 10) at 50–60 °C and then HCl (pH = 3). Although it is difficult to determine the exact structure of pectins, it has been revealed that pectins are mainly composed of linear homogalacturonan (HG, α-1,4 bound D-galactopyranosyluronic acids) and about 10 % rhamnogalacturonan II. It has been studied in several cases and revealed to be carcinogen (especially heavy metals) detoxifier agent, and it could inhibit the cancer progression (by inhibition of adhesion, angiogenesis and metastasis of cancerous cells), *in vitro* and *in vivo* in different types of cancers. The recommended dose of MCP by American Cancer Society is 800 mg, 3 times/day. In one human study, it was observed that prostate specific antigen doubling time (PSADT) was increased in 80 % of men with prostate cancer after 12 months of MCP treatment. The results showed its usefulness in prevention of tumor spreading to other organs. Generally, pectic polysaccharides have immune-modulation effect, and also can interact specially with galectin-3 (a 31–42 kDa protein on cancerous cells) to prevent their growth, survival and adhesion, inhibiting the activation of key transcriptional factors (e.g. Nf-κB and AP-1), and inducing cellular apoptosis in cancerous cells via different mechanisms of action. It has been demonstrated that esterification of pectins up to 90 % makes them more efficient in this regard. It is also demonstrated that using HG-rich pectins, pre or post administration of chemotherapeutic agent (e.g. paclitaxel, cisplatin and doxorubicin) could ameliorate the effect of the drug by targeting galectin-3 and lactosyl-L-leucine, which are

among the main causes for decreasing the sensitivity of cells to chemotherapeutic agents, and also to overcome the resistance occurred in cancer patients (Niture and Refai 2013; Leclere et al. 2013; Zong et al. 2012).

Apple oligogalactan has been reported to show preventive effects against carcinogens. There are also reports on production of biologically active pectin-derived oligosaccharides by irradiation instead of chemical treatment (Leclere et al. 2013). As a new approach, preparation of nanoparticles and nano-conjugates of natural compounds is a potential way for increasing their pharmacological activity. For instance, the gold nanoparticles of polysaccharide fraction of *Tamarindus indica* seed kernel (a polysaccharide with the backbone of galactoxyloglucan) enhanced the cytotoxic activity against different tumor cell lines *in vivo* and *in vitro* (Joseph et al. 2014).

6.3 Mushrooms as a Major Source of Anticancer Polysaccharides

Medicinal mushrooms have been used historically from ancient times for different health beneficial reasons. New efforts for demonstrating pharmacological effective parts within mushrooms have led to discovery of triterpenes and polysaccharides as the two main classes of components, while polysaccharides were found to be the major substances with anticancer and immunomodulating properties. About 200 species of mushrooms (especially from Basidiomycete class) have shown antitumor and also prophylactic activity against chemo/radio-therapeutic agents by now. A few of them have been studied in human clinical trials and some have been developed into food supplements or nutraceuticals (Chang 2007; Giavasis 2014).

They are usually described as Biological Response Modifiers (BRMs) because of their ability to non-specific modification of immune system. This change will cause some pharmacological activities such as antimicrobial, anti-inflammatory, anti-tumor, *etc*. in the host body (Giavasis 2014). A major class of anticancer fungal polysaccharides is "glucans" which could be divided into α- or β-glucans (Fig. 6.1), glycoproteins/glycopeptides and proteoglycans. Other classes of mushroom polysaccharides with anticancer effect have been identified during recent studies, too (Zhang et al. 2014; Zong et al. 2012).

