Anil K. Sharma *Editor*

Bioactive Natural Products for the Management of Cancer: from Bench to Bedside

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Preface

Natural biometabolites have been the mainstay of cancer chemotherapy, being a rich reservoir of candidate compounds for drug discovery. Recent efforts into the research and development of anticancer drugs derived from natural products have led to the identification of a variety of candidate molecules that inhibit cancer cell proliferation and metastasis via various mechanisms. With the advent of new technologies such as combinatorial chemistry and high-throughput screening, next-generation sequencing, and the ease of identifying abnormal genes, it is now possible to consider that natural products would sound the death knell for cancer. Moreover, natural products are likely to provide novel lead molecules which would be used as templates for restructuring them for potential anticancer drug candidates with enhanced biological properties. Moreover, nanomedicine-based natural products have recently shown promising therapeutic effects with better efficacy and target specificity against cancer countering drug resistance as well. Despite the increasing interest in natural product research, to our knowledge, still this area requires attention of the scientific community to explore the wide-scale mechanisms encompassing anticancer therapeutics with natural products being the lead compounds further redressing the growing problem of drug resistance against cancer. In order to fill these gaps and what kind of therapeutic roles natural products especially secondary metabolites play in the treatment and management of cancer, this book titled *Bioactive Natural Products for the Management of Cancer: From Bench to Bedside* has been able to successfully address the remarkable therapeutic potential of bioactive natural products against cancer.

The book has significant contributions in the form of book chapters by renowned authors as follows: Hala Gali-Muhtasib and his group highlighted the potential significance of anticancer alkaloids, underlying action mechanism, and clinical manifestations which were further supported by Batra and Sharma, expanding their studies to emerging alkaloids peeping into various factors and getting insight into the mechanism of action against cancer. Banerjee and his group shed light into the cancer etiology and therapeutic management by natural metabolites. Sharma et al. highlighted the potential anticancer therapeutic role of flavonoids especially flavones. Gajbhiye et al. emphasized the therapeutic properties of dietary polyphenols, flavonoids, terpenoids, and saponins in cancer chemoprevention. The same group further enlightened us with a vast immunogenic potential of natural products. Anshika Singh and S. Krishna further lead us to look into marine flora for their

immunomodulatory and therapeutic potential in the treatment of cancer. Bhattacharrya and her group enlightened us by contributing a chapter on ligand-based designing of natural products paving a way for drug discovery of novel chemical entities. In another chapter, Nag and her group tried to address the mechanism of drug resistance in cancer and the potential role of nanomedicine-based natural products in countering the menace of drug resistance.

The book holds many unique flavors as follows:

1. Recent updates on natural metabolites and their therapeutics use against cancer
2. Unique and distinctive pathways and mechanistic insight into the mode of action of the metabolites
3. The use of these metabolites and nanoparticle-augmented adjuvant therapy to counter the ever-growing problem of drug resistance
4. Ligand-based drug designing of these natural metabolites to enhance their active potential and counter adverse side effects

Once again, my sincere thanks to all the contributing authors who worked as a team to let me complete this book. Special thanks to Dr. Bhavik Sawhney who was available all the time to impart his valuable inputs and assistance. Words of appreciation also go to Mr. Daniel Ignatius Jagadisan, the Production Team, and the Editor as well.

The book is dedicated to my parents and spouse who time and again kept inspiring me to accomplish this task and complete the said manuscript timely.

Ambala, Haryana, India Anil K. Sharma

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About the Editor

Dr. Anil K. Sharma is presently at M.M. (DU), Department of Biotechnology, Mullana, Ambala (India), where he has been a Professor and Head of the department since April 2012. Previously, he worked as a Senior Research Scientist in Health Sciences (UIC Chicago, USA; 2008–2010), Postdoctoral Research Fellow in Molecular Biology (Microbiology and Immunology Department, UIC Chicago, IL, USA; 2003–2008), and Senior Research Scientist at Ranbaxy (R&D, Gurgaon, Haryana, India; 2001–2003). He has authored more than 95 publications in peer-reviewed journals and received many prestigious awards and accolades including an Eminent Scientist Award for Molecular and Microbial Science (2017 and 2018), National Achiever Award (2016), and Bharat Excellence Award (2013). In addition to editing five books, he has been the Editor-in-Chief of two journals, and lead guest editor, editorial board member or reviewer of over 30 more.

Anticancer Alkaloids: Molecular Mechanisms and Clinical Manifestations

1

Farah Ballout, Zeina Habli, Alissar Monzer, Omar Nasser Rahal, Maamoun Fatfat, and Hala Gali-Muhtasib

Abstract

Throughout history, naturally derived molecules have had countless applications in medicine, pharmacy, and biology. This rich reservoir of natural compounds demonstrated great potential in treating various diseases, mainly cancer. Alkaloids, a subfamily of secondary metabolites, are derived from a large variety of organisms including plants, animals, and marine organisms. This group of compounds has exhibited promising anticancer and chemopreventive effects and has been found to chemo-sensitize tumor cells that are resistant to conventional chemotherapy. The remarkable structural diversity of anticancer alkaloids has allowed their use as lead compounds in the treatment of cancer. Chemical derivatization and modifications of alkaloid structures led to the improvement of their therapeutic potential. Many of these second-generation alkaloids are currently commercially available or are in advanced clinical trials, and a major group is still being tested preclinically. Here we provide an overview of alkaloids that are in clinical trials and which are FDA approved. We have classified anticancer alkaloids according to their biological origin and presented an extensive discussion of their mechanism of action and clinical toxicity. The understanding of the mechanism of action and clinical manifestations of anticancer alkaloids is essential for advancing their use and enhancing their efficacy in the clinic.

Authors Farah Ballout and Zeina Habli have equally contributed to this chapter.

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Keywords
Alkaloids · Cancer therapy · FDA approved · Clinical toxicity · Plants · Marine organisms

1.1 Introduction

For millennia, cancer has been a poorly understood disease that is usually fatal. Defined as a relentless growth of cells that are capable of invading surrounding tissues and organs, cancer is an adverse disease with tremendous negative impact on individuals and society. It is one of the most common causes of mortality in developing countries and the second leading cause of death in the United States exceeded only by heart disease (Khazir et al. 2014). The World Health Organization (WHO) projects that the global number of cancer deaths will increase by nearly 80% by 2030 and predicts a rise in the number of cancer patients by 70% in the next two decades. The American Cancer Society expects more than 1.5 million new cancer cases to be diagnosed and more than 600,000 cancer deaths to occur in the United States alone in 2017.

With better understanding of the pathophysiology and natural history of cancer, the field of anticancer therapeutics has gained large popularity among scientist all over the world. At the beginning of the twentieth century, surgery and radiotherapy were solely used to treat malignancies with recovery rates not exceeding 33% (Mukherjee et al. 2001). A major breakthrough in the treatment of cancer occurred in the 1960s when chemotherapy became an adopted approach for treating this deadly disease. The use of chemotherapeutics in conjunction with the aforementioned orthodox treatment approaches opened new opportunities for cancer therapy, and since then chemotherapy became the standard clinical practice (DeVita and Chu 2008).

More than 60% of the currently used cancer chemotherapeutic and chemopreventive drugs are either natural compounds extracted from plants or animals or synthetic compounds derived from natural prototype structures (Amin et al. 2009; Khazir et al. 2014; Newman and Cragg 2016). It all started in 1955 when the National Cancer Institute (NCI) initiated a large-sale preclinical screening mission in the hope of finding promising anticancer compounds and molecules of various origins from plants, marine organisms, microbes, and animals (Nobili et al. 2009). Out of the selected 400,000 molecules, more than 114,000 compounds originating from plant species have been screened and tested (Holton et al. 1994). With this high-throughput screening and combinatorial synthesis, the quest for "safe" and selective anticancer agents was affordable and led to the discovery of compounds having growth inhibitory effects and apoptotic activities against human cancer cells with minimal toxicity to normal ones (Gordaliza 2007). Yet, the search for new improved cytotoxic agents continues to be an important approach to overcome the alarming emergence of chemotherapy resistance along with the annual increasing cancer death rates.

Nature has provided mankind with a wealth of effective agents that have immediate applications in medicine (Gordaliza 2007). Such compounds belong to several structural classes referred to as secondary metabolites (Seca and Pinto 2018). Alkaloids, the largest group of secondary metabolites, are a highly diverse collection of compounds of low molecular weight containing a heterocyclic ring structure and a nitrogen atom. About 17,000 alkaloids have displayed pronounced biological and pharmacological activities with relatively low toxicity and well-documented stability (reviewed in Habli et al. 2017). Alkaloids can be classified according to their structure and other chemical features, biological origin, as well as biogenetic origin. They can be found in a large variety of organisms including plants, especially higher plants, animals, bacteria, and fungi. They have been shown to exhibit a wide range of pharmacological properties including antimalarial, antiasthmatic, anticancer, vasodilatory, antiarrhythmic, analgesic, antibacterial, and antihyperglycemic activities (Lu et al. 2012; Iqbal et al. 2017). Currently, numerous alkaloids are being tested for their cytotoxicity or are undergoing clinical evaluation, and some have received FDA approval for cancer treatment. Their antitumor activity stems from their ability to induce DNA cleavage which is mediated by topoisomerase I and II inhibition, in addition to causing mitotic arrest, mitochondrial permeabilization, and inhibiting key enzymes involved in cell signaling and metabolism (Demain and Vaishnav 2011). In fact, the first series of chemically administered chemotherapeutics included the vinca alkaloid, vincristine, a revolution that increased the curability of children with leukemia and Hodgkin's diseases (DeVita and DeVita-Raeburn 2015). In this chapter, we focus on the various plant- and marine-derived alkaloids that are in clinical trials or that have been FDA approved for the treatment of cancer and discuss their clinical manifestations and adapted strategies to enhance their therapeutic potential.

1.2 Plant-Derived Alkaloids

Plants have played a major role in human life since ancient history. Plants are used for basic needs such as food, shelter, and clothing in addition to being used as dart poisons for hunting purposes and hallucinogens for ritualistic purposes. Plants have also been the basis of traditional medicine in various countries including China and India. Historically, the efficacy of plants was attributed to their color, name, or physiological appearance before the realization and identification of the active compounds mediating these effects (Salim et al. 2008). For example, red-colored herbs were used to treat blood diseases, liverworts were used for liver diseases, and toothworts for toothache (Sneader 2005). Morphine was the first pharmacologically active compound to be isolated from plants. The nineteenth century witnessed the extraction of various alkaloids used as drugs for the treatment of several disease conditions. These are atropine (anticholinergic), codeine (cough suppressant), colchicine (antigout), ephedrine (bronchodilator), morphine (analgesic), physostigmine (cholinesterase inhibitor), and quinine (fever-reducing, antimalarial, analgesic, and anti-inflammatory properties) (Iqbal et al. 2017). The last 200 years have

witnessed the discovery of plant-derived substances (Fridlender et al. 2015). As a result of this undertaking, various plant-isolated alkaloids with anticancer activity have been characterized (Table 1.1). This section focuses on the historical discovery and clinical use of plant-derived anticancer alkaloids that have been FDA approved or that are undergoing clinical trials, their cytotoxicity and mechanism of anticancer activity.

1.2.1 Vinca Alkaloids

Vinca alkaloids were first discovered in the 1950s by the Canadian scientists, Robert Noble and Charles Beer. Vinca alkaloids, namely, vinblastine (VBL) and vincristine (VCR), were the first plant-derived products to be used in clinical oncology. Vinca alkaloids are a versatile group of phytochemicals isolated from *Catharanthus roseus* (Apocynaceae) and are the second-most used class of cancer drugs (Verma and Singh 2010; (Moudi et al. 2013). *C. roseus* is the source of more than 130 different terpenoid indole alkaloids, some of which exhibit pharmacological activities (Mohammad Abu Taher and Ahammed 2017). The anticancer effect of these compounds was discovered by chance during an investigation for hypoglycemic agents. The plant extracts showed minimal effect on glycemia; however, it was noted that they significantly reduced white blood cell counts, caused bone marrow depression in rats, and prolonged the life of mice bearing a transplantable lymphocytic leukemia (Prakash et al. 2013). There are four major vinca alkaloids in clinical use: vinblastine, vinorelbine, vincristine, and vindesine. These alkaloids are used for the treatment of several types of cancer including breast, lung, liver, testes, and leukemia (Table 1.1). Vinca alkaloids mediate their effect by altering microtubule dynamics during mitosis, preventing the formation of the mitotic spindle, and resulting in metaphase arrest and apoptosis (Jordan et al. 1991). Vinblastine and vincristine are naturally occurring active compounds that are present in low amounts in *C. roseus* plants. A series of semisynthetic analogues of vinblastine and vincristine with improved pharmacological properties have been developed. The first semisynthetic vinca alkaloid to enter human clinical trials was vindesine in which the C(23) acetyl group in vinblastine was changed to an amido group (Fig. 1.1) (Jordan and Wilson 2004). Vindesine is used in countries such as Britain, South Africa, and several European countries, but it is not FDA approved (Khazir et al. 2014). Vinorelbine is an FDA-approved semisynthetic derivative of vinblastine in which the bridge linking the indole ring to the piperidine nitrogen has been shortened by one carbon and water has been eliminated from the piperidine ring (Fig. 1.1). This derivative showed lower neurotoxicity when compared to its precursor and has been used in combination with various drugs for the treatment of several types of cancer (Almagro et al. 2015). Vinflunine, a dihydrofluoro derivative of vinorelbine, is the first fluorinated microtubule inhibitor. Unlike other vinca alkaloids, vinflunine binds weakly to tubulin, thus showing lower neurotoxicity and enhanced tolerance. It has not been FDA approved; however, it is being actively studied in patient clinical trials for the treatment of various solid tumors (Almagro et al. 2015; (Khazir et al. 2014). Many other vinca alkaloid derivatives are

Table 1.1 FDA-approved alkaloids

Alkaloid class	Alkaloid name	Type of cancer it is effective against	Mechanism of action	References
Vinca alkaloids	Vincristine (VCR)	Acute leukemia Rhabdomyosarcoma Neuroblastoma Wilm's tumor Hodgkin's disease	Destabilize microtubules by binding to tubulin Inhibit angiogenesis	Moudi et al. (2013)
	Vinblastine (VBL)	Testicular carcinoma Hodgkin and non-Hodgkin lymphomas Breast cancer Kaposi sarcoma		
	Vinorelbine (VRL)	Breast cancer Osteosarcoma Advanced lung cancer		
Taxanes	Taxol/paclitaxel	Ovarian cancer Advanced breast cancer Non-small cell lung cancer	Stabilize microtubules in their polymerized form leading to cell death Inhibit B-cell leukemia2 (Bcl-2)	Barbuti and Chen (2015), Wink (2015) and Seca and Pinto (2018)
	Docetaxel	Breast cancer Prostate cancer Gastric cancer Head and neck cancer Non-small cell lung cancer		
	Cabazitaxel	Hormone-refractory metastatic prostate cancer		

(continued)

Table 1.1 (continued)

Alkaloid class	Alkaloid name	Type of cancer it is effective against	Mechanism of action	References
Camptothecin	Topotecan	Ovarian Small cell lung cancers	Inhibits type I DNA topoisomerase preventing DNA re-ligation during replication	Karthik Mohan (2012)
	Irinotecan	Colorectal cancers		
Homoharringtonine	Omacetaxine mepesuccinate	Chronic myelogenous leukemia	Inhibits protein synthesis by acting on ribosomes of cancer cells	Kantarjian et al. (2013) and Isah (2016)
Tetrahydroisoquinoline	Trabectedine (ET-743)	Soft tissue sarcoma Hematological malignancies Solid tumors	Decreases TAM to modulate TME (i.e., limits numbers of macrophage products promoting tumor growth) Displaces oncogenic transcription factors from their target promoter with high specificity	D'Incalci et al. (2014) and Dybdal-Hargreaves et al. (2015)
	Eribulin mesylate (eribulin) analogue of halichondrin B (E7389)	Metastatic breast cancer Soft tissue carcinoma Breast cancer Ovarian cancer Endometrial cancer Non-small cell lung cancer Prostate cancer	Depolymerizes microtubules	
Purine alkaloids	Cytarabine	Acute myelocytic leukemia Lymphocytic leukemia Meningeal leukemia Blast crisis phase of chronic myelogenous leukemia	Inhibit DNA polymerases	Matthews (2017)

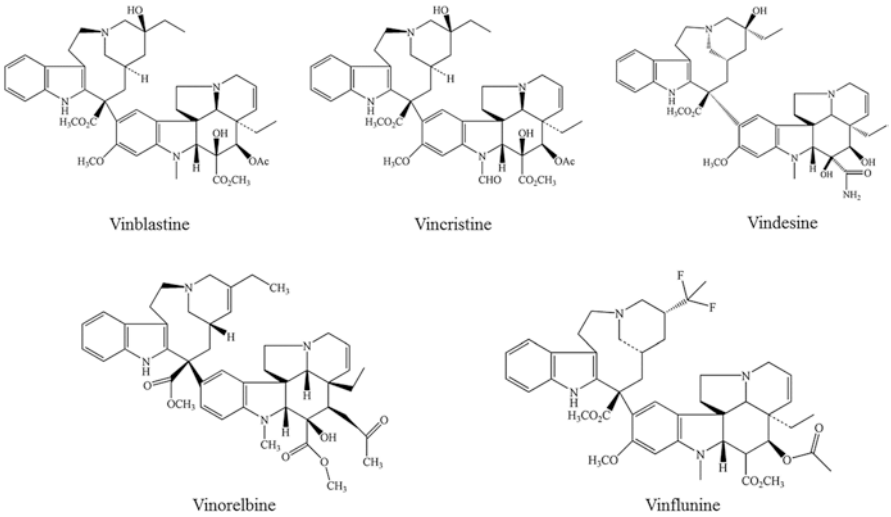

Fig. 1.1 Chemical structures of clinically used vinca alkaloids

being studied for potential use as anticancer drugs including anhydrovinblastine that has been modified from vinblastine by a 3′4′ double bond in the catharanthine moiety (Butler 2008) and is currently in phase I clinical trials. Vinblastine has also been modified by adding amino acid derivatives to facilitate its transport into cells. Vinglycinate and vintripol are examples of such derivatives that showed interesting pharmacological properties; however, they showed toxicity and no marked benefits in clinical evaluation (Khazir et al. 2014). In addition to designing various derivatives, the possibility of vinca alkaloid nanoparticle encapsulation was also tested. In 2012, the FDA approved the use of liposomal formulation of vincristine for the treatment of adult acute lymphocytic leukemia (Douer 2016). This formulation enhances the efficacy and reduces toxicity of vincristine by enabling it to circulate longer in the blood, accumulate in the tumor, and be released over an extended period of time at the tumor site (Shah et al. 2013).

1.2.2 Taxanes

The discovery of paclitaxel was the result of collaboration between the NCI and the US Department of Agriculture on a plant-screening program to identify naturally occurring compounds with anticancer activity. In 1967, Mansukh Wani and Monroe Wall isolated and identified the active ingredient from the bark of the Western yew tree *Taxus brevifolia*, named it taxol, and published its structure in 1971 (Weaver 2014). The pharmaceutical company, Bristol-Myers Squibb, then changed the name to paclitaxel. Paclitaxel entered clinical trials 22 years after its discovery in 1984. Several clinical trials were delayed due to the shortage of taxol. Paclitaxel is present in small amounts in the slow-growing *T. brevifolia*, and its extraction is a

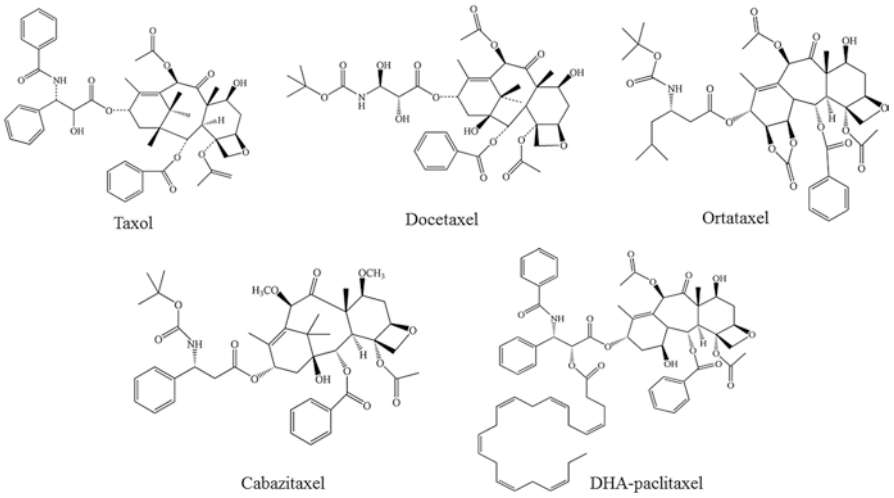

Fig. 1.2 Chemical structures of clinically used taxanes

complicated and expensive process. In addition, bark collection was restricted because the Western yew was an endangered species. Therefore, this method was not feasible to provide sufficient amounts of paclitaxel to meet the market demand. Total synthesis of the compound was established; however, it was inefficient for providing large quantities of paclitaxel (Salim et al. 2008). As the demand for paclitaxel increased, efforts were made by the government and pharmaceutical companies to increase the availability and find alternative sources of production. This led to the production of paclitaxel mimics (Fig. 1.2) including docetaxel, a semisynthetic form of paclitaxel, synthesized from 10-deacetylbaccatin III, which is isolated in large amounts from the needles of the renewable yew tree *Taxus baccata* (Salim et al. 2008; Fridlender et al. 2015). Docetaxel exhibits longer half-life, longer intracellular retention, and more rapid cellular uptake than paclitaxel (Seca and Pinto 2018). Cabazitaxel is another semisynthetic taxane with higher lipophilicity in comparison to doctaxel, which increases its intracellular accumulation, thus enhancing its cytotoxicity and effectiveness in paclitaxel-resistant patients (Seca and Pinto 2018). Bristol-Myers Squibb has also synthesized paclitaxel using plant cell cultures (Amin et al. 2009; Fridlender et al. 2015). Paclitaxel, docetaxel, and cabazitaxel are FDA approved for the treatment of various cancer types (Table 1.1).

Paclitaxel and docetaxel share the same mechanism of action; however, docetaxel has been shown to be more potent in terms of potential patient toxicity possibly due to its more rapid intracellular uptake and is co-administered with dexamethasone to prevent progressive, often disabling, fluid retention in the peripheries, lungs, and abdomen. Unlike vinca alkaloids, taxanes promote the assembly of microtubules and inhibit their depolymerization by binding specifically to the N-terminal 31 amino acids of the beta-tubulin subunit in microtubules rather than to tubulin dimers resulting in cell cycle arrest at the G2/M-phase and apoptosis (Zhang et al. 2014;

Kampan et al. 2015). Paclitaxel has been also shown to mediate its effect by inducing reactive oxygen species and activating multiple signal-transduction pathways associated with pro-apoptotic signaling including c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase, nuclear factor kappa B (NF-KB), and Janus kinase-(JAK-)signal transducer and activator of transcription factor (STAT) pathway. The induction of mitogen-activated protein kinase (MAPK) pathway, for example, results in dephosphorylation of the pro-apoptotic proteins Bad and Bax, phosphorylation of Bcl2, and induction of apoptosis (Kampan et al. 2015; Fridlender et al. 2015). The therapeutic efficacy of paclitaxel is starting to be limited due to the development of multidrug resistance (MDR), the mechanism of which is not fully understood and has been attributed to upregulation of P-glycoprotein (P-gp), alteration in tubulin dynamics, mutations in tubulin gene, changes in signaling pathways, and reduced function of significant apoptotic proteins (such as Bcl-2 and p53) (Barbuti and Chen 2015; Kampan et al. 2015). Paclitaxel is hydrophobic and had to be administrated to patients in a solution containing ethanol and polyethoxylated castor oil (Cremophor EL, CrEL) to enhance its delivery (Seca and Pinto 2018). However, the use of CrEL as drug vehicle has been associated with hypersensitivity reactions and neurotoxicity in patients (Barbuti and Chen 2015). Another issue is the rapid and extensive binding of paclitaxel to plasma proteins and its limited ability to diffuse across the blood brain barrier (Khazir et al. 2014; Kampan et al. 2015). In order to decrease the toxicity, enhance delivery and distribution, and improve efficacy and ease of administration, new paclitaxel formulations have been explored. Abraxane®, also named *nab*-paclitaxel, is an FDA-approved albumin-bound nanoparticle formulation of paclitaxel. It is soluble in saline, thus eliminating the need for the solubilizing agent Cremophor EL responsible for the hypersensitivity reactions. This formulation allows for higher intratumoral drug concentration driven by the ability of albumin to bind to its receptor, glycoprotein gp60 receptor, thus activating caveolin-1 and inducing caveolin formation, which allows nab-paclitaxel to migrate across the endothelial cell membrane into the interstitial space. In addition, Abraxane® can be administered at higher doses than paclitaxel allowing for improved efficacy (Barbuti and Chen 2015; Kampan et al. 2015). Paclitaxel poliglumex (PPX), also known as Xyotax, is a conjugate of paclitaxel and poly-L-glutamic acid developed to enhance the therapeutic index of paclitaxel (Yared and Tkaczuk 2012; Shah et al. 2013). Paclitaxel poliglumex takes advantage of the enhanced permeability of tumor vasculature and lack of lymphatic drainage to accumulate within tumor tissue, thus allowing for direct delivery to the intratumoral microenvironment and prolonged exposure to the active drug while minimizing systemic toxicities (Shah et al. 2013; Kampan et al. 2015). DHA-paclitaxel, also known as Taxoprexin, is another taxol analogue made by linking paclitaxel to the naturally occurring omega-3 fatty acid, docosahexaenoic acid (DHA). DHA is easily taken up by tumor cells, hence increasing intratumoral concentration of paclitaxel (Shah et al. 2013; Khazir et al. 2014). In addition to the abovementioned formulations, several analogues of paclitaxel and taxane derivatives are currently undergoing clinical trials including larotaxel, milataxel, ortataxel, albumin-bound docetaxel, and paclitaxel microspheres (Iqbal et al. 2017).

1.2.3 Camptothecin

Camptothecin is a quinoline alkaloid isolated from the Chinese ornamental tree, *Camptotheca acuminata.* Camptothecin is a type I DNA topoisomerase inhibitor, a critical enzyme in DNA replication and transcription (Khazir et al. 2014; Iqbal et al. 2017). Camptothecin entered clinical trials in the 1970s and was then withdrawn because of its low aqueous solubility and reports of severe bladder toxicity (Prakash et al. 2013). To overcome these limitations, several camptothecin analogues (Fig. 1.3) were synthesized and approved for clinical use including the water-soluble derivatives topotecan and irinotecan (Table 1.1). In addition to these, several analogues are in early stages of clinical trials for the treatment of various types of cancer. These include karenitecin, diflomotecan, gimatecan, elomotecan, and rubitecan. Conjugated camptothecin analogues have also been developed to enhance delivery and increase the exposure of tumor cells to camptothecin while minimizing side effects. One example is linking camptothecin to copolymers such as XMT-1001 in which camptothecin is attached to poly(1-hydroxy methyl ethylene hydroxy methyl formyl) and CRLX-101 in which camptothecin is conjugated to a linear, cyclodextrin-based polymer. These conjugated analogues are in phase I/II clinical trials for the treatment of lung cancer and solid tumors. Nanoparticle encapsulation is also being investigated to optimize delivery and improve safety of camptothecin (Khazir et al. 2014; Iqbal et al. 2017). Irinotecan has been encapsulated in a liposome formulation and is currently in phase I clinical trials for treatment of advanced cancers (https://clinicaltrials.gov/ct2/results?cond=Cancer&term=CPT11+and+liposome&cntry=&state=&city=&dist=).

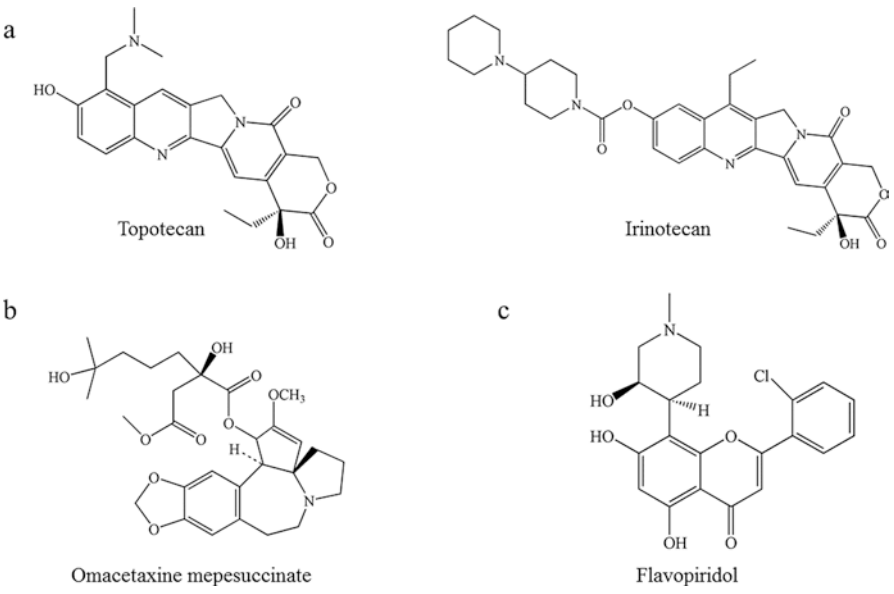

Fig. 1.3 Chemical structures of representative plant-derived alkaloids belonging to the (**a**) camptothecins, (**b**) homoharringtonine, and (**c**) rohitukine

1.2.4 Cephalotaxus

Cephalotaxus alkaloids are a group of phytochemicals originally isolated from the Chinese tree *Cephalotaxus harringtonia* (Cephalotaxaceae) (Prakash et al. 2013). Cephalotaxine itself does not display any biological activity; however, the fractionation of *Cephalotaxus* extracts resulted in the isolation of a series of esters of cephalotaxine with anticancer activity including harringtonine, isoharringtonine, homoharringtonine (HHT), and doxyharringtonine (Quintas-Cardama et al. 2009). They function by inhibiting protein synthesis through targeting initiation of protein synthesis, allowing release of nascent peptide, and polyribosome degradation (Iqbal et al. 2017). Homoharringtonine was considered one of the most effective treatments for chronic myeloid leukemia after failure of interferon-alpha therapy; however, the introduction of the tyrosine kinase inhibitor imatinib mesylate halted its clinical development. In addition, difficult production, toxicity profile of the original dose schedules, and the requirement of large quantities of bulk of cephalotaxus trees for production hampered HHT development (Kantarjian et al. 2013). Omacetaxine mepesuccinate (Fig. 1.3) is a semisynthetic purified HHT compound with 99.7% purity. It is synthesized by direct esterification of cephalotaxine extracted from dry leaves of *Cephalotaxus*, and not from the bark, which reduces the amount of *Cephalotaxus* required for extraction. Omacetaxine inhibits protein translation by preventing the initial elongation step of protein synthesis where it interacts with the ribosomal A-site and prevents the correct positioning of amino acid side chains of incoming aminoacyl-tRNAs. Omacetaxine mepesuccinate showed positive results in patients who have failed imatinib therapy, which established HHT for the second time as a valuable option in the management of CML. Omacetaxine mepesuccinate was approved by FDA in 2012 for the treatment of adult patients with chronic or accelerated phase CML after failure of two or more tyrosine kinase inhibitors. Homoharringtonine-omacetaxine probably holds the record for the longest time of development of an anticancer agent until FDA approval, almost 40 years (Quintas-Cardama et al. 2009; Kantarjian et al. 2013; Khazir et al. 2014; Iqbal et al. 2017).

1.3 Marine-Derived Alkaloids

Covering more than 70% of the earth's surface, the ocean offers a biologically rich ecosystem conferring the largest reservoir of taxonomic variety and wide chemical diversity of secondary metabolites (Huawei Zhang et al. 2017). This massive diversity of secondary metabolites helps marine organisms to withstand extreme conditions of temperature and pressure and consequently offers abundant sources of drugs that could be potential candidates for the treatment of various diseases (Romano et al. 2017). In fact, screening for marine biochemical biodiversity has just began; it is estimated that only 18% of the marine natural products have been discovered so far compared to products of terrestrial origin (Ruiz-Torres et al. 2017); thus, marine organisms represent a promising source of bioactive molecules.

However, in the history of medicine, reference to marine-based drugs is scarce, and a couple of ointments, concoctions, and cataplasms of algae and marine muds have been used for treating endless diseases, especially those involved in traditional Chinese and Japanese medicine (Hayasaka et al. 2012). For example, kainic acid was the first product of marine origin to be commercialized and used as an insecticide and anthelmintic; it was obtained from extracts of the seaweed *Digenea simplex* in 1900 (Colazingari 2013). Marine pharmacology was a new discipline that emerged in the 1950s that aimed to explore drugs and potential pharmaceuticals from sponges and marine microorganisms (Villa and Gerwick 2010). To date, only eight drugs isolated from marine organisms have been FDA approved for the treatment of various diseases. Only three of these eight compounds belong to the alkaloids family and have been approved for use as pharmaceutical drugs in cancer treatment (Table 1.1); on the other hand, two marine-derived alkaloids are in clinical trials (Table 1.2). This section focuses on the historical discovery and clinical use of marine-derived anticancer alkaloids that are undergoing clinical trials or are FDA approved, their cytotoxicity and mechanism of action.

1.3.1 Trabectedin (ET-743)

Trabectedin, ET-743, is a semisynthetic tetrahydroisoquinoline alkaloid that was initially extracted from the Caribbean tunicate *Ecteinascidia turbinata* and was currently prepared by chemical synthesis (Ruiz-Torres et al. 2017). It is the first marine-derived antineoplastic agent approved in Europe for the treatment of patients with advanced soft sarcoma and in combination with pegylated liposomal doxorubicin for the treatment of patients with relapsed platinum-sensitive ovarian cancer (D'Incalci et al. 2014). Its clinical activity is currently being evaluated in phase II/III on patients with advanced breast and hormone refractory prostate cancers. PharmaMar, a Spanish company, was first licensed to develop ET-743 for a large-scale production to provide enough materials for clinical trials. Their chemists performed an extremely elegant semisynthesis reaction by fermenting a marine-derived *Pseudomonas fluorescens* metabolite, safracin B, which led to a cGMP grade ET-743 from a 21-step synthetic process. Ultimately, the production scheme of ET-743 was licensed to Johnson and Johnson in 2001 under the brand name Yondelis, with the generic name of trabectedin (Newman and Cragg 2004). Trabectedin or ET-743 is a novel antitumor agent that has a broad spectrum of activity at pico- and nanomolar concentrations; the mechanism by which it exerts its activity has not been completely elucidated (Zelek et al. 2006). What is known so far is that ET-743 is composed of three tetrahydroisoquinoline rings containing a central carbinolamine moiety (Fig. 1.4) (Le et al. 2015). In contrast to traditional alkylated agents that bind to guanine at N7 or O6 positions, the carbinolamine moiety enables ET-743 to covalently bind to the N2 amino group of guanines in the minor groove of DNA, and through van der Waals interactions, it results in bending of the DNA toward the major groove. This allows the DNA strands to cross-link in a way that seems unique for this molecule, thus creating DNA double-strand breaks

Table 1.2 Plant- and marine-derived alkaloids in clinical trials

Alkaloid class	Alkaloid name	Type of cancer it is effective against	Clinical status	Mechanism of action	References
Taxane derivatives	Paclitaxel poliglumex	Breast Lung cancer Colorectal cancer Ovarian cancer	Phase I/II	Stabilize tubulin, resulting in the inhibition of microtubule depolymerization	Ulukan and Swaan (2002), Jones et al. (2008), Diéras V (2008) and Spigel and Greco (2008)
	DHA-paclitaxel	Non-small cell lung cancer	Phase III		
	Larotaxel	Breast cancer Urothelial tract or bladder cancer	Phase II/III		
	Ortataxel	Non-small cell lung cancer Advanced kidney cancer Refractory non-Hodgkin's lymphoma	Phase II		
Vinca alkaloid derivatives	Vinflunine	Metastatic breast cancer Non-small cell lung cancer Second-line transitional cell carcinoma of the urothelium (TCCU)	Phase I/II Phase II/III	Destabilize microtubules and bind to tubulin heterodimers	Moudi et al. (2013), Khazir et al. (2014), Reddy et al. (2007) and Butler (2008)
	Vindesine	Lung cancer Lymphoma Acute lymphocytic leukemia	Phase II/III		
	Vintafolide	Ovarian cancer Endometrial cancer	Phase II		
	Anhydrovinblastine	Advanced recurrent solid tumors	Phase I		

(continued)

Table 1.2 (continued)

Alkaloid class	Alkaloid name	Type of cancer it is effective against	Clinical status	Mechanism of action	References
Camptothecin derivatives	Rubitecan	Breast cancer Ovarian cancer Lung cancer Prostate cancer	Phase II	Inhibit type I DNA topoisomerase	Shah et al. (2013) and Quesada (2006)
	Karenitecin	Lung cancer Malignant melanoma Brain tumors Ovarian cancer	Phase II/III		
Rohitukine	Flavopiridol	Leukemias Lymphomas Solid tumors	Phase III	Targets cyclin-dependent kinases Downregulates Mcl-1 and other antiapoptotic proteins Induces mitochondrial permeability changes	Newcomb (2004)
Tetrahydroisoquinoline	Lurbinectedin (PM01183)	Relapsed ovarian cancer Breast cancer Pancreatic cancer Hematological tumors Small cell lung cancer	Phase I/II	Binds covalently to minor groove of DNA leading to double-strand break Removes DNA damage by nucleotide excision repair	J. F. M. Leal et al. (2010)
	Zalypsis (PM00104)	Uterine cervical cancer Endometrial cancer Ewing's sarcoma Primitive neuroectodermal tumor Multiple myeloma	Phase II	Binds to DNA and cause DNA double-strand breaks) Arrests cell cycle at S phase Induces apoptosis	Petek and Jones (2014)
Purine alkaloids	Elacytarabine (CP-4055)	Solid tumors (melanoma, ovarian, and lung cancer) Hematological malignancies Patients with refractory AML	Phase I/II	Inhibit DNA polymerases	O'Brien et al. (2012)

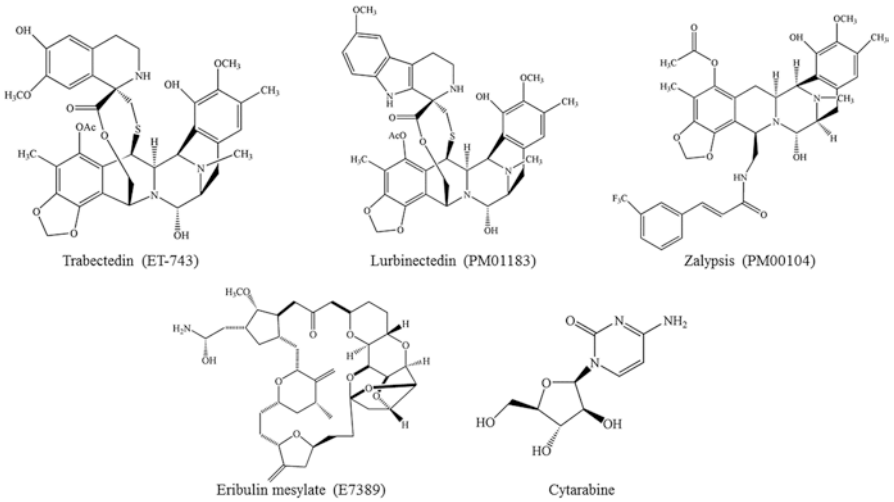

Fig. 1.4 Chemical structures of clinically used marine-derived anticancer alkaloids

(D'Incalci and Galmarini 2010). Furthermore, ET-743 interferes with DNA-binding agents such as transcription factors and DNA repair proteins altering their normal functionality and eventually leading to DNA damage, cell arrest, and cell death (Fayette et al. 2006). At the cellular level, ET-743 effectively blocks the transcription of genes that have been already activated such as HSP-70 and MDR1. It also affects promoters regulated by transcription factors that bind to the major groove such as Sp1. Of interest, ET-743 induces rapid degradation of transcribing RNA polymerase II in cells with normal transcription-coupled nucleotide excision repair, thus modulating transcription regulators (Fayette et al. 2006). It is worth mentioning that at low concentrations in vitro, ET-743 modulates cytokines and chemokines at the transcriptional level. Exceptionally, not only does it regulate tumor growth by affecting the cells directly, but it also plays a role at the tumor microenvironment level where it inhibits the production of pro-inflammatory mediators CCl2 (monocyte recruiter at tumor sites) and interleukin-6 (growth factor for several tumors) by monocytes and macrophages, thus inhibiting tumor growth and progression (D'Incalci and Galmarini 2010). The unique structure of this compound makes it a useful candidate for elucidating the complex mechanisms related to gene transcription regulation and DNA repair.

1.3.2 Lurbinectedin (PM01183)

PM01183 is a synthetic alkaloid structurally related to ecteinascidins that is in phase I clinical development for the treatment of solid tumors (Vidal et al. 2012). Like ET-743, PM01183 has a pentacyclic skeleton composed of two fused tetrahydroisoquinoline rings (subunits A and B) that recognize DNA and bind to it. An additional

third ring (subunit C) in PM01183 makes it a tetrahydro-β-carboline rather than a traditional tetrahydroisoquinoline (Fig. 1.4) (Leal et al. 2010) When it binds to DNA, ring C protrudes from the DNA minor groove, thus causing DNA double-strand breaks and interfering with normal protein machinery at the mRNA level. The accumulated DNA damage delays cell cycle progression at the S-phase and ultimately triggers apoptotic cell death (Casado et al. 2008). Preclinical studies have demonstrated that PM01183 has potent antitumor activities against a wide array of solid and liquid tumors in vitro and in vivo with manageable toxicology profile (Vidal et al. 2012). This has accelerated the clinical trial transition to determine the minimum tolerable doses using various administration methods. Up until 2017, 19 clinical trials with PM01183 have been conducted in patients with various solid and hematological malignancies either alone or in combination with other drugs. Monotreatment and combinations have shown predictable and manageable safety profiles with acceptable tolerance among patients having ovarian, small cell lung metastatic breast, or endometrial cancers. Currently, plans to incorporate PM01183 in pediatric cancers like Ewing sarcoma and neuroblastoma are being designed for future application (https://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/oncolo gicdrugsadvisorycommittee/ucm563559.pdf).

1.3.3 Zalypsis (PM00104)

PM00104 (Zalypsis) is a synthetic tetrahydroisoquinoline alkaloid that mimics natural compounds derived from mollusks. The drug is a novel chemical entity related to jorumycin (isolated from the mantle and mucus of the Pacific nudibranch mollusk *Jorunna funebris*) and ecteinascidins (isolated from the Caribbean tunicate *Ecteinascidia turbinata*) (Fontana et al. 2000; (Ciavatta et al. 2017). The high potent cytotoxic activity of jorumycin at nanomolar concentrations with the clinical success of ET-743 led to intensive chemical modifications of jorumycin's structure and eventually resulted in the development of PM00104. Similar to ET-743, PM00104 has the same cyclic structure (Fig. 1.4) and binds to DNA minor grooves via using its reactive carbinolamine group. However, unlike ET-743, PM00104 covalently binds to guanine residues with preferential affinity to G-rich codons (Leal et al. 2009). In fact, PM00104 differs slightly in one of its rings, and this alteration in structure has conferred its DNA-binding properties and nucleotide excision repair dependencies. Yet, the cytotoxic mechanism of action is similar for both drugs (Guirouilh-Barbat et al. 2009). The interaction between PM00104 and DNA leads to creation of DNA adducts which in turn inhibit early phases of transcription, causes DNA double-strand breaks, and arrests the cell cycle at the S-phase, thus driving the cell toward apoptosis. PM00104 has shown versatile anticancer potential activity in vitro and in vivo in a wide variety of solid and hematological tumors. This has led to moving PM00104 into clinical trial testing. Currently, PM00104 is in phase II clinical trials to treat Ewing sarcoma, urothelial carcinoma, multiple myeloma, and endometrial and cervical cancer (Petek and Jones, 2014).

1.3.4 Eribulin Mesylate (E7389)

Eribulin mesylate (E7389) is a synthetic macrocyclic ketone analogue of halichondrin B that acts as a non-taxane microtubule dynamics inhibitor (Dydal-Hargreaves et al. 2015). The history of this eribulin began when halichondrin B, a marine-derived alkaloid, got extracted from the marine sponge *Halichondria okadai* in 1986 (Hirata and Uemura 1986). Halichondrin B was found to have extraordinary cytotoxic activities in vitro and in murine models of solid tumors and leukemia in vivo (Dydal-Hargreaves et al. 2015). It acted through binding to a region of tubulin referred to as the "vinca domain," thus inhibiting tubulin polymerization and tubulin-dependent guanosine triphosphate (GTP) hydrolysis (Ludueña et al. 1993; Dydal-Hargreaves et al. 2015). The structural complexity made halichondrin B a mechanistically interesting molecule, but its low yield from natural resources limited its potential for clinical development. A breakthrough occurred in 1992 when chemists at Kishi laboratory (Cambridge, Massachusetts) succeeded in synthesizing over 180 analogues of this compound, one of which, eribulin, was produced with a 63-step chemical synthesis process (Yu et al. 2013). Preclinical studies showed that eribulin possessed both in vivo and in vitro antitumor activities against a wide array of solid malignancies, particularly breast and ovarian cancer. Sub- to low-nanomolar levels of eribulin inhibited cancer cell proliferation by disrupting mitotic spindles, blocking cell cycle at the G2/M-phase, and initiating apoptosis (Dydal-Hargreaves et al. 2015). In-depth studies have confirmed that eribulin inhibits microtubule dynamics through a novel mechanism that is distinct from all other tubulin-binding agents. It binds irreversibly to a unique site on tubulin resulting in the repression of microtubule polymerization without affecting the depolymerization process and thus sequestering tubulin into a nonfunctional aggregate (Dydal-Hargreaves et al. 2015). More so, mechanistic studies revealed that eribulin decreased the expression of genes associated with angiogenesis, including genes involved in Wnt, Notch, ephrin, and VEGF signaling pathways (Funahashi et al. 2014). It also triggered the reversal of epithelial-to-mesenchymal transition (EMT) in triple-negative breast cancer cell lines (Yoshida et al. 2014). In fact, drugs with the ability to inhibit or reverse EMT are highly desired as they inhibit the progression of tumors and their metastasis. In 2010 in the United States and in 2011 in Europe and Japan, the antimitotic agent eribulin received FDA approval as a third-line therapy for the treatment of liposarcoma and metastatic breast cancer patients who have been pretreated with a taxane or an anthracycline (Donoghue et al. 2012). Phase III clinical trials have demonstrated that eribulin is also effective in women with human epidermal growth factor 2 (HER2)-negative and triple-negative breast cancer (Dydal-Hargreaves et al. 2015). In addition, the California Cancer Consortium completed a phase I trial of eribulin to determine its pharmacodynamics and pharmacokinetics when administered by bolus injection. So far, results included partial responses of eribulin to several tumors with manageable toxicities, it induced morphological changes in the microtubules of peripheral blood monocytes, and its levels were maintained above the levels required for activity in the plasma for 72 h; however, when administered intravenously, it exhibited a tri-exponential elimination from

plasma of patients (Morgan et al. 2015). Recently phase I clinical trials in which eribulin and gemcitabine were combined showed manageable toxicity in patients diagnosed with advanced tumors (Lheureux et al. 2015). In addition, another phase I clinical trial of combining eribulin with cisplatin against advanced tumors resulted in well-tolerated doses with preliminary anticancer activity (Koczywas et al. 2014). Collectively, considering all the parameters, eribulin is a distinct microtubule inhibitor agent with great potential against cancer, and thus large numbers of clinical trials are still being conducted to decipher its antitumoral potential (https://clinicaltrials.gov/ct2/results?cond=cancer&term=eribulin&cntry=&state=&city=&dist=).

1.3.5 Cytarabine

The synthetic analogue of a C-nucleoside pyrimidine nucleoside cytarabine (arabinosyl cytosine or cytosine arabinoside, Ara-C) was developed from spongothymidine, a nucleoside originally isolated from the Caribbean sponge *Tectitethya crypta* (Mayer et al. 2010). Cytarabine is taken up by cells via nucleoside transporters, mainly the human equilibrative nucleoside transporter 1 (hENT1), and its primary activity stems from its intracellular conversion into cytosine arabinoside triphosphate by several deoxycytidine kinases (Li et al. 2017). Once converted, cytarabine becomes a cytotoxic compound that inhibits DNA polymerases and DNA synthesis via competition with deoxycytidine triphosphate to get incorporated into the DNA. Consequently, it causes cell death by interfering with DNA and RNA synthesis. It is also an S-phase-specific antimetabolite cytotoxic agent and may block the progression of cells from the G1-phase to the S-phase (Galmarini et al. 2002; Wang et al. 2018). Notably, cytarabine was among the first marine-derived compounds to receive FDA approval in 1969 and is still in use today to treat certain classes of leukemia affecting white blood cells mainly acute lymphocytic leukemia, acute myeloid leukemia (AML), and blast crisis phase of chronic myelogenous leukemia and meningeal leukemia (Krug et al. 2011). It is currently commercialized as either the conventional cytarabine under the trade name Cytosar-U1 or as its liposomal formulations under the trade name Depocyt1. Depocyt1 is basically a cytarabine encapsulated into multivesicular nonconcentric lipid-based vesicles that allow the sustainable release of cytarabine. It maintains cytarabine therapeutic drug concentrations in the cerebrospinal fluid for prolonged periods, and thus it has more potent potential to kill tumor cells in the meningeal leukemia (Glantz et al. 1999; (Chhikara and Parang 2010). Depocyt1 is prescribed intrathecally for the treatment of lymphomatous meningitis, whereas commercial presentation of cytarabine is designed to be administered intravenously or subcutaneously. Cytosar-U1 and Depocyt1 are marketed by Bedford Laboratories (http://www.bedfordlabs.com/) and Enzon P Pharmaceuticals (http://www.enzon.com/), respectively. Multiple clinical trials showed that patients with AML demonstrated complete remission rates of 50–60% and overall enhanced survival rates of 30–40% after the administration of cytarabine (Hagop 2016). A novel liposomal formulation of cytarabine in combination with daunorubicin (packed ratio 5:1 molar) is now in phase II clinical trials. It is

known as CPX-351, and it has shown synergistic efficacy when compared with the conventional 7 + 3 treatment of AML (100 mg/m^2 cytarabine and 60 mg/m^2 daunorubicin) (Saygin and Carraway 2017). In fact, high doses of cytarabine had the highest antileukemic efficacy among all used therapies against AML, but the mechanism of high dose of cytarabine therapy remains uncertain and needs further investigation. Although it has been the standard chemotherapy for hematological malignancies, cytarabine resistance has been emerging rapidly especially in patients with reduced expression of hENT1. Therefore, elacytarabine (CP-4055), a lipophilic 5′-elaidic acid ester of cytarabine, has been considered lately for clinical trials. Elacytarabine enters cells independently of hENT1 and has the same mechanism of action as cytarabine. Phase I clinical trials showed that elacytarabine had manageable and transient toxicities in patients with refractory AML and a pharmacokinetic profile that allowed extended exposure of cytarabine and its active metabolites to leukemic cells (O'Brien et al. 2012).

1.4 Modulators of MDR and Chemopreventive Alkaloids

Multidrug resistance (MDR) is a major obstacle in the development of chemotherapeutic agents. Various alkaloids can overcome MDR through several mechanisms including interaction with ABC-transporters and induction of apoptosis, thus enhancing cytotoxicity of chemotherapeutics. Here we provide examples of alkaloids that can serve as promising model compounds for overcoming MDR and that have shown chemopreventive effects.

