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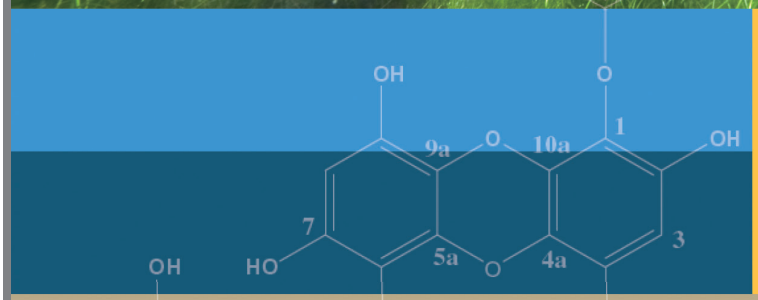

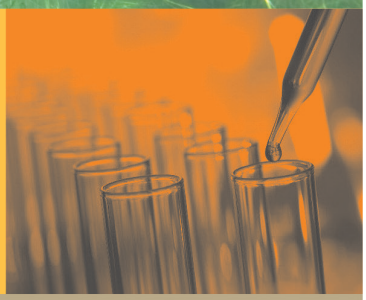

Marine Pharmacognosy

Trends and Applications

Edited by **Se-Kwon Kim**

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33 Clues for Cancer from Ocean-Derived Molecules and Role of In Silico Techniques in Anticancer Drug Discovery

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33.1 INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries, exceeded only by heart disease. The global burden of cancer continues to increase largely because of the aging and growth of the world population along with the increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries. Based on the GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. Lung cancer is the leading cancer in

males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths. Deaths from cancer worldwide are projected to continue to rise, with an estimated 13.1 million deaths in 2030 (Ferlay et al. 2010).

It is well known that genetic changes progressively convert normal cells into cancer cells. Although more than 100 distinct types of cancer exist, only six essential alterations in cell physiology cause malignant cell growth: (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) metastasis. Hanahan and Weinberg (2011) reported that these six "hallmarks of cancer" are present in almost every type of human tumor.

Chemotherapeutic agents currently approved for the treatment of invasive disease may exhibit initial efficacy; however, the development of resistance to therapy and concerns over tolerability are common and generally limit the treatment options available to physicians and patients. Novel chemotherapeutic agents are, therefore, necessary to increase survival, delay disease progression, and improve tolerability. In cancer chemotherapy, drugs administered injure rapidly dividing normal cells and, therefore, have substantial side effects when administered to patients. Anticancer agents that lack side effects and target cancer-specific molecules to eliminate cancer cells while sparing normal cells are the focus of present research (Sawyers 2004). Although advances in the field of chemopreventive and therapeutic medicine have been made regularly over the last several years, the search for novel anticancer treatments continues. New insights into mechanisms responsible for neoplastic disease are significantly changing the general philosophical approach toward cancer treatment.

Drugs derived from natural products have a giant impact on the present-day antitumor drug discovery regime. The importance of natural products in the field of therapeutics may be attributed to their high affinity to the target, little loss of entropy when binding to a protein, and bioavailability. Moreover, natural compounds are quite flexible in conformational acquisition in aqueous and lipophilic environments (Bhatnagar and Kim 2010). For many years, research has essentially focused on plants and terrestrial microorganisms, mainly because these specimens are easily available and folk traditions have described beneficial effects from their use. Recent studies in the field of cancer research have revealed promising compounds, which are isolated from natural sources, with proven anticancer activity. Three examples of such compounds are trabectedin (Yondelis®; PharmaMar, Madrid, Spain), cytarabine (Cytosar-U®; Bedford Laboratories, Bedford, OH), and eribulin mesylate (Halaven®; Eisai, Inc., New Jersey), which represent the first three described marine anticancer drugs (Schumacher et al. 2011). Indeed, almost 50% of the antitumor agents approved in the last 50 years of the twentieth century are either compounds derived from natural sources or (semi-) synthetic analogs of these products (Newman and Cragg 2007). Natural compounds remain a high-output source of promising chemotherapeutic or chemopreventive agents in current cancer research (Villa and Gerwick 2010). Currently, many pharmaceutical companies have therapeutic compounds of marine origin under development.