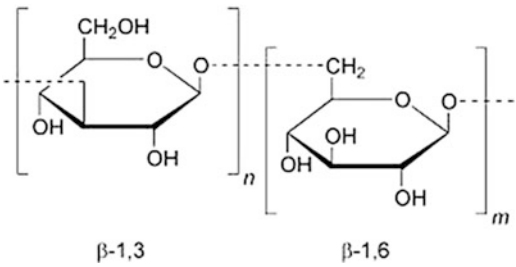

Fig. 6.1 Structure of β-1,3 and β-1,6 glucan

Agaricus blazei, *Agaricus brasiliensis, Astraeus hygrometricus, Auricularia auricular-judae, Auricularia polytricha, Boletus edulis*, *Cordyceps gunnii, Cordyceps militaris, Cordyceps sinensis*, *Cordyceps scpecocephala*, *Flammulina velutipes*, *Ganoderma* spp. *Grifola frondosa*, *Hericium erinaceum*, *Lentinus edodes*, *Penicillium jiangxiense*, *Pleurotus abalonus*, *Pleurotus ostreatus*, *Polyporus* sp., *Poria cocos*, *Inonotus* sp., *Schizophyllum communes*, *Trametes hirsuta*, *Trametes versicolor*, *Tremella* sp. and *Tricholoma aggregatum* are among the recent studied mushrooms with astonishing anticancer effect, which is assigned to their polysaccharides (Chang 2007; Zong et al. 2012; Shenbhagaraman et al. 2014; Wang et al. 2014).

Lentinan (from *Lentinula edodes*) has a backbone of β-1,3-glucan and side chains of β-1,3 and β-1,6 D-glucose residues. In 1970, it was reported that this compound has anti-tumor activity greater than other mushroom polysaccharides. Because of its poor intestinal absorption, it must be injected directly or indirectly into the blood stream. In animal testing, it has shown the stimulation of host defense system and anti-tumor activity especially by activation of dendritic cells. It has also caused prolongation of survival in metastatic gastric and colorectal cancerous patients in controlled clinical trials, when used parenterally in combination with chemotherapeutic agents, in which synergistic effects, reducing the side effects of chemotherapeutics, and enhancing the quality of life in cancerous patients have been observed. Since the glucan has shown no toxicity against human normal cells, there are adjuvant formulations/therapies of lentinan including combinations with Dendritic cell vaccine, OK-432 (lyophilized incubation mixture of group A *Streptococcus pyogenes* of human origin), thermotherapy, TACE/RFA (Transcatheter arterial chemoembolization/Radiofrequency Ablation), paclitaxel, cisplatin, *etc*. It has been prepared also as a superfine disperse lentinan (SDL) formulation (Zhang et al. 2014; Chang 2007; Zong et al. 2012; Giavasis 2014). Lentinan injection dosage form has been approved for use in gastric cancer in Japan (Zong et al. 2012). The combination of lentinan and S-1 has been evaluated in advanced oral squamous cell carcinoma patients. They have had significant inhibitory effect on tumor cells and made a promising discovery for treatment of this resistant case of cancer to typical chemotherapies (Zong et al. 2012). Moreover, Schizophyllan (from *Schizophyllum commune*) is a similar glucan (β-1,3-glucan with the side chains of β-1,6 D-glucose residues) with the same properties as lentinan, which has been tested in clinical trials and has been used as anticancer medicine (Popa 2011; Zhang et al. 2014).

PSK/Krestin, is a protein-bound polysaccharide (a β-1,4-glucan containing 25–38 % protein) extracted from the CM-101 strain of *Trametes versicolor*. It is known as an immunostimulant and is able to induce cell cycle arrest and apoptosis to protect the host from tumor by increasing natural killer and lymphokine-activated killer cells activation, as well as apoptosis prevention in circulating T cells. It is orally active and prolongs survival of patients with gastric, esophageal, nasopharyngeal, non-small cell lung and breast cancer. A formulation of PSK has been developed in Japan for treatment of gastric and colorectal cancers. Recent clinical trials indicated that combination therapy of the polysaccharide and

some chemotherapeutics (including tegafur/uracil, tegafur/gimeracil/oteracil, 5-FU/folinic acid/oxaliplatin and trastuzumab) could improve the long-term prognosis, reduced the risk of recurrence, and increase the survival rates in cancerous patients. It can increase the cytotoxicity of chemotherapeutics, too. The polysaccharide peptide (PSP) is a polysaccharide based molecule with the same structure of PSK (but with different protein structure) isolated from COV-1 strain mycelia of *T. versicolor,* which has revealed beneficial effects in gastric, lung, and esophageal cancers (Liu et al. 2004; Zhang et al. 2014). As it can interfere with the S phase of cell cycle, it can boost the cytotoxic effect of S-phase targeted drugs (*e. g*. doxorubicin, camptothecin, cyclophosphamide and etoposide) (Zhang et al. 2014; Zong et al. 2012). A new combination of two natural polysaccharides (PSP and *Astragalus* polysaccharide) has had significant anti-tumor activity, along with immunomodulatory effect, and restored the side effects of adriamycin on immune system.