1.4.1 Cinchona Alkaloids

Cinchona alkaloids are isolated from the bark of several species of cinchona trees. In the early seventeenth century, the antimalarial property of cinchona bark, mainly its active compound quinine, was discovered (Ferreira Júnior et al. 2012). Quinine and cinchonine have the potential of reversing MDR in cancer patients. Phase I/II clinical trials assessing the effect of quinine demonstrated that it could be used safely with a combination of anticancer agents, for instance, mitoxantrone, cytarabine, cyclophosphamide, or paclitaxel, enhancing the treatment of clinically resistant acute leukemias, breast cancer, or non-Hodgkin's lymphomas (Solary et al. 2003; Taylor et al. 1997; Miller et al. 1998). Unfortunately, the use of quinine in phase III clinical trials on patients with acute leukemia showed modest success (Solary et al. 2003). Other preclinical and phase I studies have shown that cinchonine is a more potent and effective anti-MDR agent in comparison with quinine (Ferreira Júnior et al. 2012). Additionally, cinchonine showed MDR-reversing activity in patients with malignant lymphoid disease in combination with cyclophosphamide, doxorubicin, methylprednisolone, and vinblastine (Solary et al. 2000). Lately, dimeric quinine linked by ester bond was also shown to be active in MDR and was capable of totally reversing the P-glycoprotein (P-gp)-mediated

paclitaxel resistance phenotype as well as inhibiting its transport in MCF-7/DX1 cells (Pires et al. 2009).

1.4.2 Dofequidar Fumarate (MS-209)

Dofequidar (MS-209) is a novel, orally active, quinolone-derived inhibitor of MDR and P-glycoprotein (Toshiaki Saeki et al. 2007). In preclinical studies, dofequidar has shown a significant reversal of MDR in P-gp- and MRP-1-expressing cancer cells in vitro. In preclinical models, MS-209 was found to enhance the antitumor activity of various anticancer agents including adriamycin, vincristine, paclitaxel, and docetaxel in multidrug-resistant tumor cell lines (Naito et al. 2002). For instance, compared to adriamycin activity alone, a combination of MS-209 with adriamycin was more effective against transplanted murine tumors, multidrug-resistant murine tumors, and human tumors transplanted to nude mice (Naito and Tsuruo 1997). Phase I clinical trials conducted to assess the safety and tolerability of MS-209 in combination with docetaxel showed no significant differences in docetaxel activity with and without MS-209, which is well-tolerated with no dose-limiting toxicities. Dofequidar in a phase II clinical trial potentiated the anticancer effect of CAF (cyclophosphamide, doxorubicin, and fluorouracil) therapy in patients with advanced or recurrent breast cancer (Saeki et al. 2005).

In addition to their role in overcoming MDR, some alkaloids have chemopreventive potential. A couple of tests done on capsaicin have shown that it inhibits mutagenicity and DNA binding of some chemical carcinogens, possibly by suppressing their metabolic activation which is accompanied with a decrease in NADH oxidase activity. It also caused an alteration in the expression of tumor forming-related genes by stimulating the overexpression of p53 and/or c-myc genes (Lewinska et al. 2015). Additionally, capsaicin inhibited the growth of xenograft prostate tumors in mice. Berberine, which is isolated from the plant *Rhizoma coptidis*, exhibited potential chemopreventive properties against colon tumor formation. It controls colon tumorigenesis by inhibiting the highly expressed enzyme cyclooxygenase-2 (COX2) in colon cancer cells (Wu et al. 2012).

1.5 Clinical Toxicity of Alkaloids

The medical adverse effects and toxicities of chemotherapeutic drugs provide insights about the unique activity of each drug and its function in the human body system. The choice of the appropriate chemotherapeutic drug is based on several considerations which include the type, size, and grade of the tumor in addition to the patients' comorbidities, age, and health status at the time of chemotherapeutic administration.

The different drugs within the vinca alkaloid family have a variable toxicity profile. Vinflunine is the latest member of the vinca alkaloid, which is evaluated in multiple trials for the treatment of metastatic and advanced urothelial cancer. A

systematic review and meta-analysis to examine the toxicity profile of vinflunine showed that fatigue (40.1%), nausea (33.9%), constipation (34.1%), and alopecia (26.0%) are the most prevalent non-hematological adverse effects, whereas anemia (56.6%), neutropenia (46.0%), and thrombocytopenia (25.5%) are the most common hematological adverse effects (Brousell et al. 2018). Neurotoxicity and axonal neuropathy are most notable with vincristine treatment, a dose-dependent toxicity that is caused by interrupting microtubules within axons (Quant 2014). However, neurotoxicity is usually reversible requiring few months for recovery (Quant 2014). Nearly all patients have neurotoxicity following vincristine treatment which is manifested as paresthesia in the feet and fingertips, cramps, and weakness often occurring weeks post treatment (Quant 2014). Vincristine-induced autonomic neuropathy is commonly displayed as abdominal pain, constipation, and paralytic ileus (Quant 2014). Vinblastine, another vinca alkaloid, is associated with less neurotoxicity but results in a dose-dependent hematological toxicity; in the same token, vinorelbine has less neurotoxicity that is reversible post treatment (Quant 2014).

Paclitaxel showed many adverse effects that were manifested during clinical trials. These include myelosuppression, neurotoxicity, musculoskeletal, and dermatological adverse effects (Walker 1993). Myelosuppression, neutropenia, and/or leukopenia was considered a major dose-limiting toxicity. In fact, results from phase I and II clinical trials showed that the administration of a recommended drug dose intravenously for 24 h every 3 weeks caused neutropenia and leukopenia in 68% and 26% of patients, respectively (Walker 1993). Thrombocytopenia was not significant when compared to leukopenia and neutropenia in paclitaxel-induced myelosuppression. Paclitaxel-induced severe neurological toxicity occurred at higher doses. Initial signs of neurotoxicity are paresthesia manifested as a burning sensation in the feet tailed by a sensation loss in a stocking-and-glove distribution followed by loss of pain sensation, vibration, temperature, and reflexes (Walker 1993). Arthralgias and myalgias are dose-dependent effects that are displayed at high paclitaxel doses causing bone and joint pain (Walker 1993). Alopecia was abrupt involving facial and body hair loss and occurred in almost all patients 2 weeks post treatment (Walker 1993).

Docetaxel can cause both acute and chronic adverse effects. Myelosuppression, cardiovascular toxicity, gastrointestinal, and dermatological side effects are some of the acute toxicities of docetaxel. On the other hand, neurotoxicity is one of the chronic side effects that can persist after the completion of the drug regimen (Ho and Mackey 2014). Unlike other chemotherapeutic regiments, febrile neutropenia could develop in patients receiving chemotherapeutic regimen containing docetaxel (Ho and Mackey 2014). Febrile neutropenia usually requires hospitalization and is associated with increased risk of developing serious infections in addition to high morbidity and mortality (Ho and Mackey 2014). In terms of the cardiovascular system, patients taking docetaxel experience fluid retention manifested as swelling in their extremities, ascites, and pericardial and pleural effusions, likely due to docetaxel-induced increase of capillary permeability and fluid leakage into the tissues (Ho and Mackey 2014). Fluid retention is a dose-dependent side effect and can be decreased by co-administration of steroids or symptomatically treated by

diuretics (Ho and Mackey, 2014). Docetaxel-induced nail toxicity consists of hyperkeratosis, subungual and splinter hemorrhages, nail growth cessation, and separation of the nail from the nail bed (Ho and Mackey 2014). Nail toxicity can be slowed down using frozen gloves and socks and can be resolved 6–12 months post treatment (Ho and Mackey 2014). Long-term neurotoxicity induced by docetaxel includes motor and sensory peripheral neuropathy, which is however less severe than paclitaxel and can be manifested as tingling, numbness, and loss of reflexes (Ho and Mackey 2014).

Irinotecan and topotecan are two camptothecin analogues that show variability in their profile toxicity. Irinotecan is used in the management of gastrointestinal tumors and therefore has mainly gastrointestinal adverse effects. On the other hand, topotecan has predominantly hematological profile toxicity (Seiter 2005). Seiter compared the toxic effects of the two drugs showing that irinotecan caused diarrhea in 20–40% of patients, while neutropenia occurred in 20–40% and 70–80% in irinotecan and topotecan, respectively (Seiter 2005). Anemia and thrombocytopenia are the other hematological toxicities occurring in patients receiving topotecan in 20–40 and 2–30%, respectively (Seiter 2005).

Omacetaxine mepesuccinate is a semisynthetic homoharringtonine approved by FDA in 2012. Clinical trials that included patients with chronic myeloid leukemia showed that hematological toxicity was the most common adverse effect of omacetaxine including thrombocytopenia, anemia, and neutropenia, in addition to other toxicities such as nausea, diarrhea, fatigue, and fever (Damlaj et al. 2016). Hematological toxicities were predictable and manageable where the highest toxicity occurred in the initial three cycles following improvement during the maintenance phase (Damlaj et al. 2016). Of note, it was impossible to conclude that the adverse effects were completely related to the omacetaxine drug versus the underlying chronic myeloid leukemia disease due to the lack of a control arm (Damlaj et al. 2016).

Trabectedin's predominant dose-dependent toxicity is neutropenia and abnormal liver function, namely, an increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as bilirubin and alkaline phosphatase probably because trabectedin is cleared by hepatic metabolism (Jordan et al. 2015). Importantly, liver biopsies post phase I trial treatment did not show serious liver damage, and thus many studies showed that liver enzyme abnormality was reversible and was not associated with significant liver dysfunction (Jordan et al. 2015). Furthermore, neutropenia was the predominant manifestation of trabectedin-induced myelosuppression during clinical studies ranging between 24% and 100% (Jordan et al. 2015). Moreover, nausea and vomiting have been reported in trials, and thus trabectedin can be best described as a moderately emetogenic agent (Jordan et al. 2015). Interestingly, rhabdomyolysis, muscle breakdown, has been described as an uncommon toxicity of trabectedin treatment with no clear mechanism of this adverse effect (Jordan et al. 2015). In contrast to other cytotoxic chemotherapeutic agents, dermal toxicity, alopecia, and diarrhea are uncommon (Jordan et al. 2015).

Eribulin is a halichondrin analogue that was approved by the FDA in 2010 for cancer treatment. Multiple studies assessed the safety profile of eribulin as a single agent, and the most common dose-limiting drug toxicity was fatigue, anorexia, and

febrile neutropenia, in addition to other toxicities such alopecia, hypophosphatemia, and hypoglycemia (Preston and Trivedi 2012). Patients with underlying renal impairment experienced liver function test abnormalities and sensory neuropathy (Preston and Trivedi 2012).

Cytarabine, a pyrimidine analogue, is used to treat many types of cancer including lymphomas, leukemias, and neoplastic meningitis when used intrathecally (Quant 2014). Little neurotoxicity is manifested when cytarabine is used in conventional doses; however, higher doses can cause acute cerebellar syndrome; the underlying pathogenesis of cerebellar syndrome is still unknown even though loss of Purkinje cells in the cerebellum is known (Quant 2014). This syndrome is likely to occur in patients who have abnormalities in their renal or liver function or if they are above the age of 40. It is characterized by somnolence and encephalopathy post few days of treatment followed by cerebellar signs such as ataxia (Quant 2014). Peripheral neuropathy such as optic neuropathy, lateral rectus palsy, Guillain-Barre syndrome, and brachial plexopathy can be occasionally caused by high doses of cytarabine (Quant 2014).

1.6 Discussion

Nature has provided mankind with many invaluable benefits mainly through its chemical products that are used as medicine to cure human diseases. The discovery of natural bioactive molecules and assessing their biological activities has been a basic scientific endeavor aimed at treating the countless maladies, specifically malignancies. The most significant challenge of all times was turning these natural products into commercialized medicines and effectively using them to eradicate tumors. In fact, the cost of moving a new drug from bench to bedside has been the main limiting factor for commercializing natural products. Developing a drug currently costs more than 2500 million dollars and only 11.8% of the drugs tested in clinical trials will eventually get FDA approved according to a new study performed by the Tufts Center for the Study of Drug Development (http://csdd.tufts.edu/news/complete_story/pr_tufts_csdd_2014_cost_study). Another major challenge is the lag time needed for these molecules to be discovered, then researched for usefulness in human trials, and later marketed, a time which is estimated to be more than 10 years of thorough testing and investigation. A further complication is related to the legislation protocols to access and utilize these natural products for human trials. All these factors have hampered the pharmaceutical industry to invest in the quest for finding natural products with anticancer potential.

Notwithstanding these obstacles and difficulties, alkaloids, being the largest subunit of naturally derived secondary metabolites, have been extensively studied and shown to carry promising antitumor potential for the treatment of cancer. Since their discovery, alkaloids have shown a rich history of pharmacological activities (Iqbal et al., 2017), which promoted their translation to clinical trials. More than 10 alkaloids are currently undergoing clinical evaluation (Table 1.2), and more than 14 survived clinical trial testing and received FDA approval for the treatment of various

diseases including but not limited to cancer (Table 1.1). The majority of FDA-approved and clinically tested alkaloids are derived from plants, with few derived from marine organisms. Alkaloids serve diverse purposes in plants including storage and transport of acids, antioxidant protection, anti-predation effects, end products of metabolism, waste products, and source for energy and reserve of nitrogen. In marine organisms, the soft bodies and sedentary lifestyle of these organisms caused the evolution of mechanisms for synthesizing or extracting toxic compounds and releasing them into surrounding environments for protection. Once released to the water, these compounds become diluted rapidly; thus, they must be very potent to mediate the desired effect (Hussain 2012). The protective role of alkaloids in both plants and marine organisms underlies to some extent their therapeutic potential. The cytotoxicity of some of these compounds resulted in their limited use in human trials; however, extensive structural modifications have and are still being developed to overcome toxicity, solubility, and availability problems and enhance potency. In fact, almost all clinically used alkaloids have been synthesized and are referred to as hybrid molecules or semisynthetic (Kaur 2015). Another limitation is the slow growth and endangered status of the majority of alkaloid-yielding plants, which necessitates the adoption of alternative approaches for the supply of these molecules. The workable alternatives include the application of biotechnology in plant cell and tissue culture or fermentation technology of alkaloid-producing microbes and metabolic engineering (Isah 2016). The generation of an adequate biomass of marine-derived molecules is still a drawback for translating marine-based drugs into the market.

Unlike plant-derived molecules, the structural complexity of marine molecules limits their total chemical synthesis by economically feasible strategies. Aquaculture of the source organisms including sponges and tunicates has succeeded in providing a supply of drug products; however, this supply is still far short from what is required for marine-based drugs to enter the market. In addition, these cultures are subject to uncertainties such as destruction by ocean pollution and improper exploitation of the undersea world. One interesting strategy for overcoming this issue is to identify the true leading producers of these bioactive compounds. To do so, maximizing the search for botanical and marine diversity is a must to find new sources from newer and hitherto unexplored areas. This plan is expected to deliver a number of new or analogous molecules with high activity and less toxicity. Development of high-throughput isolation of components from a crude natural product extract using highly automated separation techniques will enable chemists to speed up the isolation, purification, and characterization processes. These modern techniques coupled with high-throughput screening systems and the combination of complementary technologies, such as genomics, proteomics, metabolomics, metagenomics, metabolic and genetic engineering, and synthetic biology, are expected to yield a much larger number of lead structures in the near future with lower investment of time and money (Demain and Vaishnav 2011).

Unlike the long-standing historical medical uses of plants, marine organisms have a shorter history for the treatment of human diseases. In fact, it was not until the middle of the twentieth century that scientists began to systematically probe the

oceans for medicines. Advancement in drug discovery research resulted in the identification of many bioactive molecules from marine organisms. Despite that, the rich marine biodiversity has so far been explored to an extremely limited extent. This diversity is most likely capable of delivering a great abundance of secondary metabolites for research use (Hussain 2012) and might exceed those derived from plants. The extensive research on plant-derived alkaloids has deciphered major mechanisms of action of these molecules including topoisomerase inhibition, mitotic arrest, and inhibition of protein synthesis. On the other hand, most marine-derived alkaloids discovered so far have been shown to mediate their effect through inhibition of DNA polymerase and induction of double-strand breaks (Demain and Vaishnav 2011). A major problem with antimitotic drugs is that they only target cells in M-phase, leaving G1- or S-phase tumor cells refractory to the drug's cytotoxic effect. These cells can then repopulate the tumor mass once the drug is cleared. In addition, the underestimation of the human tumor doubling time in comparison to cell lines and animal models was a major challenge for advancing many of these agents into human testing. The considerable clinical efficacy of taxane, an antimitotic agent, is explained by the drug retention issue, where paclitaxel has been shown to linger in the tumor cells for a week and is thus able to exert its cytotoxicity unlike other agents that have a median half-life of approximately 13 h. In addition, paclitaxel is likely to target quiescent cancer cells because of the importance of microtubule dynamic trafficking in cells not undergoing mitosis (Chan et al. 2012). The fact that very few marine-derived alkaloids act as antimitotic agents provides an advantage for these drugs over plant-derived alkaloids. Although creating DNA double-strand breaks is an effective and a powerful mechanism by marine-derived alkaloids, there are three principal underlying factors of their bioactivities that are still not fully understood. First, what is the precise molecular link between the DNA repair systems, cell response to such DNA damage, and its effects on transcription regulation? Second, what is the role of tumor stroma interactions with the DNA-damaging alkaloids? And finally, how can we explain the context-specific induction of DNA damage in different tumor types? For example, in some tumors, the DNA-damaging anticancer activity of trabectedin stems mainly from its ability to modulate the tumor microenvironment by inhibiting tumor-associated macrophages (TAMs) that display several pro-tumoral functions such as on/off switching of neo-angiogenesis, whereas, in other tumors, the high activity of trabectedin can be related to its negative regulation of common cancer hallmark genes. It has the capacity to displace the oncogenic fusion protein FUS-CHOP (an abnormal transcription factor) from its target promoter, thus inhibiting its trans-activating ability (D'Incalci et al. 2014). Further investigations on the mechanism of action of these drugs in different tumor types could lead to a better understanding of the key molecules targeted by these drugs and ultimately allow the development of personalized treatments for cancer patients.

In terms of alkaloid toxicity, research has shown that compounds belonging to the same family have almost similar clinical manifestations probably due to structural similarity. Interestingly, both plant- and marine-derived alkaloids cause gastrointestinal adverse effects (Table 1.3). Abnormal liver function is a major manifestation

Table 1.3 Common adverse effects of clinically approved alkaloids

Alkaloid	Neurotoxicity	Cardiotoxicity/ other	Gastrotoxicity	Hematology-oncology	Musculoskeletal	Dermatotoxicity	References
Paclitaxel	Peripheral neuropathy	N/A	Diarrhea Nausea Vomiting Inflammatory disease of mucous membrane	Anemia Leukopenia Neutropenia Thrombocytopenia	Arthralgia Myalgia	Alopecia	www.dynamed.com
Docetaxel	Asthenia Neuropathy	Fluid retention Vasodilation *Reproductive:* Amenorrhea	Diarrhea Nausea Vomiting Stomatitis Inflammatory disease of mucous membrane	Anemia Leukopenia Neutropenia	N/A	Alopecia Nail changes Pruritus Rash	www.dynamed.com
Vincristine sulfate	N/A	N/A	Constipation Nausea Vomiting	N/A	N/A	Alopecia	www.dynamed.com
Vinblastine	N/A	Hypertension	Constipation	N/A	Bone pain Jaw pain	Alopecia	www.dynamed.com
Vinorelbine	Asthenia Neuromyopathy	N/A	Diarrhea Nausea Vomiting	N/A	N/A	Alopecia Injection site reaction	www.dynamed.com

Vinflunine ditartrate	Asthenia	*Endocrine:* Hyponatremia Weight loss	Abdominal pain Constipation Diarrhea Appetite loss Nausea Vomiting Stomatitis	Anemia Leukopenia Neutropenia Thrombocytopenia	Arthralgia Myalgia	Alopecia Injection site reaction	www.dynamed.com
Topotecan	Asthenia Headache	*Respiratory:* Cough Dyspnea	Abdominal pain Constipation Diarrhea Nausea Vomiting Stomatitis	Anemia Neutropenia Thrombocytopenia	N/A	Alopecia Rash	www.dynamed.com
Irinotecan	Asthenia Dizziness Parasympathomimetic	*Respiratory:* Cough Dyspnea *Endocrine:* Weight loss *Hepatic:* Increased bilirubin level	Abdominal pain Constipation Diarrhea Appetite loss Nausea Vomiting	Anemia Eosinophilia Leukopenia Neutropenia Thrombocytopenia	N/A	Alopecia	www.dynamed.com
Omacetaxine		*Endocrine:* Increase uric acid	Diarrhea Nausea	Anemia Neutropenia Thrombocytopenia	N/A		www.dynamed.com

(continued)

Table 1.3 (continued)

Alkaloid	Neurotoxicity	Cardiotoxicity/ other	Gastrotoxicity	Hematology-oncology	Musculoskeletal	Dermatotoxicity	References
Trabectidin	Headache	*Endocrine:* Hypoalbuminemia *Respiratory:* Dyspnea *Hepatic:* Increased (ALK, ALT, AST)[a]	Constipation Decrease appetite Diarrhea Nausea Vomiting	Anemia Neutropenia Thrombocytopenia	Increased creatine kinase		www.dynamed.com
Eribulin	Peripheral neuropathy	*Endocrine:* Hypocalcemia Hypokalemia	Constipation Nausea	Anemia Neutropenia	N/A	Alopecia	www.dynamed.com
Cytarabine		Thrombophlebitis *Hepatic:* Decreased liver function	Anal inflammation Diarrhea Appetite loss Stomatitis Anus ulcer Mouth ulcer Nausea Vomiting	N/A	N/A	Rash	www.dynamed.com

[a]*ALK* alkaline phosphatase, *AST* aspartate Aminotransferase, *ALT* alanine aminotransferase, *N/A* not available

in marine-derived alkaloids, most likely because these compounds are cleared by hepatic metabolism (Jordan et al. 2015).

Despite their clinical manifestations, alkaloids are valuable lead compounds for drug discovery. In addition to their anticancer activity, alkaloids have been shown to act as chemopreventive agents through modulation of various pathways and gene expression. Importantly, alkaloids have been shown to modulate MDR, which is a major reason for cancer treatment failure by chemotherapy, by restoring drug sensitivity. The combination of alkaloids with various chemotherapeutic drugs has been shown to restore chemosensitivity through various mechanisms including suppression of ABC-transporters such as P-gp, MRP1, and ABCG2 and induction of apoptosis (Aung et al. 2017). The best possible drug combinations are based on the understanding of the cancer-specific context of mutated oncogenes, tumor suppressor genes, and their regulatory pathways. This suggests that alkaloids merit further investigation as anticancer and chemo-sensitizing cancer therapeutics to improve our understanding of the molecular changes in cancer cells and provide clues about how the disease can be controlled.

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2 Emerging Alkaloids Against Cancer: A Peep into Factors, Regulation, and Molecular Mechanisms

Priya Katyal and Shivani Sharma

Abstract

Chemoprevention by the use of plant-derived alkaloids has gained attention worldwide to reduce the burden of malignantly transformed neoplastic cells on overall health and well-being. These active agents follow different molecular routes to block, suppress, and prevent the metastasis of cancerous cells. Alkaloids are plant-derived secondary metabolites that contain heterocyclic nitrogen and are well exploited as immunomodulators to prevent mutagenesis and inflammation along with antibacterial and anticancerous agents. Their mode of action includes alteration of enzymes or certain transcription factors involved at various stages of cancer progression or to deal with resistance of cancerous cells to various drugs. These alkaloids also enhance the availability of anticancerous drugs at various target sites. Alkaloids play a crucial role in regulating cell cycle by increasing cancerous cell death, angiogenesis, autophagy, alteration of mitochondrial membrane, and COX-2, Bcl-2 and Bcl-XL regulation for inducing caspase activity. Alkaloids also act as inhibitors of telomerase, STAT-3, and PI3K/Akt signaling events. Further, research is required for validation of exact anticancer mechanism of novel alkaloids for their use in combination therapy. Effective drug delivery system with clinical anticancer trials can further strengthen the research in this area.

Keywords

Alkaloids · Apoptosis · Cancer · Metastasis · Molecular mechanism

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In today's era there is an enormous increase in reported cases of cancer and it has been identified as a critical health issue causing huge economic losses in all the regions (Siegel et al. 2017). There are different factors (Fig. 2.1) contributing to cancer progression such as genetic (5–10%) and climatic factors including manner of living (90–95%). Some important ones include food habits, drug addiction, environmental mutagens, and individual physical/mental state (Parsa 2012; Katzke et al. 2015). Only a very few nutraceutical agents have been tested in human intervention trials for their chemopreventive activities (Scott et al. 2009; Hosseini and Ghorbani 2015).

A better understanding of potential risk factors in patients that trigger initiation and progression of cancer at molecular level can reduce the incidence of cancer (Sloan and Gelband 2007). Incidence of cancer can be reduced by 40–50% by using the above knowledge in designing public health strategies (Stewart et al. 2016). Among our diet there are many components which have been proved to be mutagenic and can transform healthy cells into cancerous cells (Zielinski 2014). On the other hand, many components of healthy diet have been associated with anticancerous potential (González-Vallinas et al. 2013; Rajesh et al. 2015; Baena Ruiz and Salinas Hernandez 2016). Therefore, there is a need to work out the mode of action of these dietary components in suppressing cancer before their prescription in clinical trials (Greenwald 2002). Plant-derived nutraceuticals in the form of alkaloids, flavonoids, and terpenoids have gained importance in controlling cancer progression by different molecular mechanism (Braicu et al. 2017). The significance of educating humans regarding the use of plant-based pharmaceutics in decreasing the risk of cancer is proposed (Pem and Jeewon 2015).

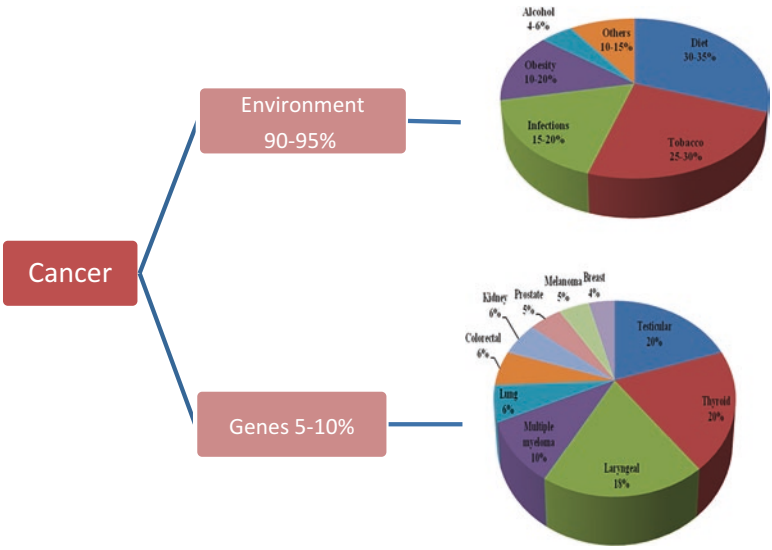


Fig. 2.1 Cancer contributing genetic and environmental factors

2.1 Cancer Development

Cancer development involves slow and dynamic changes involving initiation, promotion, and progression to transform healthy cells into a bunch of malignant cells (Cooper 2000). This transformation can be interrupted at different stages by using plant-derived chemopreventive agents (Landis-Piwowar and Iyer 2014; Donaldson 2004; Anand et al. 2008). The present chapters include the different aspects of cancer development and chemoprevention and have analyzed the chemopreventive action of an important plant-derived secondary metabolite, i.e., alkaloids, which can be helpful in exploiting alkaloids in cancer management.

2.2 Cancer Chemoprevention

For cancer prevention, different natural or synthetic agents have been tried by various researchers that can suppress various molecular/signaling pathways controlling progression of cancer (Landis-Piwowar and Iyer 2014). There are different types of chemoprevention (Fig. 2.2). Primary chemoprevention involves the blockage of premalignant zones by using blocking agents, whereas secondary chemoprevention suppresses the process of development of these zones to malignant growth (suppressing agents), and tertiary treatment prevents the dispersal of cancer by inhibiting tumor progression (Greenwald 2002; Steward and Brown 2013; Wattenberg 1985). Nowadays, multi-chemopreventive strategy is preferred, which involves utilization of low doses of combined chemopreventive agents for reducing the risk of tumor development with less side effects (Chen and Malhotra 2015; Ullah and Ahmad 2016). However, these agents have several disadvantages involving high

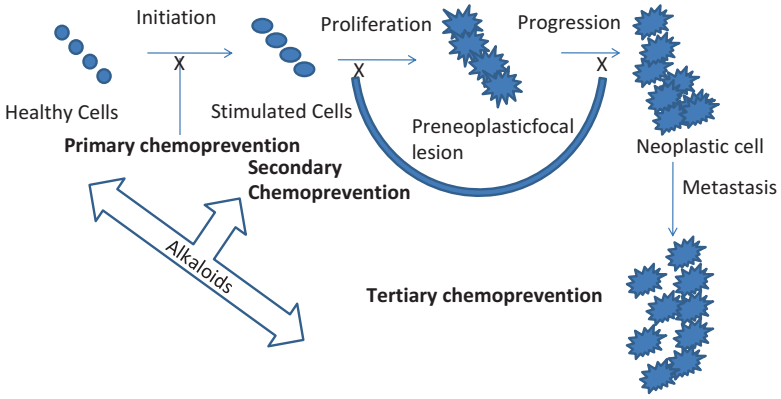


Fig. 2.2 Initiation of cancer involves the conversion of healthy cells into stimulated cell that transforms to preneoplastic and neoplastic cells. Alkaloids interfere with initiation step (primary chemoprevention), proliferation, and progression (secondary chemoprevention) to finally inhibiting the invasion and metastasis (tertiary chemoprevention)

price and more time consumption for its epidemiological, biochemical, pharmacokinetic, and pharmacodynamic properties (Rather and Bhagat 2018).

2.3 Alkaloids

Since ages, people are using botanical plants in the form of home remedies for treating various health-related illnesses and disorders (Halberstein 2005) as synthetic drugs have severe side effects and clinical risks (Bishayee and Sethi 2016). Therefore, herbs of plant origin have been gaining popularity throughout the world with therapeutic potential for treating different deadly diseases (Das and Dhanjal 2015). The remedial potential of these herbs involves the presence of bioactive ingredients that are having therapeutic properties. Alkaloids are such secondary metabolites with potential biological activity, being diverse in structure and biosynthetic pathways. Alkaloid nomenclature was coined in 1819 by W. Meisner. It means naturally present organic compounds that are alkaline in nature. Alkaloids have been described as basic compounds that contain heterocyclic nitrogen and are synthesized in plants from amino acids or their immediate derivatives in either plant or animal origin. Most alkaloids are colorless, crystalline compounds, e.g., coniines, but some such as nicotine and hygrine are liquids. Most of them are optically active, and different active forms are usually found but not in different plants.

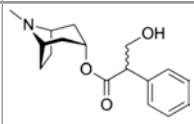
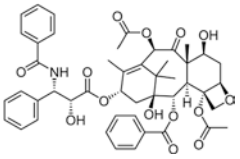
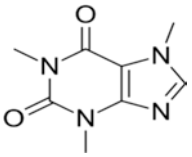
More than 17,000 alkaloids have been isolated with potential biological and pharmacological activities (Mohan and Jeyachandran 2012), and approximately 20% are vascular plant-based alkaloids (Yang and Stöckigt 2010). Among them, 80 compounds have been clinically tested, and few of them have been used for this deadly disease due to its therapeutic potential (Lu et al. 2012). These alkaloids are basically low molecular weight compounds having the nitrogen atom in the heterocyclic ring, typically alkaline in nature. On the basis of biogenesis, alkaloids are categorized in three categories mentioned in Table 2.1. Several alkaloids have been reported to have different therapeutic activities. Alkaloids have also been classified into different categories depending on their biosynthetic precursor and heterocyclic ring system (Table 2.2).

This book chapter basically deals with the important plant-derived alkaloids that are known to have anticancerous potential and are being exploited against different kinds of cancers in vitro, in vivo, and in human clinical interventions.

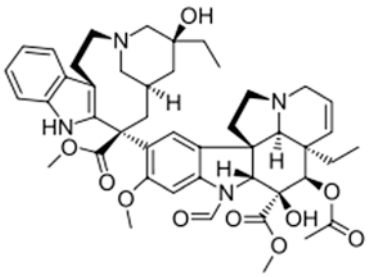
2.3.1 Vinca Alkaloids

Alkaloids derived from *Catharanthus roseus*, along with chemotherapeutic drugs, have been widely used in treating different kinds of tumors. Vinblastine, vincristine, vindesine, vinorelbine, and vinflunine are different forms of vinca alkaloids that are tried in various medical studies (Fahy et al. 2008). Only two derivatives (vindesine and vinorelbine), semisynthetic analogs of vinblastine, are recently used clinically. Antitumor activity of vinca alkaloids is mainly due to interaction with tubulin to

Table 2.1 Classification of alkaloids on the basis of their biogenesis

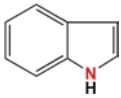
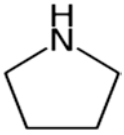
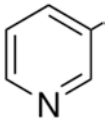
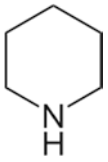
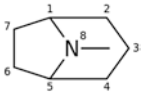
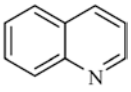
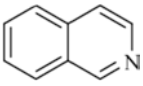
Alkaloid	Origin	Example	Biological structure	Biological significance
True alkaloid	Derived from amino acids that have nitrogen in heterocyclic ring	Atropine		Anticholinergic (Ziegler and Facchini 2008)
Proto alkaloid	Derived from amino acids that don't have nitrogen in heterocyclic ring	Taxol		Used for treating ovarian, breast, and lung cancer (McGuire et al. 1989; Zasadil et al. 2014)
Pseudo alkaloids	Not derived from amino acids but have nitrogen in their heterocyclic ring	Caffeine		Antioxidant and anti-inflammatory (Herman and Herman 2013)

interfere in the development of mitotic spindle and resultant cell division arrest in metaphase (Dumontet and Jordan 2010). By inhibiting the rate of microtubule growth and enlargement, these alkaloids basically arrest cell cycle which in turn kills diseased cells (Ngan et al. 2001). The molecular events in the cell killing by vinflunine (alkaloids) involve Bcl-2 phosphorylation with apoptosis involving activation of caspases 3/7 and c-Jun N-terminal kinase 1. Vinflunine has been reported to have antivasculature effect thereby inhibiting the vascular supply to tumor and inhibiting its growth (Holwell et al. 2001). This effect involves the disruption of vascular tissues, anatomical changes in endothelial cells, formation of deformed capillary-like structures, and obstruction in endothelial cell motility along with proliferation (Ali et al. 2012). Vinflunine is inhibitory toward bFGF-induced angiogenesis with greater superiority over vinorelbine and is effective at doses 16 times lower than maximal therapeutic dose (Kruczynski et al. 2006). In 2009, this alkaloid has been sanctioned by the European Medicines Agency as therapeutic drug for treating adult patients in advanced or metastatic transitional stages of urothelial tract carcinoma (Vaughn et al. 2009).



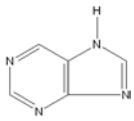
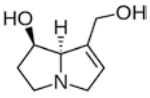
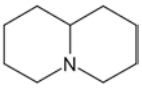
Structure of vinca alkaloids

Table 2.2 Some important groups of alkaloids based on heterocyclic ring structure and their source and activities

S. No.	Type of alkaloid	Biological structure	Examples with botanical origin	Therapeutic activities
1.	Indole (benzopyrrole)		Ergotamine, ergometrine (*Claviceps purpurea*), reserpine (*Rauwolfia serpentina*), physostigmine (*Physostigma venenosum*), vinblastine, vincristine (*Vinca rosea*), strychnine (*Strychnos nux-vomica*)	Antihypertensive, antitumor (El-Sayed and Verpoorte 2007)
2.	Pyrrolidine		Hygrine (*Erythroxylum coca*), stachydrine (*Stachys tuberifera*)	Antibacterial, antifungal, and antitubercular (Parmar et al. 2012)
3.	Pyridine		Arecoline (*Areca catechu*), ricinine (*Ricinus communis*), trigonelline (*Trigonella foenumgraecum*)	Antimicrobial (Machado et al. 2012)
4.	Piperidine		Connine (*Conium maculatum*), lobeline (*Lobelia inflata*), pelletierine (*Punica granatum*)	Antioxidant, anti-inflammatory (Herman and Herman 2013)
5.	Tropane [piperidine pyrrolidine (N-methyl)]		Atropine (*Atropa belladonna*; *Datura stramonium*), cocaine (*Erythroxylum coca*), hyoscyamine (*Atropa belladonna*)	Anticholinergic (Ziegler and Facchini 2008)
6.	Quinoline		Quinine, quinidine (*Cinchona officinalis*), cuspareine (*Cusparia trifoliata*)	Antimalarial, antibacterial, antifungal, anthelmintic, cardiotonic, anticonvulsant, anti-inflammatory, analgesic (Marella et al. 2013)
7.	Isoquinoline		Papaverine (*Papaver somniferum*), berberine (*Hydrastis canadensis*), emetine (*Uragoga ipecacuanha*), corydaline (*Corydalis aurea*)	Antihyperglycemic, antitumor, antibacterial (Nassiri 2013)

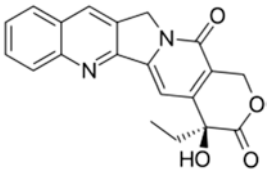
(continued)

Table 2.2 (continued)

S. No.	Type of alkaloid	Biological structure	Examples with botanical origin	Therapeutic activities
8.	Imidazole		Pilocarpine (*Pilocarpus jaborandi*)	Ophthalmic disorders (Cronemberger et al. 2012)
9.	Purine (pyrimidine imidazole)		Caffeine (*Thea sinensis*, *Camellia sinensis*, *Coffea arabica*)	Antioxidant, anti-inflammatory (Herman and Herman 2013)
10.	Pyrrolizidine		Senecionine (*Senecio vulgaris*), senneciphylline (*Senecio platyphyllus*)	Antidiabetic, anticancerous (Majik and Tilve 2012)
11	Norlupinane (quinolizidine)		Sparteine (*Lupinus luteus*, *Lupinus niger*, *Cytisus scoparius*), lupinine (*Lupinus luteus*, *Anabasis aphylla*)	Antimicrobial (Singh et al. 2011)

2.3.2 Camptothecin

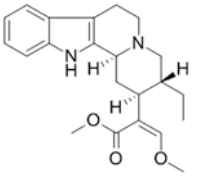
This alkaloid was first extracted from *Camptotheca acuminate* with broad spectrum against cancer. It includes both alkaloids (14) and terpenoids (3) which were extracted from a tree named as *Ervatamia heyneana* (Apocynaceae). Few camptothecin (CPT) such as 9-methoxy camptothecin coronaridine, pericalline, heyneatine, and 10-methoxyeglandine N-oxide possessed cytotoxicity effect (Mohan et al. 2012). Its first clinical trial was conducted in the 1970s, but this attempt was not successful due to its side effects like severe bladder toxicity (Potmeisel 1995). The first-generation analogs of two camptothecin alkaloids are used for treating different cancers (ovarian, colorectal, small-cell lung cancers), while several second-generation camptothecin analogs are still in clinical trials (Yu-Feng and Ruiwen 1996). Semisynthetic derivatives of camptothecin (viz., topotecan and irinotecan) are used for treating ovarian, lung, and colorectal cancers (Creemers et al. 1996; Bertino 1997) by acting on human DNA topoisomerase I (topo I) through blockage of cleavage/religation which results in formation of covalent reaction cleavage complex intermediate. Basically, CPT action involves the killing of S-phase by potentially lethal collisions between replication forks and cleavage complexes which also results in triggering the development of long-lived covalent topo-I DNA complexes for its cytotoxicity (Liu et al. 2006).



Structure of camptothecin

2.3.3 Hirsutine

Hirsutine, another major alkaloid, was extracted from plants named as *Uncaria* which has been used for treating human breast cancer cells HER2 positive, p53-mutated MDA-MB-453, and BT474 cell lines by blocking Akt (Lou et al. 2015). Its main mechanism involves the DNA damage by upregulating H2AX, a marker of DNA breakage, enhancing the p-p38 MAPK expression (Dickson and Schwartz 2009). The treatment of aggressive subtype of breast cancer (triple negative breast cancer, TNBC) involves in vitro use of hirsutine which proved that this major alkaloid modulates the survival pathways that results in DNA damage against cancerous cells (Zhang et al. 2016). This study showed that hirsutine could be used with other chemotherapeutic drugs for treating TNBC. The use of hirsutine on molecular activity has restricted only on breast cancerous cells. There would be a need to study this newly discovered metabolite to unveil its medicinal effects on different types of cancers which may result in developing an effective anticancer drug. Shih et al. (2009) found that α-tomatine, another glycoalkaloid common in *Lycopersicon esculentum*, possesses the similar mechanism like hirsutine by suppressing Akt phosphorylation and extracellular signal-regulated kinases 1 and 2 (ERK1/2) but without affecting the p38 MAPK.

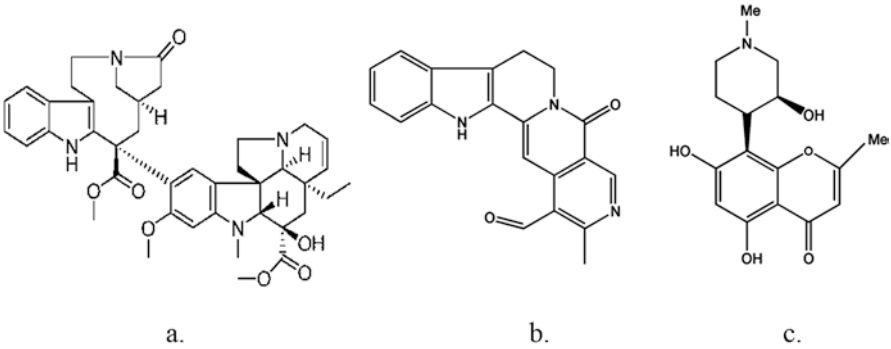


Structure of hirsutine

2.3.4 Cathachunine, Subditine, and Rohitukine

In majority of cancers, the accumulation of DNA-damaging reactive oxygen species (ROS) leads to development of neoplastic cells. These ROS are damaged by inhibiting the oxidation of proteins when the limit exceeds; hence, a balance is always maintained. But several anticancer alkaloids in cancerous cells drastically alter the ROS balance leading to ROS-induced apoptosis. Cathachunine, which is isolated

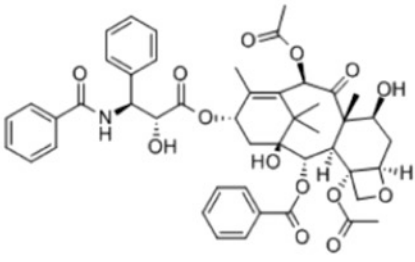
from *Catharanthus roseus*, and subditine, extracted from *Nauclea subdita*, are two newly discovered alkaloids that act by ROS-induced apoptosis in cancerous cells. These two alkaloids have been used effectively against skin and glandular cancer (Wang et al. 2016; Liew et al. 2014). Another alkaloid, rohitukine, extracted from *Dysoxylum binectariferum*, has been used for treating breast, ovarian, and lung cancer by altering the ROS balance (Kamil et al. 2015).



Structures of cathachunine (**a**), subditine (**b**), and rohitukine (**c**)

2.3.5 Taxol

Taxol (paclitaxel), a terpene alkaloid, exists as the most prominent natural chemotherapeutic agent extracted from the bark of the Pacific yew tree, *Taxus brevifolia* (Wani et al. 1971). Horwitz while studying with the tree *T. baccata* discovered that the mechanism of Taxol involves the cellular target as tubulin (Schiff et al. 1979). This alkaloid is regarded a wonderful drug for treating breast, lung, and ovarian cancer which fetches highly priced amount in the drug market more than $1 billion per year (Malik et al. 2011). This alkaloid is available in the market under the trade name Taxol® BMS [Bristol-Myers Squibb]). In 1971, its properties against cancer were found in Pacific yew tree (*T. brevifolia*) extract (Wani et al. 1971). It occurs in the form of crystalline powder of white to off-white color with a molecular formula of $C_{47}H_{51}NO_{14}$.



Structure of Taxol

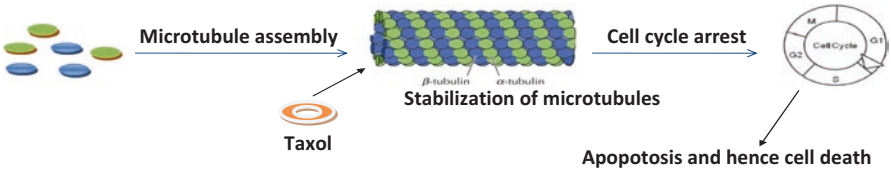


Fig. 2.3 Mechanism of action of Taxol

Microtubules within the cells play an essential role in mitotic spindle assembly that is required for M phase cell division. Basically Taxol belongs to the category of anticancer drugs that target microtubules. Unlike other antimicrotubule drug that is colchicine and vinca alkaloids that dismantle the microtubules, the Taxol binds to the protein tubulin which results in making microtubule complex highly stable but nonfunctional. As a result, it induces mitotic arrest and leads to cell division failure (Fig. 2.3). This activates apoptosis and results in cell death (Rowinsky and Donehower 1995). This drug also targets mitochondria as well as inhibits the apoptosis inhibitor protein, Bcl-2 (Ferlini et al. 2003). This drug is hydrophobic in nature; hence, there is problem in distributing it in tumor tissues. It requires a suitable carrier for efficient delivery in tumor tissues of a patient. Therefore, it is currently available in the market as anticancer drug under the brand name as paclitaxel and directed to the cancerous patients via polyethoxylated castor oil (Cremophor EL, CrEL) or albumin-bound (nab-paclitaxel, Abraxane®). Nowadays, new paclitaxel formulations are available in different formulations: nanoparticles, emulsions, liposomes, and micelles (Hennenfent and Govindan 2006).

2.3.6 Berberine

Berberine is a quaternary ammonium salt of benzylisoquinoline alkaloid isolated from different parts (roots, rhizomes, and bark) of medicinally important plants, viz., *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (*Coptis* or golden thread), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (tree turmeric), and *Tinospora cordifolia* (guduchi or giloe) (Sturm and Stuppner 1998). Different studies have proved that this alkaloid possesses antitumor properties with various mechanisms under in vitro and in vivo conditions (Jabbarzadeh et al. 2014; Ortiz et al. 2014; Sun et al. 2009). This drug was already used for treating cancers, namely, breast, prostate, and colorectal cancer (Barzegar et al. 2015). Another alkaloid that is palmatine resembles structurally with berberine as protoberberine alkaloids that are found in the same plant species. The mode of action of berberine is quite complex. It is basically a wide spectrum enzyme inhibitor, which inhibits the action of various enzymes (Fig. 2.4), namely, N-acetyltransferase, cyclooxygenase-2, and topoisomerase which results in inhibiting gene/protein expression (Sun et al. 2009). It involves the formation of ROS in tumor cells; alters mitochondrial transmembrane potential; inhibits DNA topoisomerase activity; joins with DNA or RNA and metalloproteinase regulation; prevents the action of activator protein 1, cyclins, and p53; and induces caspase activity and nuclear factor (NF-kappa B) signal activation. It

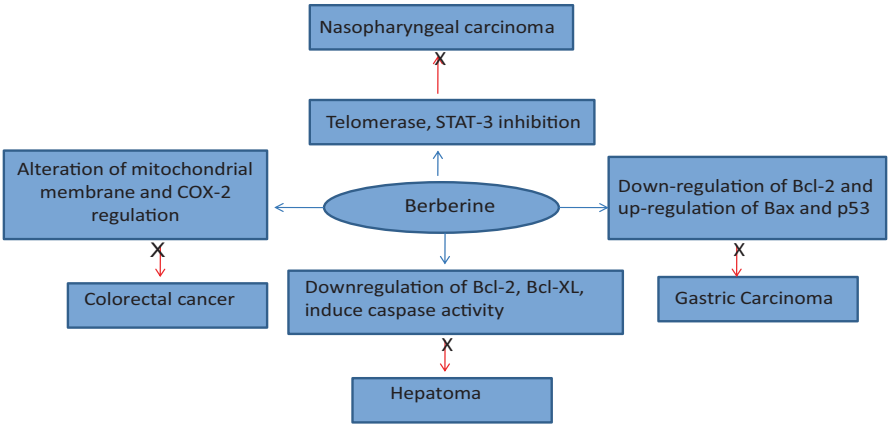
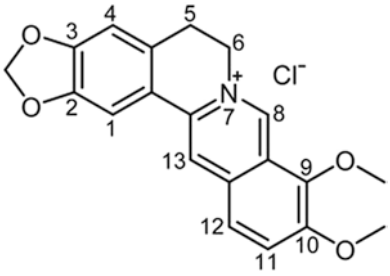


Fig. 2.4 Mechanism of action of berberine on different cancer cell types

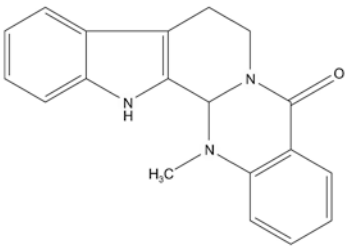
also hinders the rapid growth and multiplication of certain tumor-causing microbes and viruses, such as *Helicobacter pylori* and hepatitis B virus. Therefore, the effect of berberine through different mechanisms may lead to arrest of cell cycle progression and cause cell death via apoptosis and activate autophagy. The quaternary amine presence in berberine structure leads to poor water solubility with less absorption in the alimentary canal that restricts its use and development as an anticancerous drug. Therefore, there is a need to develop advanced mechanism for administering berberine drug in cancerous patients which improves its solubility and absorption. The development of nanoparticulate drug delivery system in the form of polymeric formulations as nanoparticles, nanocapsules, liposomes, solid lipid nanoparticles, and nanoemulsions improves the overall efficacy of berberine as anticancerous agent. The overall effect of berberine has increased along with other chemotherapeutic drugs/radiotherapy. This study has proved that berberine in combination with other drugs (cisplatin and evodiamine) could be used against different tumor cells (Youn et al. 2008). In one of the study, it was found that berberine in combination with vincristine decreased the overall toxicity toward hepatoma cells (Yu et al. 2014).



Structure of berberine

2.3.7 Evodiamine

This alkaloid is a quinazolinocarboline in nature, isolated and purified from *Evodia rutaecarpa* Bentham (Rutaceae). Different studies reported that this alkaloid exhibits hindering effects on migrating tumor cells and promotes cell death in various cancerous cells (colorectal, gastric, cervical, liver, prostate, melanoma, lung and breast, gastrointestinal, and genitourinary tract cancers (Yang et al. 2009, 2010, 2014; Wang et al. 2010; Zhu et al. 2009). It possesses antitumor activity through different mechanisms that are progression of cell cycle arrest at the G2/M phase by upregulating cyclins B1, p27, and p21 that in turn inactivate cdc2 and pRb, and another mechanism involves the induction of caspase-3, caspase-8, and caspase-9 activity which later leads to apoptosis in cancerous cells (Kan et al. 2004; Yang et al. 2014; Zhong et al. 2015). It also increased the level of lactate dehydrogenase that induces apoptosis or necrosis in carcinogenic cells (Lv et al. 2016). Its mode of action on hepatocellular carcinoma in vivo and in vitro involves the increased expression of WW domain-containing oxidoreductase (WWOX) expression (Hu et al. 2017). It was also reported by Lin et al. (2015) that antitumor activity of evodiamine was also related by inhibiting β-catenin activity. It enhances ROS production level in cancerous cells which may trigger mitochondrial permeability pores that in turn changes the membrane potential leading to cytochrome c release which activates an apoptotic signal and ultimately cell death (Gabai et al. 1998). Overactive PI3K/Akt pathway would also induce apoptosis and leads to cell cycle arrest. Therefore, the use of this alkaloid significantly affects PI3K and p-Akt expression and, therefore, considered evodiamine as a potential therapeutic natural compound for treating various cancers (Lv et al. 2016).