The rich variety of organisms in the marine environment must adapt to extreme marine environmental conditions such as high pressure; high salt concentration; low nutrient concentration; low, but steady, temperatures (except the high temperatures near underwater volcanoes and the extremely low temperatures in polar regions); limited sunlight; and low oxygen content. To acclimatize to these conditions, marine organisms possess unique characteristics that differentiate them from terrestrial organisms in many aspects, such as metabolism, behavior, information transfer, and adaptation strategies (Hu et al. 2011). The majority of marine invertebrates lack natural defense systems (e.g., innate immune systems) necessary to survive in the competitive environment; hence, they synthesize biologically active secondary metabolites. These metabolites play a role in the defense of host habitats and the adaptation of organisms to extreme environmental challenges (Schumacher et al. 2011).

Marine organisms comprise approximately half of the total biodiversity, thus offering a vast source to discover useful therapeutics. A recent census of marine life that involved the participation

of 2700 scientists from over 80 nations and assessed the diversity, distribution, and abundance of marine life resulted in the discovery of over 6000 potentially novel species (Butler et al. 2010; Fautin et al. 2010; Miloslavich et al. 2010). As a consequence of these research efforts, it is clear that the marine environment represents an important source of unknown natural compounds whose medicinal potential must be evaluated. Efforts to exploit this biodiversity through the identification of new chemical compounds have discovered approximately 22,000 natural products of marine origin so far, whereas 131,000 terrestrial natural products exist. The major sources of biomedical compounds are sponges (37%), coelenterates (21%), and microorganisms (18%) followed by algae (9%), echinoderms (6%), tunicates (6%), mollusks (2%), bryozoans (1%), etc. (Blunt et al. 2011).

Marine algae became an industrial resource much earlier than marine invertebrates and marine microorganisms (including phytoplankton). This is mainly based on the farming of edible species or the production of agar, carrageenan, and alginate. Marine macroalgae are used as foods in many places; particularly in Japan, Korea, and China, sea vegetable has been used as a crude drug for treatment of iron deficiency and diseases such as goiter, Basedow's disease, and hyperthyroidism. Also, considering their great taxonomic diversity, investigations related to the search of new biologically active compounds from algae comprise an almost unlimited field. Most species of red, brown, and green algae have been utilized on an industrial scale for nearly 100 years, which indicates that novel compounds from marine algae are more suitable as potential drugs than those from marine invertebrates (Hu et al. 2011).

Seaweeds or macroalgae belong to the lower plants, which means that they do not have roots, stems, and leaves. Instead they are composed of a thallus (leaflike structure) and sometimes a stem and a foot. Some species have gas-filled structures to provide buoyancy. The majority of the large conspicuous forms of attached marine plants are seaweeds, predominantly those in the three plant divisions Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae). Each group is characterized by specific combinations of photosynthetic pigments. Seaweeds are extremely abundant in intertidal zones, and in clear tropical waters they can extend to depths of up to 200 m. Where they are abundant, seaweeds greatly influence environmental conditions for other types of marine life by providing food, protection from waves, shade, and a substrate on which to attach. Due to their abundance in shallow waters and ease of collection, seaweeds were one of the first groups of marine organisms whose natural products chemistry was studied extensively.

In the classical Indian, Ayurvedic, and Siddha systems of medicine, little information has been reported regarding the medicinal use of seaweeds. Seaweeds have been employed as dressings, as ointments, and in gynecology (Trease and Evanes 1996). The extracts and active constituents of various algae have been shown to exhibit antibacterial activity in vitro against gram-positive and gram-negative bacteria. The production of antimicrobial metabolites was considered to be an indicator of the capability of seaweeds to synthesize bioactive secondary metabolites (Del Val et al. 2001). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibacterial (Nair, Chabhadiya, and Chanda 2007), antiviral (Richards et al. 1978), anticoagulant (Athukorala et al. 2007), and antifouling activities (Hellio et al. 2004).