Four types of polysaccharide/polysaccharide fractions have been isolated from the fruit body and mycelia of *Grifola frondosa* (Japanese name: Maitake): D-fraction and its further purified fraction; MD fraction: a heteropolysaccharide maitake; Z-fraction (MZF) and GFPPS1b: a polysaccharide peptide. They are composed mainly of protein-bound β-1,3 and β-1,6-glucans with β-1,6 or β-1,3 branches, respectively. The polysaccharide D and MD fractions have been evaluated in animal studies, and also in phase I and II trial in cancer patients demonstrating immunostimulant effects. They have also apoptotic induction effect by activation of BAK-1 gene (Zhang et al. 2014; Kodama et al. 2003; Deng et al. 2009; Nanba and Kubo 1997; Zong et al. 2012; Popa 2011). The combination therapy of MD-fraction with IFN-α2b and cisplatin has been evaluated and synergistic effects were observed in both experiments. The polysaccharide fraction also decreased cisplatin-induced immunosuppression and nephrotoxicity of cisplatin, and enhanced granulopoiesis and granulocyte colony-stimulating factor production in mice (Kodama et al. 2004; Zong et al. 2012). MZF stimulates the immune system *in vivo* and enhances the dendritic cell-based immunotherapies against cancerous cells (Zong et al. 2012).

Polysaccharides from *Ganoderma lucidum* (mainly ganoderans, a type of β-glucans) and *Cordyseps sinensis* (mainly α-glucans including WIPS and the more potent polysaccharide, AIPS) could inhibit proliferation of tumor cells by stimulating the production of cytokines by activation of mononuclear cells (Liu et al. 2004; Giavasis 2014; Lu et al. 2014). The effective fractions of polysaccharide-peptides/proteins from *G. lucidum* have also indicated anti-angiogenesis, immunomodulatory (and also prevention of immunosuppression caused by cyclophosphamide), anti-adhesion of tumor cells, and induction of cell apoptosis in cancerous cells even stronger than PSP in some cases (Pang et al. 2007).

A polysaccharide-protein complex, isolated from *Agaricus blazei,* has shown cytotoxic activity against cancerous cells *in vivo,* and prevented the leucopenia caused by 5-FU in animal study. ABP-Ia is a polysaccharide fraction with inhibitory effect on cancerous cells. Another β-glucan (LMPAB), isolated from the mushroom, exhibited immunomodulatory effect and prohibited the invasion of tumor cells, tumor growth and metastasis in animal models.

A polysaccharide derivative from *Saccharomyces cerevisiae* (sulfoethyl glucan) has shown anti-mutagenic, anti-clastogenic, DNA-protective effects and also augmentation of teniposide effect in treatment of leukemia (Zong et al. 2012). The structure-activity of β-glucans has been demonstrated in recent studied. For example, the presence of β-1,3-D-glucan structure in lentinan, grifron-D, schizophyllan, krestin and PSP is related to their anti-tumor activity. β-1,3-D-glucans in Lentian and shizophyllan form triple helical conformers which is necessary for their anti-tumor activity (Zhang et al. 2014).

6.4 Sulfated Polysaccharides as the New Anticancer Class of Secondary Metabolites

Sulfated polysaccharides such as carrageenans and agar are usually extracted from marine sources (generally algae). About 70 % of the dry matter of red algae is composed of sulfated galactans (polymers of galactose). Galactans are usually made up of a backbone of β-D-galactopyranose and α-D/L-galactopyranose residues . The anti-tumor effect of sulfated galactan oligosaccharides, polysaccharides and their depolymerized products against Ehrlich ascites cells, Meth-A tumor, and mammary adenocarcinoma as well as immunomodulation has been revealed in some studies (Delattre et al. 2011). The best examples of recently studied sources of marine polysaccharides with significant *in vitro* and sometimes *in vivo* anticancer effects are: *Capsosiphon fulvescens*, *Champia feldmannii*, *Chlorella pyrenoidosa*, *Chondrus ocellatus*, *Gracilaria lemaneiformis*, *Grateloupia longifolia*, *Monostroma nitidum*, *Porphyridium creuentum*, *Sargassum fusiforme* and *Sargassum horneri* which most of them contain sulfated polysaccharides (Zong et al. 2012; Shao et al. 2014a, b). Additionally, fucoidans and ulvans are superior sulfated polysaccharides with anticancer effect, derived from a variety of algae (*e. g. Ascophyllum nodosum*, *Cladosiphon okamuranus*, *Eclonia cava*, *Sargassum hornery*, *Costaria costata*, *Fucus evanescens*, *Fucus vesiculosus*, *Saccharinia japonica*, *Ulva* spp. and *Undaria pinnatifida*). Marine sources with anti-tumor/anticancer effects are discussed in more detail in related chapter.