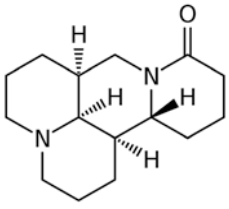
Structure of evodiamine

2.3.8 Matrine

Matrine ($C_{15}H_{24}N_2O$) is a promising phytochemical alkaloid extracted from *Sophora flavescens* roots with therapeutic potential as anti-hepatic fibrosis, antiviral, anti-arrhythmic, anti-inflammation, and antitumor (Chui et al. 2004). It exists in nature in the form of polyphenolic phytoalexin and widely used as potential chemotherapeutic drug in China. This drug is used for treating various cancers including

gastric, cervical, lung, liver, breast, and colon cancer (Li et al. 2010). This chemotherapy drug does not inhibit the cell size increase and multiplication and cell division of normal cells like other drugs, but it improves the count of white blood cells and boosts the immune system of cancer patients (Zhang et al. 2010). In 1995 the Chinese FDA (CFDA) approved the Fufang Kushen injection having matrine as main chemical ingredient as an anticancer drug in order to treat non-small cell lung cancer and liver cancer in combination with other chemotherapeutic agents (Wu et al. 2016). Antitumor property of matrine is because of amide bond present in its structure. Following the opening of D-ring and breaking of the amide bond, the antiproliferative activities of matrine are lost (Wang et al. 2012).

Matrine acts as a best chemopreventive drug for controlling multistage carcinogenesis with little toxic and side effects. Therefore, it has also been administered to cancer patients below 18 years of age. It was helpful in reducing the proliferation rate in tumor cells by different mechanisms, such as differentiation and apoptosis in tumor cells, altering cell cycle, leading to cell cycle arrest, and inhibiting telomerase activity in affected cells, and, therefore, used for treating gastric, cervical, skin cancer, and glioma cancerous cells (Zhang et al. 2001). A study by Chang et al. (2013) has proved that this drug acts on HT29 human cells in the G_0/G_1 phase of the cell cycle that leads to decrease in cell cycle progression with its antiproliferative effect. Overall, a decrease in tumor cell proliferation along with apoptosis by matrine may be one of the factors for anticancer treatment strategy. Various studies on different types of cancer with different mechanism such as inhibition of tumor growth, apoptosis in murine hepatoma cells and gastric tumors, stop of migration and adhesion in cervical cancer HeLa cells, and invasion and metastasis in human malignant melanoma A375 cells have proved matrine as anticancerous drug (Yu et al. 2011; Liu et al. 2010; Li et al. 2010; Taylor et al. 2010). This alkaloid also acts by reducing anti-apoptotic/pro-apoptotic Bcl-2/Bax ratio, releasing Cyto C from the mitochondria, and subsequently increasing caspase-3 activity and hence inducing apoptosis and ultimately cell death (Chang et al. 2013). It functions in cooperation with other anticancerous drugs for not only enhancing anticancerous effects but also reversing drug resistance with various anticancerous drugs (Huang et al. 2007). Few studies showed that it can enhance the CPT-11 (a DNA topoisomerase (TOPO) inhibitor), in colorectal cancer with less adverse effects of CPT-11 (Ren et al. 2014).



Structure of matrine

2.3.9 Podophyllotoxin and Its Derivatives

Nowadays podophyllotoxin and its derivatives become the main center of attraction in the medical world due to its extensive pharmacological properties. The development of two semisynthetic anticancerous drugs (etoposide and teniposide) has triggered its pharmacological research with more focus on its structural phenotype as effective biological inventions (Jie et al. 2012; Zhang et al. 2018). Its mode of action for anticarcinogenic activities induces cytotoxicity to overcome MDR through multiple mechanisms that include change in certain carcinogenic enzymes, apoptotic cell death by cell cycle arrest, and suppressing pathways for oncogenic signals (Fig. 2.5). Different researchers synthesized its derivatives: podophyllotoxin-piperazine acetate-like ester derivatives for in vitro antiproliferative property for human cancer cell lines (Sun et al. 2017) and N-(aminosulfonyl)-4-podophyllotoxin carbamate analogs for in vitro antiproliferative activities for human tumor cell lines such as HeLa, A-549, HCT-8, and HepG2 (Xiao-Hui et al. 2017).

2.3.10 Sanguinarine

Sanguinarine is a benzophenanthridine alkaloid extracted from the family of *Papaveraceae* (*Chelidonium majus*, *Macleaya cordata*, and *Sanguinaria canadensis* L.). It is considered as a potential chemotherapeutic agent for its anticancerous and apoptotic activities on various types of cancer, namely, epidermal, keratinocyte, prostate, cervical, breast, leukemia, lymphoma, melanoma, colon, colorectal,

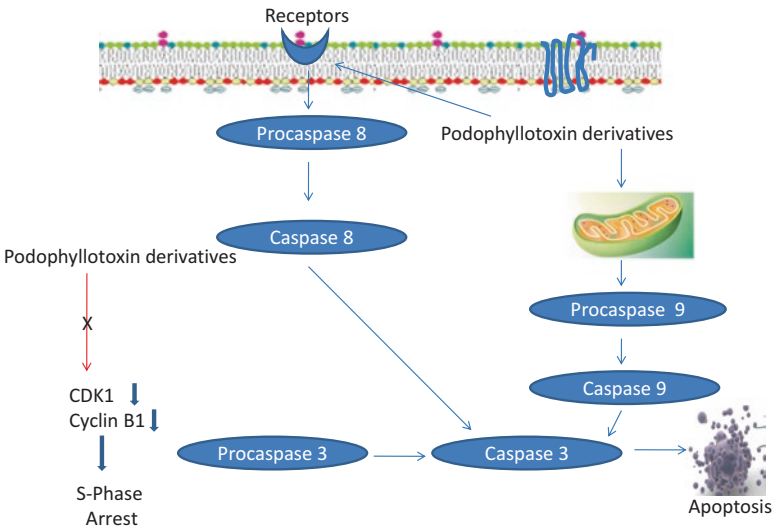
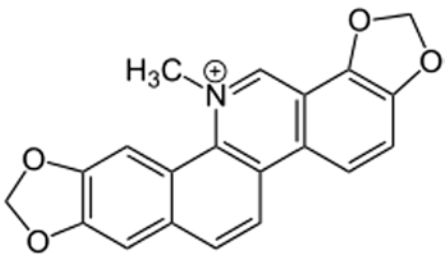


Fig. 2.5 Schematic representation of multiple drug resistance activity of podophyllotoxin derivatives

gastric, pancreatic, lung, neuroendocrine, osteosarcoma, and human neuroblastoma cells (Kalogris et al. 2014; De Stefano et al. 2009). From the various in vitro studies, dosage with concentration less than 10 μmol is quite effective as anticancerous agent. The apoptosis induced by sanguinarine involves multiple pathways that include nuclear factor-κB (NF-κB) activation and caspase activation and alters mitochondrial damage and termination of cell cycle (Adhami et al. 2003, 2004; Chaturvedi et al. 1997). This alkaloid downregulates the Bcl-2 protein expression and increase in Bax protein that results in mitochondrial damage. It also induces blockage in DNA that results in rapid apoptosis in human colon cancerous cells and in malignant melanoma cells (Hammerová et al. 2011).

Its effect on human primary effusion lymphoma cell lines involves the downregulation in the expression level of IAP family proteins cIAP1, cIAP2, and XIAP (Sun et al. 2010). It also inhibits the expression of matrix metalloproteinases (MMP-9), NF-κB, and AP-1 signaling pathways as well as suppresses tetradecanoyl phorbol myristate acetate (TPA)-induced breast cancer cell migration and invasion (Park et al. 2014). It also exhibits antitumor activity by repressing angiogenesis by inhibiting angiogenesis growth factor VEGF (Sun et al. 2010). It influences cell cycle arrest by increasing CDK inhibitors with reduction in cyclins D1, D2, E, CDK2, CDK4, and CDK6 in human prostate cancer cells (Adhami et al. 2004). This alkaloid helps in controlling breast cancer by upregulating p27 while downregulating cyclin D1 inhibiting the activation of STAT-3 (Kalorgis et al. 2014). Sanguinarine is a potential tumorigenesis inhibitor and used as a potential anticancer drug in oral squamous cell carcinoma (Tsukamoto et al. 2011).



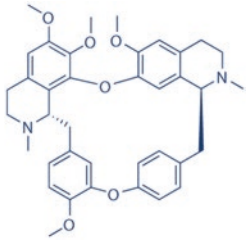
Structure of sanguinarine

2.3.11 Tetrandrine

Tetrandrine has been isolated from *Stephaniae tetrandra* roots by Kondo and Yano (1928) and later certified for its anticancerous nature by various researchers (Chen and Chen 1935; Kubota 1931). It has multiple roles, namely, potent calcium channel blocker (Wang et al. 2004), anti-inflammatory, immunosuppressant, antiallergic, antioxidant, antidiabetic, antimicrobial, anticancer, and anti-Ebola agent (Chen 2002; Dhikav et al. 2002). It is considered as a potent chemotherapy drug as it involves inhibition of various pathways involving cell cycle blockage,

Wnt/β-catenin-mediated pathway, and mitochondrial-dependent caspase activation pathway. Its anticancerous activity was found at a very low μM concentration. Several studies on tetrandrine have proved its pharmacological potential on cell multiplication, apoptosis, angiogenesis, metastasis, autophagy, and multiple drug resistance (MDR) in cancer therapy (Liu et al. 2016).

Different researchers worked on the reaction of tetrandrine on tumor and non-tumorigenic cells by inhibiting the cancer cells related to leukemia, hepatoma, lung carcinoma, and colon cancer by inducing apoptosis (Mang et al. 2004; Lee et al. 2002; Yoo et al. 2002; Lai et al. 1998). It acts as an inhibitor of cell cycle at various check points in cancerous cells that restricts its multiplication followed by cell death through different pathways that include caspase-dependent pathway or FASL-mediated pathway (Xiao et al. 2015; Chen et al. 2014; Yu and Ho 2013; Meng et al. 2004; Kuo and Lin 2003). It hinders the expression level of certain proteins (CDK4, CDK2-CycE) and prevents G1-S transition of cells in colon, endothelial, and hepatocellular cancerous cells (Xiao et al. 2015). The synergistic anticancerous activity of tetrandrine along with chloroquine has been studied in human hepatoma cell lines, human glioma cell lines, human lung cancer cell lines, and human cervical adenocarcinoma HeLa cells (Mei et al. 2015). Its antitumor activity can be enhanced when supplied with drugs such as endostar (Qian et al. 2013), sorafenib (Wan et al. 2013), fangchinoline, and doxorubicin (Sun and Wink 2014). There is a drawback of tetrandrine bioavailability due to its low solubility in polar solvents which could be enhanced by several modifications of the drug and by using nanotechnology using microspheres, emulsions, liposomes, and nanoparticles. In mice, the combined effect of tetrandrine with paclitaxel or tetrandrine-phospholipid complex in the form of nanoformulations enhanced drug delivery without damaging immune system of this therapeutic drug against cancerous cells (Xu et al. 2014).

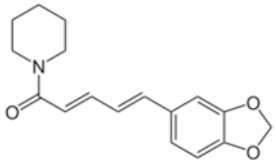


Tetrandrine

2.3.12 Piperine

1-Piperoylpiperidine belongs to amide alkaloid category and has been extracted from roots and fruits of *Piper nigrum* L. and *Piper longum* L. species (Zheng et al. 2016). In 1819, it was first time extracted from the pepper by Hans Christian Orsted (Gorgani et al. 2017). The active ingredient of black pepper is piperine that has been

used as a herbal remedy in India. Its anticancerous activity has been exploited as a natural product. It has been tested that the life of mice suffering from Ehrlich ascites tumor has been increased by piperine that inhibits the tumor development in mice (Sunila and Kuttan 2004). Its chemotherapeutic properties against melanoma, lung cancer, breast cancer have been demonstrated by focusing on the cancer stem cell-regenerating properties (Kakarala et al. 2010; Selvendiran et al. 2006; Pradeep and Kuttan 2004). As a chemopreventive agent, it involves activation of apoptotic cascades with cell proliferation inhibition. It plays an important role in arresting cell cycle and modulating redox homeostasis, ER stress, and autophagy. Several studies revealed that piperine inhibits angiogenesis and induces the production of detoxification enzymes (Manayi et al. 2017). It also targets certain transcription factors including nuclear factor-kB (NF-kB) and androgen receptor (AR) while inhibiting transcription factor (STAT-3). Piperine hinders the multiplication of prostate cancer cells with apoptosis by activating caspase-3 that initiate and execute cell death (Samykutty et al. 2013). Its inhibitory action against Akt phosphorylation suppresses angiogenesis which initiates its anticancer effect by inhibiting certain transcription factors like CREB, NF-kB, and c-Fos (Pradeep and Kuttan 2004). Tawani et al. (2016) suggested its antiproliferative, pro-apoptotic, and anticancerous properties in relation to the formation and stabilization of G-quadruplex structure at c-myc promoter region which results in downregulating the expression of cancer cells.



Piperine

2.4 Conclusion

Anticancerous activity of alkaloids against various types of cancers has been known since ages. The use of alkaloids in combination therapy can be tested in different human intervention trials as a strategy against cancer. Nowadays, these alkaloids have gained much importance due to their role in cell cycle arrest as spindle poison, cell death, angiogenesis inhibition, autophagy, alteration of mitochondrial membrane and COX-2 regulation, induction of caspase activity, downregulating Bcl-2 and upregulating Bax and p53, inhibition of telomerase, STAT-3 inhibition, and influence on signaling events. Hydrophobicity of alkaloids limits the clinical use of these plant-derived secondary metabolites. This chapter revealed that the exact mechanism involved in the chemopreventive effects of several alkaloids needs to be explored. Therefore, these important pharmaceutically active agents must be tested by stringent clinical trials to support the in vitro studies.

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3 Mechanistic Insight into Cancer Aetiology and Therapeutic Management by Natural Metabolites

Vandana Singh, Deepak Kumar, Sheemona Chowdhary, Kunal Maniar, Meenu Narwal, Rajasri Bhattacharyya, and Dibyajyoti Banerjee

Abstract

The sudden and uncontrolled proliferation of cells is a common cause of mortality. Cancer is a multifactorial disorder which involves both exogenous and endogenous factors for initiation, promotion and progression. The interplay between genes and environment induces genetic and epigenetic alterations to cause chronic diseases including cancers. The management of cancer is far from addressing genetic and epigenetic alterations. The major limitation of current therapy is that it affects the non-targeted tissues also. In this respect, anticancer drugs which are derived from natural resources are considered as good leads for drug development. The natural products have shown anticancer activity in various types of cancer. Thus, the use of the natural product in cancer chemotherapy is gaining attention. In this chapter, the recent plant-derived secondary metabolites with potential for therapeutic management of cancer are discussed.

Keywords

Malignancy · Cancer aetiology · Cancer hallmarks · Plant-derived natural products · Cancer therapies · Cancer chemoprevention

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Abbreviations

CDK1	Cyclin-dependent kinase 1
COX1	Cyclooxygenase 1
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
IGF-1	Insulin growth factor-1
TME	Tumour microenvironment
VBL	Vinblastine
VCR	Vincristine
WA	Withaferin A

3.1 Introduction

Cancer is the prime cause of mortality and morbidity worldwide (Saeki and Sugimachi 2001). It is characterized by uncontrolled proliferation of cells with the high capacity for invasion and metastasis (Martin et al. 2013). The tumours have been categorized into two types, i.e. benign (localized) and malignant (invasive), by their ability to spread to other body parts. Cancer is classified into three main types depending on the type of tissues they are affecting (Cooper 2000). Carcinoma is an epithelial cell-derived tumour. Based on cytological features, pathologists have classified it to basal cell carcinomas, adenocarcinomas, transitional cell carcinoma and squamous cell carcinomas. Lymphomas are a tumour of the lymphatic system, particularly the lymph node. Based on histopathological features, it is further subdivided into Hodgkin's and non-Hodgkin's lymphoma. *Sarcoma* is the cancer of connective tissues which includes fat, blood vessels, bones, muscles, deep skin tissues and the cartilage (Vanita et al. 2011). Malignancy occurs in several organs. Among those, lung cancer is common in males followed by breast cancer in females (Saeki and Sugimachi 2001). The pathogenesis of malignancy can be divided in three stages, i.e. initiation, promotion and progression (Oliveira et al. 2007). The initiation generally occurs with the mutation in one or more cellular genes controlling critical regulatory pathways of the cell growth. Then in the promotion phase, there is selective growth enhancement induced in the initiated cell. In the final step, i.e. progression, the tumour invades the nearby tissue or even travel at a distant place. This is called metastasis. This is a more aggressive form of cancer (Oliveira et al. 2007; Beremblum and Shubik 1947; Gutiérrez and Salsamendi 2001).

Cancer cells contain multiple genetic alterations which indicate that these alterations accumulate in cells in a stepwise manner during tumour progression (Martin et al. 2013). There are eight hallmarks of cancer which are acquired in different cancer types at various time points during multistep tumorigenesis (Fig. 3.1) (Hanahan et al. 2017; Hanahan and Weinberg 2011). These unique properties enable the tumour cells to persist, divide and disseminate (Hanahan and Weinberg 2011). It

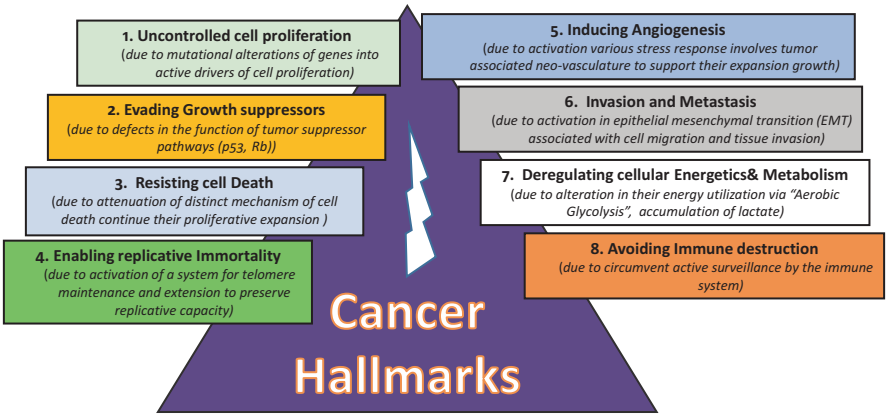

Fig. 3.1 Showing the eight biological hallmarks of cancer

involves heterotypic interactions among multiple cell types resulting in the formation of tumour microenvironment (TME), which is composed of cancer cells and a tumour-associated stroma including various subtypes of fibroblast, vascular cells and infiltrating immune cells (Hanahan and Weinberg 2011).

3.2 Aetiology of Cancer

It is well known that cancers originated from both environmental and genetic factors (Parsa 2012). Cancer is a complex multifactorial disorder, with genetics being a critical contributing etiologic factor. Mutagen is an agent that can cause mutation. It can be biological, physical and chemical. Some mutagens are carcinogenic and can alter the genetic composition of a cell (Parsa 2012). The lethal mutations can convert a normal cell to malignant one. Both the environment and lifestyle contribute to the development of cancer (Institute of Medicine (USA) Roundtable on Environmental Health Sciences et al. 2002). It is well known that epigenetics plays a vital role in the causation of cancer (Parsa 2012). Epigenetic events like DNA methylation, post-translational histone modifications and other epigenetic events regulate the gene expression and maintain cellular function. However, the unusual epigenetic event can silence the tumour suppressor gene, leading to carcinogenesis (Herceg and Vaissière 2011). Below (Fig. 3.2) some endogenous and exogenous factors are enlisted which are important for cancer development.

Genetic Factor Cancer such as breast and colon cancer often runs in families. Genetic predisposition to cancer explains the development of such cancer (Parsa 2012). The family history of cancer indicates that a person is at a higher risk (Vanita et al. 2011). There are mainly two prominent families of genes related to cancer; these are the proto-oncogenes and tumour suppressor genes. The gain of function mutation results in the conversion of proto-oncogenes into oncogenes that leads to

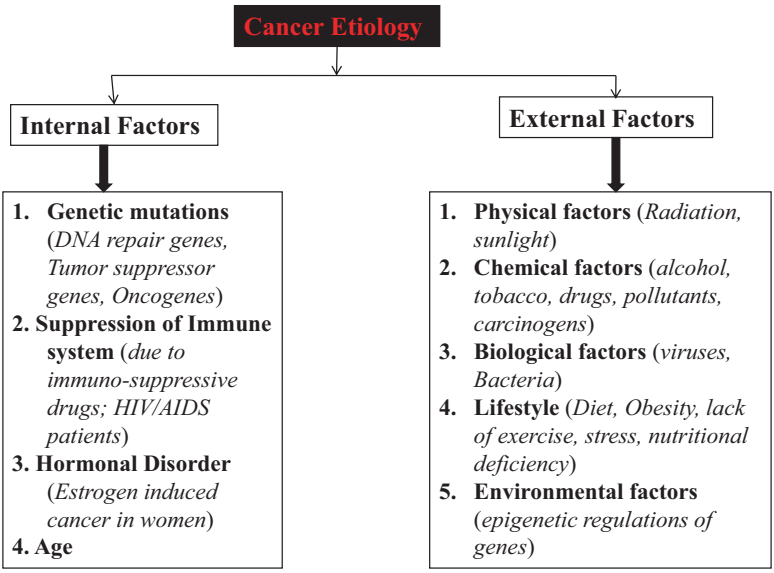

Fig. 3.2 Aetiology of cancer

uncontrolled growth and proliferation of cells (Ferreira et al. 2011), whereas the loss of function mutation in tumour suppressor genes makes them inactive thus inhibition of normal cell death process (apoptosis) (Lombardi et al. 2011). The mutation influencing the DNA repair genes, cell cycle regulators and cell death pathways are the leading genetic cause of malignancies (Vanita et al. 2011).

Ageing The most critical risk factor for cancer is ageing. The occurrence rate of cancer is higher in people over the age of 55 (Parsa 2012). The accumulated damage to the stem and progenitor cell is the cause behind the higher risk of cancer at an older age.

Hormonal Disorders Hormones like progesterone and oestrogen are believed to increase the risk of uterine and breast cancers (Parsa 2012).

Role of the Immune System There is a direct link between chronic inflammation and cancer. For example, people who have Crohn's disease (chronic inflammatory bowel disease) are at higher risk of developing colon cancer (Coussens and Werb 2002). Additionally, the suppression of the immune system is also responsible for tumour growth and progression.

Physical Factors It is known that radiations can cause mutations and chromosomal aberrations. It triggers off any steps involved in the development of carcinogenesis (Saeki and Sugimachi 2001). UV radiation causes the early ageing of the skin that leads to skin cancer. The presence of ultraviolet radiation initiates the formation of pyrimidine dimer and the production of reactive oxygen species (Rastogi et al. 2010). Exposure to ionizing radiations such as X-rays and nuclear radiations causes DNA damage that may contribute to cancer (Parsa 2012).

Chemical Factors Chemicals such as arsenic, benzene, formaldehyde, asbestos, etc. are carcinogenic agents which are dangerous in high concentrations (Saeki and Sugimachi 2001). Effect of smoking and its association with cancer is quite well known. It is believed that smoking increases the cancer risk in the lungs, head and neck, bladder, etc. The carcinogenic substance in tobacco smoke is benz(o)pyrene which causes point mutation in p53 (tumour suppressor gene) (Cook 1999). Alcohol intake has also shown to trigger carcinogenesis of the liver, oesophagus, mouth and stomach (Saeki and Sugimachi 2001). Many of the cosmetic products such as shampoo, cleanser, lotions, soap, detergents, etc. are associated with human cancers (Parsa 2012).

Biological Factors It was estimated that 18% of cancer is caused by infectious micro-organisms (viruses and bacteria). Human papillomavirus (HPV) is the prime cause of cervical cancer, whereas in liver cancer, it is hepatitis B virus (HBV) (Parsa 2012). Infection with HIV increased the risk of lymphoma and Kaposi's sarcoma. Similarly, infection with *Helicobacter pylori* causes stomach ulcers that lead to stomach and oesophageal cancer, whereas *Salmonella typhi* causes gall bladder cancer (Parsa 2012).

Lifestyle Unhealthy diet is associated with many cancers such as prostate cancer, colon cancer, etc. Obese people are at high risk of developing cancers as they produce a high amount of IGF-1 in their blood (Parsa 2012). Diet rich in red meat and other animal products is also linked to high cancer risk (Saeki and Sugimachi 2001).

Epigenetic Factors The significant epigenetic phenomena are miRNA-mediated gene silencing, DNA methylation, histone tail modification and chromatin remodelling (Shukla et al. 2014). It was found that various environmental factors have shown to be carcinogenic risk factors playing at the epigenetic level; this includes stress, social status, smoking and obesity (Parsa 2012). In cancer cells, there is dysregulation of hypermethylation of p53 gene on CpG islands. This dysregulation results in gene silencing apart from inactivation of tumour suppressor gene (Kazanets et al. 2016).

3.3 Cancer Chemoprevention

Different therapeutic approaches are used to treat cancer. However, these cause serious side effects that reduce the life quality dramatically (Baudino 2015). Despite the discovery of numerous anticancer drugs, the potency of these drugs is still less because of their toxic effects on normal tissues and genesis of drug-resistant cell population (Mokhtari et al. 2017; Baudino 2015). In cancer, chemoprevention utilizes natural or synthetic agents to slow down or halt the process of carcinogenesis (Steward and Brown 2013). In this context, considerable current interest is gained in natural medicinal compounds. They are recognized for prevention as well as therapy of cancer (Seca and Pinto 2018). The discovery of several chemopreventive bioactive molecules from natural resources encourages our research endeavours in the use of natural products for the treatment of cancer. These plant-derived products are simple, safer, eco-friendly, economical and less toxic as compared with the conventional treatment methods (Iqbal et al. 2017). The secondary metabolites present in plant extracts such as polyphenols, flavonoids and brassinosteroids have been explored for their potential use as anticancer agents (Greenwell and Rahman 2015). The phytochemicals are selective in their functions and act specifically on cancer cells without affecting the normal cells. They have the potential to target pathogenesis mechanisms such as angiogenesis, cell proliferation, immortality, tumour-promoting inflammation, apoptosis resistance, invasion and metastasis (Iqbal et al. 2017; Hanahan and Weinberg 2011). The molecules originated from dietary products are more charismatic and smarter for obvious reasons. They are expected to be non-toxic and possible to be given orally (Greenwell and Rahman 2015).

Phytotherapy is commonly used in traditional medicine worldwide. Numerous potential secondary metabolites obtained from plant sources such as herbs, spices, teas, vegetables and fruits are explored in search of anticancer molecules (Fridlender et al. 2015). The research and development of novel herbal medicine have focused on their pharmacological effect. Dietary phytochemicals through epigenetic modifications govern the cellular function thereby decreasing the risk of cancer (Shukla et al. 2014). However, low solubility and less bioavailability are two limitations of this approach which are related to its quick metabolism, poor absorption and fast systemic elimination (Fridlender et al. 2015). Therefore, with the emergence of drug discovery using computer algorithm, modification of the chemical structure of these natural compounds is done to increase its potency (Seca and Pinto 2018). Many plant-derived natural products are already being used to treat or prevent the development of cancer. Important medicinal plants and its products are discussed below.

3.3.1 Ashwagandha

Ashwagandha (*Withania somnifera*) is typically found in some parts of Asian countries and has broad therapeutic activities (Kulkarni and Dhir 2008). Leaves and roots of *W. somnifera* are rich in withanolides and related biocompounds. Withanolides are found to be cytotoxic to cancer cells apart from being

neuroprotective and immunomodulatory in function. The major active ingredient of Ashwagandha is Withaferin A (WA), which belongs to steroidal lactone metabolites that show antitumour, anti-metastatic, anti-angiogenic, anti-inflammatory and pro-apoptotic properties (vel Szic et al. 2014). It has been observed that in metastatic breast cancer model, WA decreased the cancer cell migration by disassembling vimentin, an intermediate filament protein. The WA inhibits the expression of CDK1. This results in cell cycle halt in G2/M phase thus inhibiting the growth of cancer cells. Further, its interaction with important cell signalling molecules (FOXO3a-BIM) induces apoptosis in both oestrogen receptor-positive and oestrogen receptor-negative breast cancer cells (Stan et al. 2008). WA also inhibits metastasis by a multifactorial manner (vel Szic et al. 2014). WA is well proven as a useful anticancer component, and it holds a prominent place among various anticancer medicinal products.

3.3.2 Paclitaxel

It is already a popular drug used to treat cancer. It is extracted from the outer layer of *Taxus brevifolia* (Cragg and Pezzuto 2016). It binds to the polymerized microtubules and inhibits the microtubule disassembly. This does not allow mitotic spindle to form in a dividing cell. So, the mitosis is inhibited (Seca and Pinto 2018). It is used in ovarian, lung and breast cancer therapy (Bernabeu et al. 2017).

3.3.3 Curcumin

Curcumin (diferuloylmethane) is a well-known chemopreventive agent and is used in the treatment of diseases (Iqbal et al. 2017). The yellow-orange powder obtained from dry curcumin is rich in polyphenols. It shows anticancer activities by the suppression of the NF-kB transcription factor which in turn downregulates the expression of NF-kB target genes like cyclin D1 and COX1. This leads to apoptosis (Vallianou et al. 2015). It arrests the cell cycle at various checkpoints such as G1, S and G2/M phase by upregulating the cyclin-dependent kinase (CDK) inhibitors (Seca and Pinto 2018).

3.3.4 Camptothecin

It was obtained from *Camptotheca acuminata* which is a Chinese ornamental tree. It possesses strong anticancer potential inhibiting topoisomerase I in a large number of cancers (Kim et al. 2015). It prevents DNA relegation and induces DNA damage which leads to apoptosis. It acts through the formation of a stable ternary complex by binding with the topoisomerase I-DNA binary complex (Nitiss 2009). The semi-synthetic derivatives topotecan, irinotecan and belotecan are already approved for its clinical use (Iqbal et al. 2017).

3.3.5 Gingerol

It belongs to a group of bioactive compounds isolated from the fresh rhizome of *Zingiber officinale* containing (6)-gingerol, (8)-gingerol and (10)-gingerol with known anticancer properties in colon, pancreas, ovarian and breast cancers. It down-regulates the expression of iNOS and TNF alpha through suppressing NF-kB nuclear translocation and IkB alpha phosphorylation (Park et al. 2006). It induces apoptosis in leukaemia cells by the mitochondrial pathway (Iqbal et al. 2017).

3.3.6 Capsaicin

It is obtained from red pepper. It exerts strong anticancer, anti-angiogenic, antimutagenic, anti-metastatic and chemopreventive functions in various cells, namely, pancreatic, prostatic, liver, skin, leukaemia, lung, bladder, colon and endothelial cells (Iqbal et al. 2017). Capsaicin regulates different molecular targets in breast cancer like caspase-3, reactive oxygen species (ROS), Rac1, HER-2, etc. (Chang et al. 2011). The p53 is considered as the "Grandfather of the genome." In the presence of p53 gene product, capsaicin induces apoptosis more strongly (Sarkar et al. 2015). Capsaicin produced apoptosis in breast cancer (H-Ras, MCF10A cells) by inducing ROS and Rac1 signalling pathways. Both of these pathways are induced explicitly by p38 and c-Jun N-terminal protein kinase-1 (Iqbal et al. 2017).

3.3.7 Vinca Alkaloids

The important plant-derived vinca alkaloid agents are vinblastine (VBL) and vincristine (VCR). They are obtained from *Catharanthus roseus* (Seca and Pinto 2018). Vinca alkaloids bind to the depolymerized microtubules and disrupt the microtubule assembly. VBL inhibits the process of angiogenesis (Ribatti et al. 2003). VBL is usually applied to treat breast cancer, germ cell tumours, Hodgkin's disease and non-Hodgkin's lymphoma. VCR binds to the end of the mitotic spindle and affects microtubule dynamics (Moudi et al. 2013). VCR is approved by FDA to treat acute leukaemia, Wilms tumour, neuroblastoma, Hodgkin's disease, rhabdomyosarcoma and other lymphomas (Moudi et al. 2013).

3.4 Concluding Remarks

Cancer in many cases is still not curable. However, some are manageable if diagnosed early. Many natural bioactive molecules have shown a promising effect in halting the cell cycle. Clinical trials in cancer do not test all of them. It is the time for urgent translational research to know the potential of these molecules for chemotherapeutic drug discovery.

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4

Flavones: Flavonoids Having Chemico-Biological Properties with a Preview into Anticancer Action Mechanism

Ajay Sharma, Hardeep Singh Tuli, Dharambir Kashyap, and Anil K. Sharma

Abstract

Flavones belong to the flavonoids class of plant polyphenols. Owing to their widespread distribution in plants, such as fruits, vegetables, herbs, spices, and beverages (tea, coffee, and wine), these compounds are consumed by human beings in large amounts through daily nutrition. This group of compounds occupies an inimitable position in the realm of natural, semisynthetic, and synthetic organic chemistry as well as biological sciences owing to their diversified valuable role in human health and their distinctive role in plants. Their structural features are responsible for the biochemical effects and therapeutic applications attributable to immune modulation and prevention of many diseases in humans. In this chapter, we address the requisite structural features of flavones for their biological and pharmacological significance in terms of structure activity relationship and chemical synthesis along with biosynthetic approaches and biological properties of some chemically modified derivatives. Also, the chapter highlights the mechanistic insight into the action of flavones mediating anticancer therapeutic effects.

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Keywords
Flavones · Structure-function relationship · Chemical synthesis · Biological properties · Anticancer effects · Mechanism

4.1 Introduction

Flavonoids are natural products that belong to the secondary metabolites phytochemical class having a polyphenolic structure and are currently consumed in huge quantities in daily nutrition. Among the flavonoids, flavones and flavonols delineate the enormous subgroups, and the versatile health benefits related to them have been reported in various studies. They are also liable for protecting and maintaining various properties of foods as well as plants, such as vivid color, taste, fat oxidation, vitamins, and enzyme preservation. Among these subclasses of flavonoids, flavones also play a significant role in plants and humans and possess various pharmaceutical, therapeutic, and nutraceutical properties due to their various biological actions, such as inhibition of allergic or inflammatory mediators, protection from abiotic and biotic stress conditions, inhibition of the NFκB pathway, interaction with nucleic acids and proteins, reduction of ROS, reduction of microRNA155 (miR155) expression, and agonists of ERRs (estrogen-related receptors) (Zhang et al. 2013; Moreira et al. 2017; Arredondo et al. 2015; Sharma et al. 2018a).

4.2 Structural Features

Structurally, flavone has a 2-phenylchromane nucleus (C_6–C_3–C_6) skeleton and it consists of two benzene rings (ring A and B) interrelated by a closed pyran ring (C ring) of a three carbon chain that amalgamates with ring A. Besides these features, a C_2=C_3 double bond and 4-Oxo group in ring C with the other groupings of multiple hydroxyls, –*O*-acetyl, –*O*-sulfate, –*O*-methyl, and –*C* or –*O*-glycoside group substituents are present on the basic skeleton of flavone. This class of compounds consists of aglycones, which are the basic structures of these compounds (apigenin, luteolin, chrysin, etc.), and their substituted congeners, such as sulfonated, acetylated, and methylated along with glycosylated derivatives (Fig. 4.1, Table 4.1) (Moreira et al. 2017; Arredondo et al. 2015; Singh et al. 2014; Teles et al. 2018; Correia-da-Silva et al. 2013). Apigenin, Luteolin, chrysin, and their different substituted congeners are abundantly extant in common fruits, vegetables, spices, and herbs, including parsley, onions, iceberg, celery, peppermint, thyme, celeriac, oranges, lettuce, tea, chamomile, wheat sprouts, etc. The most abundant flavones in the diet exhibit prolific biochemical effects against Cdk5 complexes, amyloid, protein kinase C, protein tyrosine kinases, adenylate cyclase, cyclooxygenases along with pharmacological effects, namely antioxidative, anticancer, anti-inflammatory, antitumor, antiviral, antibacterial, etc. (Funakoshi-Tago et al. 2011; Dundar 2015; Singh et al. 2014; Arredondo et al. 2015; Verma et al. 2012; Moreira et al. 2017;

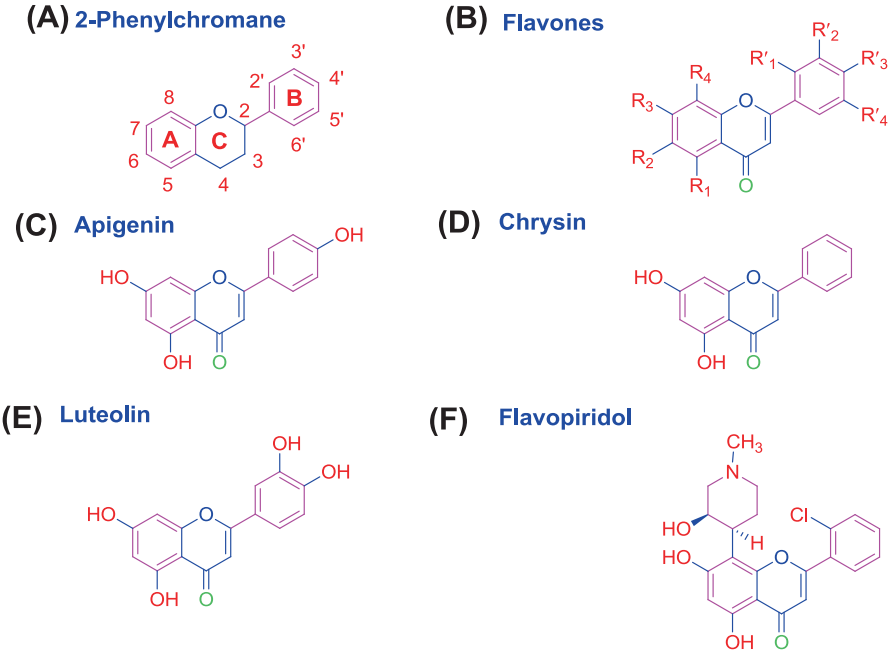

Fig. 4.1 Flavones consisting of the basic carbon skeleton (**a**) as well as substitution pattern in flavones subclass (**b**) with chemical structure of apigenin (**c**), chrysin (**d**), luteolin (**e**), and flavopiridol (**d**).

Zapata-Torres et al. 2004; Malisauskas et al. 2015; Zhang et al. 2013; Bhagwat et al. 2013; Southon et al. 1994; Teles et al. 2018; Correia-da-Silva et al. 2013). Natural flavones constitute an enormous segment of natural products and have a broad range of significant biological properties with low toxicity. The significance of flavones has led to the development of new compounds as magic bullets that possess significant biological and pharmacological properties. Flavoperidol (alvocidib) is a total synthetic flavone and is considered as a member of this family on the basis of a natural product (rohitukine) structure isolated from *Dysoxylumbinectariferum* Hook. f. (Meliaceae). It inhibits cyclin-dependent kinases CDK1, CDK2, and CDK4 and exhibits a potent inhibitory effect toward CDK9 (Cragg and Newman 2008; Zeidner et al. 2015; Zeidner and Karp 2015; Wiernik 2016). Several preclinical and clinical trials have been conducted for evaluating the significant benefits of flavoperidol alone or conjointly with other chemotherapeutic agents (thapsigargin, docetaxel, paclitaxel, gemcitabine, etc.) in treating chronic diseases (Srikumar and Padmanabhan 2016).

The chemical and structural features of flavones, such as hydroxyl (–OH) groups position, substitution of functional groups along with C_2=C_3 bond, are liable to interact with receptive sites or receptors in the tissue that are accountable for their biochemical and pharmacological properties. In general, the structural features of flavones and their relation with various therapeutic applications have been summarized as follows:

Table 4.1 The substitution pattern of hydroxyl, methoxyl, sulfate, and glycosides in different flavones

S. No	Compound	R_1	R_2	R_3	R_4	R'_1	R'_2	R'_3	R'_4
	Apigenin 7- sulfate	OH	H	OSO_3H	H	H	H	OH	H
	Apigenin 4′,7-Disulfate	OH	H	OSO_3H	H	H	H	OSO_3H	H
	Apigeninpersulfate	OSO_3H	H	OSO_3H	H	H	H	OSO_3H	H
	Acacetin	OH	H	OH	H	H	H	OCH_3	H
	Acacetin 7-sulfate	OH	H	OSO_3H	H	H	H	OCH_3	H
	Baicalein	OH	OH	OH	H	H	H	H	H
	Cirsilineol	OH	OCH_3	OCH_3	H	H	OCH_3	OH	H
	5-Demethyltangeretin	OH	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H
	Diosmetin	OH	H	OH	H	H	OH	OCH_3	H
	Diosmetin 3′ -sulfate	OH	H	OH	H	H	OSO_3H	OCH_3	H
	Dracocephaloside	OH	H	OH	H	H	O-β-D-Glucopyranoside	OH	H
	Eupafolin	OH	OCH_3	OH	H	H	OH	OH	H
	Eupatilin	OH	OCH_3	OH	H	H	OCH_3	OCH_3	H
	Eupatorin	OH	OCH_3	OCH_3	H	H	OH	OCH_3	H
	Flavone 8-acetic acid	H	H	H	CH_2CO_2H	H	H	H	H
	Gardenin B	OH	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H
	Glucolueolin	OH	H	O-β-D-Glucopyranoside	H	H	OH	OH	H
	Hispidulin	OH	OCH_3	OH	H	H	H	OH	H
	Jaceosidin	OH	OCH_3	OH	H	H	OCH_3	OH	H
	Luteolin 5-methyl ether	OCH_3	H	OH	H	H	OH	OH	H
	Luteolin 3′-methyl ether	OH	H	OH	H	H	OCH_3	OH	H
	Lutolin 7-Sulphate	OH	H	OSO_3H	H	H	OH	OH	H
	Luteolin 7,3′-disulfate	OH	H	OSO_3H	H	H	OSO_3H	OH	H
	Luteolin 7-sulfate-3′-rutinoside	OH	H	OSO_3H	H	H	O-rutinoside	OH	H

	Luteolinpersulfate	OSO_3H	H	OSO_3H	H	H	OSO_3H	OSO_3H	H
	Nobiletin	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	H
	Norwogonin	OH	H	OH	OH	H	H	H	H
	Orientin	OH	H	OH	β-D -Glucopyranosyl	H	OH	OH	H
	Rhoifolin	OH	H	O-Neohesperidoside	H	H	H	OH	H
	Skullcapflavone I	OH	H	OCH_3	OCH_3	OH	H	H	H
	Thalictiin	OH	H	O-β-D-Galactopyranoside	H	H	H	OH	H
	Tricetin	OH	H	OH	H	H	OH	OH	OH
	Tricetinpersulfate	OSO_3H	H	OSO_3H	H	H	OSO_3H	OSO_3H	OSO_3H
	Tangeretin	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H
	Wogonin	OH	H	OH	OCH_3	H	H	H	H
	Veronicastroside	OH	H	O-Neohesperidoside	H	H	OH	OH	H

1. These three key features are considered to be vital for their antioxidant activity as has been established during the structure–activity studies of flavones (Cotelle et al. 1996; Leopoldini et al. 2004):
   (a) The number of hydroxyl groups on the B ring and their configuration predicts the activity, i.e., the catechol moiety (1,2-dihydroxybenzene) or either hydroquinone moiety (1,4- dihydroxybenzene) or galloyl moiety (1,2,3-trihydroxybenzene),which causes the formation of the phenoxyl radical after the H atom donation and attributes to the high stability of the flavonoid due to the electron delocalization.
   (b) The configuration of ring C, i.e., the C_2=C_3 double bond and C_4-oxo group, permits the electron movement from the phenoxyl radicals (B ring) to the C ring;
   (c) The C_2=C_3 double bond upsurges the resonance stabilization of the molecule due to the electron displacement across it.

   Cotelle et al. have reported the antioxidant properties either by the capacity to scavenge free radicals (ring B hydroxyl groups) or to competitively inhibit xanthine oxidase (ring A hydroxyl groups). The presence of a catechol or a galloyl type moiety on the B ring appeared essential for scavenging properties, while the 3-position hydroxyl substitution with B ring substitution also displayed scavenging properties but to a lower degree. When flavones possess the hydroxyl group at position 7 in the absence of catechol or pyrogallol groups, inhibitory activity of xanthine oxidase was observed. Similarly, 6-OH substituted flavones are known to be moderately active. The existence of hydroxyl groups in the B ring, as in flavones (apigenin, luteolin), improved the nitric oxide (NO) scavenger and advanced glycation end products (AGEs) inhibition effects, while the OH group at C_3 position of ring C, as in flavonols (quercetin), was not found favorable (Crasci et al. 2018). In another report, the existence of a free catechol group in ring B and free hydroxyl (–OH) groups at positions C_5 and C_7 on ring A was found to be liable for inhibition of xanthine oxidase (XOD) activity. Luteolin showed higher XOD inhibitory activity than luteolin-6-C-glucoside, while apigenin glycoside (apigenin-6-C-glucoside-8-C-arabinoside) exhibited higher activity than the free apigenin (aglycone form). Steric effects are also found to have a stronger influence on the chemical action of flavones.
2. Casagrande and Darbon have studied the effects of various flavonoids on cell proliferation as well as cell cycle distribution in human melanoma (OCM-1) cells. The occurrence of the C_2=C_3 bond and oxy functional group at C_4 position of ring C were reported to be required for higher antiproliferative activity. Among these compounds, the existence of a hydroxyl group (–OH) at the $C_{3'}$ position of ring B (luteolin) was reported to arrest cells in the G1 phase inhibiting CDK2, while lack of this group (apigenin) blocked cells in G2 inhibiting CDK1. Both CDK2 and CDK1 were reported to be directly inhibited by flavopiridol (Casagrande and Darbon 2001).
3. The anticancer as well as anti-inflammation effects of flavonoids owing to their pro-oxidant action and electrophilic conjugation interaction with biomolecules

caused the oxidation of flavonoids into electrophilic natured quinones (*o*-quinones/*p*-quinones). These electrophilic quinone structures are very reactive toward nucleophilic groups of biomolecules, such as thiol and amino groups (proteins, glutathione). The valuable biological effects of flavonoids are also assumed due to the formation of different addition adducts by the reaction of electrophilic quinones and nucleophilic groups of biomolecules. The functionalities on the B ring, such as catechol moiety (ortho-dihyroxy), hydroquinone moiety (para-dihydroxy) or galloyl moiety (1,2,3-tridydroxy), in flavones has an imperative role in formation of electrophilic quinones through oxidation, whereas resorcinol (meta-dihydroxy) cannot readily undergo oxidization. The basic structural features, such as the occurrence of C_2=C_3 double bond, hydroxyl groups on ring A and B specifically, C_5–OH, C_7–OH, and $C_{4'}$ –OH group are liable for anti-inflammatory activity. The hydroxyl groups at ring B either on the C2′ or C3′ position reduced the activity, while the C5′ –OH group or C4′–OCH_3 on ring B abolished the activity. The hydroxy derivatives have been reported to have more potency than their corresponding methoxy derivatives (Sharma et al. 2018a; Arivudai et al. 1996; Ravishankar et al. 2013; Batra and Sharma 2013). The flavone glycosides (apigenin and luteolin 7-*O*-glucoside) were found to have no effect on TNF-α release or NF-κB activity, while the respective aglycones showed higher efficacy to reduce the above activities. Therefore, the deglycosylation enhances the absorption of dietary flavones and modulates inflammation by decreasing TNF-α and NF-κB. The compounds have the ability to inhibit NF-κB and possess both anti-inflammatory and anticancer properties (Hostetler et al. 2012). Paredes-Gonzalez et al. have described that apigenin and luteolin remarkably activate the PI3K/Nrf2/ARE system and are known to be responsible for their anti-inflammatory effects, as indicated by the suppression of lipopolysaccharide induced nitric oxide (NO), nitric oxide synthase (iNOS), and cytosolic phospholipase A2 (cPLA2). These compounds appreciably inhibited TNFα-induced NF-κB transcriptional activation, whereas they have no effect on the degradation of IκB proteins, and the nuclear translocation and DNA binding activity of NF-κB p65 was observed (Paredes Gonzalez et al. 2015).

4. Flavonoids may show a defensive role against cancer, cardiovascular diseases, and age-related degenerative diseases. They have the ability to interact with several efflux pump proteins, such as P-gp (P-glycoprotein), multidrug resistance proteins (MRP1 and MRP2), breast cancer resistance protein (BCRP), and uptake transporters, including organic anion-transporting polypeptide (OATP), organic anion transporter (OAT), and monocarboxylate transporters (MCTs) (Wang and Morris 2014).
   (a) P-gp is a member of a multidrug resistant protein family and effluxes anticancer agents from tumor cells as an energy dependent pump. The flavonoids have the ability to inhibit P-gp activity, and they are probable agents for modulation of multidrug resistance. The presence of ring B at the C_2 position and C_2=C_3double bond of ring C, as in flavones and flavonol molecules, may be responsible for the intercalation with the hydrophobic amino acid residues of P-gp. The apigenin and quercetin had greater binding affinity in comparison

with genistein, naringenin or rutin. P-gp modulators, NBD2 (C-terminal nucleotide-binding domain) contain an ATP-binding site and a close but distinctive hydrophobic steroid RU486-binding site. The SAR studies using NBD2 and cell lines recommend the presence of a double bond (planar structure), i.e., 2–3hydroxyl groups (3 and 5) and hydrophobic substituents on the A or B rings. These features are responsible for high P-gp-modulating activities, while the glycosylation causes decreased potential against the above activities (Kitagawa 2006; Zandena et al. 2005; Wang and Morris 2014). Luteolin is known to induce apoptosis in P-glycoprotein and ABCG2-expressing MDR cancer cells without any change in the transport functions of these drug transporters. It induces apoptosis and involves ROS generation, DNA damage, inhibition of NF-kB signaling pathway, activation of ATR → Chk2 → p53 signaling pathway, activation of p38 pathway, and depletion of antiapoptotic proteins. The analysis of luteolin also acknowledged specific molecular characteristics of NCI-ADR/RES and MCF-7/Mito^{R} cells that highlight their different tissue origins having therapeutic prospective to control the proliferation of MDR cancers without disturbing the physiological role of drug transporters in the body tissues (Rao et al. 2012).

(b) The structure–activity relationship regarding potency in modulating MRP1 activities specified that flavones and flavonols were more effective than flavanols, flavanolols, flavanones, and isoflavones. The glycosylation of flavonoids leads to a decline in the inhibitory activity. The required structural characteristics of flavones for high MRP1 inhibitory effectiveness are (i) the existence of two to three double bonds for a planar molecular structure, (ii) the existence of OH group at C3′ and C4′ position of the B ring, and (iii) the hydrophobic groups substitution at C4′–OH group of the B ring. The flavones with a pyrogallol group (1,2,3-trihydroxy group) on the B ring showed MRP2 inhibition (Wang and Morris 2014). Recently, Seo et al. reported that apigenin was able to oppose the drug resistance against the adriamycin-resistant breast cancer cells (MCF-7/ADR) and significantly reduced cell growth and colony formation in MCF-7/ADR cells and parental MCF-7 (Michigan Cancer Foundation-7) cells. It suppressed the mRNA expression of MDR1 and MRPs (multidrug resistance-associated proteins) along with the protein expression of P-gp (MDR1) and inhibited the production of VEGF (vascular endothelial growth factor) and MMP-9 (Matrix metallopeptidase 9), which are STAT3 (signal transducer and activator of transcription 3) target genes, in MCF-7/ADR cells. The STAT3 inhibitor S3I-201, JAK (janus associated kinase) inhibitor I, and the HIF-1α (hypoxia-inducible factor 1-alpha) inhibitor EF-24 decreased the growth of both MCF-7 and MCF-7/ADR cells (Seo et al. 2017).