Seaweeds are important sources of proteins, iodine, vitamins, and minerals and, hence, their metabolites have shown promising activities against cancer incidence. Seaweeds also contain high amounts of polyphenols such as catechin, epicatechin, epigallocatechin gallate, and gallic acid, as reported in *Halimeda* sp. (Chlorophyceae), which exhibits cutting-edge anticancer potential (Boopathy and Kathiresan 2010). Hence, drug discovery from seaweeds for the treatment of various types of cancers is highly warranted.

33.2 ANTICANCER PHARMACOLOGY OF MARINE ALGAE

Carcinogenesis is a complex process controlled by various signal transduction pathways linked to processes such as inflammation, cell differentiation and survival, and metastasis. Most of the players of these pathways are interrelated, and irregularities in their cross talk result in impairment

of cellular functions leading to tumor generation and progression (Bhatnagar and Kim 2010). To design effective drugs against cancer, it is mandatory to understand the underlying tumor physiology and the changes occurring in the tumor microenvironment.

33.2.1 Matrix Metalloproteinase Inhibitors

Zinc-dependant endopeptidases such as matrix metalloproteinases (MMPs) have been extensively studied due to their evident role in carcinogenesis and cellular invasion by catabolizing the extracellular matrix (Gill and Parks 2008). Apart from playing a major role in invasion, angiogenesis, and metastasis during tumor progression, MMPs are also important for cancer cell transformation, growth, apoptosis, signal transduction, and immune regulation. The MMP inhibitory effects of phlorotannins from the marine brown alga *Ecklonia cava* have revealed that its extract could specifically inhibit both MMP-2 and MMP-9 activities significantly ($p < 0.001$) at a concentration of 10 μg/mL in human dermal fibroblasts and HT1080 cells by fluorometric assay. In addition, the *E. cava* extract did not exert any cytotoxic effect even at 100 μg/mL, proposing its potential use as a safe MMP inhibitor (Kim et al. 2006). Expression of MMP-1 was dramatically attenuated by treatment with eckol or dieckol, which were purely isolated from *Ecklonia stolonifera*, indicating that these compounds are active principles to inhibit MMP-1 expression in human dermal fibroblasts (Joe et al. 2006).

33.2.2 Nuclear Factor-κB Inhibitors

Nuclear factor-κB (NF-κB) is a ubiquitous transcription factor, a dimer of proteins of the Rel family including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel, whose deregulated expression may lead to cancer (Keutgens et al. 2006). It is noted that NF-κB is activated by various stimuli, including tumor necrosis factor-α (TNF-α), interleukin-1, and lipopolysaccharides (LPSs). Extracts from three species of Alariaceae, *Eisenia bicyclis*, *E. cava*, and *E. stolonifera*, show strong inhibition of both NF-κB and activator protein 1 (AP-1) reporter activities (Joe et al. 2006). Phlorofucofuroeckol A isolated from the edible brown alga *E. stolonifera* inhibited the activation of Akt and p38 mitogen-activated protein kinase (MAPK) in LPS-treated RAW 264.7 cells; it also regulates inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions through the NF-κB-dependent transcriptional control associated with inhibition of multiple signaling proteins, suggesting potential candidates of phloroglucinol derivatives for treatments of inflammatory diseases (Kim, Lee, and Shin 2011).

33.2.3 Hypoxia-Inducible Factor Inhibitors

Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor that is composed of a hypoxia-inducible α subunit (HIF-1α and HIF-2α) and a constitutively expressed β subunit (HIF-1β). The HIF mediates the adaptation of cells and tissues to low oxygen concentrations. Tumor progression is associated with not only increased microvascular density but also intratumoral hypoxia (Hockel and Vaupel 2001). Loss of HIF-1 activity has been shown to have immense negative effects on tumor growth, vascularisation, and energy metabolism in xenograft assays (Semenza 2001; Kung et al. 2000). Thus, a number of HIF inhibitors have been designed with the aim of finding a new direction for tumor therapy. Laurenditerpenol, which was isolated from bioassay-guided fractionation of the lipid extract of the red alga *Laurencia intricata* Lamouroux (Rhodomelaceae), yielded the first marine natural product that inhibited HIF-1 activation (Mohammed et al. 2004). It was shown to inhibit HIF-1 activation by blocking hypoxia-induced HIF-1α protein accumulation and suppressed mitochondrial oxygen consumption at electron transport chain (ETC) complex I at a 50% inhibitory concentration (IC_{50}) value of 0.8 μM.