Animals are another source of sulfated polysaccharides. A protein-attached polysaccharide (GSPP), extracted from *Gekko swinhonsis,* has shown significant anticancer effect *in vitro*. It can inhibit the proliferation of cancerous cells, induce their differentiation, decrease cell migration, and recover dendritic cells by changing immunological condition but fortunately shows no direct toxicity on normal cells. *Ommastrephes bartrami* is a source of another sulfated polysaccharide named TBA-1. It is able to inhibit the invasion and migration of tumor cells as well as angiogenesis *in vivo* (Zong et al. 2012). In addition, bacteria are the new sources of sulfated polysaccharides. An O-sulfated polysaccharide, isolated from *Escherichia coli* k5 (K5PS), is an inhibitor of tumor metastasis in animal models perhaps by inhibition of heparanase (Zong et al. 2012). It has been demonstrated that in specific cases, the anticancer effect of sulfated polysaccharides is not positively related to the level of sulfate content of the molecule (Shao et al. 2014a, b).

6.5 Polysaccharides Isolated from Animals with Anticancer Effect

Besides some sulfated polysaccharides isolated from animal sources (mentioned in earlier section), a short-chain polysaccharide, extracted from pig's cartilage, has been reported to possess *in vitro* and *in vivo* anticancer effects. It can inhibit the proliferation of some human cancer cells (including breast, liver and blood cells) by induction of apoptosis with little effects on normal cells. It has also the property of enhancing the immunogenicity of a tumor vaccine (Zong et al. 2012).

SIP, isolated from *Sepiella maindroni,* is a heteropolysaccharide with relatively different backbone (containing N-acetylgalactosamine, mannose and fucose). It has reduced the frequency of micro-nucleated polychromatic erythrocytes caused by cyclophosphamide, and is a strong anti-mutagen agent. Specific polysaccharides have been isolated from *Cyclina sinensis* (containing a polysaccharide named CSPS-3), *Strongylocentrotus nudus* eggs (named SEP) *Hyriopsis cumingii*, and *Misgurnus anguillicaudatus*, and revealed to possess significant inhibitory effect against tumor cell growth *in vitro* (Zong et al. 2012).

6.6 Carbohydrate-Based Vaccines in Cancer Therapy

With astonishing developments in glycoscience, carbohydrate-based vaccines are now a new approach, and an important part of future vaccination system in the modern medicine, which can prevent the diseases caused by microorganisms (Liao et al. 2013; Zhang et al. 2014). Alongside other changes in cancerous cells, the cell surface alteration, especially in carbohydrate fraction of glycoconjugates is usually observed. These special altered carbohydrates on tumor cells, referred to TACAs (tumor-associated carbohydrate antigens) are a valuable tool in cancer diagnosis and producing immunotherapeutic agents (Freire and Osinaga 2012; Hudak and Bertozzi 2014). Upon administration of the antigen (usually co-administered with carrier proteins and adjuvants for better immunogenicity and safety profile), it may stimulate an immune response especially by receptors of adaptive immunity and produce cytotoxic T-lymphocyte response against the carbohydrate expressing tumor cells, and thereby decreasing tumor cell proliferation. A diverse range of carbohydrate motifs on cancerous cells have been detected, and synthetically defined and developed as carbohydrate vaccines since then (Freire and Osinaga 2012). Globo-H hexasaccharide 1 (Globo-H) is one example of these TACAs (Tsai et al. 2013; Jeon et al. 2009).