(c) The higher BCRP inhibitory potential of flavonoids is because of the planarity of molecular structure owing to the existence of two or three double bonds. In addition to this, the OH group at the C_5 position of ring A and absence of this group at position C_3 of ring C as well as the ring B bonding site at C_2 position of ring C causes the enhancement of potential against inhibition of BCRP. However, the hydroxyl (OH) groups at 6, 7, 8- or 4′

position substituted with hydrophobic groups also increase inhibitory potency against BCRP, while the glycosylation reduces the BCRP-inhibiting activities (Wang and Morris 2014).

From the highlights of SAR studies, the presence of free hydroxyl groups, 4-oxo group along with the C_2=C_3 double bond of flavones are requisite features for their enzyme inhibitory activity as well as for the antioxidant activity, which suggests that this class of compounds could be attractive leads for anticancer therapies.

4.3 Biosynthesis

Biosynthetically, flavones are synthesized through the phenylpropanoid metabolic pathway (Ibrahim 2001a) from cinnamoyl-CoA, 4-coumaroyl-CoA, and caffeoyl-CoA produced from the amino acid phenylalanine, which is synthesized via the shikimate pathway (Morreel et al. 2006; Herrmann and Entus 2001; Ibrahim 2001b). CHS is a pivotal enzyme in the biosynthetic pathway of flavonoids to produce the main backbone intermediate of flavonoids commonly entitled chalcone. The CHS enzyme causes the condensation of the malonyl-CoA with either cinnamoyl-CoA, 4-coumaroyl-CoA or caffeoyl-CoA leading to the respective pinocembrin, naringenin, and eriodictyolchalcones. These are common intermediates that stereospecifically and spontaneously cyclize into respective pinocembrin, naringenin, and eriodictyol by the action of CHI. Naringenin was also converted into eriodictyol by the action of F3′H, which carried out the hydroxylation at C3′ position of naringenin. In the last step, these derivatives transformed into respective flavones by the origination of a double bond between the C_2 and C_3 positions of ring C catalyzed by FNS (Martens and Mithofer 2005; Mizuno et al. 2016; Winkel-Shirley 2001) (Fig. 4.2). The biosynthesis of flavones in *Escherichia coli* has been successfully reported by Miyahisa et al. The four genes of *Escherichia coli* cells, i.e., phenylalanine ammonia-lyase (PAL), cinnamate/4-coumarate-CoA ligase (CNL/4CL), chalcone synthase (CHS), chalcone isomerase (CHI), and acetyl-CoA carboxylase (ACC), have been used for the production of naringenin from tyrosine and pinocembrin from phenylalanine. The flavones synthase I gene from *Petroselinum crispum*, apigenin from naringenin, and chrysin from pinocembrin were successfully isolated previously (Miyahisa et al. 2006). The biosynthesis of luteolin in *Escherichia coli* and *Saccharomyces cerevisiae* has been reported from p-coumaric acid and malonate, or caffeic acid, respectively (Leonard et al. 2005; Leonard et al. 2008). Marin et al. have also reported the biosynthesis of apigenin from naringenin and luteolin from the apigenin by the action of F3′H hydroxylase in *Streptomyces albus.*

In 1939, Hutchins and Wheele reported the first reliable chemical synthesis of chrysin, apigenin, and luteolin. This method involves the reaction of substituted 2-hydroxy-4,6-dimethoxyacetophenone (I) and benzaldehyde(II a-c) in ethanolic KOH, which resulted in the formation of intermediate chalcones (III). The bromination of compounds (IIIa-c) gave rise to brominated ketones (IVa-c) by using bromine and carbon disulfide. The brominated ketones undergo cyclization by the action of potassium cyanide or at higher temperature. The demethylation and

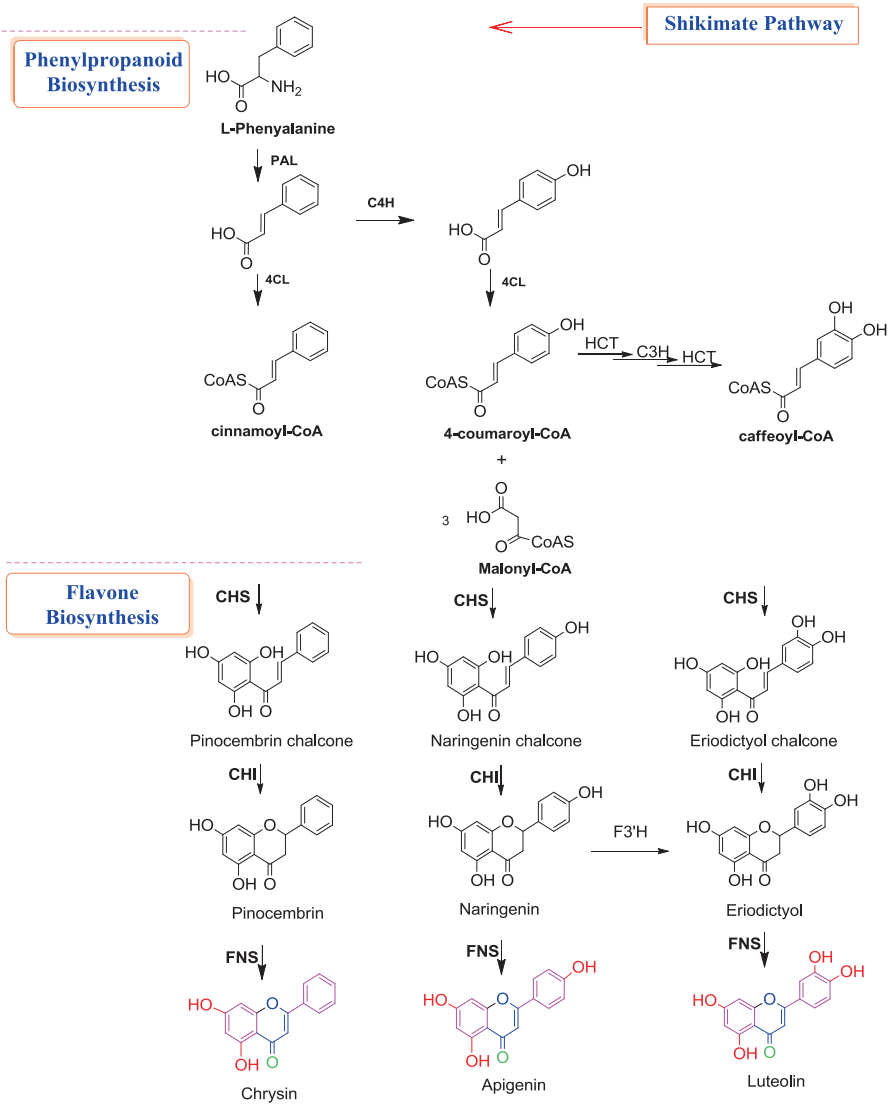

Fig. 4.2 Flavones biosynthesis pathway. The enzymes involved in the biosynthesis; phenylalanine ammonia-lyase (PAL); cinnamate-4-hydroxylase (C4H); 4-coumaroyl-CoA-ligase (4CL); *p*-coumarate 3-hydroxylase (C3H); *p*-hydroxy cinnamoyl-CoA: shikimate/quinate *p*-hydroxycinnamoyl transferase (HCT); chalcone synthase (CHS); chalconeisomerase (CHI); flavone synthase, cytochrome P450 flavone synthase (FNS)

debromination of cyclized products (Va-c) were carried out with hydroiodic acid in acetic anhydride which resulted the desired flavone products (Figs. 4.3 and 4.4). Yang and his group have also reported the synthesis of flavone (chrysin, apigenin, and luteolin) through the chalcone intermediate pathway without the bromination step. The cyclization of chalcones resulted the methylated derivative by the action

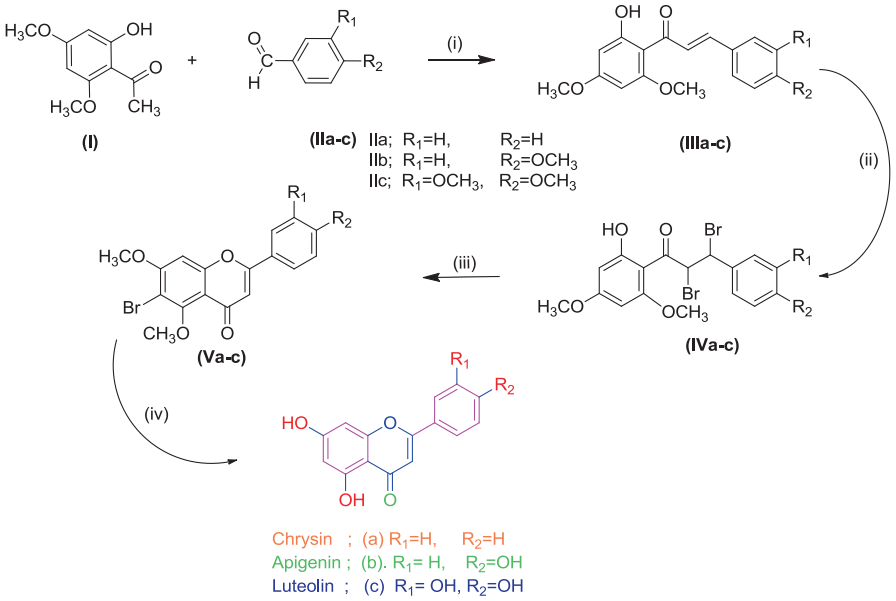

Fig. 4.3 General synthetic reaction scheme of flavones (chrysin, apigenin, luteolin) via Hutchins and Wheele's approach using 2-hydroxy-4,6-dimethoxyacetophenone (I) and benzaldehydes(IIa-c) as starting materials; (i) KOH, C_2H_5OH, (ii) Br_2, CS_2, (iii) 195 0C or KCN, C_2H_5OH, (iv) Ac_2O, HI, reflux

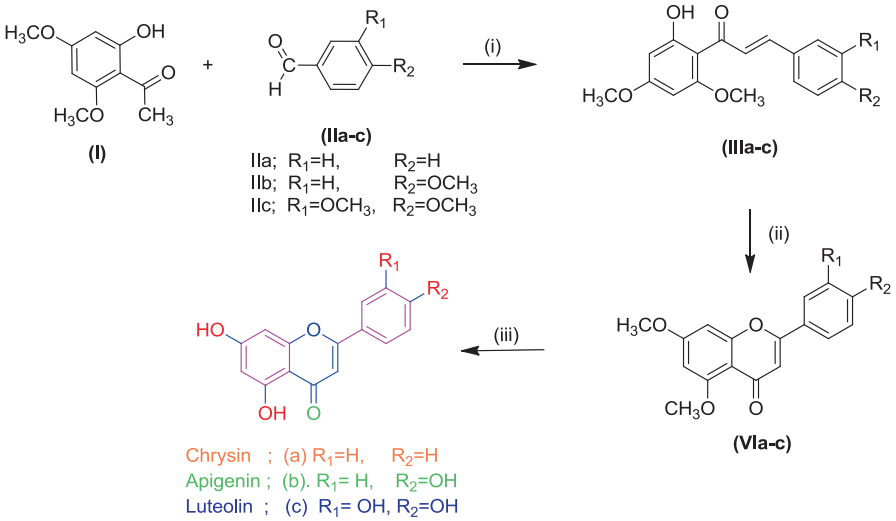

Fig. 4.4 General synthetic reaction scheme of chrysin, apigenin, and luteolin by using 2-hydroxy-4,6-dimethoxyacetophenone (I) and benzaldehydes (IIa-c) as starting materials; (i) KOH rt., (ii) DMSO, I_2, 120 °C, (iii) Py·HCl, 180 °C

of iodine with dimethylsulfoxide and then demethylation using pyridine hydrochloride led to aglycones (Wang et al. 2015; Liu et al. 2014; Zhang et al. 2014). The synthesis of flavopiridol was proposed on the basis of the reports in which compounds had a methyl group in place of a chlorophenyl ring. The proposed scheme involves the replacement of a methyl group with a chlorophenyl ring. The first step involves the reaction of trimethoxybenzene with *N*-methylpiperidone. After this step, the hydroxylation and stereocenter generation at position 3 of the piperidine moiety of trimethoxybenzene-1-methylpiperidine were carried out by using various reaction conditions, such as action of diborane, sodium borohydride, hydrogen peroxide, etc. The acetylation at 3 position of benzene ring of the intermediate was achieved by acetic anhydride and then treated with methyl 2-chlorobenzoate. The deprotonation of methoxy groups of ring A into hydroxyl groups was done by using a mixture of pyridine hydrochloride and quinolone (Kattiger et al. 1990; Naik et al. 1994) (Fig. 4.5). However, there is still a need to develop novel methods and use of existing synthetic procedures (Sharma et al. 2014a, b; Khare et al. 2016) along with reagents (Sharma et al. 2014c, 2015) for modifying the structure of bioactive molecules with improved pharmacological significance. The apigenin derivative (Ap1) possessed the strongest activity with IC50 values of 2.03 ± 0.22 μM against

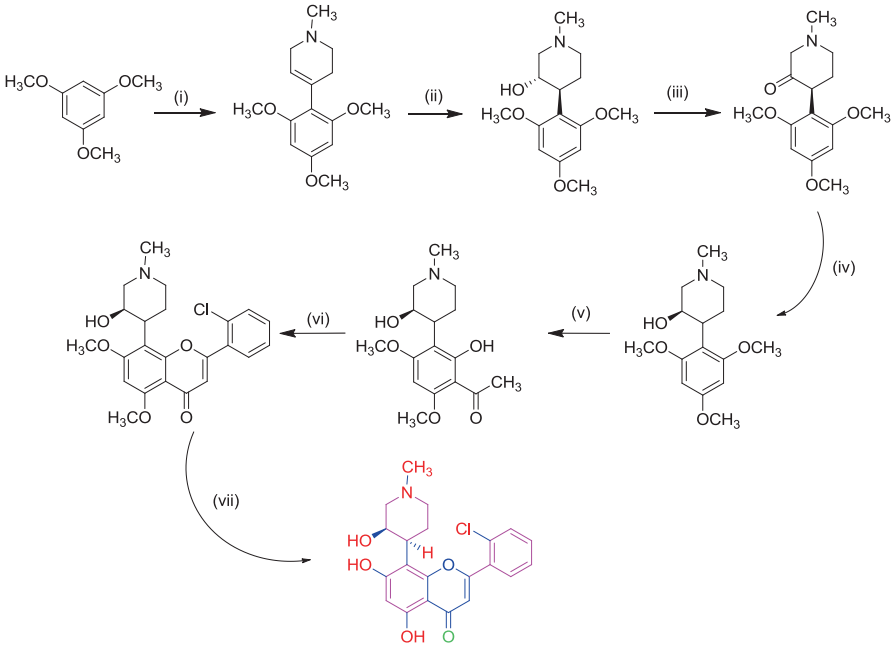

Fig. 4.5 General synthetic scheme of flavopiridol from the trimethoxy benzene via multistep synthesis; [(i); (**a**) *N*-methylpiperidone, CH_3CO_2H, (**b**) HCl gas]; [(ii); (**a**) BF_3 –OEt, Diglyme, $NaBH_4$, HCl (**b**) NaOH, H_2O_2]; (iii) CO_2Cl_2, CH_2Cl_2, DMSO, N_2, $(C_2H_5)_3N$ (iv) $NaBH_4$, C_2H_5OH (v) BF_3–OEt, CH_2Cl_2, Ac_2O (vi) methyl-2-chlorobenzoate, NaH, dioxane (vii) pyridine hydrochloride, quinolone

colorectal adenocarcinoma (HT-29) cell line and 2.25 ± 0.42 μM against leucocythemia (HL-60) cell line, which are better than 5-FU (12.92 ± 0.61 μM, 9.56 ± 0.16 μM) (Zheng et al. 2014). Liu et al. reported the chemically modified apigenin derivative (Ap2), which showed notable antiproliferative activity against human cervical (HeLa), human breast (MCF-7), human lung (A549), and human hepatocellular liver (HepG2) cancer cells lines, with the lowest IC_{50} values compared to apigenin. The chrysin derivatives (Ch1, Ch2, and Ch3) displayed the strongest activity in vitro against SGC-7901 (human gastric adenocarcinoma) and HT-29 (colorectal adenocarcinoma) cell lines with the lowest IC_{50} values (Zheng et al. 2003). Zhang et al. reported the phosphorylated chrysins ($IC_{50}$of Ch4= 10.3 μM and $IC_{50}$of Ch5=9.8 μM) were more potent and inhibited proliferation as well as induced apoptosis in HeLa cells compared to chrysin (IC_{50} =14.2 μM) (Zhang et al. 2004). In another report, the chrysin derivative (Ch6) possessed stronger activity when tested in vitro against HCT-116 (human colon), Hela (human cervical carcinoma), DU-145 (human prostate), K562 (human leukemia), and SGC-7901 (human gastric) cancer cell lines compared to 5-flourouracil and chrysin (Fig. 4.6).

4.4 Mechanistic Insight into Flavones Mediated Anticancer Effects

In the past few decades, the scientific community has revealed the immense potential of flavonoids in the treatment of dreadful diseases such as cancer (Kashyap et al. 2016a, b, 2017, 2018a, b; Sharma et al. 2018a, b). It is necessary to understand the interactions of such natural molecules with the recognized cellular target (Kashyap et al. 2016c, d, e, f, 2018b, c, d; Kashyap and Singh Tuli 2018). Flavones, such as apigenin, luteolin, chrysin, and flavoperidol, have been known to mediate both intrinsic (mitochondrial) as well as extrinsic (Fas/FasL) apoptotic cell death in cancer cells. In a study, chrysin was found to induce apoptosis in U937 cells by activating caspase 3 and the protein kinase B (Akt) signal pathway (Woo et al. 2004). Similarly, flavopiridol has shown promising in vitro anticancer activity against human chronic (CLL) lymphocytic leukaemia cells via activation of caspase-3, independently of Bcl-2, interleukin-4 (IL-4), or p53 modulation (Byrd et al. 1998). Using human hepatoma HepG2 cells, Lee et al. (2005)investigated the apoptosis inducing effect of luteiolin via translocation of Bax/Bak as well as via activation of c-Jun N-terminal kinases (JNK). Previous studies have suggested the role of these bio-metabolites to arrest the cell cycle by regulating the expression of cyclin dependent kinases (CDKs) in addition to apoptosis. It was found that flavones and flavonols caused G2/M arrest by enhancing the expression of growth arrest and DNA-damage-inducible gene β (GADD45β), 14-3-3σ and suppressing cyclin B1 in OE33 cells (Zhang et al. 2008). In another study, Zhang et al. (2009), investigated dose and time-dependent anticancer effects of flavones (luteolin, apigenin, and chrysin) and flavonols (quercetin, kaempferol, and myricetin) in human oesophageal squamous cells (KYSE-510). Mechanistic insight revealed that higher expression of p63 and p73 proteins was found to be associated with modulation of cell

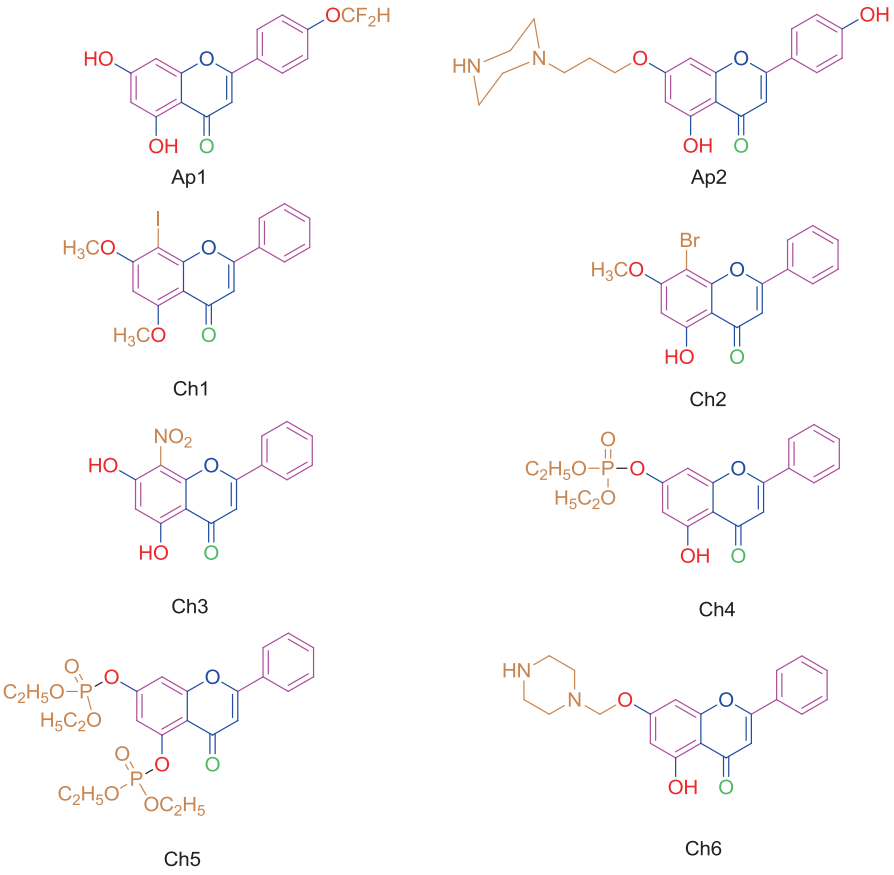

Fig. 4.6 Some of the chemically modifying derivatives of apigenin and chrysin possessing significant anticancer activities

cycle regulation via p21waf1, cyclin B1, and PIG3. Also, the expression of metastatic proteins, including matrix metallo-proteases (MMPs), has been down-regulated by these bio-metabolites. Results revealed that flavones treatment on oral squamous cell (OSCC) carcinoma led to down regulation of the expression of MMP-2 and urokinase plasminogen activator (u-PA) along with modulation of their endogenous (TIMP-2 and PAI-1) inhibitors (Yang et al. 2008). Similarly, using triple negative breast cancer (TNBC) cells, Yang et al. (2014) investigated the anti-metastatic effect of crysin via MMP-10, epithelia to mesenchymal transition (EMT), and phosphatidyl inositol 3-kinase (PI3K)/Akt pathway. Metastasis is further supported by angiogenesis, and these metabolites are well documented to inhibit neovascularization in the microenvironment of tumors. Chrysin suppresses IL-6-induced angiogenesis through modulation of the sIL-6R/gp130/JAK1/signal transducer and activator of transcription 3 (STAT3)/vascular endothelial growth factor (VEGF) signaling pathway (Lin et al. 2010). The mechanism-based antiangiogenic potential of

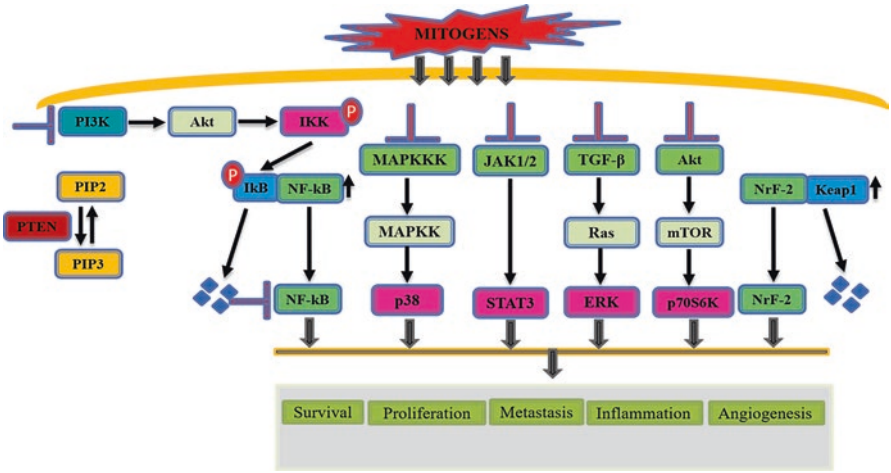

Fig. 4.7 Signaling mechanisms governed by flavones in cancer. Flavones modulate various growth factors that are involved in the signaling of both intrinsic and extrinsic apoptosis, cell cycle arrest, antimetastasis, antiangiogenesis, and anti-inflammation

other flavonoids was also observed as vascular endothelial growth factor receptor (VEGFR) and multi-kinase inhibitors of endothelial cells (Geetanjali et al. 2014). Another antitumor aspect of these metabolites can be correlated with their inhibitory effects on inflammatory mediators, such as IL-6, IL-8, interferon γ (IFN-γ),inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrotic factor-α (TNF-α) (Chen et al. 2014; Kanai et al. 2016; Lee et al. 2016). Exploring the mechanistic insight on the mode of actions of such bioactive metabolites will help to understand the biology of cancer and further stimulate the scientific community to design novel anticancer strategies in the near future (Fig. 4.7).

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Cancer Chemoprevention by Dietary Polyphenols, Flavonoids, Terpenoids, and Saponins

5

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Abstract

Cancer chemoprevention invokes the adoption of natural or man-made agents for the inhibition, delay, or reversal of carcinogenesis before an invasion. It is predicted that roughly one-third of all cancer deaths might be prevented through proper dietary alteration. Chemopreventives should be defined by low toxicity in therapeutic drugs and the possibility of an oral administration. Several epidemiological studies and preclinical evidence indicate that various nutraceuticals and dietary supplements display chemopreventive properties, which is well supported by in vitro and animal studies. Diet derived compounds widely investigated for their chemopreventive activity mostly belong to a class of polyphenols, flavonoids, terpenoids, or saponins. A well-balanced diet is an excellent source of macronutrients, micronutrients, and phytochemicals and can diminish the risk of cancer as well as provide cancer preventive activity.

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Keywords
Dietary phytochemicals · Cancer · Chemoprevention · Epidemiology · Epigenetics · Apoptosis

5.1 Cancer Chemoprevention by the Dietary Phytochemicals

Cancer ranks as one of the top leading causes of morbidity and mortality worldwide, with roughly 18.1 million new cancer cases and 9.6 million cancer deaths in 2018. It is also expected that the number of cases will increase by about 70% over the ensuing two decades reaching 22 million annual cases (Bray et al. 2018; McGuire 2016). Carcinogenesis is a mechanism by which a normal cell is transformed into a cancer cell. This is due to the mutation and epimutation of the genetic material of normal cells, which agitates the harmony between proliferation and apoptotic cell death. This causes uncontrolled cell proliferation and the formation of cancer. Chemoprevention is action taken to thoroughly cut-off or lower the chance of getting cancer by actively intervening in the course of carcinogenesis (Zhang et al. 2017; Al Rabadi and Bergan 2017). Scientists are investigating many different ways to support the prevention of cancer, including the following:

- Avoiding or controlling cancer causing things.
- Managing changes in diet and lifestyle.
- Early detection of precancerous conditions that may lead to cancer.
- Chemoprevention (medicine to stop or reduce cancer conditions from the initial stage).
- Risk-reducing surgery.

An abundance of data suggests that lifestyle factors, along with exposure to chemical carcinogens, diet, and lack of physical activity play a pivotal role in the advancement of common cancers. It has been predicted that appropriate lifestyle modifications could prevent more than two-thirds of human cancers.

Diet is closely linked to the incidence and prevention of different cancer types and dietary behavior has been diagnosed as one of the most significant modifiable lifestyle impetus of cancer risk. Human cancer deaths attributable to diet make up nearly 10–70% (average 35%). Therefore diet, together with a healthy lifestyle, can reduce cancer incidence by 30–40%. In fact, most authors agree that there is consistent epidemiological evidence to suggest that a diet rich in fruits or vegetables significantly rolls back the risk of certain disorders, such as cancer and cardiovascular diseases (De Stefani et al. 2000).

In consequence, several international organizations, like the World Cancer Research Fund (WCRF), American Institute for Cancer Research (AICR), and other various cancer research foundations, endorsed a boost in the ingestion of certain fruits, vegetables, and grains as their incorporation in diet is associated with a reduced risk for the spreading of certain tumors and cancers (Mosby et al. 2012; Gapstur et al.

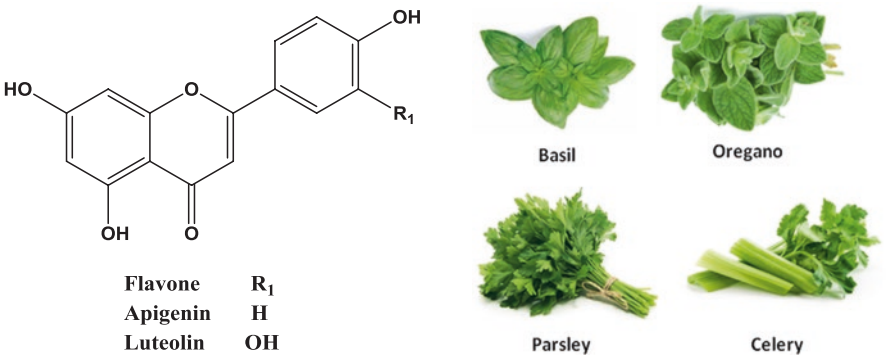
2018). The National Cancer Institute (NCI) has picked out about 35 plant-based foods with cancer-preventive properties. Foods and herbs possessing these qualities include garlic, cabbage, soybeans, licorice root, ginger, and the umbelliferous vegetables (including celery, carrots, coriander, parsnips, and parsley). Additional foods with anticancer activity include citrus, onions, flax, turmeric, cruciferous vegetables (Brussels sprouts, broccoli, cabbage, and cauliflower), sweet peppers and tomatoes, brown rice, oats, barley, whole wheat, various herbs, such as rosemary, mints, thyme, sage, oregano, and basil, cantaloupe, cucumber, and berries (Wiseman 2018; Surh 2003). Plenty of secondary plant products, such as polyphenols (Thomasset et al. 2007), terpenoids (Rabi and Gupta 2008), saponins (Raju and Mehta 2009), flavonoids (Galati and O'Brien 2004), carotenoids (Tanaka et al. 2012). etc., which are substantial constituents of our daily food, have thus transformed themselves from being considered as non-nutritive constituents to possibly cancer preventive ones.

5.2 Occurrence of Polyphenols, Flavonoids, Terpenoids, and Saponins in Diet

Dietary phytochemicals are divided into four main classes according to their chemical structures: polyphenols, terpenoids, alkaloids, and sulfur compounds. Among them, dietary polyphenols and flavonoids are the rich antioxidants in human diets (Table 5.1). They are further subdivided into groups based on the number of

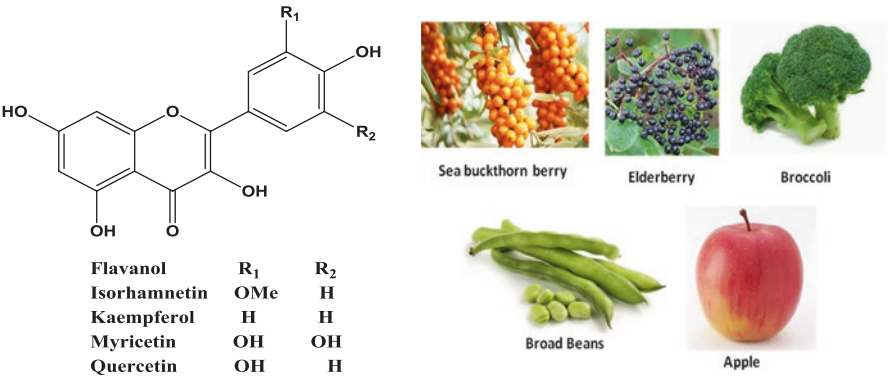
Table 5.1 Dietary sources of polyphenols

Subclass	Compounds	Primary source
Flavonols	Quercetin, myricetin, kaempferol, rutin, isorhamnetin	Vegetables: capers, chives, celery, onions, red onions, lettuce, dock leaves, broccoli, hartwort leaves, kale
		Fruits: apricots, apples, grapes, plums, bilberries, blueberries, blackberries, cranberries, olive elderberries, black currant juice, currants, cherries, apple juice
		Drink: red wine, tea (green and black)
Flavanones	Hesperin, hesperidin, naringin, eriodictyol, naringenin	Citrus fruits and juices: orange, orange juice, lemon, lemon juice, lime juice, grapefruit, tangerine juice
Flavan-3-ols	Catechin, epicatechin, galloylated derivatives	Tea, apple, plums, cranberries, berries, chocolate
Flavones	Luteolin, apigenin	Fruits: olives, celery
		Vegetables: hot peppers, fresh parsley, celery hearts
		Spices and herbs: oregano, dry parsley, rosemary, thyme
Anthocyanins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin (mostly as glycosides)	Fruits: Cherries, blackberries, black currants, blueberries, elderberries, black grape, strawberries, plums, cranberry, raspberry, pomegranate juice



Flavone	R_1
Apigenin	H
Luteolin	OH

Fig. 5.1 Chemical structure of flavones and their dietary sources



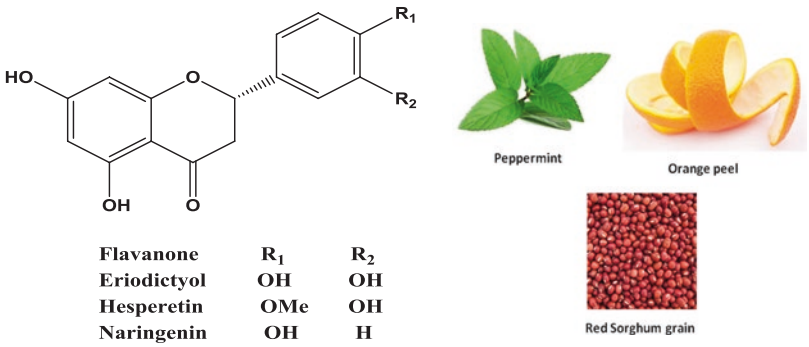
Flavanol	R_1	R_2
Isorhamnetin	OMe	H
Kaempferol	H	H
Myricetin	OH	OH
Quercetin	OH	H

Fig. 5.2 Chemical structure of flavonols and their dietary sources

phenolic rings that link the structural elements: (1) The phenolic acids, which have subclasses derived from different hydroxybenzoic acids, such as gallic acid and hydroxycinnamic acids, containing ferulic, caffeic, and coumaric acids; (2) the giant flavonoid subclass, which consists of the flavones (Fig. 5.1), flavonols (Fig. 5.2), flavanones (Fig. 5.3), flavanols (Fig. 5.4), and anthocyanidins (Fig. 5.5); (3) the stilbenes; and (4) the lignans and their polymers (Lin et al. 2016).

The richest flavonoids in the diet are flavanols (catechins plus proanthocyanidins), anthocyanins, and their oxidation analogues. The leading dietary sources of polyphenols include some common fruits and beverages (tea, coffee, fruit juice, wine, beer, and chocolate) and, to a lesser extent, dries legumes, vegetables, and cereals as shown in Table 5.1 (Bhagwat et al. 2013).

Terpenoids (Figs. 5.6 and 5.7) and Saponins (Fig. 5.8) occur in a wide range of plants but only a few of them are frequently used as food by humans. The more regularly consumed ones are spinach, soybeans, chickpeas, and peanuts. Many different saponins can exist within a sole plant species. Common dietary sources of saponins are soybeans (5.6%), lentil (4%), broad bean (3.7%), chickpeas (3.6%), garden peas (2.5%), and lucerne/alfalfa (2–3%) (Guclu-Ustundag and Mazza 2007).



Flavanone	R_1	R_2
Eriodictyol	OH	OH
Hesperetin	OMe	OH
Naringenin	OH	H

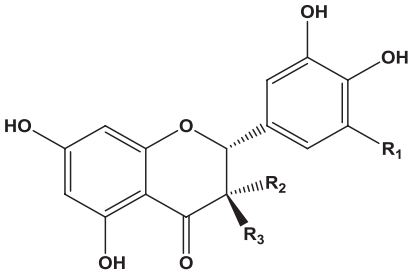
Fig. 5.3 Chemical structure of flavanones and their dietary sources

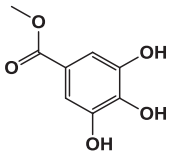
5.3 Dietary Phytochemicals and Cancer

5.3.1 Epidemiological Evidence

Epidemiological studies and systematic analyses have shown the response of diet on health, and the relation between the utilization of certain foods and a marked down risk of some chronic diseases like cancer. In this view, many exercises have proven the potential of dietary phytochemicals as anticarcinogenic agents during study using different cell lines, animal models, and human epidemiological data (Scott et al. 2009; Kapinova et al. 2018). Dietary phytochemicals may inhibit numerous stages in the carcinogenesis mechanism and hence prevent or hold up tumor development. Although induction of apoptosis looks to be rather specific for the cancer cells, it may perhaps be mentioned that certain human studies have exposed no useful effects (Table 5.2). The Korean Population Cohort Study showed that a higher intake of flavonols and flavan-3-ols can slow down the risk of colorectal cancer (Cho et al. 2017). In a Netherlands Cohort Study involving 20,852 people, decreased colorectal cancer risk in normal wt. women was observed on consumption of a flavonol and catechin rich diet (Simons et al. 2009). Similarly, a decreased risk for esophageal, gastric, and prostate cancers have been reported after the consumption of anthocyanidin (Petrick et al. 2015). Also several epidemiological studies showed the pull down of different types of cancer risk after consumption of quercetin, myricetin, catechins, green tea, soy and many more dietry phytochemicals.

In summary, epidemiological and nutritional intervention studies data suggest that high consumption of dietary foods rich in polyphenols, flavonoids, terpenoids, and saponins may shorten the flourishing risk of several types of cancers (breast, colon, rectal, gastric, pancreatic, lung, ovarian, and prostatic cancer). This promising finding has motivated scientists to explore the molecular mechanisms involved in the antitumor actions in order to validate the value for cancer treatment. Various in vitro and in vivo investigations have provided broad evidence to prevent carcinogenesis and to suppress tumorigenesis over different molecular mechanisms.





Gallate

Flavan-3-ol	R_1	R_2	R_3
(+)-Catechin (C)	H	H	OH
(+)-Catechin-3-gallate (CG)	H	H	Gallate
(-)-Epicatechin (EC)	H	OH	H
(-)-Epicatechin-3-gallate (ECG)	H	Gallate	H
(-)-Epigallocatechin (EGC)	OH	OH	H
(-)-Epigallocatechin-3-gallate (EGCG)	OH	Gallate	H
(+)-Gallocatechin (GC)	OH	H	OH
(+)-Gallocatechin-3-gallate (GCG)	OH	H	Gallate

Barley

Cocoa beans

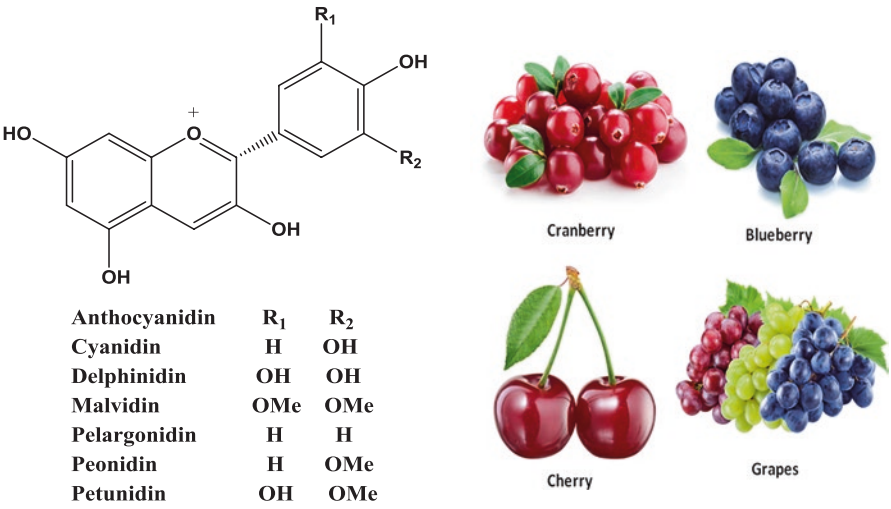
Green tea

Acacia Catechu

Fig. 5.4 Chemical structure of flavan-3-ols and their dietary sources

5.3.2 Dietary Phytochemicals Effects in Xenograft Models

The in vivo effect of dietary phytochemicals has been studied using subcutaneous xenografts in mice. A nude mouse model of breast cancer xenogratfs can reduce the primary tumor growth using combined treatment with resveratrol, quercetin, and catechin (Schlachterman et al. 2008). Anthocyanidins (cyanidin, malvidin, petunidin, peonidin, and delphinidin) at 1.5 mg/mouse inevitably inhibit the development



Anthocyanidin	R_1	R_2
Cyanidin	H	OH
Delphinidin	OH	OH
Malvidin	OMe	OMe
Pelargonidin	H	H
Peonidin	H	OMe
Petunidin	OH	OMe

Fig. 5.5 Chemical structure of anthocyanidins and their dietary sources

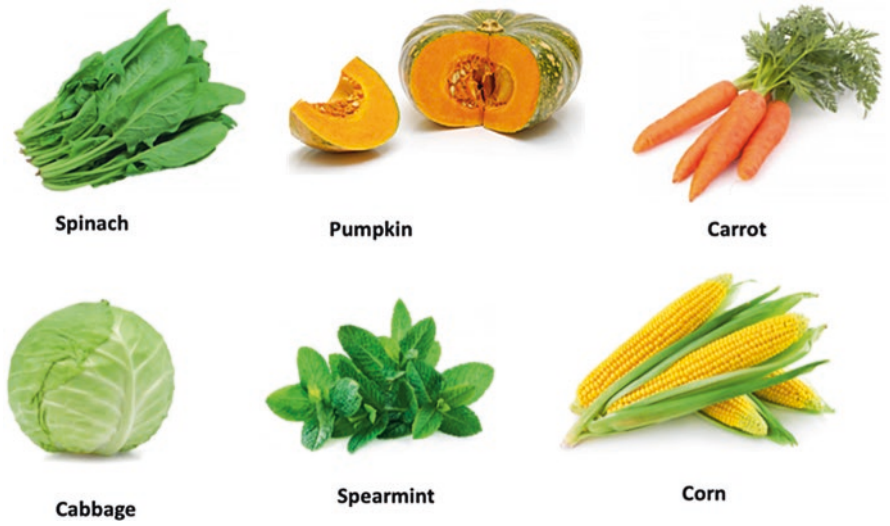


Fig. 5.6 Common dietary sources of terpenoids

of H1299 xenografts in nude mice. Cyanidin, the most active anthocyanidin, reduced the growth by ≈60% and inhibited the growth of xenografts in nude mice (Liu et al. 2018). Significant reduction of tumor growth in a T24 bladder cancer xenograft model has been observed after treatment with the flavonol myricetin (Sun et al. 2012). Similarly, hepatocellar carcinoma xenograft growth is inhibited by flavone apigenin in nude mice (Gao et al. 2018).

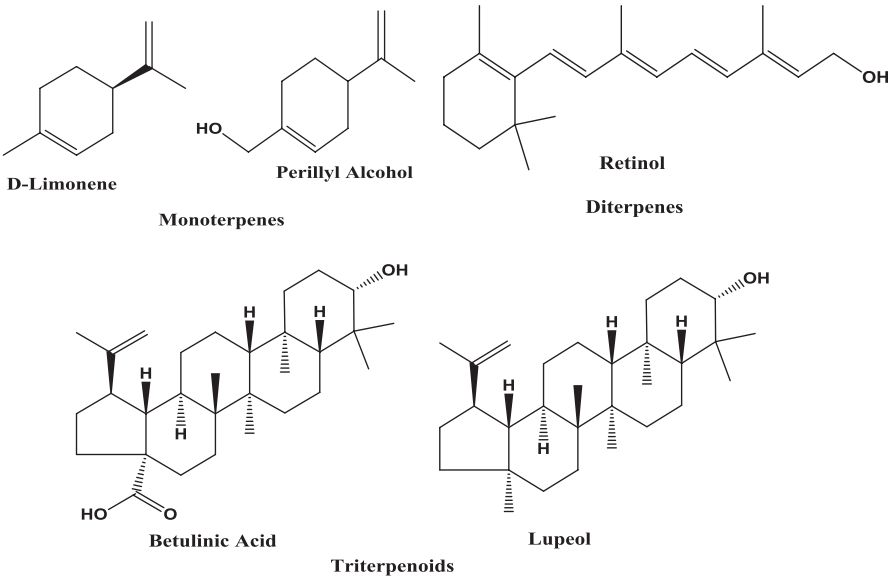


Fig. 5.7 Structure of some common dietary terpenoids

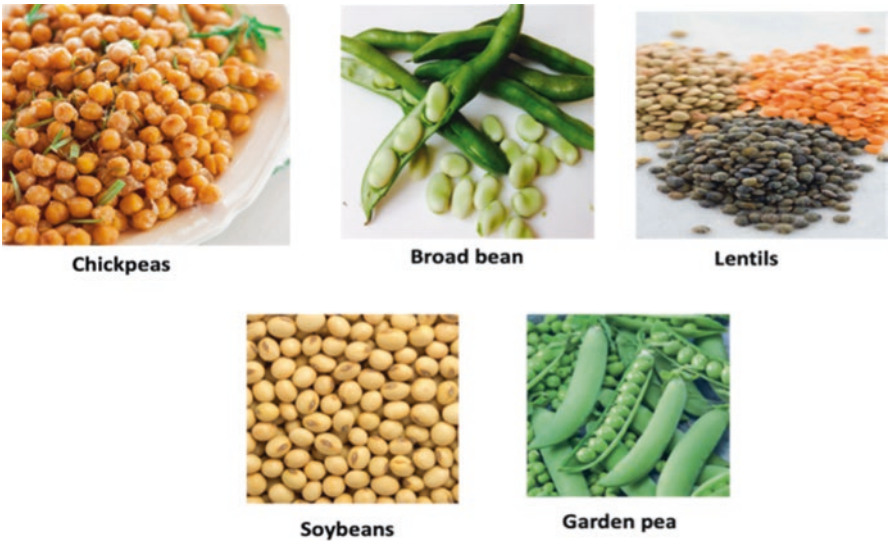


Fig. 5.8 Common dietary sources of saponins

Dietary saponins, such as hederagenin, and the triterpene oleanolic acid also inhibit the tumor growth in a mouse xenograft model (Kim et al. 2017; Niu et al. 2018). Similarly, dietary diterpene carnosol (Johnson et al. 2010), monoterpene geraniol (Kim et al. 2012), and triteppene betulinic acid suppress cancer cell growth in xenograft tumor models (Cai et al. 2018).

Table 5.2 Epidemiological studies: corporation between flavonoids or foods affluent in phenolic compounds and cancer

	Effect	Sample size	References
Flavonoids	Dropped off cancer risk in entire sites combined	9959 men	Knekt et al. (1997)
	Deteriorated cancer risk in the oral cavity, larynx, pharynx and esophagus	540 people	De Stefani et al. (1999)
Quercetin, onions, white grapes	Decreased recurrence of lung cancer	582 people	Le Marchand et al. (2000)
Quercetin	Decreased incidence of lung cancer	10,054 men	Knekt et al. (2002)
	Decreased incidence of colon cancer	264 people	Kyle et al. (2010)
Quercetin, kaempferol	Decreased risk of gastric cancer	354 people	Garcia-Closas et al. (1999)
Catechins	Decreased incidence of epithelial cancer	939 men	Arts et al. (2001)
Tea	Decreased risk of colon cancer	12,170 people	Su and Arab (2002)
Green tea	Slow down risk of prostate cancer	49,920 men	Kurahashi et al. (2008)
	Diminished risk of recurrence breast cancer and metastasis	472 women	Inoue et al. (2001)
Flavonoid intake and black tea	Declined risk of prostate cancer	58,279 men	Geybels et al. (2013)
Soy	Decreased risk of lung cancer	999 men	Wakai et al. (1999)
	Decreased risk of breast cancer	34,759 women	Key et al. (1999)
Anthocyanidin	Reduced risk of oesophageal and gastric cancer	615 people	Petrick et al. (2015)
Flavonol and catechin	Decreased colorectal cancer risk in normal wt. women	120, 852 women	Simons et al. (2009)
	Reduced risk of prostate cancer	118 people	Geybels et al. (2013)
Epicatechin, catechin, quercetin, and kaempferol	Decreased lung cancer risk among tobacco smokers	558 people	Cui et al. (2008)

5.3.3 Dietary Phytochemicals Effects on Apoptosis

Programmed cell death is defined by morphological and biochemical modifications in cells (e.g., DNA fragmentation) (Debatin 2004). Dietary phytochemicals induce apoptosis in various cancer cell lines, namely human breast cancer (Valcic et al. 1996), lung cancer (Yang et al. 1998), gastric cancer (Horie et al. 2005), colon cancer (Tan et al. 2000), and prostate cancer (Brusselmans et al. 2003). Recent investigations have shown that the dietary flavonol quercetin induces apoptotic cell death in various types of cancers, such as leukemia (Chen and Jiu-Hong 2005; Mertens-Talcott and Percival 2005), prostate cancer (Huynh et al. 2003), breast cancer

(Hakimuddin et al. 2004), lung cancer (Nguyen 2003) and hepatoma (Chi et al. 1997). Moreover, these flavonols have also been shown to promote morphological mutation and DNA cleavage in leukemia (Csokay et al. 2005) and rat pancreatic carcinoma cells (BSp73AS) (Mouria et al. 2002).

Genistein has been found to restrain the growth of various cancer cells by the modulation of genes that are related to the control of apoptosis or to another mechanism like cell growth or signal transduction pathways, after all this isoflavonoid is a magnificent inhibitor of protein tyrosine kinases (Lian et al. 1999). It can promote apoptosis in prostate cancer (Kumi-Diaka et al. 2000), breast cancer (Li et al. 1999), head and neck squamous cell carcinoma (Alhasan et al. 1999), lung cancer cells (Lian et al. 1999), and stomach cancer cells (Yanagihara et al. 1993).

Kaempferol exerts a direct effect on the apoptosis extrinsic pathway, which is based on the presence of death receptors on the cell surface able to recognize death inducing substances. These death receptors comprise tumor necrosis factor alpha (TNF-α), FAS, and TRAIL (Lee et al. 2014).

Anthocyanidins have been found to activate morphological change and DNA fragmentation in hepatoma cells (Shih et al. 2005). Cell apoptosis was detected by DNA agarose gel electrophoresis when lung cancer cells NCI-H460 were treated with anthocyanidin (Zhang et al. 2005). Theaflavin digallate and epigallocatechin inhibited growth and promoted apoptosis in COLO 320DM cells (Hsu et al. 2012). Morphological observation of the tissue displayed apoptotic bodies in treated human stomach cancer KATO III cells (Hibasami et al. 1998). Viability, apoptosis, and DNA fragmentation assay indicated that the merger of EGCG and bleomycin potentiated apoptosis (Bimonte et al. 2015). Epigallocatechin-3-O-gallate provoked dose-dependent cell propagation inhibition, cell cycle detention at the G0/G1 stage, and DNA cleavage in HT-1080 cells, suggesting the induction of apoptosis (Lee et al. 2011). Synergistic apoptosis of HCT 15, HCT 116, as well as Hep G-2 cells by curcumin and catechin have been observed efficiently (Alam et al. 2018). Anthocynin rich bilberry extract has been shown to induce apoptosis in HL60 cells and nucleosomal DNA fragmentation (Katsube et al. 2003). Triterpenes oleanolic acid and ursolic acid induced apoptosis in four cancer cell lines of human liver. Completion of apoptosis was proved microscopically by observing escalation in plasma membrane permeability and detecting the fragmentation of DNA (Shyu et al. 2010).

5.3.4 Epigenetic Markers Effects

Dietary factors play a pivotal role in many natural biological courses of action and are also convoluted in the surveillance of pathological breakthroughs. Environmental and dietary circumstances can influence diseases linked to genetic and epigenetic modifications. Recently, an increasing number of nutritional components that have an inherent epigenetic activity have been identified. These micronutrients are able to control gene expression by carrying out an inheritable DNA (or DNA-associated proteins) modification without altering the DNA sequence. The posttranslational modification of histone proteins is the most well-known epigenetic mechanism by histone

deacetylases (HDACs). Synthetic HDACs cause harmful side effects like atrial fibrillation, questioning their applicability. Therefore, the discovery of new HDACs inhibitors (HDACIs) is of great interest as potential anticancer drugs (Berger et al. 2013).