33.2.4 Deoxyribonucleic Acid Methyltransferase-1 Inhibitors

The compound halomon [6(*R*)-bromo-3(*S*)-bromomethyl)-7-methyl-2,3,7-trichloro-1-octene] was first isolated from the red alga *Portieria hornemannii* (Lynbye) collected in the Philippines in 1992. Halomon exhibited strong differential cytotoxicity to brain-, renal-, and colon-derived cell lines in the *in vitro* human tumor cell line screen of the National Cancer Institute (NCI), Maryland. On the basis of its unprecedented cytotoxicity profile, halomon was selected by the NCI for preclinical drug development (Fuller et al. 1992). However, research and development of halomon as an anticancer lead has been limited by the lack of a reliable natural source and the failure to show in vivo effects. Andrianasolo et al. (2006) rediscovered the red alga *P. hornemannii* at Madagascar. The organic extract possessed a potent inhibitory activity to the DNA methyltransferase-1 (DNMT-1) isoform. The DNMT-1 causes methylation of cytosine phosphodiester-linked guanine dinucleotide (CpG) by catalyzing the transfer of a methyl group from *S*-adenosylmethionine to the 5′ position on cytosine residues residing at CpG sites. In many cancers, promoters of tumor suppressor genes are silenced by hypermethylation at CpG sites and, thus, the inhibition of DNMT-1 could potentially reverse tumor growth. Halomon and (3*Z*)-6-bromo-3-(bromomethylidene)-2-chloro-7-methylocta-1,6-diene were tested for DNMT-1 enzyme inhibition assay and were found to have activities of 1.25 and 1.65 μM, respectively (Andrianasolo et al. 2006).

33.2.5 Deoxyribonucleic Acid Polymerase Inhibitors

At least 19 different DNA polymerases have been identified in eukaryotic cells. Ohta et al. (1998) found that the sulfolipid metabolite sulfoquinovosyldiacylglycerol (KM-043) isolated from a marine red alga, *Gigartina tenella*, inhibited eukaryotic DNA polymerases α and β (IC_{50} values of 0.25 and 3.6 μM, respectively) and HIV-reverse transcriptase type 1 but did not influence the activities of prokaryotic DNA polymerases. 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol and its methyl ether were isolated from the marine red alga *Symphyocladia latiuscula*, which completely inhibited 1.5 units of Taq DNA polymerase at 0.5 μg and 5 μg respectively (Jin et al. 2008).

33.2.6 Teleomerase Inhibitors

Telomerase is a ribonuclear protein that is detected in more than 90% of primary cancer tissues using a telomeric repeat amplification protocol, which is an early marker for cancer detection. Moreover, telomerase is upregulated in 95% of breast carcinomas but not in adjacent normal tissues by reducing the telomere length, which makes it an ideal target for anticancer drug development. Kanegawa et al. (2000) screened 304 marine algae samples that were collected from various coasts of Japan. In particular, the MeOH extract from the green alga *Caulerpa sertularioides* strongly inhibited telomerase activity when added to a MOLT-4 cell culture. Screening of specific secondary metabolites from marine algae by bioinformatic tools against telomerase enzyme would probably result in more evidence for telomerase inhibitors in the future.

33.2.7 Inosine 5′-Phosphate Dehydrogenase

The enzyme inosine 5′-phosphate dehydrogenase (IMPDH) catalyzes the NAD-dependent oxidation of inosine 5′-phosphate (IMP) to xanthosine 5′-monophosphate and is the key enzyme in de novo guanosine 5′-triphosphate (GTP) biosynthesis (Carr et al. 1993). The two substrates of IMPDH bind in an obligate order: the IMP precedes NAD, and the products also dissociate in an obligate fashion, with NADH preceding xanthosine 5′-monophosphate. The activity of IMPDH is tightly linked with cell proliferation and the inhibition of IMPDH has anticancer, antiviral, and immunosuppressive effects (Jackson et al. 1975). Gerwick and coworkers (1994) at Oregon State University, Corvalis, Oregon, evaluated over 500 extracts of marine microalgae (primarily cyanobacteria) and

macroalgae for their ability to inhibit IMPDH. This assay yielded 24 active extracts and resulted in the isolation of the bromophenolic compound isorawsonol (IC_{50} = 18 μM) from the tropical marine green alga *Avrainvillea rawsonii* (Chen et al. 1994).