A synthetic vaccine containing prostate and breast cancer-associated carbohydrate antigens (*i.e.* Globo-H, GM2, STn, TF, and Tn) that conjugated to the carrier protein has been developed and is now in clinical trials (Hudak and Bertozzi 2014). Other examples are "Globo H-KLH immunostimulant OPT-822" and a conjugation of Globo-H to diphtheria toxoid, administered alongside with α-galactosylceramide

for using in breast cancer (Page et al. 2014). Some patents describe new adjuvants for producing immunologically improved Globo-H and its related anticancer vaccines (Wong et al. 2010; Seeberger et al. 2013). Tumor vaccines are preventive agents but they may also be used in combination with other common cancer treatments (*e.g.* chemotherapy), which has revealed a better result in cancer treatment (Emens 2008).

6.7 Glycosylated Anticancer Agents

In recent years, new efforts are being made to improve the efficacy, decreasing the side effects and improving the partition coefficient of chemotherapeutics (Cao et al. 2011; Popa 2011). One of the solutions is the smart drug delivery, which could be maintained by different methods. Linking the anticancer medications to carbohydrates and making "Glycoconjugates" is among important strategies for targeted drug delivery. The "Warburg effect" indicates high intake of glucose by cancerous cells, and has been noted as one of these ways, after the synthesis and evaluation of a carbohydrate-conjugated anticancer drug in 1995. In this case, glycoconjugate must be substrate for the insulin-independent glucose transporter (GLUT-1) that is usually overexpressed in cancerous cells' surface. Therefore, the conjugate can enter the abnormal cells more than normal ones. Glucofosfamide (a conjugate between β-D-glucose and the anti-neoplastic agent, isofosfamide) is the first in this class to pass phase I and II of clinical trials in treatment of pancreas and non-small cell lung cancers and recurrent glioblastoma multiforme. However, it has not been followed yet for commercial production because of modest activity compared to common drugs. This type of conjugates could also cause hemolytic anemia due to the high expression of GLUT-1 on erythrocytes (Calvaresi and Hergenrother 2013). The other anticancer drugs, subjected to glyco-conjugation by different natural or semisynthetic monosaccharides, are chlorambucil, busulfan, benzylguanine, doxorubicin, geldanamycin (a heat shock protein 90 inhibitor), duicarmycin SA, nordihydroguaiaretic acid and methan sulfonate (Calvaresi and Hergenrother 2013). There are also other examples of glyco-conjugated anticancer drugs like paclitaxel and docetaxel, conjugated with glucose, galactose, glucuronic acid, mannose, *etc*. for making prodrugs with fewer side effects and better water solubility (Popa 2011; Roy et al. 2014). They are discussed in details in "Anticancer terpenoids" chapter.

In special cases of colon cancers, pectin based vehicles are good candidates for colon drug delivery, because they are not ingested in the gastrointestinal track until they reach the microflora in colon to release the carried medication. A new type of sustained release dosage forms has been studied during development of calcium-pectinate beads for delivery of chemo-preventive drugs. For instance, coupling resveratrol with calcium pectinate beads resulted in delaying the release of resveratrol in lower GI track (Niture and Refai 2013). Pectin-derived biocompatible hydrogels or pectin nanoparticles, as a part of a delivery system, contain anticancer agents including doxorubicin, methotrexate, gemcitabine and 5-fluorouracil, and

have been developed to be controlled release or enhanced anticancer effect in both *in vitro* and *in vivo* models (Leclere et al. 2013).

Chirosan is another example of polysaccharides used for drug delivery in cancer. Chitosan-gadopentetic acid complex nanoparticles have been considered suitable for injection in the case of solid tumors (Rinaudo 2006). Generally, it seems that achievement of a potent carbohydrate-conjugate anticancer product with a few side effects is the main goal of future studies considering the smart drug delivery system as a novel strategy.

6.8 Conclusion

In the glycomic era, we will encounter the vast application of carbohydrates, as the new tools for treatment of diseases. While new studies have revealed the possible application of them as the new class of compounds for treating cancer, clinical trials are going to demonstrate the real (in human) effect of previously examined components. Fortunately, astonishing effects for improving the conventional treatment systems and the overall quality of life of the patients have been observed in some cases. Overall, it seems that by different favorable mechanisms of action, alongside minor toxicity for normal cells, carbohydrates (especially polysaccharides) could be a future functional food and chemotherapeutic of choice in cancer therapy, alone or in combination with conventional methods.

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