Dietary polyphenols and flavonoids have a custodial role against diseases and have found an important place in cancer prevention (Yang et al. 1998; Tan et al. 2000). In fact, various mechanisms have been found that aid in demonstration of the preventive nature of polyphenols, along with their ability to amend the epigenome by chromatin remodeling or by reactivating silenced genes in cancer cells (Bag and Bag 2018). Their chemopreventive potential can be defined by their ability to restrain DNMTs and also act as histone modifiers. The epigenome of cancer cells could be changed significantly by both of these properties, and they are viewed as interesting possibilities for anticancer therapeutics (Fig. 5.9).

The DNMT inhibitory activity of a green tea catechin and epigallocatechin 3-gallate (EGCG) was described by Morris et al. (2016). More than 50% of effective compounds in green tea are EGCG. Flavones apigenin and luteolin, flavanone hesperetin, and anthocyanidin cyanidine inhibit DNMT activity when tested in vitro. The flavone apigenin shows chemopreventive properties against prostate cancer by inhibiting HDAC (Ganai 2017). The dietary flavone luteolin influenced apoptosis of HL-60 cells, is associated with c-Jun activation, and expressed the histone H3 acetylation-mediated by Fas/FasL (Wang et al. 2018). It also cut down protein levels and the enzyme actions of epigenetic modifying enzymes, such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), in HCT116 cells (Zuo et al. 2018). Delphinidin, a dominant anthocyanidin compound found in diversified fruits, promotes p53-mediated apoptosis by abolishing HDAC activity and stimulating p53 acetylation in human prostate cancer LNCaP cells (Jeong et al. 2016).

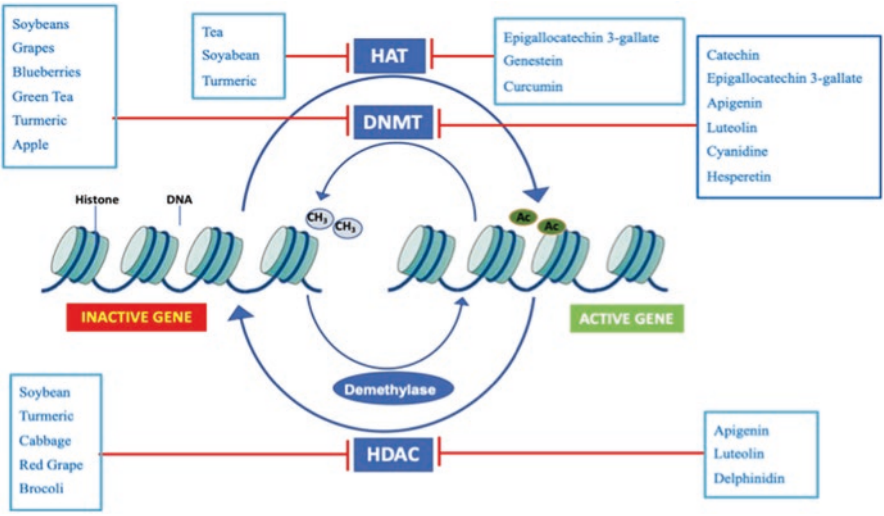


Fig. 5.9 Epigenetic modifications by dietary phytochemicals. (Idea adapted from Szyf 2015)

5.3.5 Importance of Dietary Phytochemicals in Co-therapy

Used in combination, dietary phytochemicals also have great potential to enhance the therapeutic effects of antitumor drugs, a practice known as co-therapy. The combination of the flavonol kaempferol with classical chemotherapeutic agents results in greater cytotoxic effects than those achieved by each of them separately (Luo et al. 2010). It is also able to sensitize the cytotoxic effects of 5-fluorouracil to the tumor cells, cytarabine (Mohan et al. 2013) and doxorubicin (Sharma et al. 2007).

Combination of autophagy inhibitors with the flavone apigenin inhibits the cell proliferation and induces autophagy by way of suppressing the PI3K/Akt/mTOR pathway (Yang et al. 2018).

A synergistic cytotoxic effect by theaflavin-3,3′-digallate, a black tea polyphenol, and cisplatin (CDDP) was shown in cisplatin resistant ovarian cancer cells A2780/CP70 and OVCAR3 (Pan et al. 2018).

Another polyphenol, epigallocatechin-3-gallate, found in tea combined with cisplatin significantly shortened the size of the tumor (Zhou et al. 2014). It also potentiated the effect of adriamycin in CaEs-17 cells (Fantini et al. 2015), fluorouracil in Hep G2 cells (Yang et al. 2012), and ponatinib in chronic myeloid leukemia cells (Goker et al. 2014). Combination of capecitabine with (-)-epigallocatechin-3-gallate inhibits tumor growth and angiogenesis with gastric cancer xenografts in nude mice (Wu et al. 2012). Green tea catechins augmented the antitumor properties of doxorubicin for chemoresistant liver cancer in a mouse model (Liang et al. 2010).

The anthocynidin delphinidin (DPN) in combination with 5-aza-2-deoxycytidine (AzaC) showed the highest inhibition of cell growth in human glioblastoma LN18 and U87MG cells (Chakrabarti and Ray 2015). Anthocyanins also potentiated the activity of trastuzumab in human epidermal growth factor receptor 2-positive breast cancer cells in vitro and in vivo (Liu et al. 2014).

Luteolin with lapatinib inhibited the growth of breast cancer cells (Zhang et al. 2017) and the doxorubicin-induced autophagy in human osteosarcoma U2OS cells (Zhang et al. 2015). The combination of the oncolytic adenovirus CD55-TRAIL with luteolin significantly decreased cytotoxicity in lung/bronchial normal epithelial cells compared with single treatment (Xiao et al. 2017). Co-treatment of tamoxifen and naringenin could inhibit cell proliferation more effectively in ER+ breast cancer cells (Xu et al. 2018). Combination of myricetin with 5-fluorouracil chemotherapy can enhance tumor chemosensitivity of esophageal cancer EC9706 cells, and hence myricetin could be a potential chemosensitizer for esophageal cancer therapy (Wang et al. 2014).

Combination of afromosin with soyasaponin I enhanced their antitumor promoting activity. Consequently, many active compounds were found that might be valuable chemopreventive agents (Konoshima et al. 1992).

Limonene enhances the antitumor effect of docetaxel against prostate cancer cells without being toxic to normal prostate epithelial cells (Rabi and Bishayee 2009). Geraniol in combination with gemcitabine induced BXPC-3 cell apoptosis (Jin et al. 2013). Co-treatment 5-fluorouracil with triterpenoid lupeol induced

apoptosis by upregulating the expressions of Bax and p53 and downregulating the expressions of survivin and Bcl-2 (Liu et al. 2016).

5.4 Concluding Remarks

In the past few decades, several studies have been performed that support the concept of cancer chemoprevention dietary polyphenols, flavonoids, terpenoids, and saponins. Several epidemiological studies have corroborated that dietary consumptions have a huge impact on cancer prevalence. Owing to these encouraging observations, research efforts all across the globe have focused on identifying, characterizing, and providing the scientific basis behind the chemopreventive properties of dietary supplements. The results have shown that fruits and vegetables represent an untapped reservoir of various nutritive and nonnutritive phytochemicals that when incorporated into a healthy lifestyle can be a very useful step toward cancer chemopreventive.

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Immunogenic Potential of Natural Products

6

Rahul L. Gajbhiye, Sanjit K. Mahato, Anushree Achari, Parasuraman Jaisankar, and V. Ravichandiran

Abstract

The immune system is the most complex and important biological system of the human body, and good health requires it to be strong and well-functioning. Imbalance or malfunction of the immune system leads to a wide range of chronic diseases, including allergies, cancers, autoimmune diseases, and others. Immunogenicity mainly refers to any changes in the immune response and may involve amplification, induction, expression or inhibition of different components of the immune response. Immunomodulators can be broadly classified into three categories: immunoadjuvants, immunostimulants, and immunosuppressants. Natural products-based immunogenic agents provide a safe alternative for treatment in order to avoid the unwanted side effects of synthetic drugs. A wide range of structurally diverse plant secondary metabolites, such as alkaloids, polysaccharides, lectins, glycosides, phenolic compounds, flavonoids, anthocyanins, tannins, saponins, terpenoids, and sterols can improve and/or activate mac-

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rophage immune responses, leading to immunomodulation, antitumor activity, wound-healing, and other therapeutic effects.

Keywords
Immunomodulators · Natural products · Plant secondary metabolites · Cytokine · Innate and adaptive immunity · T regulatory cell · Th1 · Th2

6.1 Introduction

The immune system of the body acts as a controlling weapon against pathogens. Malfunctioning of the system can lead to hyper-reactivity of the machinery. This may even give rise to autoimmune diseases, like rheumatoid arthritis (RA) or Crohn's disease (Davidson and Diamond 2001; Burnet 1959). Immunosuppression involves a decrease of the activation of the immune system and is considered a useful approach for the amelioration of such conditions. T-lymphocytes play a pivotal role in the defense response of the immune system; therefore, immunosuppressive approaches tend to condition these cells (Abbas et al. 1996). Cyclosporine A (**1**), a cyclic nonribosomal peptide of fungal origin, is a well-known and clinically tested immunosuppressive drug used to treat or repress the overactivity of lymphocytes, particularly after transplantation surgery or in cases of severe RA. However, the compound has several, sometimes severe, side effects (Marshall and Kearns 1999). Other vital problems may also arise when trying to suppress the immune system. For example, cells may be held in an inactive state where they are viable but not capable of growing and proliferating. This is an essential requirement to identify a compound as immunosuppressant; otherwise, cytotoxicity would lead to side effects. The other important drawbacks of many therapeutic peptides are their low oral bioavailability consequent upon fast degradation in the system and the poor drug permeation that is a consequence of their hydrophilic nature (Thell et al. 2014; Srinivas et al. 2005). It is therefore necessary to provide improved means and methods to suppress the immune system so as to be able to treat or prevent disorders of the system. It is relevant in this context to point out that there are some cyclotides which possess the ability to decrease or else arrest the proliferation of (activated) immune cells [for example peripheral blood mononuclear cells (PBMC) or T-lymphocytes] (Grundemann et al. 2013; Weidmann and Craik 2016).

Immunomodulators or immunogenic agents are the substances of biological or synthetic origin capable of stimulating, suppressing or modulating either or both of the innate and adaptive arms of the immune system. From a clinical standpoint, these agents are usually considered to be a member of the following classes:

Immunoadjuvants: These substances enhance the efficiency of vaccines and may be described as specific immune stimulants that can be the true modulators of the immune response and may act as a discriminator between cellular and humoral helper T1 (Th1) and T2 cells (Th2) and between immunodestructive, immunoprotective, and reaginic [immunoglobulin E (IgE) versus IgG] type immune responses, which pose a real challenge to vaccine designers (Cox and Coulter 1997).

Immunostimulants: These are naturally nonspecific and are only supposed to act as enhancements to a body's resistance to infection. They can act through both adaptive as well as innate immune responses. Immunostimulants act as prophylactic and promoter agents (immunopotentiators) in healthy individuals by increasing the basic level of immune response but act as immunotherapeutic agents in patients with impairment of immune response (Hadden 1993).

Immunosuppressants: These comprise a structurally and functionally diverse group of drugs and are often administered as combination regimens to deal with various types of organ transplant rejection and autoimmune diseases (Liu et al. 2009).

The immune system functions by guarding the host from invading pathogens and thus protecting it from the effects of diseases (Malone et al. 2013; Metzger 2014). Formerly, the target was to suppress the immune system and permit allotransplantation. The system may be activated by "self" antigen (autoantigen) or "nonself" antigen (alloantigen). The pathway usually involves processing of the antigen by the phagocytic cells, such as macrophages, monocytes, or related cells (Geissmann et al. 2010). There are two types of immune responses found in human body:

I. Innate immune response
II. Adaptive immune response
    (a) Humoral immunity
    (b) Cellular immunity

6.1.1 Innate Immune Response

Efficient interactions among innate (natural and nonspecific) as well as acquired (adaptive and specific) components of the immune system produce immune responses. The first line of defense against invading pathogens is the innate immune response, and it depends on the body's ability to acknowledge such preserved features of pathogens that are absent in the healthy or uninfected host. The products are specific for the pathogen in question and are identified by plant's Toll-like receptor proteins as well as in invertebrate and vertebrate animals (Aderem and Ulevitch 2000; Ahmad et al. 2000). Microbial surface molecules are also activate complement in vertebrates; groups of these blood proteins act together and disrupt the microorganism membrane, making them vulnerable to phagocytosis caused by neutrophils and macrophages, and hence generate an inflammatory response (Figueroa and Densen 1991). The phagocytic cells kill the invaders by employing a combination of antimicrobial peptides, reactive oxygen species (ROS), and degrading enzymes (Wink 2003). They may give rise to an inflammatory response by releasing signaling molecules and also harness the army of the adaptive immune system. Interferons (IFN) generate after cells become infected with viruses, culminating in a series of cellular responses that stop viral replication and activate the killer instincts of natural killer (NK) cells (Vivier et al. 2008) and cytotoxic T-lymphocytes (Martin et al. 1988).

6.1.2 Adaptive Immune Response

An immunological memory, as well as stronger immune response, comes from the adaptive immune system, where each pathogen is identified by a specific marker antigen. B- and T-lymphocytes are the special types of leukocytes cells of this system. While T-cells produce a cell-mediated immune response, B-cells produce a humoral immune response. Both these cells carry receptor molecules capable of identifying specific targets (Medzhitov and Janeway 1997). A T-cell can identify a nonself target, such as a pathogen, only when antigens (small fragments of pathogen) have been processed and presented along with the self receptor known as the major histocompatibility complex (MHC) molecule. Antigens get recognized by killer T-cells coupled to Class I MHC molecules, whereas helper T-cells will recognize antigens coupled to Class II MHC molecules (Iwasaki and Medzhitov 2004). The gamma-delta T-cells form a third, minor subtype that identifies intact antigens (**8**). In contrast, a B-cell antigen-specific receptor is an antibody molecule on a B-cell surface with the capability to identify all pathogens devoid of any antigen processing. Every lineage of the B-cell expresses a different antibody; a complete set of the B-cell antigen receptors represent all antibodies that the body can generate. Replication of T-cells and B-cells following their activation gives rise to offspring, some of which turn into the long-lived memory cells. These memory cells memorize each specific pathogen encountered during the lifetime of an animal and remain capable of posing a strong response to future challenges (Gross et al. 1989).

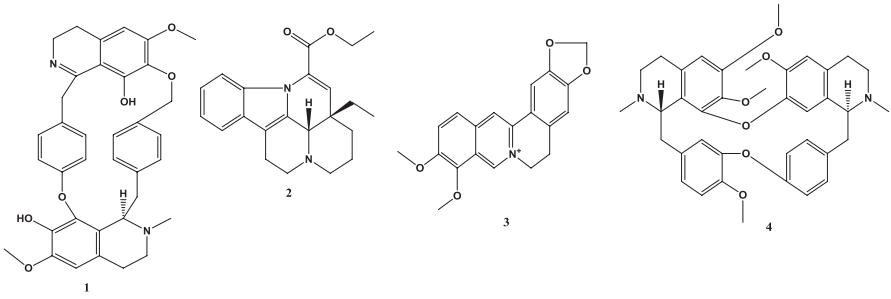
6.2 Natural Products as Immunogenic Agents

Plant derived phytochemicals are naturally occurring compounds often endowed with bioactive potentials. The primary metabolites of plants are important for growth and reproduction. In addition, a number of the secondary metabolites synthesized by plants are generally not important for the survival of the plant. These consist of several classes of compounds, such as flavonoids, alkaloids, glycosides, coumarins, gums, tannins, polyphenols, polysaccharides, terpenes, and terpenoids (Zhong 2001). Production of these phytochemicals sometimes depends on response to external stimuli, such as infection, nutritional or climatic challenges, and they can accumulate only in certain parts of the plant. They thus function as a natural defense system for the host. In addition, they contribute to the color, aroma, and flavor or even act as signaling molecules. More than 4000 such products have been identified to date. Interestingly, these phytochemicals are also recognized to be endowed with curative potentials, such as antioxidant, memory enhancing, antidiabetic, cholesterol lowering, anticancer, adaptogenic, and immunomodulatory activity. Natural products having immunostimulating potential can be either high- or low-molecular weight compounds. Polyphenolic compounds, terpenoids, and alkaloids mainly constitute the low-molecular weight ones, while the high-molecular weight type is dominated by polysaccharides (Kayser et al. 2003).

6.2.1 Alkaloids

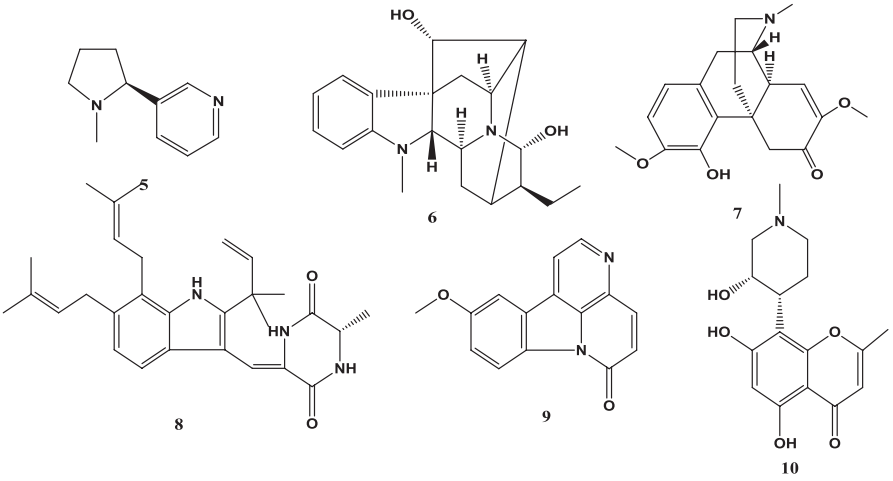
Alkaloids are the most effective and therapeutically significant plant substances (13). With nearly 5500 alkaloids, they are the largest single class of secondary plant metabolites that contain one or more nitrogen atoms, generally as part of a cyclic structure (Guha et al. 1979). When administered to animals, they exhibit distinct physiological activity. Alkaloids can be used for their antitumor (vinblastine and vincristine) (Owellen et al. 1972), analgesic (morphine) (Siegel 1976) or antimicrobial (cepharanthine) properties. They are also able to enhance immune responses, and most of them are being tested for their immunostimulating properties (Kondo et al. 1992). Warifteine (**1**), an alkaloid isolated from *Cissampelos sympodialis,* shows immunomodulatory activity in a chronic allergic rhinitis model by reducing antigen-specific IgE levels, eosinophil infiltration, and lung hyperactivity (Lima et al. 2014). Vinpocetine (**2**), a vasoactive *Vinca* alkaloid extracted from the periwinkle plant, shows promising immunomodulatory activity when tested against plasmacytoid dendritic cells (pDCs) isolated from C57BL/6 mice. It significantly downregulates the expression of CD40, CD80, and CD86 on pDCs and inhibits the Toll-like receptor 9 signaling pathway, thereby reducing the secretion of related cytokines in pDCs through TSPO (Feng et al. 2017).

The immunomodulatory effect of berberine (**3**), an alkaloid found in *Berberis* and *Coptis,* was investigated for the myosin-induced myocardial injury in rats. It differentially modulated the activities of p-STAT1, p-STAT3, and p-STAT4 to suppress Th17 and Th1 cell differentiation (Liu et al. 2016). Tetrandrine (**4**), a potent immunomodulator isolated from the Chinese herb Han-Fang Chi, can effectively suppress cytokine production and proliferation of CD28-costimulated T cells (Ho et al. 2004). It also suppresses the mitogen induced lymphoproliferative response and antibody production by B cells (Seow et al. 1988).



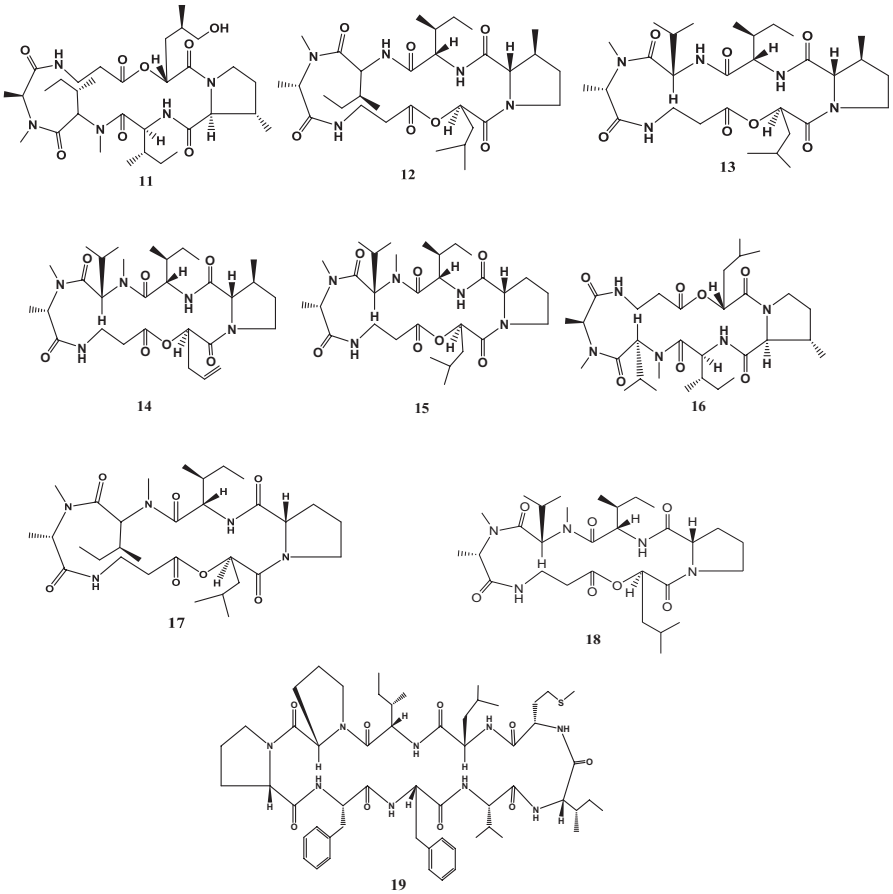
Nicotine (**5**), isoquinoline alkaloids like cepharanthine, and indole alkaloids like ajmaline (**6**) inhibit a TAK-induced activation of polymorphonuclear leukocytes or PMN (Kinoshita et al. 1992). Sinomenine (**7**) decreased IFN-γ, IL-1β, TNF-α, IL-12P70, and IL-6 production by inhibiting the TLR/NF-κB pathway (Xiong et al. 2017). The tryptophan-derived alkaloid cristatin A (**8**), isolated from the plant *Lepidagathis cristata* Willd, shows immunosuppressive activity by inhibiting the

Con A (T-cells) and LPS (B-cells)-induced proliferation in mouse splenic lymphocytes (Ravikanth et al. 2001). LPS-induced production of proinflammatory cytokines, such as IL-1β, TNF-α, and IL-6, was inhibited by 10-methoxycanthin-6-one (**9**) in LPS-stimulated macrophages from Balb/c mice. A significant inhibition of iNOS and COX-2 and of cytokine gene expression in LPS-stimulated macrophages was also observed (Siveen and Kuttan 2012). The chromone alkaloid rohitukine (**10**), isolated from the plant *Dysoxylum binectariferum,* inhibited the immune-complex mediated inflammation.



6.2.2 Cyclodepsipeptides

Cyclodepsipeptides are secondary metabolites with unique structures composed of unusual amino acids and nonamino acid moieties and are produced mainly by marine and terrestrial organisms. They have generated considerable interest among the scientific community because of their novel structural features and the wide spectrum of biological organisms, but it is quite difficult to isolate adequate quantities of these metabolites for pharmacological and toxicological testing. Therefore, structural elucidation and their total synthesis are required to make their use more rational and affordable. Cyclodepsipeptides trichomide A (**11**), trichomide B (**12**), trichomide C (**13**), homodestcardin roseotoxin B (**14**), destruxin A5 (**15**), roseocardin (**16**), homodestruxin B(**17**), and destruxin B (**18**) belong to the destruxin family and inhibit the proliferation of lymph node cells (Zhang et al. 2013).



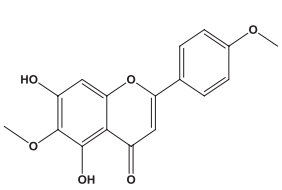
A new cyclic nonapeptide cyclolinopeptide B (**19**) has been isolated from the seeds of *Linum usitatissimum*, which exerts an inhibitory effect on the mitogen (concanavalin A)-induced response of human peripheral blood lymphocytes (Krauss et al. 1999).

6.2.3 Flavonoids

Flavonoids are among the most abundant plant products found in both the free-state and as glycosides. Most of them are water-soluble compounds. The basic skeleton for their chemical structure is the carbon skeleton C6–C3–C6. Flavonoids are potent free radical scavengers and super antioxidants, which prevent oxidative cell damage and also have strong chemopreventive activity against all stages of carcinogens. They are known to reduce the risk of heart diseases (Hertog et al. 1995). They also slow down the initiation, promotion, and progression of tumors

(Brownson et al. 2002). Plant flavonoids have attracted the attention of researchers recently as a dietary supplement for cancer patients because of their chemoprotective effect (Ramos 2007).

Pectolinarigenin (**20**), isolated from the leaves of *Clerodendrum volubile,* significantly inhibited T-cell proliferation and modulated the respiratory oxidative burst in phagocytes (Erukainure et al. 2017). Responsiveness of T and B lymphocyte subsets for antibody synthesis has been increased by administration of dietary supplementation genistein (**21**) and hesperidin (**22**). This augmented the humoral immune response in chicken in both lipopolysaccharide-challenged and -unchallenged broiler chickens. This improved immune status can be effective against infectious agents, particularly for gram-negative bacterial pathogens (Kamboh et al. 2016).



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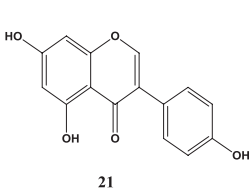

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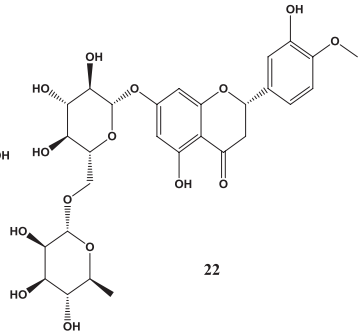

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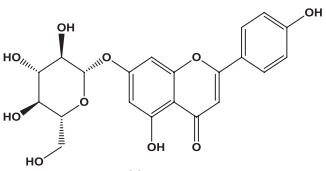
Teucrium ramosissimum Desf. isolates apigenin-7-glucoside (**23**), genkwanin (**24**), and naringenin (**25**) significantly improved splenocyte proliferation. These compounds distinctly increased the killing activity of natural killer (NK) cells and cytotoxic activity of T lymphocytes (CTL) isolated from splenocytes. These experimental outcomes suggest its use in modulation of immune cell functions in physiological and pathological conditions (Nasr-Bouzaiene et al. 2016).



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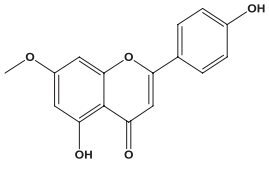

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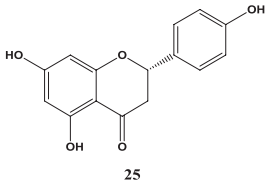

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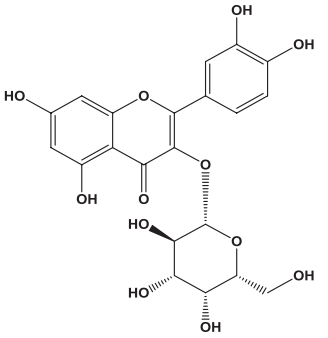
Hyperoside (**26**), a 3-*O*-galactoside of quercetin, significantly promotes the LPS and lectin-stimulated splenocyte proliferation, which leads to potential activation of lymphocytes B and T enhancing humoral and cellular immune responses. Moreover, when tested on B16F10 cells, both ethyl acetate extract and hyperoside could enhance the activity of NK and T lymphocytes cells, as well as the macrophage-mediated cytotoxicity (Mustapha et al. 2016).



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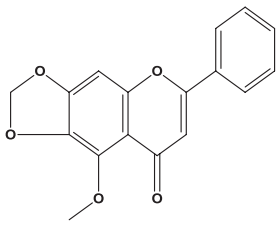

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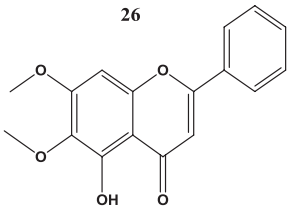

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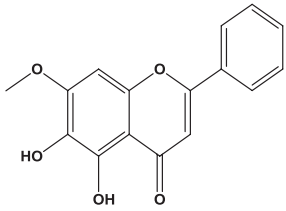

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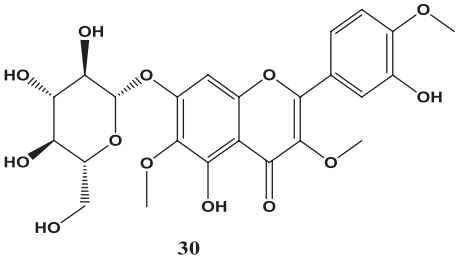
Dose-dependent inhibition of TNF-α, IL-1β, and iNOS levels in the supernatant of mouse macrophage cell line J774A has been observed by three flavones, 5-methoxy-6,7-methylenedioxyflavone (**27**), mosloflavone (**28**), and negletein (**29**), isolated from *Actinocarya tibetica* Benth (Kilani-Jaziri et al. 2016). The flavonoid centaurein (**30**), from *Bidens pilosa,* augmented IFN-γ promoter activity, probably via NFAT and NFκB in T cells (Chang et al. 2007). Eupalitin-3-*O*-β-D-galactopyranoside (**31**), isolated from the alcoholic extract of *Boerhaavia diffusa* root, inhibits the PHA-stimulated proliferation of peripheral blood mononuclear cells, two-way MLR, and NK cell cytotoxicity as well as LPS induced NO production by RAW 264.7 cells (Pandey et al. 2005).



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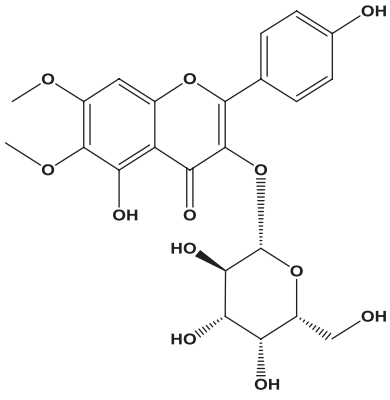
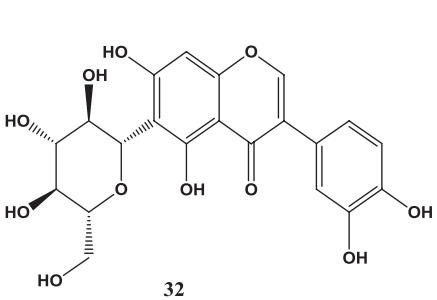


31

Orobol 6-*C*-glucoside (**32**) and orobol 8-*C*-glucoside (**33**), isolated from the bark of *Dalbergia monetaria,* showed colony-stimulating factor (CSF)-inducing activity in a dose-dependent manner (Kawaguchi et al. 1998). Luteolin 7-O-glycoside (**34**), obtained from the aerial parts of *Celosia argentea* Linn., showed inhibition of lipopolysaccharide-induced inflammatory responses through modulation of NF-κB/AP-1/PI3K-Akt signaling cascades in RAW 264.7 cells (Park and Song 2013).



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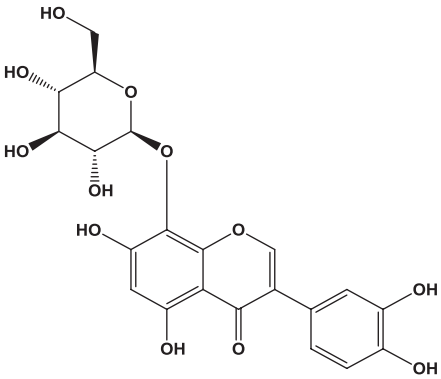

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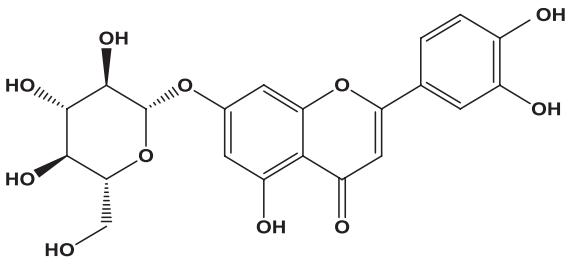
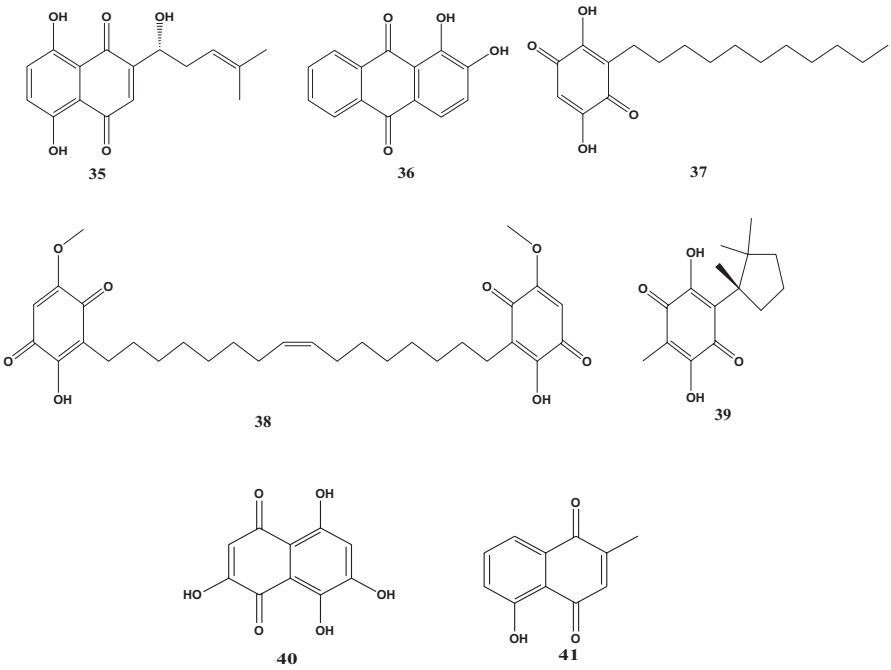


34

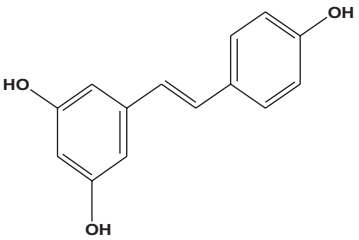
6.2.4 Naphthoquinones and Benzoquinones (Quinones)

The naphthoquinone shikonin (**35**) exerts its inhibitory role on human GMCSF promoter activity, thereby inhibiting cytokine production (Su et al. 2008). Several naturally occurring quinines, such as alizarin (**36**), embelin (**37**), ardisiaquinone A (**38**), helicobasidin (**39**), and mompain (**40**), showed inhibition of the TAK-induced PMN activation (Kinoshita et al. 1992). A hydroxy-1,4-naphthoquinone, plumbagin (**41**), exerts an immunosuppressant effect by selectively inhibiting IFN-γ and IL-17 production by CD4+ T cells, which was mediated through abrogated phosphorylation of JAK1 and JAK2 (Jia et al. 2011).



6.2.5 Polyphenols

Resveratrol (**42**), a polyphenol found in over 70 plants, possesses both immunomodulatory and anticancer effects. A decrease in the production of proinflammatory cytokones and nitric oxide has been observed when tested in a *Porphyromonas gingivalis*-ligature-induced periodontitis model in diabetic mice (Correa et al. 2017). Resveratrol administration in IL-10$^{-/-}$ mice induces immunosuppressive CD11b^{+} Gr-1^{+} MDSCs in the colon (Singh et al. 2012). It also enhances the function of peritoneal macrophages and CD4+ cell count in peripheral blood in an immunosuppressive mice model (Zhong et al. 2006). Catechin (**43**), a natural phenolic compound abundant in tea, cocoa, and berries, displays immunosuppressive activity by inhibiting T-cell proliferation and suppressing oxidative burst (Koko et al. 2015).



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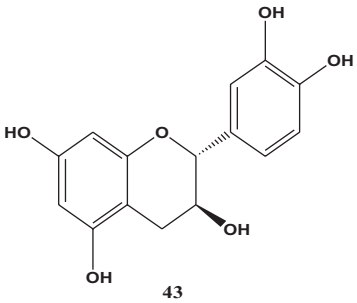
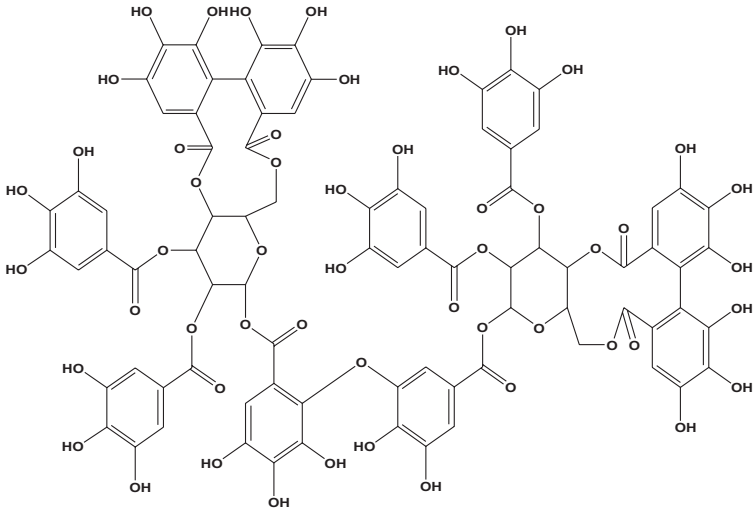


43

The dimeric antitumor ellagitannins cofiariin A (**44**) and agrimoniin (**45**) induce TNF-α production when incubated with h-PBMC's (Feldman et al. 1999).



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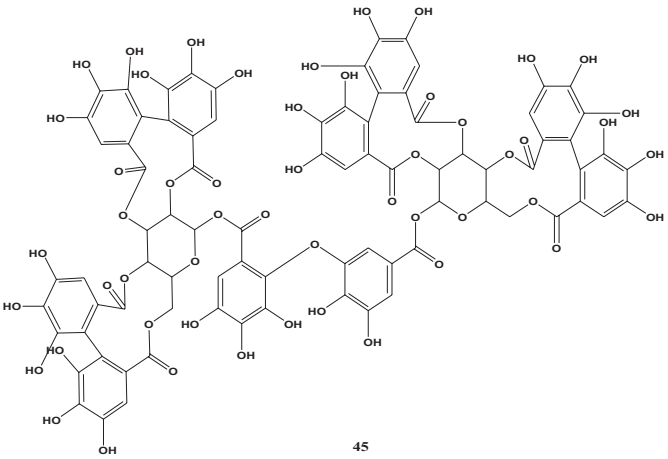
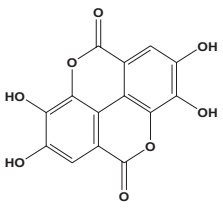


45

Ellagic acid (**46**) and rosmarinic acid (**47**) are natural immunomodulatory and antiinflammatory compounds that may prevent or limit the UVB-induced inflammatory cascade through reduction of proinflammatory mediators and increment of IL-10 with its protective function (Lembo et al. 2014).



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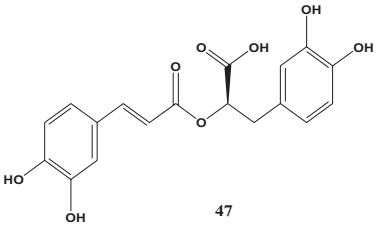

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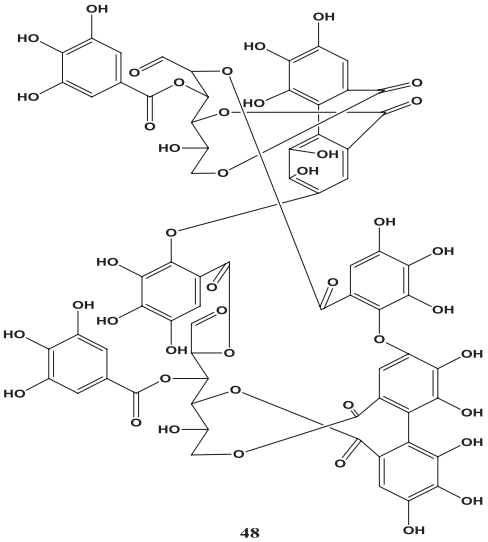


48

A polyphenol, oenothein B (**48**), isolated from *Epilobium angustifolium,* stimulates the innate lymphocytes, including bovine and human T cells and NK cells, resulting in either increased CD25 and/or CD69 expression and thereby exhibits immunomodulatory properties (Ramstead et al. 2012).

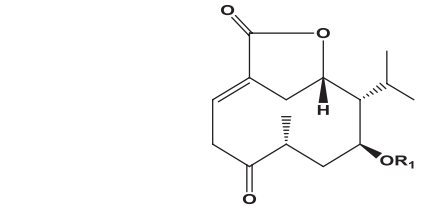
6.2.6 Polysaccharides

Plant polysaccharides have been a topic of study for a very long time, but mainly for their industrial use because of their physical properties. Over the past two decades, interest among the scientific community has been increased to evaluate the biological activity of these molecules. As a result, over 300 types of bioactive polysaccharides have been identified from diverse natural sources (Li et al. 2018). They can be mainly divided into five categories based on their sources, namely fungal (Giavasis 2014), lichen (Olafsdottir and Ingolfsdottir 2001), algal (Jiao et al. 2011), bacterial, and higher plant polysaccharides (Lerouxel et al. 2006). They constitute one of the main classes of bioactive substances present in various traditional herbal medicines and have been shown or implicated to confer a spectrum of pharmacological activities, especially immunomodulatory (Tzianabos 2000), antitumor (Wong et al. 1994) or cancer chemopreventive effects (Gamal-Eldeen et al. 2009). A large volume of studies have reported that various plant polysaccharides can confer potent immunomodulatory activities by regulating the specific functions of various immune cells,

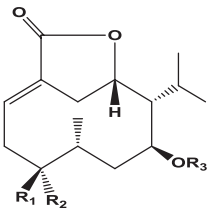
including monocytes, macrophages, NK cells, DCs, T lymphocytes, B lymphocytes, and others (Ferreira et al. 2015). The α-D-glucan RR1 polysaccharide, isolated from *Tinospora cordifolia,* exhibits significant activation of NK cells and complement activation of Th1 pathway associated with cytokine profile, together with a low level of nitric oxide synthesis and absence of oxidative stress (Nair et al. 2004; Aranha et al. 2012). The polysaccharide G1-4A, isolated from *Tinospora cordifolia,* causes death of *Mycobacterium tuberculosis* by modulating host immune responses in a TLR4 dependent manner (Gupta et al. 2016). A water-soluble polysaccharide from *Erythronium sibiricum* significantly enhanced nitric oxide (NO) production and also showed a dose-dependent enhancement of TNF-α and IL-1β in RAW 264.7 cells (Kasimu et al. 2017). The alkaline-soluble polysaccharide (ALP) and water-soluble polysaccharide (WAP) of *Aloe arborescens* act as immunomodulatory agents by increasing T-lymphocyte proliferation, thereby increasing the release of IL-2 and IL-12 (Nazeam et al. 2017). Several immunological polysaccharides from the tubers of *Butea superba* Roxb enhance the T-lymphocyte proliferation, which confirms their immunostimulating property (Burana-Osot et al. 2010). Lichen-derived polysaccharides could also upregulate the production of proinflammatory cytokines, such as IL-12 and TNF-α (Shrestha et al. 2015). Polysaccharides originating from *Salicornia herbacea* possess potent immunomodulatory activity on monocyte/macrophage lineage cells (Im et al. 2006). The mucilage polysaccharide from *Dendrobium huoshanense* exhibited definite functions in murine splenocytes. The mucilage induced various cytokines, including IFN-γ, IL-6, IL-10, and IL-1α and hematopoietic growth factors GCSF and GM-CSF (Hsieh et al. 2008). Numerous other reports on polysaccharides isolated from various plants suggested their efficacy to modulate the immune system in disease condition (Yamassaki et al. 2015; Xing et al. 2015; Bao et al. 2015; Li et al. 2014; Zheng et al. 2014).

6.2.7 Terpenoids

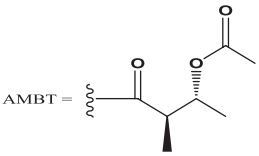
Germacrane sesquiterpenoids, Scapiformolactones A, C, D, F, G, H, I (**49**–**55**), isolated from *Salvia scapiformis,* significantly inhibited proliferation of LPS-induced murine B cells (Lai et al. 2013).

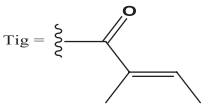


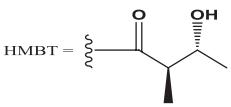
	R_1
49	AMBT
50	Tig



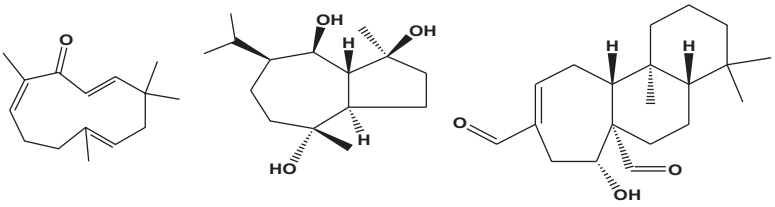
	R_1	R_2	R_3
51	OH	H	AMBT
52	OH	H	HMBT
53	H	OH	HMBT
54	OH	H	Tig
55	H	OH	Tig





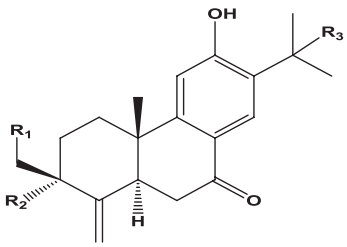


Zerumbone (**56**), a cyclic sesquiterpene and a natural plant dietary compound, exerts an immunostimulatory effect by inducing expression of interleukin-12p70 cytokine in human peripheral blood mononuclear cells. It also activates mice thymocytes, splenocytes, and human peripheral blood mononuclear cells (PBMC) in a dose-dependent manner (Haque et al. 2017). The guaiane sesquiterpene teuclatriol (**57**), isolated from *Salvia mirzayanii,* showed a significant antiproliferative effect on human activated-peripheral blood lymphocytes (Ziaei et al. 2011). The labdane diterpene galanal (**58**) competitively inhibited the expression of IDO1 mRNA induced by the IFN-γ-dependent pathway (Yamamoto et al. 2014).

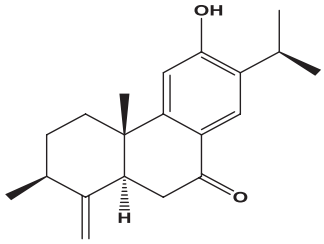


56 **57** **58**

Scrodentoids A–E, the Abietane diterpenoids (**59**–**63**) isolated from the whole plant of *Scrophularia dentate,* showed immunosuppressive activity by inhibiting Con A-induced splenocyte proliferation (Zhang et al. 2015).

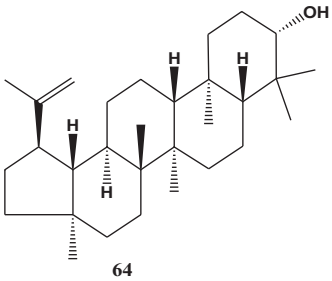


	R_1	R_2	R_3
59	H	H	H
60	H	H	OH
61	H	OH	OH
62	OH	H	OH



63

Several lupane triterpenoids, such as lupeol (**64**) (Das et al. 2017; Ogechukwu et al. 2011) and lupeol-based triterpenoid esters (**65**), isolated from different plants are found to posses immunomodulatory abilities (Ogechukwu et al. 2011).



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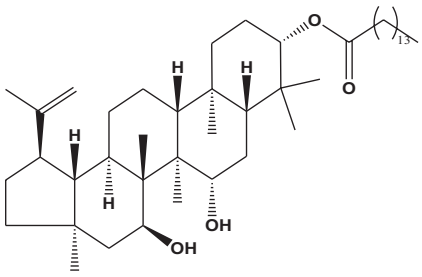

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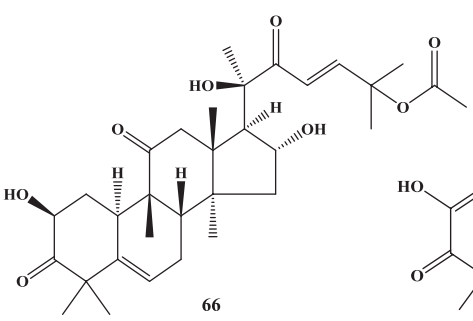

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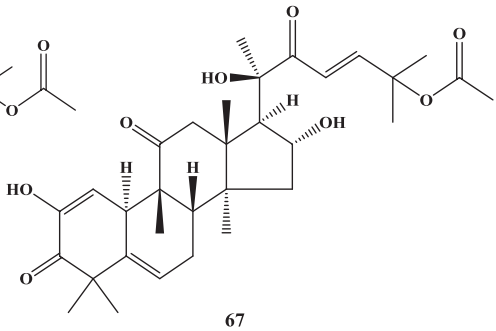
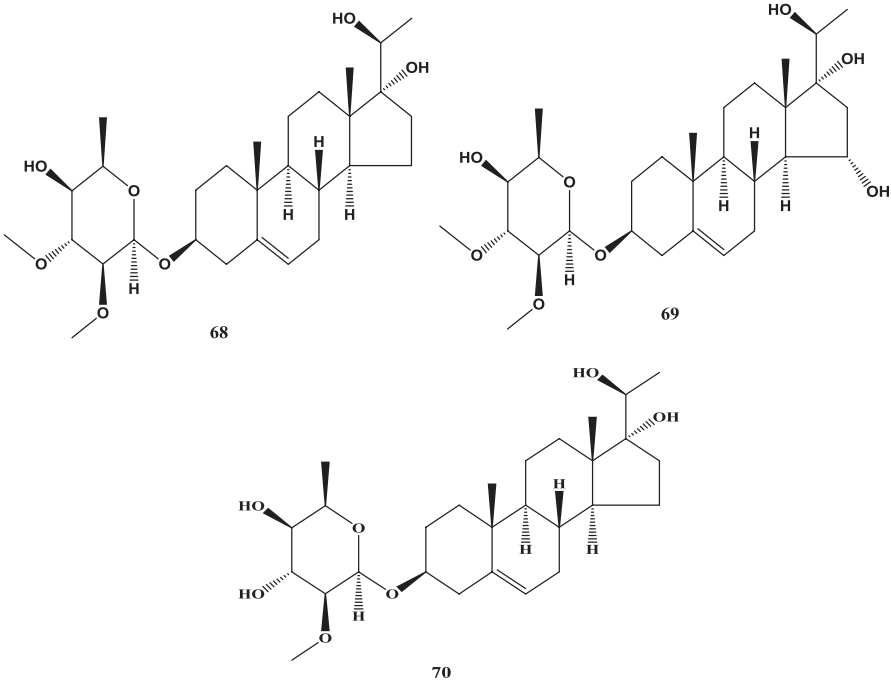


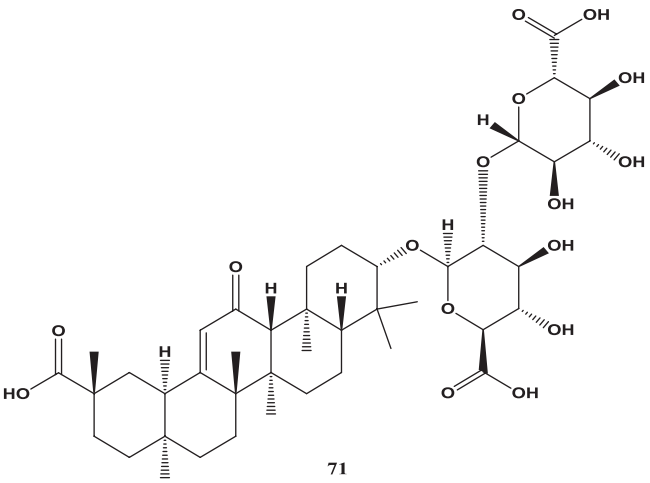
67

Triterpenoids cucurbitacin B (**66**) and E (**67**), isolated from cucurbitaceae plants, exhibit an immunosuppressive effect. They attenuate critical cytokine expression by downregulating the NF-κB signaling pathway and inhibiting PBMC activation by PHA (Wang et al. 2015). Deltostim, a mixture of furostanol glycosides protodioscin (68) and deltoside (69), displayed high immunomodulatory activity on cultured lymphocytes. It inhibited the phytohemagglutinin induced lymphocyte proliferation (Vasil'eva and Paseshnichenko 1996).