33.2.8 Apoptosis

Apoptosis represents a universal and efficient form of cell death that is executed through a highly ordered intrinsic cellular suicide program. Mutations that cause uncontrolled cell growth and those that lead to insufficient cell death occur commonly in neoplasia and contribute to the etiology of cancer. Elucidation of apoptotic pathways and an increased understanding of the importance of apoptosis in the development and progression of cancer have provided impetus for the development of apoptosis-targeted therapies (Nagle et al. 2004). Thyrsiferyl 23-acetate is a cyclic ether that contains a squalene carbon skeleton. Thyrsiferyl 23-acetate was isolated as a potent cytotoxin (Effective dose $(ED)_{50}$ of 0.3 ng/mL against P388 cells) from the marine red alga *Laurencia obtusa* collected in Japan (Suzukia et al. 1985). In serum-deprived Jurkat cells, thyrsiferyl 23-acetate (10 μM) induced chromatin condensation and DNA fragmentation, which are the hallmarks of apoptosis. Although thyrsiferyl 23-acetate has been shown to selectively inhibit serine/threonine phosphoprotein phosphatase 2A (PP2A) (Matsuzawa et al. 1994), its apoptotic activity is not dependent on the inhibition of PP2A (Matsuzawa et al. 1999).

A glycoprotein from the brown alga *Laminaria japonica* displayed several apoptotic features, such as DNA fragmentation, sub-G1 arrest, caspase-3 activation, and poly ADP ribose polymerase (PARP) degradation, in HT-29 colon cancer cells. The mechanism of apoptosis may be mediated via multiple pathways, including the Fas signaling pathway and the mitochondrial pathway, and cell cycle arrest (Go, Hwang, and Nam 2010). Similarly, porphyran, a sulfated polysaccharide from marine red algae, *Porphyra haitanesis*, showed apoptotic activity by following the mitochondrial pathway on AGS human adenocarcinoma cell line (Kwon and Nam 2006). Fucoxanthin, a carotenoid from the edible seaweed *Undaria pinnatifida* induces apoptosis and enhances the antiproliferative effect of the PPARγ ligand, troglitazone, on human colon cancer cells lines, Caco-2, HT-29 and DLD-1 (Hosokawa et al. 2004).

33.2.9 Antimitotic Agents

Antimitotic agents are classified as tubulin-interactive agents, that interfere with the polymerization or depolymerization of tubulin. Actin inhibitors are those that interfere with the polymerization or depolymerization of actin, and kinesin inhibitors are those that disrupt the function of kinesin motor proteins. The compound 14-ketostypodiol diacetate from brown algae, *Stypopodium flabelliforme*, inhibited microtubules by delaying the lag period associated with nucleation events during assembly and significantly decreased the extent of microtubule polymerization in DU-145 human prostatic cells. It also inhibited cell proliferation by affecting the protease secretion and the in vitro invasive capacity, both properties of cell for metastases (Depix et al. 1998).

33.2.10 Multidrug Resistance

Multidrug resistance (MDR) is one of the main causes for the failure of chemotherapeutic cancer treatments. It is noted that MDR was first described by Biedler and Riehm (1970), based on investigations in resistant cell lines derived from a Chinese hamster lung tissue–derived cell line (DC-3F) and a Chinese hamster fibroblastic cell line (CLM-7). The 170 kDa surface glycoprotein P-glycoprotein (P-gp) membrane transporter acts as an ATP-dependent drug efflux pump that actively removes a variety of structurally diverse xenobiotics and natural product–based drugs with

different cellular targets and mechanisms of action (Juliano and Ling 1976). A novel marine terpenoid, dehydrothyrsiferol, isolated from a Canarian red alga, *Laurencia viridis*, showed growth inhibition in oral squamous carcinoma cells with S-phase arrest but no apoptosis (Pec et al. 1998). The IC_{50} values of dehydrothyrsiferol against the P-gp overexpressing multidrug-resistant KB-8-5 cells were about 2.6 times greater in the nonresistant KB-3-1 cells relative to the resistant KB-8-5 cells. Studies conducted in a fluorescence-based efflux system measuring the interference of a test compound with MRP1 (multidrug resistance-associated protein 1)-mediated drug extrusion suggested that dehydrothyrsiferol did not inhibit MRP1-mediated drug transport (Pec et al. 2002).