The pregnane glycosides epigynosides E-G (**68–70**), isolated from *Epigymum auritum,* displayed significant immunosuppressive activities by inhibiting splenocyte proliferation in Con A-stimulated mice (Gao et al. 2017).



Glycyrrhizin (**71**), a principle component of the plant *Glycyrrhiza glabra,* upregulates the allostimulatory activity of professional antigen presenting DCs and thereby conducts immune responses toward a T helper 1 response (Bordbar et al. 2012).



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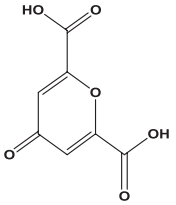
6.2.8 Miscellaneous Products

Chelidonic acid (**72**) is an organic acid having the pyran skeleton found in *Chelidonium majus* (Celandine) and several other *Papaver* species. It prevented ovalbumin induced mast cell degranulation and reduced blood eosinophile counts and serum IgE levels. It also inhibited the histamine release from rat peritoneal mast cells (RPMC) in vitro in a dose related manner (Singh et al. 2016). Essential oils, such as eugenol (**73**), found in clove oil, cinnamon, nutmeg, and basil leaves exhibit a dose-dependent antileishmanial activity against *L. donovani* promastigotes as well as the intramacrophagic amastigotes by restoring the immune response in *L. donovani*-infected BALB/c mice. It elevated the levels of lymphoproliferation, DTH, IFN-γ, IL-2, and NO and maximally reduced the Th1 suppressive cytokines (IL-4 and IL-10), in a concentration-dependent manner. It also strengthened the development of central memory (CD62L-high CD44-high) in CD8+ T lymphocytes (Islamuddin et al. 2016). Phenylpropenes, such as trans-anethole (**74**) and estragole (**75**), exert immunosuppressive effects on SRBC-induced delayed-type hypersensitivity response (DTH). They increase the level of interleukin 10 (IL-10), a cytokine important in the suppression of T helper cell 1-response (TH1), thereby decreasing the levels of TH1-type cytokines, such as interleukin 2 (IL-2) and TNF-α.



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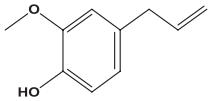

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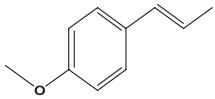

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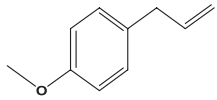
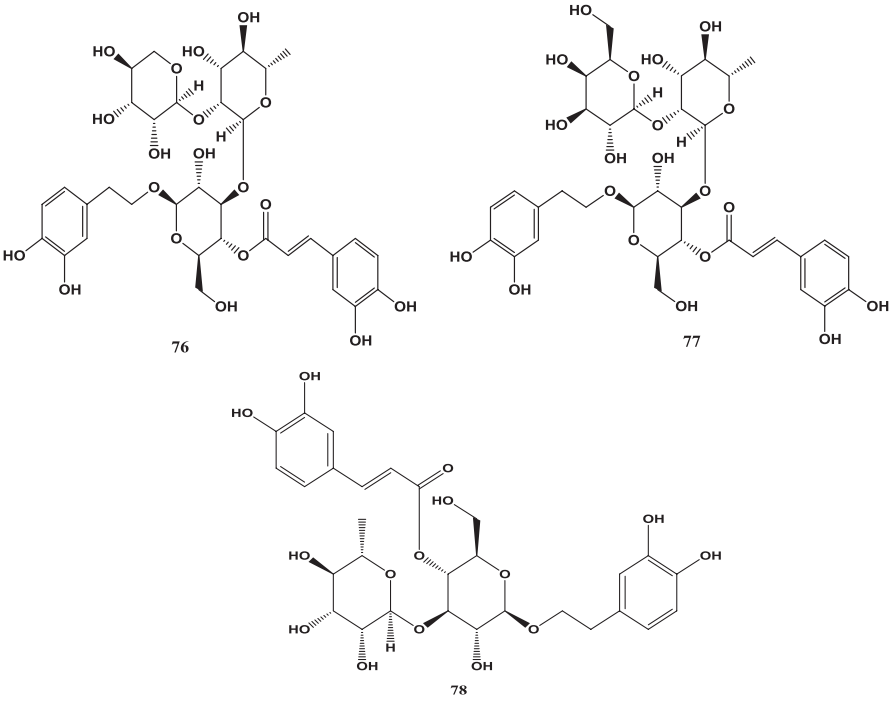
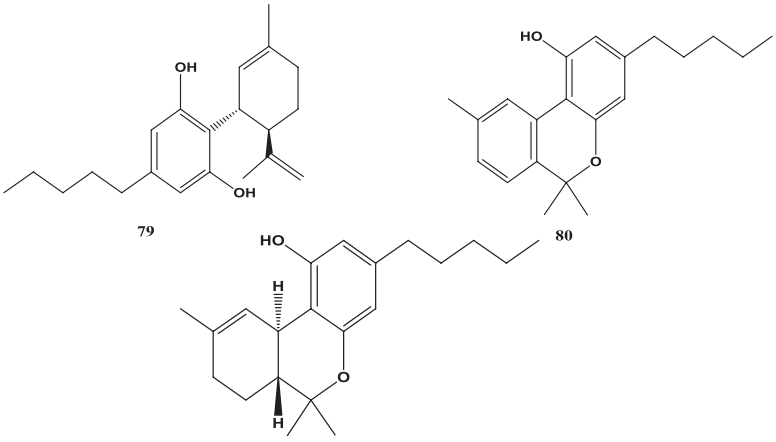


75

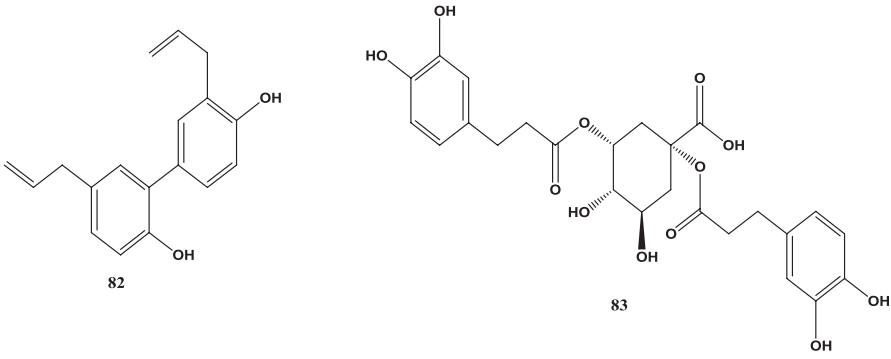
Teucrioside (**76**), lamiuside A (**77**), and verbascoside (78), caffeoyl phenylethanoid glycosides found from *Teucrium chamaedrys* and *Nepeta cataria,* inhibit alkaline phosphatase enzyme calcineurin, which is a main regulator of T-cell activation (Prescott et al. 2011).



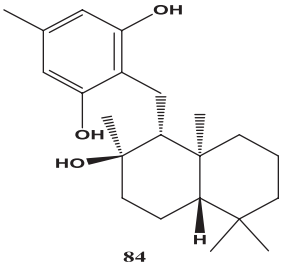
Cannabinoids, such as cannabidiol (CBD, **79**), cannabinol (CBN, **80**), and tetrahydrocannabinol (THC, **81**), decrease TNF-α production in human NK cells and peripheral blood mononuclear cells (PBMC); THC also increases TNF-α production in human monocytes. At low doses both THC and CBD stimulated interferon (IFN)-γ production, while at high dose they suppressed formation of this cytokine suggesting that the response of these cannabinoids on immune system is dose dependent (Jenny et al. 2010; Jan et al. 2003; Aguayo et al. 2005).



Honokiol (**82**), a lignin mainly found in trees from the genus Magnolia, has the ability to combat ultraviolet radiation-induced immunosuppression through inhibition of inflammation and DNA hypermethylation in mouse skin (Prasad et al. 2017). The hydroxycinnamic acid cynarine (**83**) displays an immunosuppressive effect by downregulating the CD28-dependent interleukin-2 (IL-2) expression in a T-cell culture line (Dong et al. 2006).



Albaconol, a prenylated resorcinol (**84**), suppresses NF-κB activation and enhances SOCS1 expression, leading to inhibition of the macrophage function and thereby significantly inhibiting LPS-induced TNF-α, IL-1β, IL-6, and NO production in RAW264.7 cells (Liu et al. 2008a, b). Thymol (**85**), a monoterpenoid phenol derivative of cymene, showed immunosuppressive effects against lymphocytes in a concentration-dependent manner (Amirghofran et al. 2011).



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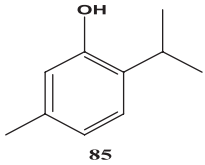
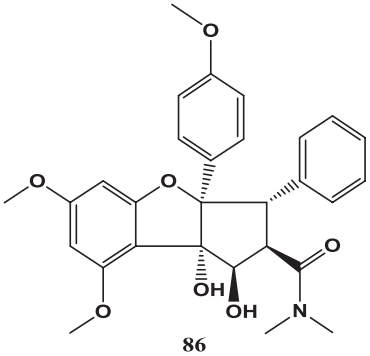


85

Rocaglamide (**86**) is a potent immunosuppressive agent that can suppress IFN-γ, TNF-α, IL-2, and IL-4 production in the peripheral blood T cells. It also inhibits the cytokine gene expression at the transcriptional level (Proksch et al. 2005). Xanthonoid mangiferin (**87**), a natural immunomodulator mainly found in the mango tree, can exhibit both chondrogenic and chondroprotective effects on damaged MSCs and mediates these effects by targeting the multiple aspects of the Smad and SOX9 signaling pathways (Huh et al. 2014). It also enhances recognition memory by increasing the levels of the nerve growth factor (NGF) and tumor necrosis factor (TNF)-α in vitro in human U138-MG glioblastoma cells (Pardo Andreu et al. 2010).



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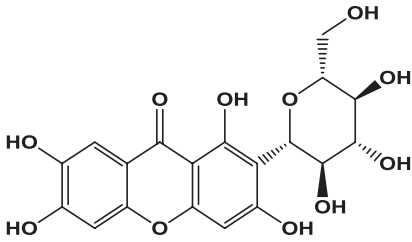

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6.3 Concluding Remarks

Research on exploring the immunogenic potential of plant derived natural products has increased owing to the growing awareness about immune system modulation and the desire to achieve positive effects on disease prevention. Many plant remedies well-known in traditional medicine or refined natural products exert their anti-infective effects not only by directly affecting the pathogen but also by stimulating natural and adaptive defense mechanisms of the host. Also, owing to diverse chemical complexes, plant-based natural products could provide appropriate combinations of synergistic moieties useful in immune drug discovery.

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Immunomodulatory and Therapeutic Potential of Marine Flora Products in the Treatment of Cancer

7

Anshika Singh and Sudhir Krishna

Abstract

Marine floras, constituting over 90% of the oceanic biomass, are rich sources of potent chemicals predominantly belonging to polyphenols and sulphated polysaccharides. They act as potential sources of drugs with various pharmacological properties (such as antioxidant, anti-inflammatory, antibiotic, immunostimulatory, and anticancer). Although human history depicts the use of marine floras as medicine and nutrient supplement, the search for novel pharmaceutical compounds has mostly been limited to terrestrial floras. Marine floras are taxonomically diverse, largely productive, biologically active, and chemically unique, thereby offering great scope for discovery of new anticancer drugs. The mode of action of marine flora products is through activation of macrophages, induction of apoptosis, and prevention of oxidative damage of DNA, thereby controlling carcinogenesis. This chapter focuses on the immunomodulatory and therapeutic properties of marine flora-derived products in the context of increasing cancer incidences and demand for cheaper, safer, and potent anticancer drugs. It highlights the excellent therapeutic potential of the known marine flora-derived anticancer compounds, thereby encouraging researchers to actively participate in the marine natural product drug discovery for treatment and prevention of cancer.

Keywords

Marine flora · Cancer · Immunomodulators · Cytotoxicity · Apoptosis · Antioxidative · Angiosuppressive properties

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7.1 Introduction

About 71% of our planet is covered by oceans, which accounts for roughly 97% of the earth's total water and approximately 50% of total biodiversity (Ray 1988). The highly dynamic and competitive nature of marine environment creates a selection pressure for the development of specific biochemical and physiological systems amongst its living beings, thereby leading to the production of unique chemical entities with diverse structural and functional features (Haefner 2003; Kathiresan et al. 2008). Oceans offer a huge amount of novel bioactive compounds (such as bryostatin-1, panobinostat, plitidepsin, marizomib, and plinabulin) with potential to become drug candidates as they have reached phase I, phase II, and phase III clinical trials (Wang and Miao 2013). However, most part of the oceans has been unexplored, causing lack of ethnomedical history and under-representation of marine compounds in the current pharmacopoeia. Over the last few decades, there has been an upsurge in the search for identifying new marine-derived natural products especially from faunal species, by both pharmaceutical companies and academic institutions (David et al. 2015). Marine flora accounting for more than 90% of oceanic biomass has emerged out as one of the richest sources of diverse bioactive compounds with potential health benefits. Several reports have demonstrated the role of marine flora-derived bioactive compounds as antimicrobial, antioxidative, antihypertensive, anticoagulant, or anticancer agents in functional foods, nutraceuticals, and pharmaceuticals (Fenical et al. 1999; Kim and Wijesekara 2010; Ngo et al. 2011; Wijesekara et al. 2011).

A recent report from WHO predicts 50% rise in the global cancer rates by 2020 (http://www.who.info). Increasing evidences suggest that oxidative processes inside the cellular environment promote carcinogenesis, although the underlying mechanisms are not well understood (Reuter et al. 2010). Under such oxidative stress, the antioxidants may cause the regression of premalignant lesions and inhibit their development into cancer. There is a positive correlation between the increased dietary intake of natural antioxidants and the reduced coronary heart diseases, longer life expectancy, and recovering from cancer (Noguti et al. 2013). Moreover, angiosuppressive properties of marine flora products have also been useful in inhibiting cancer by cutting down the nutrient supply required for the active metabolism (Fan 1994). Angiosuppression also prevents the metastasis of malignant tumour cells through the newly formed blood vessels. Thus, potential antioxidant and anticancer properties of marine flora extracts or isolated products of marine flora origin can possibly be explored for developing the anticancer drugs. Up to now, two of four approved marine-derived drugs (cytarabine and ecteinascidin) have been approved for cancer treatment in 1969 and 2007, respectively (Abraham et al. 2012; Mayer et al. 2010). However, there is no full proof cure for cancer until today, due to various side effects of currently available drugs. There is a continuous demand for the discovery of novel anticancer compounds with greater efficacy and specificity from unexplored sources such as marine environment (Montaser and Luesch 2011).

7.2 Immune System and Immunomodulators

The immune system provides the ability to identify and resist various infectious and harmful microorganisms (pathogens), thus preventing diseases and organ/tissue damage. It comprises of immune stem cells, which originate in the bone marrow and later migrate to different body sites for maturation. In many species, the immune system can be classified into two distinct classes with overlapping mechanisms to fight infections, (a) humoral immunity (= the antibody-mediated defence system) and (b) cellular immunity (= the cell-mediated defence system). The immune system provides multilayered defences of increasing specificity. The first line of defence is the skin, which acts as a physical barrier. This is followed by inflammation which alters the physiological conditions (temperature and pH) of the body, resulting in inappropriate living conditions for foreign organisms. However, if the pathogens cross the physical barrier, they are tackled by the innate (nonspecific) and/or the acquired or adaptive (specific) immune system. Both systems consist of a multitude of cells and molecules interacting in a complex manner to identify and eliminate various pathogens. Recognition and elimination of pathogen are mediated by chemical bonding between the specific receptors of immune system cells and epitopes of the pathogens, thus activating the complex signalling system that mediates the immune response. The term "immune modulation" refers to any process in which the immune response is altered (either increase or decrease) to a desired level and the substance, either of biological or synthetic origin, which can stimulate, suppress, or modulate any of the components of the immune system is known as an immunomodulator. Clinically, immunomodulators are classified into the following three categories:

(i) *Immunostimulants* are the substances which enhance the body's resistance to infection in a nonspecific manner by acting through both innate and adaptive immune responses. When administrated to healthy individuals, they act as prophylactic and promoter agents by enhancing the basic level of the immune response. On the other hand, in the immune-compromised individuals, they can act as immunotherapeutic agents by boosting up the immune system (Kumar et al. 2012).

(ii) *Immunoadjuvants or immunopotentiators* are the substances which are used to enhance the efficacy of vaccines in a specific manner. Immunoadjuvants hold the promise of being the true modulators of the immune response. For instance, Freund's complete adjuvant, which is composed of the inactive or dried antigen of *Mycobacterium bovis*, emulsified in mineral oil (Azuma and Seya 2001).

(iii) *Immunosuppressants* are a structurally and functionally heterogeneous group of drugs, which are often concomitantly administered in combination with regimens to treat various types of organ transplant rejections and autoimmune diseases (Kovarik and Burtin 2003). Examples from marine-derived immunosuppressants include (a) microcolin A (immunosuppressant for concanavalin A, phytohemagglutinin, and lipopolysaccharide-induced prolifera-

tion of murine splenocytes) and (b) MS14-a marine herbal medicine (immunosuppressant for autoimmune encephalomyelitis) (Kalan et al. 2014; Zhang et al. 1997).

7.3 Pharmacology of Marine Flora Products in the Treatment of Cancer

Several marine flora products exhibit not only immunomodulatory activity but also a wide range of other biological activities (such as antioxidant, antiasthmatic, anti-arrhythmic, anti-inflammatory, hepato-protective, hypo-cholesterolemic, antifungal, cardiotonic, diuretic, etc.). Some of the anticancer properties of marine flora products fall into the following mode of action (Fig. 7.1):

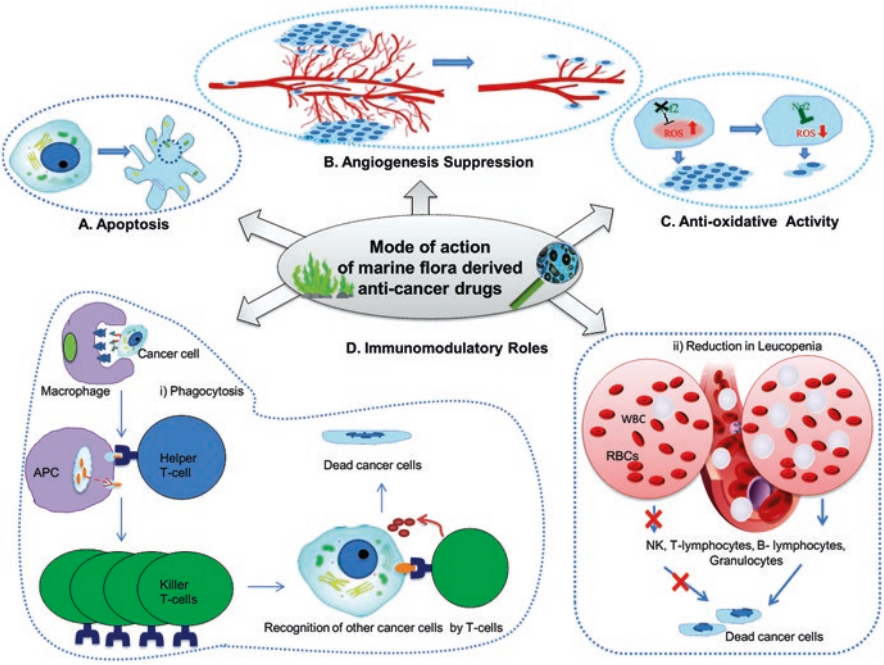

Fig. 7.1 Mode of action of marine flora-derived anticancer drugs: the systematic representaion of the various mode of action of anticancer compounds derived from marine flora. A) Apoptosis or programmed cell death of cancerous cells, B) Suppression of angiogenesis to cut-off the nutrient supply of cancerous cells, C) Antioxidative activity leading to reduction of reactive oxidative species in the vicinity of cancerous cells and D) Immunomodulatory roles such as i) phagocytosis by macrophages and antigen presentation by APCs, leading to destruction of other cancerous cells by T-cells and ii) reduction in leucopenia (loss of WBCs), leading to enhanced immune response to cancerous cells

(i) *Immunomodulatory effects*: Marine flora products can act as immunostimulants, immunoadjuvants, and immunosuppressants by affecting the effector arm of the immune response. Their immunomodulatory action mostly occurs through the following processes such as (a) phagocytosis; b) activation of macrophages or lymphoid cells; c) immunostimulation of peritoneal macrophages and enhancement of cell-mediated immunity and nonspecific immune response, mediators, and natural killer cells; (d) production of antigen-specific immunoglobulin; and (e) reduction of chemotherapy-induced leukopenia by increasing WBCs and IL-2. The immunomodulatory potential of marine flora extracts provides major health benefits to both normal and unhealthy people. In the case of cancer patients, they can activate host defence mechanisms, thereby providing supportive therapy to conventional chemotherapy. Most anticancer drugs with immunosuppressive action can affect the bone marrow and its ability to regenerate new blood cells, thereby resulting in thrombocytopenia and leucopenia. In such a scenario, marine flora-derived immunomodulators with improved pharmacological activity and limited toxicity can provide better chances of recovery for patients on chemotherapy.

(ii) *Apoptotic effects*: Apoptosis (also known as programmed cell death process) is a complex process, which is characterized by a multitude of changes in the dying cells (such as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and DNA fragmentation). It is regulated by various anti- and proapoptotic proteins and caspases. The proapoptotic proteins of Bcl-2 family (such as Bim, a BH3) induce apoptosis by inhibiting the function of antiapoptotic proteins (such as Bcl-XL and Bcl-w). The proapoptotic protein Bim (along with caspase 8) causes cytochrome C release from the mitochondria and executes cell death by cleavage of proteins, vital for cell survival (Levine et al. 2008). Thus, apoptosis maintains cellular homeostasis as its impairment leads to the manifestation of several diseases (such as autoimmune diseases, degenerative disorders, and cancer). Cancer cells become resistant to apoptosis by (1) dysregulation of the mitochondrial pathway; (2) inactivation of caspases; and/or (3) deficiency of death signals (tumour necrosis factor (TNF) superfamily) (Lowe and Lin 2000; Reed 1999). Induction of apoptosis is one of the active strategies to treat cancer. The apoptosis-inducing effect of marine flora products is due to upregulation of immune surveillance and activation of macrophage and death-inducing signal complex, which is discussed in detail in Sect. 7.4.

(iii) *Antioxidative effect*: Free radical damage to DNA is considered to be one of the most important steps leading to cancer. Reactive oxygen species (ROS) overproduction results in genomic instability and cellular damage leading to carcinogenesis (Sabharwal and Schumacker 2014). Free radicals are the product of tissue metabolism, and their potential cellular damage is minimized by the antioxidant capacity and repair mechanisms within the cell. Several endogenous antioxidant enzymes (such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and exogenous free radical scavengers (such as vitamins E and C and carotenoids) defend against free radicals and other

reactive oxygen species (ROS) in the human system (Devasagayam et al. 2004). Several essential minerals (such as selenium, copper, manganese, and zinc), required for the catalytic activity of these antioxidant enzymes, are taken as food supplements. Thus, nutrition plays a key role in maintaining the body's enzymatic defences against free radicals. Seaweeds are excellent food supplements due to the presence of various vitamins A, Bl, B12, C, D and E, riboflavin, niacin, pantothenic acid, and folic acid as well as minerals such as Ca, P, Na, and K (Fleurence 1999). Marine flora products can modulate ROS generation through their antioxidative properties, thus playing vital roles in cancer therapy (Sithranga Boopathy and Kathiresan 2010).

(iv) *Angiosuppressive effects*: Angiogenesis is defined as the formation of neovessels (new blood vessels) from pre-existing vasculature. It is a complex process which involves multiple steps such as (a) activation of endothelial cell (EC) by angiogenic growth factors, (b) protease secretion to digest the extracellular matrix, (c) EC chemotactic migration and invasion, (d) EC tube formation, and (e) neovessel stabilization. Angiogenesis is essential for an adequate supply of oxygen and nutrients to proliferating cancer cells and removal of waste products from its vicinity. Moreover, new blood vessels are also required for the metastatic spread of cancer cells (Carmeliet and Jain 2000). The process of angiogenesis is regulated by a series of angiogenic stimulators and inhibitors. In order to treat cancer and reduce its metastatic spread, the anticancer compounds with angiosuppressive activities are the most popular candidates for drug development. Several marine-derived compounds can exert inhibitory effects on neovessel formation in tumours, thereby acting as potent anticancer drugs (Yue et al. 2017).

7.4 Marine Flora-Derived Products: Their Chemical Composition and Immunomodulatory and Therapeutic Action Against Cancer

Marine floras include microflora (bacteria, actinobacteria, cyanobacteria, and fungi), microalgae, macroalgae (seaweeds), and flowering plants (mangroves and other halophytes). Historical data from India, China, the Near East, and Europe reveal the use of marine floras for medicinal purposes since ancient times (Bandaranayake 1998). Traditional use of seaweeds especially brown algae as food rich in iodine has been found in China, Japan, and other maritime countries to reduce the cases of goitre and glandular diseases. Seaweeds have also been used for the treatment of venereal disease, coronary heart disease, hypertension, gout, and wounds (Mshigeni 1990). Marine floras are a rich source of bioactive metabolites with immunomodulatory and therapeutic potential due to the presence of wide ranges of chemical constituents such as polysaccharides (carbohydrates, uronic acids, and sulphates), polyphenols, terpenoids, steroids, etc. Amongst these, polyphenols and polysaccharides are the most predominant group of compounds with antioxidant and anticancer activities (Thomas and Kim 2011; Wijesekara et al.

2011). Polyphenolic compounds regulate the activity of several xenobiotics metabolizing enzymes responsible for activation of potential carcinogens, thereby acting as anticancer agents. Some polyphenols (such as flavonoids) can inhibit cancer cells by altering hormone production (such as inhibiting aromatase oestrogen synthetase needed for the production of oestrogen) (Steele et al. 2001). The mode of action of anticancer activity of polyphenols is through hindrance in the cellular division during the telophase stage of mitosis. Phenolics reduce the amount of cellular protein and mitotic index and the colony formation in cancer cells (Steele et al. 2001). As far as polysaccharides are concerned, the active components are always the sulphated ones (Jiao et al. 2011; Wang et al. 2014a; Wijesekara et al. 2011). Sulphated polysaccharides increase the tumouricidal activities of macrophages and natural killer cells (NKs), enhancing the innate immune response (Wang et al. 2014a). It can also enhance the adaptive (or specific) immune response by turning tumour cells into antigen-presenting cells (APCs), which activates T-helper cells and produces cytokines (IL-1β and TNF-α). As a result, T-helper cells promote the activity of cytotoxic T cell and NKs to destroy tumour cells. Not only sulphated polysaccharides can recognize a range of cell adhesion systems (CAMs), but they can also bind to various CDs (such as CD2, CD3, and CD4) in T lymphocytes, enhancing their proliferation (Chen et al. 2008). However, most of the research works have been confined to marine faunal species, and floral species have been largely ignored. Recent reports suggest that microbial floras associated with these invertebrates (such as sponges, soft corals, sea fans, sea hares, nudibranchs, bryozoans, and tunicates) are the actual producers of medicinal compounds. Thus, this vast and relatively unexplored marine floral resource acts as a hidden treasure for the discovery of new drugs.

(i) *Marine Bacteria*: Marine bacteria act as a great source for the discovery of secondary metabolites with new drug properties and target sites. Several novel anticancer agents (such as bryostatins, discodermolide, eleutherobin, and sarcodictyin) have been isolated from marine bacteria associated with sponges, corals, and fungi (D'Ambrosio et al. 1987; Davidson et al. 2001; Partida-Martinez and Hertweck 2005; Piel et al. 2004). Some of these marine bacteria (such as lactobacilli and bifidobacteria) are used as dietary supplements (probiotics), thereby controlling colon cancer through their immunostimulatory and immunosuppressive behaviour (Burns and Rowland 2000; Wollowski et al. 2001). These probiotics modulate the mucosal immune system by (a) increasing production of anti-inflammatory cytokines (such as IL-10) and host defence peptides (such as β-defensin 2), (b) enhancing IgA defences, (c) influencing dendritic cell maturation, (d) increasing cell proliferation and apoptosis through cell responses to short-chain fatty acids, and (e) reducing the production of pro-inflammatory cytokines through actions on NFκB pathways (Devine and Marsh 2009). Not only nutrient supplements (probiotics) but also toxins from marine bacteria can help to cure cancer. For instance, the marine bacteria associated with the dinoflagellate *Noctiluca scintillans* secrete a major compound **macrolactin-A** which can inhibit murine melanoma cancer (B16-

F10) cells (Jaruchoktaweechai et al. 2000). **Kahalalide F** (KF), a depsipeptide isolated from the symbiotic bacteria of the mollusc *Elysia rufescens*, can induce cytotoxicity by blocking the G1 phase of the cell cycle in a p53-independent manner. In vitro assay on solid tumours showed that KF can selectively kill prostate cancer cells, thereby allowing this drug molecule to reach phase 1 clinical trials (Rademaker-Lakhai et al. 2005). In addition, the extensive in vivo experimental results support the role of KF as potent anticancer against breast and colon cancers (Janmaat et al. 2005; Suárez et al. 2003). **Aburatubo lactams**, acyl tetramine derivatives, isolated from the marine bacteria *Streptomyces* sp., SCRC-A20 inhibit TPA-induced superoxide anion generation by activation of human neutrophils, thereby acting as a potent drug against inflammation, cancer, and ageing (Bae et al. 1996; Kim 1998). However, its action mechanism is currently under investigation. **Streptopyrrolidine,** a benzyl pyrrolidine derivative, isolated from the deep-sea marine bacteria *Streptomyces* sp. KORDI-3973 does not show cytotoxicity but can significantly suppress capillary tube formation of human umbilical vein endothelial cells (HUVECs), thereby acting as potent antiangiogenic drugs for cancer therapy (Shin et al. 2008). The marine bacteria belonging to the order *Actinomycetales* are commonly called actinomycetes. Due to their high diversity, the actinomycetes are treated as important sources of useful bioactive compounds. For instance, compounds of anthraquinone family (such as **parimycin**, **trioxacarcins**, and **gutingimycin**), isolated from marine *streptomycete* isolate B8652, showed antitumour activities at different degrees (Maskey et al. 2002, 2004a, b). **B-1**, a sulphated polysaccharide from the marine bacteria *Pseudomonas* sp., induces apoptosis of human leukaemic cells (U937) (Matsuda et al. 2003). Marine actinomycetes from the family *Micromonosporaceae* are found to be potent anticancer agents by targeting the proteasome function of the cancer cells. For instance, **thiocoraline**, a novel bioactive depsipeptide isolated from *Micromonospora marina* belonging to this family, selectively acts against lung, colon, and melanoma cancer cell lines by inhibiting RNA synthesis (Romero et al. 1997). Interestingly, the compound can act through p53-independent pathways as shown by its preferential antiproliferative effects on p53-depleted colon cancer cell lines (Erba et al. 1999). Moreover, thiocoraline can be biosynthesized and expressed in the heterologous host by specialized nonribosomal peptide-synthetase (NRPS) gene cluster, and it is the best illustration of solving the problem of drug supply through coculture engineering (Lombó et al. 2006).

(ii) *Marine Fungi*: Marine fungi are natural sources of antioxidants and anticancer compounds. **Phosphosulfomannan (PI-88)**, a sulphated oligosaccharide, isolated from the marine yeast *Pichia holstii* induces apoptosis of pancreatic islet carcinoma and also acts as an angiosuppressive drug (Ferro et al. 2007). **Nidurufin**, a hydroxy derivative of anthraquinone (averufin), isolated from marine fungus *Penicillium flavidorsum* SHK1-27, can induce in vitro cell cycle arrest at G_2/M transition in the leukaemia K562 cell line in a dose- and time-dependent manner (Ren and Liu 2011). Another compound **emodin**

(1,3,8-trihydroxy-6-methylanthraquinone) with multiple biological activities including anticancer activity acts as a specific inhibitor of the protein tyrosine kinase p65lck (Srinivas et al. 2007). Several **quinofuracins** (anthraquinone derivatives containing β-D-galactofuranose) can induce apoptosis by both downregulating miR-200c in pituitary adenoma cells and causing cytotoxicity in radiosensitive and radioresistant nasopharyngeal carcinoma cells via miR-205-PTEN-Akt pathway (Fouillaud et al. 2016). Recently, a novel anthraquinone derivative, **aspergiolide A**, isolated from the marine fungus *Aspergillus glaucus*, kills several cancer cell lines (A-549, HL-60, BEL-7402, and P388) by targeting topoisomerase II and inducing apoptosis via a caspase-dependent pathway (Wang et al. 2014b). In vivo studies on this compound demonstrated its anticancer activity in hepatocellular carcinoma xenografts (Wang et al. 2014b).

Lichens: Lichens are the composite organisms formed by symbiotic association of (a) algae or cyanobacteria and (b) fungi. Traditional uses of lichen as medicine were documented by Pacific Islanders due to its antimicrobial and anticancer agents (Bugni et al. 2009; Marante et al. 2003). **Atranorin** (phenolic acid) from lichen *Parmotrema saccatilobum* and *Lethariella canariensis* inhibits cyclooxygenase enzymes (COX-1 and COX-2) and biosynthesis of leukotriene B4 (LTB4) in bovine polymorphonuclear leukocytes (Bugni et al. 2009). It was also found cytotoxic against HL-60 leukaemia cells (Marante et al. 2003). **Diploicin**, a chlorinated depsidone, isolated from the lichen *Diploicia canescens* shows cytotoxic activity against B16 melanoma cells (Millot et al. 2009).

Epiphytic fungi: The fungi which grow upon or are attached to the living plants (such as seaweeds, seagrass, halophytes, etc.) are called epiphytic fungi. They act as a good source of anticancer compounds. For instance, **communesins A**, an alkaloid from the epiphytic fungus *Penicillium* sp. on the green alga *Enteromorpha intestinalis*, has cytotoxic properties (König and Wright 1996). The peptide **dictyonamide A** isolated from the epiphytic fungus *Cocodinium* sp. on red alga *Ceradictyon spongiosum* inhibits CDK4 (Bowers 2017; Kobayashi and Tsuda 2004). The macrolides (**sporiolides A** and **B**) from the epiphytic fungus *Cladosporium* sp. on the brown alga *Actinotrichia fragilis* exhibit cytotoxicity against L1210 leukaemia cells (Murti and Agrawal 2010). The cyclic **pentadepsipeptide sansalvamide** from the epiphytic fungus *Fusarium sp*. growing on the same seagrass species shows cytotoxic against COLO 205 colorectal adenocarcinoma cells (Belofsky et al. 1999). The polyketide glycoside (**cladionol A**) isolated from fungus *Gliocladium* sp. epiphytic to the seagrass *Syringodium isoetifolium* shows cytotoxic activity against L1210 and KB cells (Kasai et al. 2005). However, the mode of action for these compounds is not known.

Endophytic fungi: Marine endophytic fungi (which grow inside the seaweeds and halophytes) are a source of novel bioactive compounds including several anticancer drugs. For instance, the compound **2,3-dihydro-2-hydroxy-2,4-dimethyl-5-trans propenylfuran-3-one**, from the endophytic obligate

fungus *Ascochyta saliconiae* residing inside the green alga *Ulva* sp., acts as potent anticancer compound due to its protein tyrosine kinase inhibitory action (Folmer et al. 2010). The alkaloid **halimide** from the fungus *Aspergillus* sp., which is endophytic to the green alga *Halimeda copiosa*, inhibits the growth of colon carcinoma cells and ovarian carcinoma cells by arresting G2/M phase of the cell cycle (Fenical et al. 2000). **Leptosin A**, from endophytic fungus *Leptosphaeria* sp. isolated from the brown alga *Sargassum tortile*, shows cytotoxic activity against P-388 leukaemia cells by inhibiting topoisomerase II (Takahashi et al. 1994). The chromone **pestalotiopsone F**, from the fungus *Pestalotiopsis* sp. endophytic to the mangrove plant *Rhizophora mucronata*, acts as potent anticancer compound against L5178Y lymphoma cells (Xu et al. 2009). The macrolide **hypothemycin**, isolated from the fungus *Aigialus parvus* endophytic to marine mangroves, shows cytotoxicity against several cancers (such as P-388, L1210 leukaemia cells, C-26 colon cancers, KB, and BC-1 solid tumour cells) (Tanaka et al. 1999). **G503**, an anthraquinone compound, isolated from mangrove endophytic fungus *Halorosellinia* sp. No. 1403, induces apoptosis in gastric cancer cells through the intrinsic mitochondrial pathway and/or reticulum apoptosis pathway via caspase-4 cleavage (Huang et al. 2014). **Alterporriols**, bianthraquinone derivatives, isolated from the endophytic mangrove fungus *Alternaria* sp. ZJ9-6B can kill human breast cancer cell lines (MDA-MB-435, MCF-7) by either apoptosis or necrosis. Moreover, they also cause the generation of the reactive oxygen species (ROS) and shift in mitochondrial membrane potential by altering cytosolic free calcium level (Huang et al. 2011; Huang et al. 2012). **Rhein** (anthraquinone metabolite) from endophytic fungus *Rheum palmatum* can act a potential antagonist of the purinergic P2X7 receptor, which is a potential therapeutic target for inflammatory diseases (You et al. 2013). It is reported that rhein can inhibit the purinergic P2X7 receptor-mediated macrophage responses in mice model, resulting in a cascade of immune responses such as (a) intracellular cytosolic calcium concentration, (b) pore formation in cellular membrane, (c) reactive oxygen species production, (d) attenuation of phagocytosis, and (e) cell apoptosis (Tsang and Bian 2015). Several **hydroxyanthraquinone derivative**s, from the mangrove associated fungi *Guignardia* sp. No. 4382 and *Halorosellinia* sp. No. 1403, have shown potent cytotoxicity to parental drug-sensitive KB and KBv200 cancer cell lines by inducing apoptosis via mitochondrial dysfunction, rather than intercalating itself into cellular DNA (Fouillaud et al. 2016). **SZ-685C**, an anthraquinone isolated from marine endophytic fungus *Halorosellinia* sp. No. 1403, inhibits the growth of various cancer cell lines such as KB, KBv200, MMQ, human glioma, hepatoma, prostate cancer, and breast cancer cell lines (MCF-7 and MDA-MB-435) by inducing apoptosis via the Akt/forkhead box protein pathway (Xie et al. 2010). Several anticancer compounds have been isolated from fungi associated with several invertebrates such as gorgonian, corals, and sponges. For instance, **cladosporilac-**

tam A, a novel bicyclic lactam isolated from gorgonian-associated endophytic fungus *Cladosporium* sp., shows anticancer activity against multiple cancer cell lines (Cao et al. 2015). **Hirsutanol**, a novel sesquiterpene compound isolated from coral *Sarcophyton tortuosum*-associated endophytic fungus, causes apoptosis and autophagy in human hepatocellular carcinoma and breast cancer cells via mitochondrial-independent ROS production (Yang et al. 2010, 2012). **Sorbicillactone A**, an alkaloid isolated from various species sponge-associated fungi, demonstrates cytotoxicity against several cancer cell lines (Bringmann et al. 2005). Reports suggest that anticancer compounds from *Mycale plumose* sponge-associated endophytic fungus of the genus *Penicillium* selectively target leukaemia cells without any visible cytotoxicity to normal cells (Bringmann et al. 2005).

(iii) *Microalgae*: This group comprises a diverse class of microscopic organisms including diatoms, dinoflagellates, and blue-green algae (cyanobacteria). Amongst various marine microalgae, blue-green algae (cyanobacteria) outstand as one of the richest sources of known and novel bioactive compounds for pharmaceutical applications (Burja et al. 2001).

Diatoms: A very few bioactive diatom-derived natural products have been studied until today. The lipid **hasla-6(17), 9, 13, 23 tetraene**, isolated from the diatom *Haslea ostrearia*, inhibits lung cancer (NSCLC-N) and leukaemia (P-388) cells by arresting G1/S phase of the cell cycle (Rowland et al. 2001).

Dinoflagellates: Many of the dinoflagellate-derived compounds have beneficial anticancer properties. For instance, the sulphated polysaccharide **GA3P**, isolated from *Gymnodinium* sp., induces apoptosis in K562 leukaemia cells by competitively inhibiting DNA topoisomerases I and II. GAP3 mimics the structure of DNA and binds to the positively charged locus of the enzyme topoisomerases I and II, thereby preventing its binding to DNA (Sogawa et al. 1998; Umemura et al. 2003). The sulphated polysaccharide, **p-KG103**, which is purified from the marine microalgae dinoflagellates *Gyrodinium impudium*, prevents tumour cell growth both in vitro and in vivo. Immunomodulatory action of this compound is via ROS (nitric oxide) production in a JNK-dependent manner leading to the production of cytokines (interleukin-1 (IL-1), IL-6, and TNF-α) in macrophages (Guo et al. 2017). The macrolides **amphidinolide H** isolated from *Amphidinium* sp. is extremely cytotoxic against L1210 murine lymphoma, KB cervical cancer, and P-388 leukaemia cell lines by disrupting actin organization in the cells (Kobayashi et al. 1991; Usui et al. 2004). Other macrolide **carbenolide** from the same species *Amphidinium* sp. is cytotoxic against HCT-116 colon carcinoma cells; however, its mode of action is not known (Folmer et al. 2010).

Cyanobacteria: The marine cyanobacteria-derived products act as powerful anticancer compounds by either inducing cell apoptosis or affecting the cell signalling via activation of protein kinase-c (PKC). For instance, the lipopeptide **mitsoamide** from *Geitlerinema* sp. is cytotoxic against NCI-H460

cells (Andrianasolo et al. 2007). **Scytonemin**, a protein serine/threonine kinase inhibitor isolated from the marine cyanobacterium *Scytonema* sp., inhibits cell division in human fibroblast, Jurkat, and endothelial cells by regulating mitotic spindle formation and cell cycle kinases (CDKs and cyclins) (Pallela et al. 2010; Zhang et al. 2013). Its mode of action is via inhibition of the cell cycle kinase human polo-like kinase 1, which plays an important role in the progression through the G2/M phase of the cell cycle (Frenz et al. 2004; Nagle et al. 2004). **Patellamide D**, isolated from symbionts *Prochloron didemni* of didemnid ascidians, is capable of reversing MDR in the human leukaemia (CEM/VLB100) cells (Williams and Jacobs 1993). **Calothrixins A and B**, pentacyclic metabolites isolated from marine cyanobacterium *Calothrix* sp., inhibit cervical cancer (HeLa) cells in a dose-dependent manner (Bernardo et al. 2007). **Curacin A**, isolated from the marine cyanobacterium *Lyngbya majuscula*, inhibits the cell division in colon, renal, and breast cancer-derived cell lines by preventing the polymerization of the tubulin during the cell cycle (Blokhin et al. 1995). In addition, the lipopeptide **microcolin A**, isolated from the same species *Lyngbya majuscula*, induces apoptosis in lymphocytes and murine leukaemia (P-388) cells (Koehn et al. 1992). **Largazole**, a unique chemical scaffold, isolated from tropical marine cyanobacterium *Symploca* sp., acts as an efficient anticancer drug against chemoresistant melanoma, colon cancer (HCT-116), and cervix carcinoma (HeLa) cell lines (Bowers et al. 2008; Taori et al. 2008). Its mode of action is through the inhibition of class I histone deacetylases (HDACs) (Bowers et al. 2008; Montero et al. 2009). **Apratoxin A**, isolated from marine cyanobacterium *Lyngbya boulloni*, can affect a variety of cancer cell lines (including adenocarcinoma cell lines) at nanomolar concentrations. Its mode of action is through cell cycle arrest at the G1 phase and blocking the fibroblast growth factor receptor (FGFR) pathway by preventing the phosphorylation of STAT3 (Luesch et al. 2006). However, due to its high toxicity, it was not considered as a good drug candidate for the clinical trials. **Dolastatins**, initially isolated from the sea hare *Dolabella auricularia*, are actually the metabolite produced by its symbiotic cyanobacteria *Symploca* sp. (Frenz et al. 2004; Tan 2007). It acts as a cytotoxic agent against lymphocytic leukaemia cells by inhibiting tubulin polymerization by binding to the tubulin vinca domain (Molinski et al. 2009; Tan 2007). Dolastatin analogues (**symplostatin 1** and **belamide A**), isolated from *Symploca* sp., act as potential inhibitor of cancer cells by depolymerization of microtubule assembly (Luesch et al. 2001; Simmons et al. 2006). A synthetic dolastatin analogue, **ILX-651** (**tasidotin**) (Genzyme Corporation, Cambridge, MA), a potent anticancer drug, has successfully reached phase II clinical trials (Mayer et al. 2010). **Coibamide A**, a cyclic depsipeptide, isolated from marine cyanobacterium *Leptolyngbya*, exhibits high cytotoxicity against lung cancer (NCI-H460) and mouse neuroblastoma (neuro-2a) cells (Hau et al. 2013; Medina et al. 2008). **Borophycin**, a boric acid containing polyketide, isolated from marine cyanobacterial

strains of *Nostoc linckia* and *Nostoc spongiae*, exhibits potent cytotoxicity against human epidermoid carcinoma (LoVo) and human colorectal adenocarcinoma (KB) cell lines (Scorei 2013). **Cryptophycin and its analogues**, isolated from marine cyanobacteria *Nostoc* sp. ATCC 53789 and GSV 224, exhibit potent cytotoxicity against drug-sensitive and drug-resistant murine and human solid tumours, KB and LoVo cells (Smith et al. 1994). However, due to their high toxicity, these compounds could not enter clinical trials. Its mode of action is through induction of hyperphosphorylation of antiapoptotic protein Bcl-2. Similar to dolastatin 10, it can bind to the tubulin vinca domain (Smith and Zhang 1996). Its synthetic analogue (cryptophycin-52) (LY355703) (Eli Lilly & Co.) had progressed into phase II clinical trials, but the compound could not go further due to its dose-limiting neurotoxicity (Edelman et al. 2003).

(iv) *Macroalgae (Seaweeds)*: Marine algae have been widely used as medicine by ancient Chinese, Egyptians, South Americans, Indians, and Italians for about 2000 years (Chatterji et al. 2010; Dillehay et al. 2008; Liu et al. 2012; Manivannan et al. 2008). Seaweeds, being the important sources of protein, iodine, vitamins, and minerals, were amongst the first organisms investigated by marine natural product researchers in their quest for novel pharmaceutical compounds. Due to their high polyphenolic content (such as phenolic acids, flavonoids, anthocyanidins, lignin, tannins, catechin, epicatechin, epigallocatechin, and gallic acid), seaweeds display various biological functions as antioxidant, anticancer, antiviral, and anti-inflammatory agents (Damonte et al. 2004; Gupta and Abu-Ghannam 2011; Senthilkumar et al. 2013). They are used as food supplements in patients with coronary heart disease, cancer, and several other diseases due to their antioxidative and metal chelating activities (Dring 2005; Jiménez-Escrig and Sánchez-Muniz 2000). Polyphenols regenerate α-tocopherol (a type of vitamin E) through reduction of the α-tocopheroxyl radical, which has antioxidative and anticancer properties (Haslam 1996). The crude extracts from various seaweeds (such as red alga *Acanthophora spicifera*, green alga *Ulva reticulata*, red alga *Gracilaria foliifera*, and brown alga *Padina boergesenii*) from the Gulf of Mannar (GoM), India, have also been reported to exhibit cytotoxic activity (Kolanjinathan et al. 2014).

Brown algae: The first use of seaweed as the anticancer drug was done in the 1960s using a phytocolloid **Algasol T 331**, isolated from brown alga. The intramuscular injections of Algasol T331 were found to cure over 68% of oncologic patients in Italy (Claudio and Stendardo 1966). **Heterofucans**, which are composed of fucose, glucose, glucuronic acid, galactose, and sulphate, show both anti-proliferative and immunomodulatory functions. For instance, heterofucans from the brown alga *Dictyota menstrualis* inhibit leukocyte migration with a related decrease in the levels of pro-inflammatory cytokines (Albuquerque et al. 2013). Heterofucans from *Sargassum filipendula* exhibit anti-proliferative effects on cervical, prostate, and liver cancer cells (Telles et al. 2018). **Fucoidans** (sulphated polysaccharides) exhibit several biological functions such as antitumour, anticancer, antimetastatic,

anticoagulant, antithrombotic, antiviral, immunomodulatory, anti-inflammatory, antioxidant, anticomplementary activities, and fibrinolytic properties (Cumashi et al. 2007; Li et al. 2008). Fucoidans from various brown algae act as immunostimulants in immunosuppressed individuals by acting directly on macrophage and T lymphocyte. For instance, fucoidans from brown alga *Laminaria japonica* can promote the recovery of immunologic function in irradiated rats by the arrest of lymphocyte apoptosis (Huang et al. 2010; Wang et al. 2008). Its mode of action is via activation of the production of interleukin-1 (IL-1), interferon-γ (IFN-γ), T lymphocyte, B cell, macrophage, and natural killer cell (NK cell). In addition, it also promotes the primary antibody response to sheep red blood cell (SRBC) (Itoh et al. 1995). High-molecular-weight fucoidan, from edible brown algae *Okinawa mozuku*, promotes an increase in the proportion of murine cytotoxic T cells (Shimizu et al. 2005). Fucoidan, from the brown alga *Fucus vesiculosus*, acts as an immunostimulating agent by causing maturation of antigen-presenting cells such as dendritic cells (DCs) in the bone marrow via nuclear factor-κB (NF-κB) pathway (Kim and Joo 2008). However, sulphation is critical for the biological activity of fucoidan as desulphated fucoidan does not cause angiogenesis (Koyanagi et al. 2003) or induce immature CD34+ cell mobilization (Irhimeh et al. 2007). In the case of the anticancer activity, the degree of sulphation may also have an important role. For instance, fucoidan isolated from brown algae *Ascophyllum nodosum* shows an anti-proliferative effect on both normal and malignant cells, including fibroblasts (Hamster Kidney Fibroblast CCL39), sigmoid colon adenocarcinoma cells (COLO320 DM), and smooth muscle cells (Jiang et al. 2010). It causes apoptosis effects on human colon cancer cells (HCT116) by activation of caspases 3 and 9 and the PARP cleavage, thereby causing changes in the permeability of mitochondrial membrane (Foley et al. 2011). Fucoidan from the brown alga *Ecklonia cava* decreased cyclooxygenase-2, nitric oxide, and prostaglandin E2 levels by acting as anti-inflammatory agent (Kang et al. 2011; Lee et al. 2012). It also induces apoptosis in human lymphoma (HS-Sultan) and colon cancer (HT-29 and HCT116) cells by activation of caspase-3 and downregulation of extracellular signal-regulated kinase pathway (Ahn et al. 2015). Fucoidans from the brown algae *Cladosiphon okamuranus* show anti-proliferative activity on myeloid leukaemia cell lines by inducing cell apoptosis and production of ROS (Atashrazm et al. 2016). Fucoidan from the brown algae *Saccharina gurjanovae* inactivates the epidermal growth factor receptor (EGFR), an important player in cell transformation, differentiation, proliferation, and cancer prognosis (Nicholson et al. 2001; Vishchuk et al. 2013). Fucoidan from *Cladosiphon okamuranus* also inhibits *Helicobacter pylori*, the main cause of gastric cancer in rodent *Mongolian gerbils* (Shibata et al. 2003).

Fucoidans and carotenoids, extracted from brown algae or seaweed (e.g., mozuku, kombu, bladderwrack, wakame, and hijiki), modulate angiogenesis by enhanced HUVEC migration and fibroblast growth factor-2 (FGF-2)-

induced vascular tube formation (Delma et al. 2015; Matou et al. 2002; Matsubara et al. 2005). For instance, the carotenoids, **siphonaxantin** and **fucoxanthinol**, from brown algae (edible seaweed such as *Undaria pinnatifida* and *Hijikia fusiformis*), exhibit inhibition on HUVECs' proliferation and tube formation by reduction of microvessel outgrowth from rat aortic fragments (Sugawara et al. 2006). **Stylopoldione**, metabolite isolated from brown algae *Stypopodium* sp., kills cancer cells by halting mitotic spindle formation during the cell division (Varshney and Singh 2013). **Meroterpenes** and **usneoidone**, isolated from brown algae *Cystophora* sp., control tumorigenesis in several cancer cell lines (such as murine L929, human MCF7 breast adenocarcinoma, PA1 ovary teratocarcinoma, and PC3 androgen-resistant prostate carcinoma cells) by the radical scavenging antioxidant mechanism (Taskin et al. 2010). **Phloroglucinol** and its different polymers, isolated from the brown alga *Eisenia bicyclis,* suppress metastasis of breast cancer cells by inhibition of epithelial-mesenchymal transition (EMT) (Hussain et al. 2016). This compound also downregulates a transcription factor SLUG (SNAIL-related zinc-finger transcription factor), which is crucial for EMT, via inhibition of PI3K/AKT and RAS/RAF-1/ERK signalling (Kim et al. 2015). **CphF**, crude polyphenolic fractions from the marine brown alga *Ecklonia cava*, inhibits the growth of mouse colon cancer cells (Athukorala et al. 2006). **Dieckol** isolated from the same algae shows anti-inflammatory and antitumour activity on ovarian cancer cells (A2780 and SKOV3) and also inhibits tumour xenograft in a mouse model. Its mode of action is through induction of caspase-dependent apoptosis via ROS production and the regulation of AKT and p38 signalling. Besides anticancer activity, dieckol also exerts potent angiosuppressive effects by inhibiting VEGF-induced EA.hy-926 human endothelial cell proliferation and migration by suppressing MMP-2 and MMP-9 gene and protein expression. The mode of action is through inhibition mitogen-activated protein kinase (MAPK) signalling pathway molecules (ERK and p38) (Li et al. 2015). **Dioxinodehydroeckol**, a phloroglucinol derivative from the same algal species, causes apoptosis in MCF7 breast cancer cells by activating caspase-3 and caspase-9 and deactivating DNA repair enzyme PARP (Kong et al. 2009b). **Laminaran (or laminarin)**, a polysaccharide from brown algae *Laminaria* sp., shows inhibitory effect on colon cancer (HT-29 and LOVO) cells by modulating ErbB and insulin-like growth factor-IR signalling pathways and increasing intracellular ROS and Ca levels (Ji et al. 2012; Park et al. 2013). This compound induces cell death in HT-29 cells in a dose-dependent manner by decreasing mitogen-activated protein kinases (MAPK) and ERK phosphorylation and inhibiting the heregulin-stimulated phosphorylation of ErbB2 (Park et al. 2013). Moreover, S-laminaran (sulphated) reduces metastasis by inhibition of heparanase, an enzyme required for metastasis process (Alessandra Gammone et al. 2016). Crude extract from the brown seaweed *Sargassum thunbergii* inhibits metastasis in the rat mammary adenocarcinoma cells (13762 MAT) (Zhuang et al. 1995).

Phloroglucinol and its polymers such as eckol (a trimer), phlorofucofuroeckol A (a pentamer), dieckol, and 8,8-bieckol (hexamers), isolated from brown algae *Eisenia bicyclis*, show both antioxidative and inhibitory effects on enzyme hyaluronidase (HAase), thereby acting as potent anticancer compounds (Shibata et al. 2002).

Red algae: Several red seaweeds are also used as nutrient supplements due to its antioxidative and anticancer activities. For example, the edible red algae *Palmaria palmata* inhibits cancer cell proliferation in patients due to its antioxidative activities (Burtin 2003). The crude extracts isolated from several seaweeds have also shown antioxidative and anticancer properties. For instance, the extract of the red alga *Acanthophora spicifera* exhibits tumouricidal activity on Ehrlich-Lettre ascites carcinoma (EAC) cells in mice model (Lavakumar et al. 2012). This extract demonstrates an immunomodulatory action through cellular apoptosis involving membrane blebbing and vacuole formation. **Condriamides A and B**, novel indolic metabolites from red algae *Chondria* sp., act as a potent anticancer agent against colorectal and nasopharyngeal cancer cells (Palermo et al. 1992). The alkaloids **lophocladine A** and **lophocladine B**, isolated from a red alga *Lophocladia* sp., act as an anticancer agent against various cancer cell lines (Zhang et al. 2007). **Terpenoids** from various red algae show differential cytotoxic profiles based on their active functional groups. **Polyhalogenated monoterpenes** such as 28-anhydrothyrsiferyl diacetate, l5-anhydrothyrsiferyl diacetate, magireol A, magireol B, and magireol **C**, from marine red alga *Portieria hornemannii*, exhibit differential cytotoxicity against the NCI-60 human tumour cell lines, depending on its chemical constituents (Fuller et al. 1992). **Laurene-type sesquiterpenes**, isolated from the red alga *Laurencia obtusa*, possess both antimicrobial against *Candida albicans* and antitumour activities against Ehrlich as cites tumour cells (Alarif et al. 2012). **Cytotoxic sesquiterpenes**, from the red algae *Laurencia obtusa* and *Laurencia microcladia*, show cytotoxicity against chronic myelogenous leukaemia-derived K-562 cells (Kladi et al. 2006). Diterpenes (**amijiol**, **amijiol acetate, 10-diacetate**) isolated from the red sea brown alga *Dictyota dichotoma* var. *implexa* show high cytotoxicity against WI-38, HepG2, and MCF-7 cell lines by causing DNA damage (Ayyad et al. 2011). Several **brominated diterpenes**, isolated from the organic extract of red alga *Sphaerococcus coronopifolius*, show differential cytotoxicity profiles against the human lung cancer (NSCLCN6-L16 and A549) cell lines, depending on their chemical compositions and active functional groups (Smyrniotopoulos et al. 2010). Squalenoid-derived triterpenoids (such as **laurenmariannol** and (**21a)-21-hydroxythyrsiferol**), isolated from the marine red alga *Laurencia mariannensis*, display significant cytotoxic activity against murine leukaemia (P388) cells (Ji et al. 2008b). Some **squalene derivatives** (such as squalene-derived brominated triterpenes – dehydrothyrsiferol, isodehydrothyrsiferol, and 10-epidehydrothyrisiferol), isolated from *Laurencia viridis*, show potential anticancer activities against

cancer cell lines (Fernández et al. 1998; Norte et al. 1996). **Polyethers** (such as iubol, 22-hydroxy-15(28)-dehydrovenustatriol, 1,2dehydropseudodehydrothyrsiferol, and secodehydrothyrsiferol) from the same alga exhibit significant cytotoxic activity against a panel of cancer cell lines (Pacheco et al. 2011). Other compounds, **16-hydroxydehydrothyrsiferol**, **thyrsenol A**, and **thyrsenol B**, from the same red seaweed *Laurencia viridis*, cause cytotoxic effect against P388 cell line by inhibiting enzyme protein phosphatase (Souto et al. 2003). Two **sterol glycosides** (19-O-b-D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one and 19-O-b-DN-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one) from red alga *Peyssonnelia* sp. inhibit several cancer cell lines such as breast (MDA-MB-468) and lung (A549) cancer cells (Lin et al. 2010). The sulfolipid sulfoquinovosyldiacylglycerol **(KM-043**) from *Gigartina tenella* inhibits DNA polymerases a and b, thereby acting as a potent cytotoxic agent (lohn Davis and Kumar 2012). Crude extracts of some red algae are also cytotoxic in nature. For instance, the crude extracts of red alga *Gracilaria tenuistipitata* show anti-proliferative effects on Ca9-22 oral cancer cells by inducing cellular apoptosis, DNA damage, and oxidative stress (Yeh et al. 2012), whereas crude extracts of red algae *Plocamium telfairiae* inhibit growth of HT-29 colon cancer cells by causing caspase-dependent apoptosis (Kim et al. 2007).

Green algae: The bromophenolic compound **isorawsonol**, isolated from green alga *Avrainvillea rawsonii* and the glycolipids **nigricanosides A** and B, isolated from *A. nigricans,* induce mitotic arrest in several cancer cell lines (Chen et al. 1994; Williams et al. 2007). **Caulerpenyne**, a sesquiterpene isolated from the green algae *Caulerpa* sp., shows anti-proliferative activity against tumour cell lines SK-N-SH by modifying the microtubular network during cell division and migration (Barbier et al. 2001). Some carbohydrates (polysaccharides) derived from the green algae are reported to effectively block carcinogenesis. For instance, **CRP**, a crude polysaccharide from the green alga *Caulerpa racemosa*, shows anticancer activity on melanoma cells and hepatoma (H22) tumours transplanted in mice by activation of pro-inflammatory cytokines (IL-2, IL-12, and INF-α), natural killer cells, Toll-like receptor-4, CD-14, and competent receptor 3, thereby leading to nitric oxide release and apoptosis (Ji et al. 2007, 2008a). The polysaccharide **DAEB**, isolated from the green alga *Enteromorpha intestinalis*, acts as an immunostimulant by enhancing phagocytosis and secretion of TNF-α and nitric oxide in peritoneal macrophages as well as concanavalin A-induced lymphocyte proliferation in mice model (Jiao et al. 2009). Several polysaccharides from the green alga *Ulva rigida* activate the production of nitric oxide and immunostimulate the release of cytokines (interleukin-1 IL-1, IL-6, and TNF-α) in macrophages in a JNK-dependent manner (Leiro et al. 2007). The sulphated glycoproteins from the green alga *Codium fragile* can act as the potent anticancer drugs with immunostimulatory properties. The mode of action of this compound is through the prolif-

eration of murine macrophage cells (RAW264.7) via NF-κB and MAPK pathways to release free radical NO that could possibly kill the cancer cells (Tabarsa et al. 2015). Various **steroids** from marine green alga *Tydemania expeditionis* show significant anticancer activity on the prostate cancer cell lines (DU145, PC3, and LNCaP) (Zhang et al. 2012). However, the exact mechanism of action is still under investigation. **Astaxanthin**, a carotenoid from *Haematococcus pluvialis* and *Chlorococcum* sp., acts as a potent antioxidant, apoptosis promoter, NF-kB inhibitor, and growth inhibitor against various cancer cell lines (Liu and Lee 1999; Palozza et al. 2009; Seon-Jin et al. 2003). Other two carotenoids, **siphonaxantin and fucoxanthinol**, from green alga *Codium fragile* exhibit inhibition on human umbilical vein endothelial cells' (HUVECs) proliferation and tube formation in a dose-dependent manner (Li et al. 2014). The mode of action of both compounds is through the reduction of microvessel outgrowth from rat aortic fragments (Ganesan et al. 2010).

(v) *Mangroves and halophytes*: Medicinal values of mangroves have long recognized by fisherman communities to treat various diseases (Bandaranayake 1998; Kathiresan 2000). Several mangroves and halophytes have been suggested as potent sources of anticancer drugs, based on traditional knowledge and preliminary scientific work. For instance, **brugine** (A 1,2-dithiolane), a sulphur-containing alkaloid isolated from *Bruguiera sexangula* (commonly called upriver orange mangrove), acts as an antitumour agent against sarcoma 180 and Lewis lung carcinoma (Kathiresan and Qasim 2005). Another compound, **tannin**, from this plant also showed anticancer activity against lung carcinoma. A ribose derivative (2-**benzoxazoline)** isolated from *Acanthus ilicifolius* shows anticancer and antiviral activities against several cancers (Kathiresan and Qasim 2005). **Tea extract** from the mangrove plant *Ceriops decandra* prevents the (DMBA) dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis (HBPC) by enhancing beneficial bacteria in its oral cavity (Boopathy et al. 2011). **Tetranortriterpenoids such as xylogranatins A–D**, isolated from the mangrove *Xylocarpus granatum*, are cytotoxic against various cancer cell lines (Yin et al. 2006), whereas **limonoids and granaxylocarpins A and B** from the same plant show cytotoxic effect against the P-388 leukaemia cells (Yin et al. 2007). **The naphtoquinones 3-chlorodeoxylapachol** and **stenocarpoquinone B** from *Avicennia germinans* and *A. marina* act as the cytotoxic agents against a wide range of cancer cell line (such as K562 and HeLa) (Han et al. 2007; Jones et al. 2005). The three cardenolide glycosides (such as **2′-O-acetyl cerleaside A**, **17b-neriifolin**, and **cerberin**), isolated from *Cerbera odollam*, are cytotoxic against a range of cell lines (KB and NCI-H187) (Laphookhieo et al. 2004). The flavonoid **luteolin** from the seagrass *Zostera marina* possesses strong antioxidant and inhibitory activity on matrix metalloproteinase-1 (MMP-1) (Kim et al. 2004). Another flavonoid **isorhamnetin 3-O-b-D-glucopyranoside** from halophyte *Salicornia herbacea* acts as a strong antioxidant by preventing ROS-induced cellular damage in TNF-α stimulated myeloid cells (Kong et al. 2009a).

However, the exact mode of action of anticancer products from mangroves and halophytes has not been described in detail.

7.5 Conclusion

Marine floras are potential sources of various novel anticancer compounds due their diverse chemical ecology which act as a selection pressure for production of secondary metabolites with unique characteristics and extraordinary diversity. Marine flora products possess immunomodulatory potential with desired pharmacological activity and a limited toxicity. Through their ability to regulate NF-κB, WBC production, IL-2, NKs, ROS, ER stress, phagocytosis, macrophages or lymphoid cells, cell-mediated immunity, specific/nonspecific immune response, apoptosis, and angiogenesis, these compounds act as the suitable candidates for anticancer drug development. Advancement in microbial fermentation techniques and aquafarming provide effective and economical ways for mass cultivation of marine microbes and seaweeds for the sustainable development of anticancer compounds. Moreover, through improved functional metagenomics, it has been now possible to discover novel natural product from uncultured microbiota. With these exciting new technologies, it is likely to expect that in the future, more novel compounds will be isolated from marine flora and subsequently developed as anticancer drugs in a comparatively short span of time.

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Ligand-Based Designing of Natural Products

8

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Abstract

Cancer is the second leading cause of mortality according to the current WHO report, which states that 30–50% of cancer cases can be prevented by maintaining a healthy lifestyle. One of the ways to maintain a healthy lifestyle is by consumption of healthy food containing antioxidant molecules and enzymes. Curcumin and polyphenolic compounds are now proven as anticarcinogenic molecules. The structures of these molecules are the reason for their anticarcinogenic property. There are many compounds available in nature that may be used as anticancer or antioxidant molecules. Some of these molecules have similar chemical structures and some have different structures. Moreover, in the absence of the known 3D structure of the natural compound, design and optimization of lead molecules are based on physicochemical properties and quantitative structure activity relationships. In this chapter, ligand-based designing of naturally available anticarcinogenic molecules is discussed.

Keywords

Phytochemicals · Cancer · Computer-aided drug design and discovery · Structure-based method · Ligand-based method · Chemoprevention

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Abbreviations

EGCG	Epigallocatechin gallate
GSH	Reduced glutathione
GST	Glutathione-S-transferase
LPO	Lipid peroxidation
QSAR	Quantitative structure activity relationship
ROS	Reactive oxygen species
SOD	Superoxide dismutase

8.1 Introduction

Cancer is still one of the significant causes of mortality in the world (Ferlay et al. 2008). The most common feature of cancer is the rampant cell proliferation that cannot be controlled by the normal cell checkpoint regulators (Devi 2004). The management of cancer involves several conventional therapies, including radiotherapy, chemotherapy, hormonal therapy, and immune therapy, along with bone marrow transplantation and surgery as the treatments of choice (Rahman 2016). Currently, these main treatments for cancer have been accompanied by several unwanted effects, such as hair loss, suppression of bone marrow, and drug resistance, and a large number of patients suffer from poor prognosis (Rajesh et al. 2015). Therefore, the search for new anticancer drugs with better efficacy and fewer side effects has continued. It has been seen that natural compounds are a good source for the treatment of cancer as they are reported to have anticancer effects (Demain and Vaishnav 2011). They have been shown to decrease cell proliferation, induce apoptosis, retard metastasis, and inhibit angiogenesis (Rajesh et al. 2015). Epidemiological studies have revealed that a diet rich in fruits and vegetables reduces the risk of several types of cancer, and it prevents diseases associated with oxidative stress conditions (Boeing et al. 2012). For example, the discovery and development of Paclitaxel, isolated from *Taxus brevifolia* bark, was the first anticancer drug originated from a natural product (Cragg 1998).

The natural products have become an essential area of drug discovery, and they are used in the treatment of various diseases. For example, diets rich in fiber, vitamins A, C, and E, beta-carotene, retinols, alpha-tocopherol, polyphenols, flavonoids, and minerals like selenium and zinc have cancer chemopreventive effects (Borek 2004). The anticarcinogenic activities of these natural products are gaining attention as they can be a cost-effective alternative for cancer treatment (Rajesh et al. 2015).

The development of new technologies and the harnessing of the various sources of information facilitate the development of new drugs that modulate the protein target (Prada-Gracia et al. 2016). Computer-aided drug discovery and design are used to provide computational tools and sources for the storage, management, analysis, and modeling of compounds. They have been used in every stage of drug

discovery from target identification to lead identification, validation, and even preclinical trials (Prada-Gracia et al. 2016).

8.2 The Development of Cancer: Carcinogenesis

Carcinogenesis is a multistage process (Fantini et al. 2015) and some intrinsic and extrinsic factors have been associated with the development of tumors (Podolskiy and Gladyshev 2016). It develops as a function of age, environment, diet, and genetic makeup. It may result from the action of any one or a combination of physical, chemical, biologic, or genetic insults to cells (Oliveira et al. 2007). The transformation of a normal cell into a cancer cell involves three distinct phases: initiation, promotion, and progression (Hosseini and Ghorbani 2015). The first stage of carcinogenesis involves an irreversible genetic alteration in somatic cells, such as transition, transversion, deletion, etc., that arises spontaneously or is induced by exposure to a carcinogen. It predisposes the affected cell, and its progeny, to develop neoplastic transformation (Devi 2004). The activation of one or more oncogene is vital for the development of neoplastic transformation, thus proving the two-hit model for cancer development (Hutchinson 2001). The mutations such as a gain of function observed in proto-oncogene leading to abnormal proliferation and loss of function mutation in the tumor suppressor gene are responsible for suppression of cell differentiation and apoptosis leading to cancer development (Hall and Giaccia 2006). This results in dysregulation of genes involved in biochemical signaling pathways. In the second stage of carcinogenesis, promotion affects the altered expression of the genome and requires repeated and prolonged exposure to promoting stimuli (Devi 2004). The final, irreversible stage of progression involves the successive changes in neoplasm and gives the malignant growth of a tumor (Pitot 1993). As a tumor grows in size, the cell may undergo further mutations that result in increased heterogeneity of the cell population. As the tumor progression advances, the cells lose their adherence property, and they detach from the tumor mass and invade the neighboring tissues (Valastyan and Weinberg 2011). It is this metastatic effect that is mainly responsible for the lethal effect of human tumors. The tumor vasculature results in impaired vessels and prevents the infiltration of immune cells in the tumor microenvironment (Barker et al. 2015). The anticarcinogenic roles of different phytochemicals at different stages of cancer are listed in Table 8.1.

8.3 Chemopreventive Agents

There are extensive studies where different compounds from fruits, vegetables, spices, tea, herbs, and extracts from medicinal plants have exhibited anticarcinogenic activities (Rahman 2016; Huang et al. 2009). Many herbal medicines contain bioactive constituents possessing antioxidant properties that protect cells against the damaging effect of reactive oxygen species (ROS) (Lobo et al. 2010). Phytochemicals have been considered as complementary medicine in the treatment of these

Table 8.1 A list of currently known phytochemicals along with their important anticancer properties and mechanism of action(s)

S. no.	Phytochemicals	Source	Properties	Mechanism of action
1.	Curcumin (diferuloylmethane)	Present in rhizomes of turmeric	Antioxidant and anti-inflammatory properties	Inhibits cyclooxygenase-2, lipoxygenase (LOX), and inducible nitric oxide synthase. Inhibits carcinogen bioactivation via suppression of specific cytochrome P450 isozymes (Menon and Sudheer 2007; Surh and Chun 2007)
2.	Resveratrol (trans-3, 5, 4-tryhydroxystilbene)	Present in skin of red grapes and red wine	Antioxidant, anti-inflammatory, and antiproliferative effects	Inhibits tumor necrosis factor-α-mediated matrix metalloproteinase-9 expression in HepG2 cells by downregulation of the nuclear factor-kB signaling pathway (Yu et al. 2008)
3.	Apigenin (plant flavone)	Present in common fruits and vegetables	Antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antigrowth, and antiprogression properties	Promotes metal chelation, scavenges free radicals, and stimulates phase II detoxification enzymes (Middleton et al. 2000)
4.	Quercetin (dietary flavonoid)	Present in foods, including apples, berries, brassica vegetables, grapes, onions, shallots, tea, and tomatoes as well as many seeds, nuts, barks, and leaves	Potent antioxidant, anticancer agent	Inhibits oxidative species generating enzymes, such as xanthine oxidase, LOX, and nicotinamide adenine dinucleotide phosphate oxidase, tyrosine kinase inhibition (Rajesh et al. 2015)
5.	Genistein (isoflavone)	Present in soybean and related products, such as tofu, soy milk, and soy sauce	Cancer chemotherapeutic agent	Increases apoptosis, including cell cycle delays and modulating intercellular signaling pathways (Rajesh et al. 2015)

(continued)

Table 8.1 (continued)

S. no.	Phytochemicals	Source	Properties	Mechanism of action
6.	Ursolic acid (pentacyclic triterpene)	Present in food, medicinal herbs (Tulsi), apple peel	Antioxidant, antitumor, anti-inflammatory activities	Enhances the activity of liver detoxification enzymes, which deactivates toxic chemicals (Cohen 2014)
7.	Isothiocyanates	Present in cruciferous vegetables, such as watercress, brussel sprouts, broccoli, cabbage, horseradish, radish, and turnip	Potent chemo protective effect, anticarcinogenic activity	reducing the activation of carcinogens and increasing their detoxification (Rajesh et al. 2015)
8.	Epigallocatechin gallate (polyphenol)	Present in green tea	Potent antiproliferative effect and chemopreventive effect	Induces significant cell apoptosis and cell cycle arrest (Du et al. 2012)

diseases. It has been claimed that phytochemicals isolated from natural compounds are more effective as anticancer drugs than synthetically manufactured drugs (Rahman 2016). Phytochemicals belong to a class of non-nutritive agents that have shown great potential in fighting cancer and other diseases. The combined effect of curcumin and quercetin provides antioxidant and anticarcinogenic properties (Liu et al. 2015). It significantly decreases the levels of LPO and ROS and decreases the activity of drug metabolizing enzymes (cytochrome P_{450} and b5) in a lung carcinogenesis induced model. It improves the levels of SOD, GSH, and GST resulting in enhancement of activity of the detoxification system which in turn protects against the damaging effect of carcinogens (Liu et al. 2015). In Table 8.1, phytochemicals along with their mechanism of action against targeting tumor cells are listed.

8.4 Computer-Aided Drug Designing and Discovery

In the discovery of new drug leads, the structure-based drug designing is an integral part of most industrial drug discovery programs (Baldi 2010). The computational approach of drug design is based on two types of methods: (1) ligand- and (2) structure-based methods (Martis and Somani 2012). In ligand-based methods, the existing knowledge of active compounds is used against the target to predict the new molecule representing similar behavior (Prada-Gracia et al. 2016). In contrast, the structure-based methods rely on targeting structure information to determine

whether a new compound is likely to interact and bind with the target (Anderson 2003). In this method, no prior knowledge of active ligands is required. Recently, the combination of ligand- and structure-based methods has become a common approach in virtual screening (Prada-Gracia et al. 2016).

The drug discovery process involves the improvement of an existing drug or discovery of a new chemical entity, which should be more potent than the existing drug (Martis and Somani 2012). This process of drug discovery (Fig. 8.1) includes the following steps: (1) **Identification of targets**: this involves the identification of the function of a possible therapeutic target (gene/protein) and its role in the disease (Prada-Gracia et al. 2016). This is done by data mining using databases. (2) **Validation of biological target**: in this step the molecular target which is directly involved in the disease process will be validated and the possible modulation to target molecule will show therapeutic effect that will be noted down for further screening of lead (Martis and Somani 2012). (3) **Lead structure search**: in this step, small molecule hits from a high throughput screening are evaluated (Martis and Somani 2012). (4) **Lead optimization**: this involves the synthesis of lead compound with improved potency, reduced off target activities, and physiochemical and metabolic properties (Lounnas et al. 2013). This optimization is achieved by chemical modification of Hit molecule structure and by employing knowledge of the structure–function relationship and is also based on ADMET tools (Verma et al. 2010). (5) **Preclinical studies**: this step is necessary to ascertain the safety of newly developed molecules and to study the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the molecule along with the toxicology data of the molecule. (6)

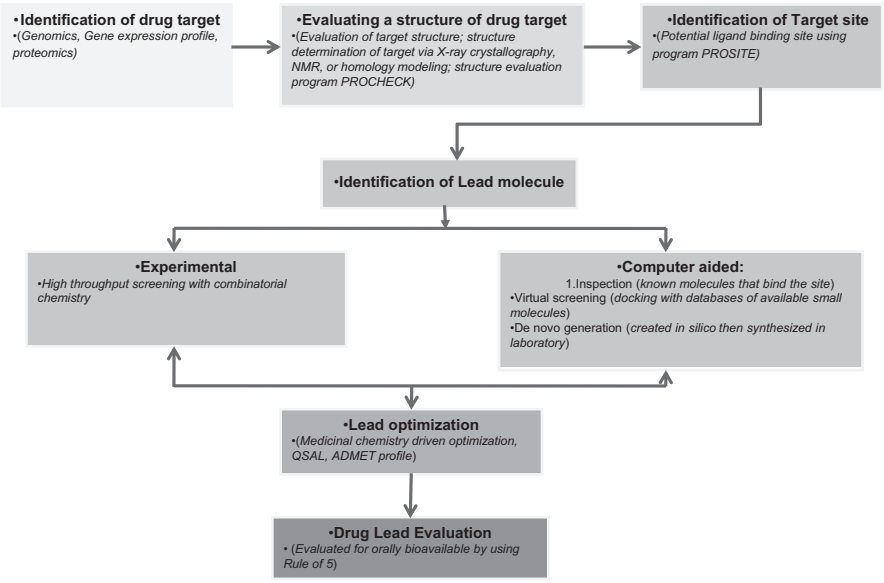

Fig. 8.1 The process of drug designing using modern techniques and algorithms

Clinical trials: this step involves actual testing of the molecule in human volunteers for assessing the safety and efficacy of the new molecule (Martis and Somani 2012).

Today, the computational method has become the crucial component for the drug discovery program. Discovering a novel drug is an expensive time-consuming process as it requires around 10–15 years (Leelananda and Lindert 2016). Therefore, the development of new strategies to make the process of drug development more efficient and less time consuming involve computer programs combined with the prior knowledge and experience of researchers (Prada-Gracia et al. 2016). The process of screening more compounds in less time with low cost is only possible by virtual screening of compounds from large databases by using computational tools rather than physically screening each of them (Xue and Bajorath 2000).

Owing to the absence of the 3D structure of potential targets, the ligand-based method has become an important tool in drug discovery (Martis and Somani 2012). Methods such as pharmacophore modeling and quantitative structure activity relationship (QSAR) provide information about target–ligand interaction that in turn results in predicting a suitable model for lead discovery and optimization (Prada-Gracia et al. 2016). The QSAR has been applied to find a statistically significant correlation between physiochemical properties of chemical substances and their biological activities (Verma et al. 2010). This methodology of drug discovery is known as an indirect method as it is usually used when the 3D structure of the target is unknown and cannot have been predicted. The physiochemical properties of the active ligand molecule are used as descriptors to compare the reference molecule or a set of molecules present in a library at very low cost (Prada-Gracia et al. 2016). In contrast to ligand-based methods, structure-based methods work directly with the 3D structure of a molecular target and target–ligand complex (Prada-Gracia et al. 2016). In this, the target is used as a mold, where the interaction with the small molecule present in the library is computationally simulated. The ones that show the better fit in the binding site are selected. The best possible way of drug design using the structure-based method is by using docking algorithms (Lounnas et al. 2013), which is a molecular simulation technique used to research the interaction between the ligand and target.

The chemopreventive effect of green tea, which is one of the most common beverages consumed around the world, is because of antioxidants (Cabrera et al. 2006). The major constituents in green tea are polyphenols, which possess anticancer activities. There are ten polyphenols present in green tea and out of them Epigallocatechin gallate (EGCG) showed the most potent antiproliferative effect (Du et al. 2012). The increased antiproliferative effect is due to the presence of gallic acid, which significantly enhanced the anticancer potential of catechin. EGCG has also been seen to enhance the antiproliferative effect of panaxadiol on human colorectal cancer cells (Du et al. 2012). The antiproliferative effect of EGCG is by cell cycle arrest as it increased the percentage of cells in the G1 phase, which resulted in cancer cell growth inhibition. EGCG also shows its effect by inducing significant cell apoptosis in cancer cells (Gupta et al. 2004). The results from this study will provide more information regarding the use of natural products in the treatment of cancer and to develop novel anticancer agents using both structure- and ligand-based drug designing techniques.

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9 Drug Resistance in Cancer and Role of Nanomedicine-Based Natural Products

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Abstract

Cancer is an age-old malady that has claimed millions of lives across the globe and the death toll is ever increasing. Despite intensive research for over a decade, contemporary anticancer treatment regimens still suffer from certain shortcomings, with drug resistance posing as a major hurdle. In this aspect, natural anticancer products have attracted attention as suitable chemopreventive agents over other synthetic compounds. However, the potential application of such natural compounds has been restricted due to their low bioavailability, poor efficacy amongst other limitations. An exciting advancement in the field of medicine has been the advent of nanoparticles that have reformed the usage of natural products as innovative anticancer therapeutics. This chapter elaborates the role of nanoparticle based natural products as potent and efficacious therapeutic agents for treatment and management of cancer.

Keywords

Nanomedicine · Nanoparticle · Nanoscience · Cancer · Natural products · Drug resistance · Anti-cancer · Therapy

9.1 Introduction

In the combat against a deadly menace popularly known as cancer, natural products have had a massive contribution, especially over the past few decades. Natural anticancer products have procured preference as particularly suitable candidates for chemoprevention over other synthetic compounds, largely because the associated adverse side effects are reported to be minimal. However, such natural anticancer

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products come with their own limitations, such as low bioavailability, requirement of higher dosage for their effectivity, and other issues. With the advent of nanoscience and nanotechnologies, there has been breakthrough transformation in its application in research areas, including innovation of novel pharmaceutics that has yielded development of successful nanodrugs. Nanoparticles (NPs) have revolutionized the implementation of natural products as therapeutics by circumventing their shortcomings and enhancing their targeted efficacy by impressive folds. This chapter basically provides an overview of natural anticancer products and the exceptional promise of their nanoformulations for improved treatment of cancer.

9.2 Overview on the Current Status of Drug Resistance in Cancer

Drug resistance is a global crisis that threatens the health benefits of most of the drugs. It is primarily driven by inappropriate use of drugs and paucity of new drugs. The concept of resistance against chemotherapeutics emerged when bacteria failed to respond to certain antibiotics, but over time similar mechanisms were realized in other diseases, including cancer.

Cancer therapy is a broad area with multiple treatment options and combinations available. For solid tissue tumors, such as liver cancer, surgical resection is a good option, although there is a risk of cancer recurrence in case it is a metastatic tumor. Radiation therapy is a common treatment modality, but it comes with a huge list of inadvertent side effects, since it lacks the ability to distinguish cancer cells from their neighboring normal population. Some newer methods that have come up in recent years after decades of research include immunotherapy, targeted therapy, hormone therapy, stem cell transplant, and precision medicine; however, these are relatively less frequently employed treatment options, owing to their cost, time, and other criteria. Overall, nevertheless, for a significant number of metastatic cancers, the most effective, and often the only, treatment regimen is usage of anticancer drugs for improved quality of life and/or palliate the symptoms, rather than to cure the patient.

Back in 1961, a clinical trial administering high-dose four-drug combination was set up by Frei and Freireich for the treatment of pediatric leukemia (Frei et al. 1965). The initial success proved to be short-lived as almost all the patients eventually exhibited reemission of cancer, and what was a matter of concern was that these cancer cells were no longer responsive to chemotherapy. This was the first reported incident of resistance to chemotherapy, and over the following years, multidrug resistance has become an incredibly arduous impediment in the treatment and cure of cancer. But how do cancer cells develop resistance to available drugs? Tolstoy had mentioned in the opening lines of Anna Karenina, “normal cells are all alike in their response to drugs, but cancer cells each respond in their own way.” The genetic makeup of one cancer cell may vary from another, and this is dependent on a vast array of factors, including tissue origin, differential expression pattern of oncogenes and tumor suppressors, and, most importantly, random mutations. The consequent phenomenon is an enormous amount of heterogeneity when it comes to drug resistance. For the past millennium, extensive research has been able to accumulate the

potential mechanisms by which cancer cells can become resistant to anticancer drugs (Cree and Charlton 2017). The common mechanisms usually constitute of loss of a cell surface receptor or transporter for the drug, changes in metabolism of the drug, or alteration of the specific drug target owing to mutation. But since this usually results in resistance development against one particular drug or its related group, use of a multidrug regimen that employs alternate mechanisms can overcome its infirmities. However, the more notorious mechanism of resistance is multidrug resistance (MDR) that confers simultaneous resistance to multiple structurally and functionally unrelated drugs (Zahreddine and Borden 2013). The causative effects of MDR are diverse (Fig. 9.1) ranging from cellular changes that constrain accumulation of drugs within cells by limiting their uptake, enhancing their efflux, or affecting membrane lipids, resulting in hindered apoptosis which is the primary mechanism of action of cell cytotoxicity engaged by most anticancer drugs, activation of general response mechanisms for drug detoxification and repair of the original damage stimuli, and amendments in cell cycle that render the cells relatively resistant to the cytopathic effects of drugs on cancer cells.

The most noted cause of MDR is attributed to drug efflux resulting in drug accumulation that is accomplished by different cell membrane transporter proteins (Cree and Charlton 2017). The best characterized transmembrane proteins implicated in cancer drug resistance are multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP). The most extensively studied among these is MDR1, better known as the P-glycoprotein (p-gp) (Chen et al. 1996), that is actively involved in the transport of

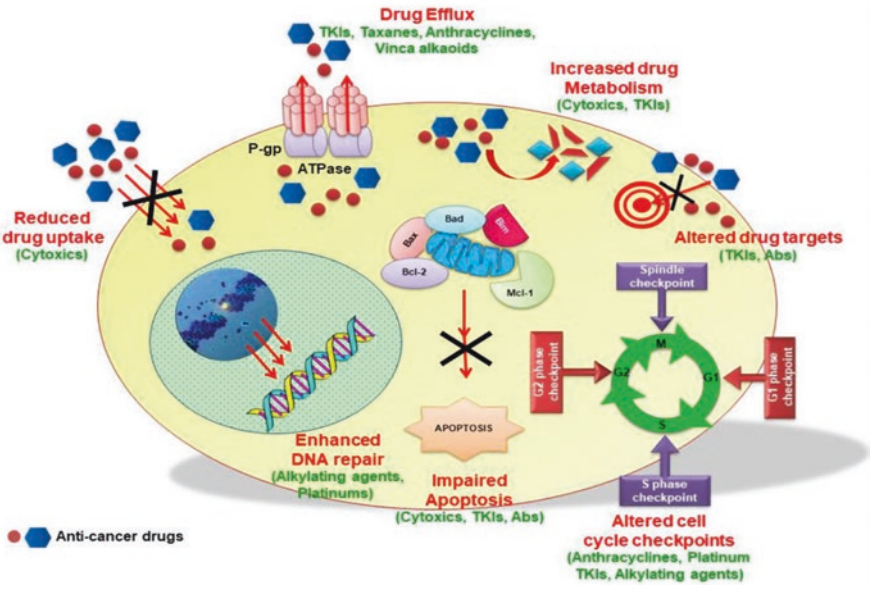


Fig. 9.1 Resistance mechanisms adopted by cancer cells to chemotherapeutics. Cancer cells adopt an array of mechanisms to develop resistance against chemotherapeutics that involve multiple pathways and a cascade of molecular targets

several compounds, including anticancer agents, from cytosol to the extracellular space of a cell. Overexpression of such proteins is documented to cause cancer resistance and thereby poor clinical outcome in patients suffering from neuroblastoma, lung cancer, and breast cancer (Gottesman 2002). MDR1 overexpression has been correlated with chemoresistance in carcinoma of the breast, lung, prostate, kidney, colon, and liver and in leukemia and lymphomas. Erlotinib, imatinib, sunitinib, and nilotinib are substrates of MDR1 and are being reported for drug resistance (Housman et al. 2014).

The uptake of drugs by the cell is mediated through various groups of transporters that are normally involved in different physiological processes, and cancer cells are often reported to develop resistance to drugs by maneuvering these transporters. Resistance to methotrexate is due to mutation in the folate transporters. Cisplatin resistance has several diverse mechanisms, but reduced uptake of cisplatin is a common phenomenon in cisplatin-resistant cells (Gottesman 2002; Housman et al. 2014).

An important criterion that affects the efficacy of a drug is its molecular target and any alteration, mutation, or modification in that target or the signaling cascade involved can adversely influence the drug activity and ultimately result in resistance. The best example is of the anticancer drugs that act by inhibiting topoisomerase II. These drugs act by stabilizing the usually transient complex formed between topoisomerase II and DNA, causing DNA damage and halting the mitotic phase. Cancer cells can become resistant to such drugs by mutating the topoisomerase II encoding gene (Holohan et al. 2013). Imatinib resistance in leukemic patients is brought upon by mutations in the *ABL* gene or amplification of the fused *BCR-ABL* gene (Housman et al. 2014). Resistance to paclitaxel and other taxanes found across ovarian cancer cells is predominantly due to drug target alterations (Giaccone and Pinedo 1996). Many patients who were initially found to respond well to Herceptin treatment of HER2-positive breast cancer later developed resistance, associated with various changes in the signal transduction process, including PI3K/Akt pathway activation. Tumor resistance is also acquired through *HER2* amplification that can cause tamoxifen resistance in breast cancer patients (Giaccone and Pinedo 1996).

Many anticancer drugs undergo metabolic activation to exert their cytotoxic effects. Cytarabine (Housman et al. 2014), used in leukemic treatment, requires a phosphorylation event in order to get activated. Cancer cells have developed mechanisms to manipulate the expression of the enzymes involved in the activation of this compound and acquire drug resistance. Other examples include methotrexate and 5-fluorouracil.

The highly prevalent mode of action of cytotoxicity caused by anticancer drugs is through induction of apoptosis. Any dysregulation in the expression of the major proteins involved in apoptosis, such as Bax, Bcl2, Bad, etc., can thus impede the process of apoptosis and contribute to tumor drug resistance (Holohan et al. 2013). Tumors with mutant p53 are less sensitive to a diverse spectrum of anticancer drugs, such as doxorubicin, cisplatin, and 5-fluorouracil.

Anticancer drug resistance is strongly shaped by DNA damage response, and this is especially applicable for drugs that act directly or indirectly by damaging DNA. The efficiency of such drugs is therefore dependent on the failure of the DNA

damage repair methods of the cancer cells. Cisplatin, for example, stimulates cancer cell apoptosis by damaging the DNA. However, nucleotide excision repair and other DNA damage repair mechanisms can reverse the platinum damage and give rise to resistance to platinum-based therapy (Housman et al. 2014).

Resistance development is posing an extreme challenge since the efficacy of several promising drugs that otherwise demonstrate strong tumor-suppressive effect is hampered by this rising menace. Many chemotherapeutic drugs that have been in use in treatment of different cancers are now being eliminated due to tumor cell resistance. A new drug has to undergo a time-consuming and cumbersome process for clearance and approval for medical use, hence causing a marked delay in the therapy of cancer patients. Efforts are underway for better comprehension of the mechanisms of drug resistance to come up with effective options for therapy that are tailor-made for patients with lower chance of cancer reemission. Rational drug design and agents that selectively target different signaling pathways offer more efficient options as they have much lesser avenues for acquiring tumor drug resistance. High-throughput techniques like microarray and next-generation sequencing and discovery of biomarkers have enabled stratification of patients into categories based on their differential sensitivity to certain chemotherapy or combination therapy. In this regard, natural products that exhibit anticancerous properties have gained popularity, owing to their safety profile and lower chance of resistance procurement.

9.3 Natural Products: A Rising Army in the Fight Against Cancer

The application of natural products in the cure for cancer is not a novel area of research, and there is an overwhelming rate of successful eviction of cancer using natural product-based therapy that is worth applause. The increasing attraction garnered by plant-derived natural products for use in cancer chemotherapy is due to their higher safety profile and minimal off-target effects (Mishra and Tiwari 2011). According to a report, more than 3000 plant species are documented to possess anticancer properties, and of the isolated compounds, several are being tested for clinical usage. Various classes of plant-derived metabolites, including polyphenols, flavonoids, and brassinosteroids, have been under thorough research since a long time for their potential use as anticancer agents, with considerable number of them progressing into clinical trials. The most common mechanism of tumor suppression induced by plant-derived anticancer drugs includes induction of apoptotic cell death, either dependent or independent of pathways involving caspase and p53 (Gali-Muhtasib et al. 2015). Recent studies have also hinted toward the role of autophagy, necrotic cell death, mitotic catastrophe, and senescence leading to cell death, as alternative modes of action. This section gives an overview on some well-known plant-derived natural compounds with promising anticancer effects.

As emphasized before, plants have been a popular source of *avant-garde* traditional medicine system with the earliest records of usage of plant extracts for alleviating symptoms of inflammation and certain infections dating to several centuries ago. The Chinese civilization has been a resilient follower of nature-based

traditional medicines, and even in this modern era, a mammoth proportion of their chemotherapeutics are actually based on the age-old traditional medicine system. Likewise, the Indian Ayurveda is another classic archetype, which boasts of implementing natural extracts for therapeutic purposes. Interestingly, in 1985, the World Health Organization had documented the utilization of plant-based traditional medicines by 65% of the global population for primary healthcare. Mention must be made here of *Papaver somniferum* that provided us with valuable painkillers, such as morphine that proved exceptionally pragmatic in several sectors of healthcare. Another noteworthy drug from traditional medicinal plants is the antihypertensive agent, reserpine, from the plant *Rauwolfia serpentina*. Nevertheless, the discovery and development of effective antimalarial, namely, quinine and artemisinin, is a brilliant achievement when it comes to natural product-based therapy, since malaria used to wreak havoc in older times and could somewhat be brought under control with the isolation of these drugs, although it continues to be a confrontational health challenge in developing countries even today (Greenwell and Rahman 2015).

It is intriguing to know that about three-quarters of antitumor compounds currently used in medicine are natural products or related to them (Basmadjian et al. 2014). The most commonly used practices for treating solid tumors are surgical removal of the affected area with adjuvant radiotherapy and chemotherapy. But these inadvertently come with severe side effects and dramatically reduce the quality of life of patients. Additionally, the toxicity of some treatments is a major hurdle in their use and effectiveness. The cost of chemotherapeutic drugs is exorbitantly high as well, and their effectiveness is limited due to the exhaustingly long clinical testing procedure before they are available in the market. The most severe impediment in most cases is emergence of resistance of the tumor to a particular drug, or even more dangerous, a combination of several drugs. The dire need of the hour, therefore, is development of novel and efficient anticancer drugs with reduced off-target effects and who better than nature herself as a promising source for such entities. Coming to factsheets, recent analysis reports that 36% of the first-in-class small molecules approved by the US Food and Drug Administration between 1999 and 2008 alone were natural products or their derivatives (Newman and Cragg 2016) and 60% of the currently available anticancer drugs have their source from natural products. Plant-based anticancer therapeutics (Mann 2002) started with the introduction of vinblastine and vincristine (Greenwell and Rahman 2015; Cragg and Pezzuto 2016), isolated from *Catharanthus roseus*, in the late 1960s for effective treatment of childhood leukemia, testicular teratoma, Hodgkin's disease, and many other cancers. This product was shown to increase patient survival rate to 80% (Evans et al. 1963). Vincristine functions primarily by inhibiting tubulin polymerization resulting in the disruption of the mitotic spindle assembly and thereby inducing mitotic suppression (Almagro et al. 2015). Etoposide has shown great promise in providing relief to patients suffering from testicular teratoma and small-cell lung cancer (Demain and Vaishnav 2011) primarily by targeting DNA topoisomerase II activities that culminates in DNA breakage and ultimately deregulated cellular metabolisms (Montecucco et al. 2015). Nonetheless, the discovery of paclitaxel, from *Taxus* sp., proved a milestone event as it showed efficacy against refractory breast, lung, and ovarian cancers (Cragg and Pezzuto 2016) and is one of the

bestselling anticancer drugs in contemporary times (Greenwell and Rahman 2015). The blockbuster success of this drug, sold under the brand name Taxol® since 1963, was followed by extensive studies for the design and development of its synthetic analogues. The basic mode of its antitumor activity is through targeting DNA repair, mitotic spindle assembly formation, and cell proliferation (Weaver 2014). More recent times witnessed a spike in the unearthing of several essential phytochemicals in conventional day-to-day Indian spices. The combination of phenethyl isothiocyanate and curcumin, a polyphenol present in the rhizomes of turmeric, is nowadays being used for prostate cancer treatment as this particular product is reported to regulate tumorigenesis through multiple cell signaling pathways that control cell proliferation, cell survival, caspase activation, tumor suppression, and mitochondrial and protein kinase pathways (Demain and Vaishnav 2011). The therapeutic efficacy of curcumin has been well-established in tumors of the brain, pancreas, lung, breast, liver, prostate, colorectum, head and neck, and skin and leukemia (Perrone et al. 2015). Curcumin functions mainly by executing cell cycle arrest (Dasiram et al. 2017), apoptosis (Schwertheim et al. 2017), and caspase-dependent mitotic catastrophe (Gali-Muhtasib et al. 2015) and by inhibiting tubulin polymerization (Haris et al. 2017). This compound was also documented to act as a chemosensitizer for several clinically used anticancer drugs, such as gemcitabine, paclitaxel, and 5-fluorouracil, and displays a synergistic effect when combined with other natural products, including resveratrol, honokiol, and others, thus implying a superior therapeutic index for curcumin in combination for better anticancer therapy (Di Martino et al. 2017). Resveratrol (Demain and Vaishnav 2011; Juarez 2014), a naturally found phytoalexin concentrated in red grapes, possesses antioxidant, anti-inflammatory, and antiproliferative properties on a diverse group of cancers. In fact, resveratrol has been identified as an efficient potential candidate for cancer prevention of liver by inhibiting cellular events associated with cancer initiation, promotion, and progression (Rauf et al. 2018). Soybean and its products such as tofu, soy milk, and soy sauce are a rich source of an isoflavone compound genistein with promising tumor-suppressive action (Cragg and Pezzuto 2016) that is achieved through modulation of genes involved in cell cycle regulation and apoptosis, angiogenesis, and metastasis (Sarkar and Li 2002). Honokiol is one more bioactive natural product extracted from *Magnolia* spp. (Juarez 2014). This polyphenol promotes apoptosis of countless human cancer types, including carcinoma of the lung, colon, liver, and breast (Arora et al. 2012).

Reports have revealed anti-inflammatory, anti-angiogenic, antioxidative, and anticancer properties of honokiol by targeting multiple signaling pathways, including nuclear factor kappa B (NF-κB), signal transducers and activator of transcription 3 (STAT3), epidermal growth factor receptor (EGFR), and mammalian target of rapamycin (m-TOR) (Arora et al. 2012). The well-known antimalarial, artemisinin, isolated from the plant *Artemisia annua L.*, is now a sought-after anticancer drug (Slezakova and Ruda-Kucerova 2017). This super herb is now recorded to demonstrate remarkable improvement in the prognosis of cancer patients, particularly by inhibiting cancer proliferation, metastasis, and angiogenesis (Crespo-Ortiz and Wei 2011). And since it utilizes a mechanism that gives it a selective preference for attachment to cancer cells, the side effects are negligible, while the tumor

suppression is superb. It is important to remember here that we have just mentioned a relatively small number of members of plant-derived anticancer therapeutics, and the list is impressively long, more of which are enlisted in Table 9.1.

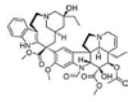
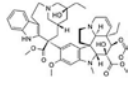
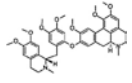
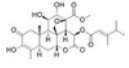
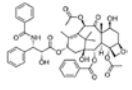
Some anticancerous natural molecules are currently under investigation for their application as chemotherapy. Flavopiridol, isolated from *Dysoxylum binectariferum*, with broad antitumor activity against tumors, leukemia, lymphomas, and solid tumors, is currently under phase 1 and phase 2 clinical trials. A derivative of olomucine, roscovitine, derived from *Raphanus sativus*, is in phase 2 and phase 3 clinical trials in Europe. Combretastatin from *Combretum caffrum* is documented to have suppressive effects for lung and colon cancers and leukemia. Betulinic acid isolated from *Zizyphus* spp. has selective cytotoxicity against human melanoma cell lines. Silvesterol isolated from the fruits of Meliaceae family is known to act significantly against breast and lung cancer cells. Apart from such natural products, there are several common plants that have proved effective in exhibiting anticancerous properties in *in vitro* and *in vivo* systems, but their medical usage is still a thought of the future. A commonly encountered roadside herb in our country is *Achyranthes aspera* Linn. (family, Amaranthaceae) whose methanol extract is reported to show pronounced cytotoxic activity in cancer cells. A component of raw garlic, allicin, has displayed antitumorigenic effects in human primary fibroblasts and a tumorgenic lymphoid cell line. Extract of *Andrographis paniculata* contains several phytochemical compounds with potent tumor inhibitory functions in cancer cell lines. An intriguing observation was obtained with acetogenins from *Annona muricata*; it targets the pump involved in removal of anticancer drugs from the cell, allowing chemotherapy to be more effective in various cancer cell lines, including carcinoma of the lung, breast, pancreas, prostate, colon, lymphoma, and liver (Prakash et al. 2013). Other examples include *Bidens pilosa*, *Bolbostemma paniculatum*, *Mangifera indica*, *Nervilia fordii*, *Salvia miltiorrhiza*, *Oroxylum indicum*, *Rubia cordifolia*, *Silybum marianum*, *Taraxacum officinale*, *Terminalia chebula*, *Zingiber officinale*, *Vernonia amygdalina*, *Cannabis sativa*, *Centaurea ainetensis*, and *Camellia sinensis* (green tea) (Prakash et al. 2013).