Hormone-unresponsive breast cancer is associated with poorer prognosis than hormone-receptor expressing malignant mammary tumors. Estrogen-negative breast cancer cells were more sensitive to dehydrothyrsiferol than their receptor-positive counterparts and induction of apoptosis might be transduced through more than one effector pathway. Initial studies suggested that dehydrothyrsiferol may modulate MDR, but modulation of these proteins has subsequently shown to be false. (Pec et al. 1998). Also, dehydrothyrsiferol significantly reduced the adhesion of breast cancer cells through the very late activation antigen (VLA) integrins α2β1 and α5β1 by an apoptosis, when studied on low amounts of the extracellular matrix. Since the activation state of integrins is recognized as an essential factor in metastasis formation, the action of dehydrothyrsiferol in regulating integrin affinity may be a potential therapeutic strategy in cancer therapy (Pec et al. 2007).

33.3 INTERNATIONAL SCENARIO

As mentioned earlier there are numerous secondary metabolites of marine origin wherein marine algae contribute to about 9% (Blunt et al. 2011). Moreover, the role of marine macroalgae specifically in cancer pharmacology around the globe is promising. Adding to the excitement, Parish and associates (Coombe et al. 1987; Parish et al. 1987; Parish and Snowden 1988) discovered that sulfated polysaccharides, including fucoidin and carrageenans, inhibit tumor cell–derived heparanases. Nagumo (1983) discovered that sulfated polysaccharides from *Sargassum kjellmanianum* inhibited mouse S 180 tumor growth, and Matsumoto et al. (1984) reported that carrageenan, which is not active alone, significantly potentiates the effect of mitomycin against leukemia L-1210 ascites tumor in mice. Red algae of the genus *Laurencia* (Rhodomelaceae) are cosmopolitan species with a wide distribution throughout the world. Their secondary metabolites include sesquiterpenes, diterpenes, triterpenes, and acetogenins, which are usually characterized by the presence of one or more halogen atoms in their structures. Due to their relatively high degree of halogenation, many of these molecules either are biologically active or play an ecological role in their ecosystems, often exhibiting antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, cytotoxic, antifouling, antifeedant, ichthyotoxic, and insecticidal activities (Lhullier et al. 2010). All three cuparene sesquiterpenes isolated from *Laurencia microcladia* were found to exhibit significant cytotoxic activity against two lung cancer cell lines (Kladia et al. 2005). Although cytotoxic activity cannot be correlated with the presence or absence of specific functional groups, it was probably influenced by a combination of factors, including the overall three-dimensional (3D) structure of the molecules and the spatial orientation of their substituents (Lhullier et al. 2010).

Oxygenated desmosterols of the red alga *Galaxaura marginate* exhibited significant cytotoxicity toward several cancer cell lines such as P388, KB, A549, and HT-29 (Sheu et al. 1996). Bromoditerpenes from the red alga *Sphaerococcus coronopifolius* exhibited cytotoxic activity on NSCLC-N6-L16 and A549 human lung cancer cell lines (Smyrniotopoulos et al. 2008). Bromophycolide A was cytotoxic against several human tumor cell lines by specific induction of apoptosis (Kubanek et al. 2005). Bromophycolides C–I from the Fijian red alga *Callophycus serratus* displayed modest antineoplastic activity against a range of human tumor cell lines. The most selective of these was bromophycolide H, and its strongest activity was against the breast tumor cell line DU4475 ($IC_{50} = 3.88$ μM) (Kubanek et al. 2006).