9.4 Hurdles in Application of Natural Products

The method of development of new products from natural sources, however, is not entirely free of obstacles. For instance, it is often difficult to access the source of the samples, obtaining appropriate amounts of the sample is a big issue as well, identification and isolation of the bioactive compound out of a vast array of constituents in the sample is a lengthy and labor-intensive process in itself, and there are several problems in synthesizing the necessary amounts of the compound of interest.

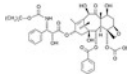
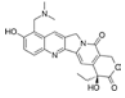
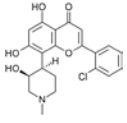
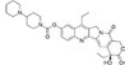
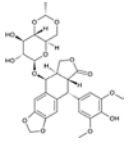
Although intensive research is still a need of the hour to conquer all the limitations of natural products, nanotechnology has helped in ruling out many of the obstacles. Most of these natural compounds happen to be highly lipophilic in nature and are not ideal for drug delivery since they have extremely low solubility in the bloodstream. These compounds are thus characterized with a low bioavailability,

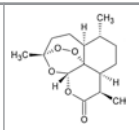
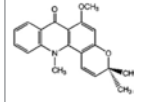
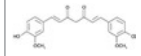
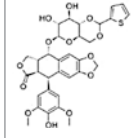
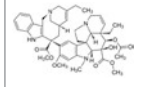
Table 9.1 Natural products as anticancer therapeutics

Name of the natural product	Structure	Plant source	Brand name	Indications	FDA approved/ clinical trials	References
Vincristine (leucristine)		*Catharanthus roseus* 	Oncovin	Acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin's disease, neuroblastoma, and small-cell lung cancer	Approved	Andre (2014), Yoshida et al. (2011), Tu et al. (2013), and Gatzemeier et al. (1992)
Vinblastine		*Catharanthus roseus* 	Velban	Breast, lymphoma, germ-cell and renal cancer	Approved	Ruf et al. (2018) and Fossa et al. (1992)
Thalicarpin (thaliblastine)		*Camellia sinensis* 	–	Breast cancer and ovarian cancer	Preclinical/ phase 1	Chen et al. (1996) and Stevigny et al. (2005)
Bruceantin		*Brucea antidysenterica* 	–	Leukemia, lymphoma, and myeloma	Preclinical/ phase 1	Cuendet and Pezzuto (2004)
Paclitaxel		*Taxus brevifolia* 	Taxol	Lung, breast, ovarian, and liver cancers and leukopenia	Approved	Yamori et al. (1997), Crown et al. (2004), and Clegg et al. (2002)

(continued)

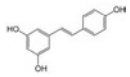
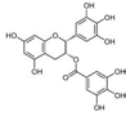
Table 9.1 (continued)

Name of the natural product	Structure	Plant source	Brand name	Indications	FDA approved/ clinical trials	References
Docetaxel		*Taxus baccata* 	Taxotere	Breast cancer, head and neck cancer, stomach cancer, prostate cancer, and non-small-cell lung cancer	Approved	Chen et al. (2018), Horn et al. (2017), Leung et al. (2018), and Liu et al. (2017)
Topotecan		*Camptotheca acuminate* 	Hycamtin	Cervical, ovarian cancers, small-cell lung cancer and lung carcinoma Lung carcinoma	Approved	Cormio et al. (2011) and von Pawel (2003)
Flavopiridol (alvocidib)		*Amoora rohituka* 	–	Acute myeloid leukemia	Approved as orphan drug by FDA	Zeidner and Karp (2015)
Irinotecan		*Camptotheca acuminate* 	Camptoser	Colon cancer and small-cell lung cancer	Approved	Fuchs et al. (2006) and Zhang et al. (2015)
Etoposide		*Podophyllum hexandrum* 	Etopophos/ Toposar	Kaposi's sarcoma, Ewing's sarcoma, lung cancer, testicular cancer, lymphoma, nonlymphocytic leukemia, and glioblastoma multiforme	Approved	Gatzemeier et al. (1992), Tas et al. (2013), and Ozaki et al. (2015)

Artemisinin and derivatives		*Artemisia annua* 	–	Liver and colon cancer, breast cancer, oral cancer, gastric cancer	In research	Blazquez et al. (2013), Wu et al. (2013), Ricci et al. (2011), Sun et al. (2013), and Michaelis et al. (2010)
Acronycine		*Agathymus baueri* 	–	Leukemia and melanoma, lung, ovarian and colon cancers	In research	Guilbaud et al. (2002) and Kluza et al. (2002)
Curcumin		*Curcuma longa* 	–	Prostate cancer, head and neck cancer, breast cancer, liver cancer, cervical and oral cancer, breast, pancreatic and colorectal cancers, and multiple myeloma	In research	Chen (2015), Gao et al. (2012), Darvesh et al. (2012), and Deguchi (2015)
Teniposide		*Podophyllum hexandrum* 	Vumon	Acute lymphocytic leukemia (ALL), Hodgkin's lymphoma, bladder cancer	Approved	Grem et al. (1988), Muggia and Kelley (1992) and Muggia (1994)
Vinorelbine		*Catharanthus roseus* 	Navelbine	Breast cancer and non-small-cell lung cancer	Approved	Fumoleau et al. (1993) and Group ELCVIS (1999)

(continued)

Table 9.1 (continued)

Name of the natural product	Structure	Plant source	Brand name	Indications	FDA approved/ clinical trials	References
Resveratrol		Grapes, blueberries, cranberries, mulberries, lingonberries, peanuts, and pistachios 	Resveratrol	Skin cancer, breast cancer, colorectal cancer, liver cancer, pancreatic cancer, prostate cancer, and ovarian cancer	Approved	Jang et al. (1997), Carter et al. (2014), and Vergara et al. (2012)
Epigallocatechin-3-gallate (EGCG)		Green tea *gyokuro* 	–	Colorectal cancer, head and neck cancer, breast cancer, prostate cancer, and pancreatic cancer	Preclinical/ phase I	Du et al. (2012), Shin et al. (2016), Pianetti et al. (2002), Albrecht et al. (2008), and Qanungo et al. (2005)

This table gives a brief insight into the commonly used natural products with potent antitumorigenic properties, some of which are currently undergoing clinical evaluation, while many are in clinical use as anticancer therapeutics

and therefore large quantities of the compounds need to be administered in order to achieve the desired therapeutic effects. Such high doses can lead to toxicity or low patient compliance. Encapsulation of these highly lipophilic compounds can improve the bioavailability and lower the dose needed to obtain a therapeutic effect. One more major benefit in utilizing NPs in drug delivery of natural compounds is their ability to target specific tissues or organs that enhances drug bioavailability and reduces toxic side effects of the drug as it is mainly being released in a localized area of the body. A third gain that NPs provide is controlled release of the drug. Also, one can tailor-make these NPs to make them more favorable for our intentions. The important technological advantages that NPs deliver are high stability, high carrier capacity, and feasibility of incorporation of both hydrophilic and hydrophobic substances and the choice of variable routes of administration, including oral application and inhalation. Particularly as nanomedicines, NPs confer benefits such as protection of the loaded drug from the harsh environment of the GI tract, release of the drug in a controlled manner at target sites, prolongation of the residence time in the gut by mucoadhesion, and inhibition of efflux pumps to improve the drug accumulation. In the field of cancer, researchers aim to selectively and accurately target the tumor cells avoiding interaction of the NPs with the healthy cells. Above all, NPs assist therapeutic agents to pass through biologic barriers, mediate molecular interactions, and identify specific molecular targets.

9.5 Nanoparticles: Good Things Come in Small Packages

Modern nanotechnology is an interdisciplinary approach that deals with the tiniest of particles and their special chemical, physical, and mechanical properties at the convergence of physics, chemistry, biology, medicine, electronics, and information technology. The terminology "nano" originated from the Greek word "nanos," which means petite or extremely small. NPs typically possess three distinct layers on the basis of its structure: the outer most layer is the surface layer to which various molecules, tags, or proteins are added; the middle layer serves as the shell; and the core constitutes the actual volume of the NP. These tiny particles are steadily becoming an indispensable part of our life. A well-known application of early nanotechnology is the ruby red color that was used for making attractive stained glass windows during the Middle Ages – as found in many Gothic European cathedrals. Presently, the application of NPs is not only restricted to the field of science; it has diverse applications in a multitude of arenas. For instance, zinc oxide NPs have found utility in imparting protection to wood and plastic, whereas iron NPs are being used to clean up carbon tetrachloride pollution in groundwater. In the field of electronics, coating anodes of lithium-ion batteries with silicon NPs have improved battery power and reduced recharge time (Khan et al. 2017). NPs have also emerged as a cutting edge technology in the field of medicine recently (Zhang et al. 2008).

Designing of NPs needs careful consideration of several crucial factors that can influence their overall behavior. These properties of NPs are important for determining the degradation rate and drug release kinetics (Gaumet et al. 2008). Factors such

as size and polydispersity index (PDI) are assessed using electron microscopy. The smaller the size of the NPs, the larger the surface area, and this results in fast drug release. However, smaller particles also tend to aggregate during storage and transportation of NP, thereby limiting its potential. Therefore, a balance between maximum stability and the size of the NP is necessary. Likewise, PDI tells about the homogeneity of the NP population. The lower the PDI, the more similar are the NPs sizes within a population. Correspondingly, the shape of the NP influences its circulation in the body of the organism. For example, spherical-shaped NP tends to move more freely compared to the other tubular- or irregular geometry-shaped NPs. Surface charge is another important parameter that is known to influence the interaction of NPs with the biological environment as well as their electrostatic interaction with bioactive compounds that in turn modulates their stability.

When it comes to classification, NPs exist in various forms; here we have discussed about a few. The different types of NPs have varying applications, depending on their specific properties.

1. Polymeric micelles are the primitive type of NP. They are self-assembled core-shell nanostructures formed in an aqueous solution consisting of amphiphilic block copolymers that associate when they cross the critical micelle concentration (CMC) (Zhang et al. 2014a). They provide an alternative to other nanosystems due to some inherent properties like biocompatibility, nonimmunogenicity, nontoxicity, and biodegradability. They are the ideal candidates for cancer therapy, delivery of vaccines, contraceptives, and delivery of targeted antibiotics (Elsabahy and Wooley 2012). An example of this is Genexol-PM that has shown promising results under FDA study against advanced malignancies (Lee et al. 2008).
2. Liposomes are small vesicles of spherical shape that can be produced from cholesterols, nontoxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and even membrane proteins (Davis and Shin 2008). These are closed structures that can carry both hydrophobic and hydrophilic drugs on the membrane and inside the vesicle, respectively. They have been successfully exploited in tumor therapy, asthma problems, ophthalmic drug delivery, leishmaniasis, pulmonary delivery, and so on (Saad et al. 2012). There is a study wherein scientists have discovered the potential role of combination therapy of the natural agents curcumin and resveratrol given in combination with liposomes in treatment of prostate cancer (Narayanan et al. 2009).
3. Biodegradable/biocompatible NPs are usually prepared from proteins, polysaccharides, and synthetic biodegradable polymers. Being biodegradable, they are protected from the immune response of the host patient (Mahapatro and Singh 2011). They have gained increased attention for their property to be used as delivery molecule for carriers such as drugs, vaccines, and genes. Recently, a biodegradable calcium phosphate NP has been developed for efficient delivery of small interfering RNA (Li et al. 2010) used as anticancer therapeutic regimen.

4. Dendrimer is usually a single core formation of central shell that is followed by repetitively branched treelike structure known as dendrons. The exterior of a dendrimer can be functionalized using various surface groups that contribute to drug targeting, solubility, and chelation (Madaan et al. 2014). They are used in drug delivery, diagnosis of disease, chemotherapy, gene therapy, treatment of cancer, and anti-retroviral therapy (Saad et al. 2012; Pan et al. 2007; Tomalia et al. 2007). A novel approach using dendrimers has been established where biotin-dendrimer conjugates are shown to have substantially higher cellular uptake in cervical cancer HeLa cells as compared to the uncongujated one (Yang et al. 2009a).
5. Artificial DNA nanostructures are complex mesoscopic structures based solely on DNA. It is self-assembled by the complementary binding of the sticky ends of the DNA molecules. The lattice so formed serves as a scaffolding material for biological molecule (Sun and Kiang 2005). Apart from their clinical significance, they have been found to be extensively used as biosensors (Pei et al. 2010). Interestingly, aptamer-conjugated DNA icosahedral nanocarriers carrying the drug doxorubicin proved significantly effective in treatment of epithelial cancer (Chang et al. 2011).
6. Drug-NP conjugates are useful for simultaneous targeting of multiple subcellular organelles in the target cells to improve the therapeutic efficacy of the free drugs. This drug delivery system greatly decreases the leakage of drug in intravascular system, while the NP is in circulation (Qi et al. 2017). This has been shown by Basu et al. wherein they have prepared dual drug conjugate that can simultaneously target the mitochondria and nucleus. These were internalized into the acidic lysosomal compartments of cervical cancer HeLa cells and induced cancer cell cytotoxicity through enhanced apoptosis (Mallick et al. 2015).
7. Stimuli-based drug-releasing NPs help in controlled release of the encapsulated material in response to stimuli such as pH, temperature, light, redox, and others. This reduces the toxic effect of the drug and also enhances the drug efficacy (Tang et al. 2018). The studies related to stimuli-based NPs are in initial stages, but one striking example is the chitosan-grafted copolymers that have been improved by adding magnetite that responds to the magnetic field. Presently, only the drug release kinetics has been thoroughly studied for such nanoformulations (Yuan et al. 2008), but they certainly show promising properties that can be exploited in therapeutics.
8. Silica NPs are a recent addition to the field of nanotechnology. They are promising for biological applications due to their higher stability and low toxicity and ability to be functionalized with a range of molecules and polymers (Ryu et al. 2014). Research is in the initial stage to study their toxic response upon normal cells; nevertheless, they possess potential in acting as delivery vehicle for antibodies, antibiotics, and enzyme delivery (Bhatia 2016).
9. Metal NPs are generated from metal atoms such as Au, Ag, Cu, Pt, Pd, Ru, and Re. These metal atoms are obtained from bulk metals that are allowed to coalesce to NPs. These NPs have also shown to act against resistant strain of

bacteria (Slavin et al. 2017). They have been used in active delivery of bioactive and various drugs, bioassays, detection, and imaging (Bhatia 2016). Taratula et al. showed that simultaneous delivery of doxorubicin and Bcl2 siRNA in such NPs can enhance the efficacy of chemotherapy even in MDR cases (Chen et al. 2009).

10. Carbon NPs are nanosized carbon elements created through various methods including carbonization, heating, activation, and grinding. Being the most abundant element in the universe, carbon has gained attention as a drug delivery system (Zhang et al. 2014b). They are also used in gene silencing and diagnostic purposes. Studies have suggested that delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes considerably suppresses tumor growth (Sinha and Yeow 2005).
11. Magnetic NPs can be manipulated using magnetic field. They are usually made up of iron, nickel, and cobalt and a chemical component that has functionality (Akbarzadeh et al. 2012). Based on the stability and the size of these NPs, they serve various industrial functions in biotechnology, biomedical, material science, engineering, and environmental areas. For example, magnetic cobalt spinel ferrite NPs coated with biocompatible polygalacturonic acid was functionalized to target ovarian cancer cells. With the help of magnetic field, such NPs are able to target and extract exclusively malignant cells (Scarberry et al. 2008).
12. Targeting ligand-modified NPs are basic NPs that have been modified to target a particular ligand. This selective targeting of cells helps in intracellular delivery of therapeutics overcoming a major challenge of pharmaceutical intervention in cancer (Jahan et al. 2017). Such nanoformulations are presently being explored as a link for diagnostics to therapeutics for the development of personalized medicines (Jain 2006). Additionally, there are reports where magnetic iron oxide NPs have been developed conjugated with urokinase-type plasminogen activator (uPA) that specifically targets uPA receptor, which is overexpressed in breast cancer tissues (Yang et al. 2009b).
13. Protein-drug nano-conjugates are conjugates between a protein and a NP that selectively binds to the interacting partner of the protein. A popular example of such type of NPs is the gold NPs that have gathered great attention due to their high affinity for sulfhydryl group that can be readily conjugated for antibodies and proteins. Such nano-conjugates are not only used for drug delivery but also to study the function of the conjugated protein and diagnostic studies (Munir et al. 2017). For instance, sensors based on gold NPs are being developed that can distinguish between the breath of lung cancer patients and healthy individuals (Peng et al. 2009).
14. Nanospheres/nanocapsules – Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly

dispersed (Cavalli et al. 2014). Among all the nanocapsules, carbon nanotubes are the most extensively studied. They are not only used as drug delivery vehicles but also in semiconductor devices, energy storage, and conversion devices and in probes and interconnects (Baughman et al. 2002). Moreover, nanospheres are increasingly being used for anticancer therapy, such as the curcumin-loaded PLGA nanosphere is being recently studied for tumor therapy (Mukerjee and Vishwanatha 2009).

In order to utilize such varied NPs in the field of medicine, a crucial step is their delivery at the target site of action and that is usually mediated through active or passive means of delivery (Elkhodiry et al. 2016). Most nanomedicine products attempt to reach the target sites passively which relies on nonspecific accumulation in diseased tissue through the enhanced permeability and retention (EPR) effect – a greatly pursued quality to overall increase drug efficacy (Shi et al. 2017). It is well known that tumor cells grow and proliferate faster than normal cells. This cellular proliferation is associated with an increased metabolic rate that necessitates more nutrients and oxygen supply. In order to compete for the nutrients, the architecture of normal cells becomes disrupted as well as displaced by tumor cells. Passive targeting allows NPs to accumulate in the neoplastic tissue through EPR effect that includes increased permeability of blood vessels in combination with poor lymphatic drainage. The normal vasculature is impermeable to molecules of size >2–4 nm (Bertrand et al. 2014), whereas tumors have leaky vasculature facilitating the retention of NPs in the circulation due to its high density-associated outer defective porous vasculature structure.

Active targeting of the therapeutic molecules is accomplished by coupling the drug or nanocarrier with cell-specific targeting moiety called ligands. For targeted therapeutics, the internalization process should accumulate higher amount of drug in the tumor cells following recycling of the receptor back onto the cancer cells. Ligand-based NP, hypoxia-targeted NP, and magnetic, ultrasound, and temperature- and pH-sensitive NP systems are few examples of newly engineered NPs that have been developed to provide additional physical stimuli to achieve targeted therapy (Zhou and Kopeček 2013). The actively targeted NPs, however, face a major challenge of being exposed to the reticuloendothelial system (RES) due to which they get majorly hoarded in unwanted organs such as liver and spleen. Loss of NPs in the RES leads to extravasation of the drugs with no effective response at the tumor site. To minimize this, scientists have developed "stealth" NPs that have an inert coating of polyethylene glycol (PEG). PEG coating is believed to shield the NPs from aggregation, opsonization, and phagocytosis, thereby prolonging their circulation time. In conclusion, PEG is believed to overcome cancer drug resistance and to minimize the interaction of NPs with RES (Markman et al. 2013). Although NPs have established a toehold in the clinical practice, it requires more effort to occupy the market with full-fledged successful research.

9.6 Nanomedicine: A Boon for Modern Medicine

Cancer is now addressed as a high profile disease in both the developed and developing nations, but its treatment is still a struggle. The chemotherapeutics in clinical use are limited due to their toxic side effects on nontargeted tissues that majorly hinder the quality of life of the patients, apart from the possibility of MDR and disease recurrence. This has created a demand for alternative treatments, and this is where naturally derived anticancer agents are regarded as the best choice. Intriguingly, studies have verified that natural compounds have the ability to inhibit P-gp, which is usually overexpressed in tumor cells and cause efflux of chemotherapeutic drugs from targeted tissues, consequently reducing the efficacy of cancer thereby by several folds (Di Pietro et al. 1999). Natural products like flavonoids, such as quercetin and genistein, are known to inhibit P-gp and MRP1 (Borska et al. 2012). Administration of a combination of genistein, quercetin, morin, and kaempferol in pancreatic carcinoma line resulted in disruption of MRP1-mediated transport (Nguyen et al. 2003). However, such natural agents lack in their clinical maturity due to the reasons discussed previously in this chapter. Herein comes the utility of potent drug carriers that impart high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, opportunity of variable routes of administration, and sustained drug release. Thanks to advances in the field of nanotechnology, the use of NPs, as a delivery system for drugs to reach target sites, is the novel approach being adopted by many (Casals et al. 2017). NPs are perceived as promising drug delivery system in the field of medicine as they are able to convert poorly soluble, inadequately absorbed, labile biologically active substances with low therapeutic index into promising deliverable substances (Ahmad et al. 2016). NPs in their use for anticancer treatment are of growing interest and show promise as an effective alternative to current treatments. Nanomedicines combine nanotechnology with therapeutic agents to provide controlled biodistribution, enhanced efficacy, minimal toxicity, high drug loading capacity, feasibility of functionalization with ligands, controlled drug release capacity, biocompatibility, storage stability, and flexibility in the route of administration. Nanomedicine is a young field, which has been researched merely since the 1990s. There is no clear definition of nanomedicine yet, but such medicines are taken as nanoscale or nanostructured materials within 300 nm size (Etheridge et al. 2013). Despite a rising number of scientific articles describing nanotherapeutics, the number of marketed nanomedicine products only represents one-tenth of them (Fornaguera and García-Celma 2017). During the past two decades, a growing number of nanomedicines have received regulatory approval (Pillai 2014), and many more show promise for future clinical translation (Bregoli et al. 2016).

The advantages furnished by nanoformulations of chemotherapeutics include a wide spectrum (Fig. 9.2), as stated, and the pharmaceutical industry has a strong intention of applying nanomedicines for cancer therapy for enhanced efficacy of treatment. Combination of targeted methods of treatment with potent drug delivery systems, such as NPs, lowers the likelihood of propagation of cancer cells, including resistant populations. Targeted nanomedicines enhance the therapeutic index of

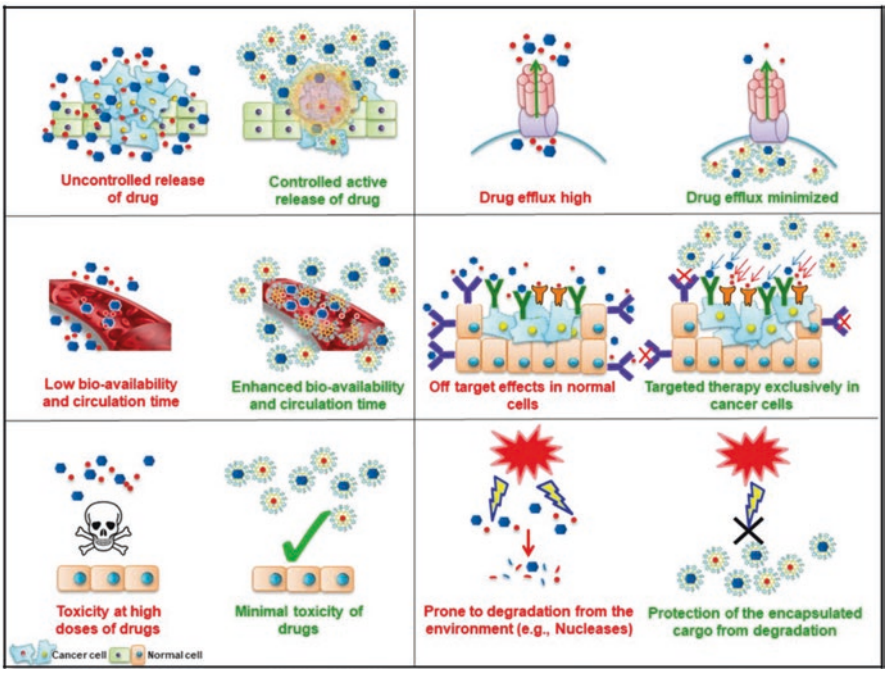


Fig. 9.2 Benefits of encapsulation of drugs in nanoformulations. Among the several advantages offered by nanomedicines over free drugs, a few essential features have been highlighted here, such as more targeted effect in cancer cells with minimal impact on surrounding normal cells, higher circulation time thereby presenting better bioavailability, higher drug retention with lower clearance rate, and the possibility of usage of higher dosage of drugs without the associated toxicity

chemotherapeutics by specific targeting of the drug through ligands or through improved pharmacokinetics consequent to their privileged accumulation in tumors via EPR effect and also by reducing drug efflux and other resistance mechanisms. The characteristic specifications like prolonged systemic circulation, reduced non-specific cellular uptake, active and passive targeting potential, controlled drug release, and multidrug encapsulation are few of the studied modes that permit nanomedicines to hinder MDR (Ahmad et al. 2016). Extensive research centered around drug delivery systems has concluded that surfactant-based formulations, liposomes, polymer and lipid nanocapsules and NPs, polymer-drug conjugates, and micelles can successfully overcome P-gp-mediated drug resistance and enhance drug uptake in tumor cells (Dong and Mumper 2010). Also, the use of more than a single chemotherapeutic agent ensures targeting of multiple oncogenic pathways, which is achieved using multiple payload nanocarriers. Targeted therapy brought upon by nanoformulations also allows usage of much lower doses of the drugs while administering the same efficacy specifically in tumor cells. Doxil was the first FDA-approved nanodrug back in 1995 (Barenholz 2012), and the following years have

witnessed several nanoformulations receiving clinical approval and others still undergoing the procedure.

Bromelain, isolated from *Ananas comosus*, a well-known anticancer phytotherapeutic agent, exhibited higher efficacy in its anticancer mechanisms when administered in formulation with NPs than its free form (Sameer et al. 2016). Cellular uptake of the nanoformulation was severalfold higher in different carcinoma cell lines compared to the free drug, and its activity was preserved upon oral administration (Bhatnagar et al. 2014). The study strongly concluded that NPs furnish a safe and biocompatible method that allows sustained release of drugs at target sites while prolonging the shelf life of the drug. The particular research also construed that the bromelain-loaded NPs induced apoptosis of benign cells in mice more vividly than free bromelain (Bhatnagar et al. 2014).

Despite the long history of clinical application of vincristine as an anticancer therapeutic, three factors diminish its impact in its tumor-suppressive role: (i) its antitumor mechanism is cell cycle-specific, and the duration of its exposure to tumor cells can significantly affect its antitumor activity; (ii) the pharmacokinetic behavior of vincristine in human blood characteristically shows a very fast initial distribution half-life followed by a longer elimination half-life, and it has a large volume of distribution, suggesting diffuse distribution and tissue binding; and (iii) it may induce temporary or permanent peripheral neuropathy, influenced by several variables such as age, race, genetic profile, and administration method, and older children, in particular Caucasian, seem to be more susceptible. Some of these factors could be mitigated by encapsulation of vincristine into liposomes, which is intended to increase the circulation time, optimize delivery to target tissues, and facilitate dose intensification without increasing toxicity. One study depicted that nanoformulation of vincristine significantly upregulated its cellular uptake and imparted greater cytotoxicity in MCF-7 and MCF-7/Adr cells compared to its free form through enhanced drug accumulation in the resistant tumor population by escaping the P-gp-mediated drug efflux. Thus, vincristine in conjugation with a nanocarrier proved beneficial to overcome MDR (Wang et al. 2014).

FDA-approved liposomal anthracyclines, pegylated liposomal doxorubicin (Doxil®/Caelyx®) (Kapse-Mistry et al. 2014), which is the first FDA-approved nanodrug for cancer treatment, and liposomal daunorubicin (DaunoXome®), are currently used for the treatment of Kaposi's sarcoma due to their preferential localization in tumor tissues through the abovementioned EPR effect that is an asset to mend drug resistance. At present, liposomes of paclitaxel, camptothecins, and vincristine are in clinical development (Kapse-Mistry et al. 2014). Liposome coencapsulating doxorubicin and verapamil conjugated with human transferrin had higher cytotoxicity, uncanny targeting, and reversal of P-gp-mediated drug resistance in resistant leukemia K562 cell line. Doxorubicin liposomes resulted in rapid internalization and release of the potent drug inside the cells that led to significantly higher cytotoxicity on HL60 cells and vincristine-resistant HL60 cells (Gokhale et al. 1996). According to a study by Robert Lee, there was a remarkable surge in uptake of folate-PEG-liposomal doxorubicin by KB cells than nontargeted liposomal doxorubicin (Lee and Low 1995). An interesting finding was that doxorubicin

in conjugation with liposomes succeeded in disabling drug resistance even in brain tumors due to endothelial P-gp efflux mechanism (Zhou et al. 2002).

Polymeric NP based nanodrugs are known to prolong circulation time and cap toxicity to normal limits by enabling targeted release of drugs within tumor cell endosomes. NPs accelerated drug accumulation in drug-resistant tumor cells and renders chemotherapeutic agents more effective to overcome MDR. A recent study by Yang et al. unveiled a P-gp blocking imidazole derivative, loaded in combination with vincristine into polymeric micelles, led to a fivefold higher re-sensitization of resistant KB-V200 cells (Yang et al. 2008). Paclitaxel given in polymeric NP formulation was reported to have better target specificity and therefore refined ability to inhibit carcinogenesis with reduced side effects. Co-delivery of Paclitaxel and survivin shRNA NPs enhanced efficacy of Paclitaxel induced apoptosis and cell cycle in Paclitaxel resistant A549/T lung cancer cells (Shen et al. 2012). The nanoformulations had raised drug accumulation in tumor cells that reduced the apoptosis threshold of drug-resistant cells and helped to overcome MDR. Docetaxel loaded NPs had an increased drug uptake and improved cytotoxicity in Docetaxel-resistance human breast cancer cell line compared to its free form, indicating its potential to overcome MDR (Mei et al. 2009). The poor solubility and minimal systemic bioavailability of Curcumin was resolved by encapsulating it in a polymeric NPs (Kapse-Mistry et al. 2014), thus proffering an opportunity to exploit the clinical repertoire of this valuable nanodrug in targeting MDR cancer. In 2010, a considerable inhibition of tumor growth, suppression of P-gp and mdr1 gene levels and overcoming of prevalent drug resistance was achieved in KB-A-1 cells implanted in Balb/c-nu/nu mice using folic acid conjugated antisense oligodeoxynucleotides-hydroxypropyl-chitosan NPs (Das and Sahoo 2012). RNAi-mediated silencing of P-gp expression in human drug-resistant breast MCF-7/ADR cancer cells utilizing polymeric NPs loaded with iMDR1-pDNA plasmid displayed heightened transfection efficiency and lower cytotoxicity. There was a remarkable boost in cellular drug accumulation and cytotoxic effects of Vincristine (Zhang et al. 2011) when loaded in nanoassemblies and a subsequent rescue from MDR through clathrin and caveolae mediated endocytic pathways as reported in MCF-7 and MCF-7/ADR cells. Docetaxel loaded NPs augmented drug uptake and elevated cytotoxicity in DOX-resistance human breast cancer cell line strengthening its potential to overcome MDR in breast carcinoma. A combination NP formulation of Doxorubicin with Curcumin resulted in vanquishing MDR in multiple *in vivo* models including multiple myeloma, acute leukemia, prostate and ovarian cancers. Pramanik et al. showed that these NPs were found to have negligible cardiac toxicity and bone marrow suppression compared to free Doxorubicin (Pramanik et al. 2012). NPs loaded with doxorubicin and chemosensitizer (GG918) bespoke nuclear drug localization and effective anticancer activity toward MDR breast cancer cells. Currently, two polymers, polylactide (PLA) and poly (lactideco-glycolide) (PLGA), serve as polymeric biodegradable nanoplatforms used for synthesis of FDA-approved nanomedicines, with many undergoing clinical trials.

Doxorubicin in PLGA NPs exhibited significantly higher drug accumulation and improved nucleus targeting in MCF-7 cells and P-gp overexpressing resistant

MCF-7/ADR cells and, more importantly, reversed P-gp-mediated drug resistance in human breast cancer (Li et al. 2012). Doxorubicin in conjugation with solid lipid NPs had a good potential in overcoming P-gp-induced MDR as it exhibited a lower IC_{50} value, longer survival duration, higher drug accumulation, enhanced cellular uptake, and intense cellular apoptosis, all of which negatively affected MDR in P388/ADR leukemic mice. Similar observations were seen in ADR-resistant human breast cancer cell lines using the same formulation. Additionally, solid NPs led to reduction in drug cell resistance by augmenting cellular internalization and cytotoxicity of doxorubicin and paclitaxel in leukemic HL60 and breast carcinoma MCF-7 cells (Miglietta et al. 2000). The cellular uptake of doxorubicin NPs was multiple times better than its free form, thus indicating its potential in diminishing P-gp-mediated drug efflux (Kang et al. 2010). Docetaxel use in brain cancer therapy is hindered due to P-gp efflux at the brain that has been circumvented using NPs loaded with docetaxel and a P-gp inhibitor (Vinay Kumar et al. 2013). Docetaxel administered in combination with hepatome-targeting solid lipid NPs had increased cellular uptake, better biodistribution, heightened drug accumulation, and better efficacy in murine models of metastatic hepatocellular carcinoma (Xu et al. 2009).

Micelles are widely known drug carriers that are characterized by EPR effect, active internalization, endosomal-triggered release, and drug escapade that aid in inhibiting MDR. Micelles loaded with anticancer drugs include Genexol®-PM, NK105, NC-6004, NC-4016, NK012, NK911, and SP1049C, all of which are currently in clinical trials (Varela-Moreira et al. 2017). Doxorubicin micelles had high cytotoxicity in doxorubicin-resistant MCF-7 cells due to internalization via folate receptor-mediated endocytosis to overcome P-gp. Micelles co-encapsulating paclitaxel paraded greater cytotoxicity and higher cellular uptake in drug-resistant MCF-7 and MDR MCF-7/ADR cells through efflux mechanism to overcome drug resistance in tumor cells (Wang et al. 2011a). Doxorubicin loaded in polymeric micelles boosted cell accumulation and cytotoxicity in MDR ovarian cells (Lu et al. 2011). Micelles containing methotrexate curbed tumorigenesis in KBv MDR cells (Chen et al. 2013). Vincristine sulfate nanocarriers ameliorated cellular uptake, cytotoxicity in MCF-7, and P-gp overexpressing MCF-7/ADR-resistant cancer cell (Zhang et al. 2011) through bypassing the P-gp mechanism. A combination of camptothecin and doxorubicin in nanoformulation had a synergistic effect in overcoming tumor drug resistance in breast cancer through enhanced cellular drug uptake (Chen et al. 2015).

Several clinical trials are underway for the application of dendrimer to deliver paclitaxel for the suppression of carcinoma of the breast, lung, and pancreas. DOX has also been used in combination with dendrimer that proved tenfold less toxic than free DOX toward colon carcinoma cells. A combination of doxorubicin and dendrimer demonstrated high cytotoxicity against both sensitive and resistant strains of human ovarian adenocarcinoma cell (Lim and Simanek 2012; Webster et al. 2013).

A novel variety of anticancer compound containing both tumor-targeting antibodies and NPs, called fullerenes, allows loading of several molecules of an anticancer drug, thereby magnifying the potential to carry multiple drug payloads, such

as Taxol in addition to other chemotherapeutic drugs. This innovative immunotherapy gives the exceptional advantage of overcoming drug resistance against one drug, since multiple drugs are being administered simultaneously. Modern approaches to nanotubes include the incorporation of drugs such as doxorubicin and paclitaxel, but this technology is still in its virgin state, requiring full-fledged research to reach clinical trials. DOX carbon nanotubes resulted in controlled and sustained release of DOX that successfully mitigated MDR in resistant human leukemia K562R cells (Gao et al. 2011).

Of the different array of NPs available, the application of metallic NPs, especially gold NPs (Au NPs), ranks topmost as they offer several advantages over others, such as biocompatibility, high stability, tissue permeability, and their nontoxic nature. Recently, TNFα used in conjugation with colloidal gold as a therapeutic option for advanced solid tumors, for instance, sarcomas and melanomas, is being considered for practical usage and is in clinical trial (Shenoi et al. 2013). Au NPs of DOX has yielded increased intracellular drug uptake in resistant liver cancer HepG2-R cells (Gu et al. 2012). DOX Au NPs has a higher intracellular uptake and minimized efflux, triggering a significantly greater cytotoxicity in resistant breast cancer MCF-7/ADR cells due to uptake of NPs by the caveolae- and clathrin-mediated endocytosis, thus avoiding the P-gp pathway and subsequent release of the drug into the tumor cells (Wang et al. 2011b).

Given the emerging conspicuous contributions of natural products as potent anticancer agents, it is not surprising that several nanomedicines for cancer treatment have been generated utilizing natural molecules. A major portion of the anticancerous natural compounds, including curcumin and resveratrol, are highly lipophilic in nature and therefore not ideal for drug delivery, due to the associated low bioavailability and the requirement for high doses to achieve the desired therapeutic effects, culminating in acute toxicity and low patient compliance. Encapsulation of such products in NPs helps to improve solubility of the drug and overcome the accompanying shortcomings. Improved pharmacokinetic properties of the nanoformulations of natural products result in better therapeutic effect, without high-dose-induced toxicity. The major natural product-based nanomedicines that have either been clinically approved for use or those that have their clinical evaluation underway have been highlighted in Table 9.2 to provide a comprehensive insight into the clinical significance of natural product-based nanomedicines in providing an effective intervention alternative and in overcoming drug resistance in cancer.

9.7 Future Prospects

Although nanomedicine is a new discipline, its translation into clinics has been exponential. Nanomedicine is no longer simply a tool; it is a mushrooming field that can be exploited as a revolutionary cutting-edge platform for development of future medicine. There is tremendous research underway for generation of NP-based chemotherapeutics that is expected to completely change our perception about cancer treatment, by not only providing the market with novel drugs but by enhancing the efficacy of

Table 9.2 Recent advances in natural product-based nanomedicine

Nanomedicine	FDA approved/ clinical trials	Indication	Product/company	Significant outcome	References
Etoposide (ETPS)-loaded solid lipid NPs					
Solid lipid NPs	Ongoing research	Glioblastoma cancer	–	Exhibited higher cytotoxicity to U87MG cells and permeability across the blood-brain barrier	Kuo and Lee (2015) and Pooja et al. (2015)
		Small-cell lung cancer		Demonstrated significantly higher anticancer activity manifested by antiproliferative mechanisms, morphological changes, and induced apoptosis in A549 cells	
Irinotecan NPs					
Hyaluronic acid (HA)-modified layer-by-layer NPs (NPs)	Ongoing research	Gastric carcinoma	–	Displayed enhanced antitumor activity	Gao et al. (2017)
	Approved	Metastatic pancreatic cancer	Onivyde (liposomal irinotecan injection)	Showed significantly prolonged disease-free survival and overall survival	Kipps et al. (2017)
Liposomes	Phase 1	Colorectal, non-small-cell lung, small-cell lung, prostate, ovarian, and gastric cancer cells	IHL-305 [Yakult Honsha]	Revealed superior antitumor activity against a wide range of tumors at lower doses than free CPT-11	Matsuzaki et al. (2012)
PEG-drug conjugate	Phase 3	Breast cancer, ovarian cancer, colorectal cancer	NKTR-102 (Etirinotecan pegol) [Nektar]	Unveiled better drug release performance and prolonged tumor cell exposure to the drug	Balasubramanian et al. (2014) and Awada et al. (2013)

Liposome	Phase 2	Colorectal cancer	CPX-1 [Celator]	Presented longer and stable maintenance of plasma concentrations of infused drugs	Batist et al. (2009)
Topotecan NPs					
Liposome	Phase 1	Solid tumors	INX-0076 (Brakiva) [Tekmira]	Demonstrated improved pharmacokinetics with enhanced efficacy in both murine and human tumor models	Tardi et al. (2000)
Artemisinin NPs					
Magnetic liposome using chitosan	Ongoing research	Breast cancer	–	Exhibited significant efficacy in inducing apoptosis even at a very low drug concentration	Mughees et al. (2018) and Natesan et al. (2017)
Curcumin NPs					
PLGA NPs	Ongoing research	Prostate cancer, cervical cancer, and breast cancer	–	Showed efficient internalization of the formulation in prostate cancer cells with sustained release of drug	Yallapu et al. (2014), Zaman et al. (2016), and Jin et al. (2017)
				Effectively inhibited cervical cancer cell growth through induction of apoptosis and cell cycle arrest	
Liposome	Phae 1b	Advanced cancer	Lipocurc [SignPath pharma]	Unveiled potent anti-angiogenic effect in tumors	Ranjan et al. (2016)
Vincristine NPs					
Liposome	Approved	Acute lymphoid leukemia	Marqibo® [Talon]	Found to be more efficacious than the equivalent dose of single drug and can be used as a substitution for vincristine in combination treatment	Dong et al. (2016) and Rodriguez et al. (2009)

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Table 9.2 (continued)

Nanomedicine	FDA approved/ clinical trials	Indication	Product/company	Significant outcome	References
Liposome	Phase 2/3	Non-Hodgkin lymphoma	Onco-TCS [Inex/ Enzon]	Manifested potential role in the combination therapy	Sarris et al. (2000)
Camptothecin NPs					
Polyglutamic acid-drug conjugate	Phase 1/2	Colon cancer, ovarian cancer	CT-2106 [CTI biopharma]	Displayed manageable toxicity and prolonged release of drug for a longer time	Homsi et al. (2007) and Davis (2009)
Hydrophobically modified glycol chitosan (HGC) NPs	Ongoing research	Breast cancer	–	Showed prolonged blood circulation and higher accumulation in tumors	Min et al. (2008)
HPMA drug conjugate	Phase 1	Solid tumors	MAG-CPT (PNU166148/ Mureletecan) [Pfizer]	Pharmacia made a strategic decision to discontinue further clinical development of MAG-CPT after considering its toxicity and antitumor activity	Bissett et al. (2004)
PEG-drug conjugate	Phase 2	Gastric cancer	Pegamotecan (EZ-246) [Enzon]	Revealed prolonged circulating half-life, tolerability, and passive tumor accumulation	Scott et al. (2009)
Fleximer-drug conjugate	Phase 1	Gastric cancer, lung cancer	XMT-1001 [Mersana]	Prolonged exposure of drug with greater antitumor response was evident as lower levels of CPT in the urine and less bladder toxicity were observed	Yurkovetskiy and Fram (2009) and Walsh et al. (2012)

Cyclodextrin NPs	Phase 1/2	Solid tumors, renal cell carcinoma, rectal cancer, non-small-cell lung cancer	CRLX-101 (IT-101) [Cerulean pharma]	Presented higher inhibition of tumor cell proliferation and angiogenesis as the expression of topoisomerase-1, Ki-67, CaIX, CD31, and VEGF decreased after CRLX101 treatment	Gaur et al. (2014)
Epigallocatechin-3-gallate (EGCG) NPs					
Poly(L-lactide)-poly(ethylene glycol), (PLA-PEG)	Ongoing research	Prostate cancer	–	This formulation had a much higher biological effectiveness in terms of its pro-apoptotic and angiogenesis inhibitory effects	Siddiqui et al. (2009)
Gelatin NPs	Ongoing research	Breast cancer	–	Exhibited inhibition of hepatocyte growth factor (HGF)-induced intracellular signaling in breast cancer cell line	Shutava et al. (2009)
PLGA encapsulation	Ongoing research	Lung carcinoma, cervical carcinoma and acute monocytic leukemia	–	Demonstrated improved antiproliferative effects and enhanced the anticancer potential of cisplatin	Singh et al. (2015)
Resveratrol nanoparticle					
Poly(epsilon-caprolactone)-, poly(D,L-lactic-*co*-glycolic acid)-, and poly(ethylene glycol)-based NPs	Ongoing research	Prostate cancer	–	Displayed significantly higher cytotoxicity than free drug	Sanna et al. (2013)
BSA NPs	Ongoing research	Ovarian cancer	–	Manifested significantly retarded growth of carcinomas by regulating expression of caspase-9 and caspase-3, suggesting activation of the mitochondrial apoptotic pathway	Guo et al. (2010)

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Table 9.2 (continued)

Nanomedicine	FDA approved/ clinical trials	Indication	Product/company	Significant outcome	References
Biodegradable NPs	Ongoing research	Glioma cancer	–	Potentially higher cell death compared to equivalent dose of free drug was observed	Shao et al. (2009)
Gelatin NPs	Ongoing research	Non-small-cell lung cancer cells; lung cancer	–	Elevated expression of p53, p21, caspase-3, Bax, Bcl-2, and NF-κB, indicating oxidative mechanism in NCI-H460 cells was found	Karthikeyan et al. (2013)
				Higher loading efficiency and superior efficacy in NCI-H460 cells was procured	
Targeted NPs	Ongoing research	Lung adenocarcinoma	–	Induction of apoptosis via mitochondria signaling pathway resulted in treatment of resistant lung cancer	Wang et al. (2011c)
Liposome	Ongoing research	Breast cancer	–	Exhibited higher cytotoxicity against the drug-resistant MCF-7/ Adr tumor cells and enhanced bioavailability and tumor retention of the drugs	Meng et al. (2016)
Paclitaxel NPs					
NPs albumin-bound	Approved	Breast cancer, pancreatic cancer, non-small-cell lung cancer	Abraxane® (ABI-007) [Abraxis/Cel gene]	Complete regressions, longer time to recurrence, longer doubling time, and prolonged survival was witnessed	Desai et al. (2006)

PEG-PLA polymeric micelle	Approved	Breast cancer	Genexol-PM®(IG-001) [Samyang Biopharm]	Demonstrated significantly higher tumor drug concentrations and reductions in tumor volume	Lee et al. (2008)
		Lung cancer			
		Ovarian cancer			
HPMA drug conjugate	Phase 1	Solid tumors	PNU166945 [Pfizer]	Offered improved water solubility and stability resulting in minimized hydrophobicity of paclitaxel for intravenous administration	Terwogt et al. (2001)
Docosahexaenoic acid-drug conjugate	Phase 2/3	Melanoma, liver cancer, adenocarcinoma, kidney cancer, non-small-cell lung cancer	Taxoprexin [Protarga]	Better performance of the formulation was observed with enhanced cytotoxic activity and improved therapeutic ratio	Bedikian et al. (2010)
Polyglutamic acid drug conjugate	Phase 3	Lung cancer and ovarian cancer	Xyotax, Opaxio (CT-2103) [Cell Therapeutics]	Improved therapeutic index and better tolerability was found as compared to free paclitaxel	Sabbatini et al. (2004)
Liposomes	Phase 2	Triple negative breast cancer	Endo-Tag-1 (MBT-0206) [Medigene]	Demonstrated better tolerance and more potent antitumor efficacy	Awada et al. (2014)
Liposomes	Phase 1/2	Ovarian cancer, breast cancer, lung cancer	LEP-ETU (PNU-93914) [NeoPharm/ Insys]	Reduced toxicity while maintaining similar efficacy as the free drug was seen	Slingerland et al. (2013)
Liposomes	Phase 2	Solid tumors, gastric cancer, metastatic breast cancer	Lipusu [Luye Pharma]	Found to be as effective as free paclitaxel, but with significantly reduced adverse reactions	Xu et al. (2013)

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Table 9.2 (continued)

Nanomedicine	FDA approved/ clinical trials	Indication	Product/company	Significant outcome	References
Polymeric NPs	Phase 1	Peritoneal neoplasms	Nanotax [CritiTech]	Presented higher and prolonged peritoneal paclitaxel levels with minimal systemic exposure and reduced toxicity	Williamson et al. (2015)
Polymeric micelle	Phase 1	Advanced breast cancer	Nanoxel [Fresenius Kabi Oncology]	More efficient delivery of drug to target cells as compared to conventional formulation resulting in better intracellular uptake	Madaan et al. (2013)
PEG-PAA polymeric micelle	Phase 2/3	Gastric cancer, breast cancer	NK-105 [NanoCarrier Nippon Kayaku]	Modest activity and tolerability in patients with advanced gastric cancer after failure of first-line chemotherapy was observed	Kato et al. (2012)
Polymeric micelle	Phase 3 (orphan drug status)	Ovarian cancer	Paclical [Oasmia Pharmaceuti cal]	Displayed similar effectiveness and safety but with shorter infusion time as paclitaxel, without standard use of premedication	Vergote et al. (2015)
Docetaxel NPs					
Albumin NPs	Phase 2	Metastatic breast cancer, prostate cancer	ABI-008 [Abraxis/Cel gene]	Offered improved efficacy and safety for first-line treatment of metastatic breast cancer.	Hawkins et al. (2008)
PEG-PLGA polymeric NPs	Phase 1/2	Non-small-cell lung cancer, prostate cancer	BIND-014 [Bind therapeutics]	Exhibited prolonged tumor growth suppression and longer blood circulation half-life	Hrkach et al. (2012)

Polymeric NPs	Phase 1	Non-small-cell lung cancer	Docetaxel-PNP [Samyang Biopharmaceuticals]	Demonstrated delayed tumor growth, higher solubility in water, active internalization into cells, and higher anticancer effect compared to free taxanes	Jung et al. (2012)
Liposomes	Phase1	Solid tumors	LE-DT [NeoPharm/ Insys]	Found to be well tolerated by patients with expected toxicities of neutropenia, anemia, and fatigue, but without neuropathy or edema	Deeken et al. (2013)
Liposomes	Phase 1	Solid tumors	ATI-1123 [Azaya therapeutics]	Acceptable tolerability and favorable pharmacokinetics profile in patients with solid tumors was shown	Mahalingam et al. (2014)
PEG-drug conjugate	Phase 1/2	Solid tumors, ovarian cancer	NKTR-105 [Nektar]	Exhibited sustained release of drug to improve its efficacy, safety, and tolerability profile that improved its antitumor activity and pharmacokinetic profile	Calvo et al. (2010)

This table enlists the natural product-based nanomedicines that are currently being evaluated for cancer therapy, of which many possess the capacity for translation from bench to bedside

established therapeutics. Increased circulation time, precise multiple targeting mechanisms, enhanced drug accumulation at the tumor site, targeted delivery to the site of action in cancer cells, and ability to carry combinations of therapeutic payloads are few of the several opportunities that nanomedicines have offered us with. Nanomedicines have especially proved invaluable for treating MDR patients. Well-defined criteria and comprehensive strategies for both regulatory approval and large-scale industrial production are a need of the hour to make nanomedicine available to the mass. Although the recent focus of the scientific organizations has been centered around cancer therapy, the only objective for application of nanomedicines is expected to cater to a broad range of diseases, including neurodegenerative diseases. Here, penetrating the blood-brain barrier is the ultimate challenge, which can actually be circumvented using nanodrugs; metabolic diseases, such as diabetes, to provide a less invasive platform for treatment; and certain rare and viral-borne diseases.

However, apart from the unequivocal advantages, there still exist certain shortcomings in this virgin field. For instance, broader-reaching efforts are necessary for a distinct definition of "nanomedicine," track key data, and facilitate coordination among agencies in this complex arena. A set of paradigms are in need for toxicogenomic and nanotoxicological data in order to predict drug toxicities and comprehend the mechanisms of toxicity, so that more specific therapeutic targets which are essentially devoid of side effects could be selected. This requires more in-depth understanding of the molecular mechanisms underlying carcinogenesis. Similar to small molecule inhibitor library, the design of safe and effective nanomedicine could be assisted by the development of safety assessment tests and high-throughput screening platforms that would yield predictive information about structure-activity relationships. It is absolutely essential to biologically characterize the NPs and evaluate their health hazards before clinical use. Therefore, detailed investigations on the behavior of these NPs inside the human body are the need of the hour.

Yet, nanoscience is now ushering in a long-awaited era of personalized cancer treatment that appears closer to reality than ever before. Personalized medicine is attributed as a healthcare strategy focused at development of specific intervention strategies customized for each patient or a group of patients, considering the genetic, phenotypic, and environmental factors that could influence the outcome of the therapy.

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