33.4 INDIAN SCENARIO

Considerable work has been done on the chemical aspects of seaweeds of the Indian coast of which those up to 1970 have been reviewed by Umamaheshwara Rao (1970). Kesavo Rao (1992) has compiled the elemental composition of Indian marine algae. Utilization of agar and algin by many industries has led to research on cultures of agarophytes and alginophytes (Oza et al. 1994; Sivakumar and Rengaswamy, 2000; Kaladharan et al. 2001; Oza et al. 2001). Although the interest in marine pharmacology of institutions and laboratories around the world has been increasing over the last three decades, in India only sporadic work has been done in this field comprising mainly preliminary studies done by institutions in coastal areas on the marine organisms they come across (Agshikar et al. 1979; Naik et al. 1980). A systemic effort toward pharmacological exploration of the marine wealth of India began in the 1980s with the collaboration of a number of universities. Their study involved the broad pharmacological screening of approximately 500 marine samples in which various activities, such as antifertility, antiviral, hypotensive, and CNS-stimulant activities, were detected (Bhakuni 1991). The leads were so promising that it was decided to have a broad based project and, thus, a national multicenter project, "Development of Potential Drugs from the Ocean," funded by the Department of Ocean Development, Government of India, New Delhi, was started in 1990, which continues to this day. Keeping in mind the number of cytotoxic chemicals of marine origin, a few seaweeds, *Acanthaphora spicifera, Ulva reticulate, Gracilaria folifera*, and *Padina boergesenii*, from the Gulf of Mannar region were screened for their cell viability. It was identified that the alcoholic extracts of the seaweeds exhibited cytotoxic activity (Vasanthi 2002). Further to the screening studies, Hannah R. Vasanthi and coworkers tested the alcoholic extracts of the red alga *Acanthaphora spicifera* from the vicinity of the Mandapam coast, Tamil Nadu, India, for its tumoricidal effect in Ehrlich's ascites carcinoma cells developed in mice. The species *A. spicifera* exhibited tumoricidal activity at a dose of 20 mg/kg comparable to the standard drug 5-flurouracil. This was evidenced by an increase in mean survival time, a decrease in tumor volume, and viable cell count (Vasanthi, Rajamanickam, and Saraswathy 2004). Turbinaric acid from brown algae, *Turbinaria ornate*, was reported to have cytotoxic property against tumor cells (Asari, Kusumi, and Kakisawa 1989). Similarly, monoterpenoids, sargol, sargol-I, and sargol-II, isolated from the brown alga *Sargassum tortile* exhibited cytotoxic activity (Numata et al. 1991). Simultaneously, a linear cytotoxic diterpene bifurcadiol, which was isolated from the brown alga *Bifurcaria bifurcate* by Guardia et al. (1999), was found to exhibit cytotoxicity against various cultured human tumor cell lines, such as A549, SK-OV-3, SKL-2, XF-498, and HCT. It has been identified that heparin and other proteoglycans have a role in neoplasia in regulating the growth of endothelial cells and controlling the proliferation of other cells through its interaction with growth factors.

An extract of *Laurencia brandenii* from the southwest coast of India was evaluated for brine shrimp cytotoxicity and hatchability assay using *Artemia salina*, where the petroleum:chloroform (6:4) fraction showed an LD_{50} value of 93 µg/mL; at 200 µg/mL, 100% hatching inhibition was achieved. Recently, methanolic extracts of seven brown seaweeds occurring in the Indian coastal waters were screened and reported for their cytotoxic and antioxidant properties (Vinayak, Sabu, and Chatterji 2011).

33.5 IN SILICO METHODS IN CANCER DRUG DISCOVERY

Computational screening (also known as virtual screening) refers to the use of a computer-based method to select compounds from a library or database of compounds in order to identify the ones that are likely to possess a given activity, such as the ability to inhibit the action of a particular therapeutic target. Virtual screening has an inherent advantage over traditional, and even experimental, high-throughput screening (HTS) due to its massive parallel-processing ability; using virtual screening, millions of compounds per week can be tested.

Compound–target interactions validated experimentally are available in the scientific literature, and this information is in varied interests of biological, physical, or pharmacological research. Rapid development of information and communication technologies during the previous few decades has dramatically changed our capabilities of collecting, analyzing, storing, and disseminating all types of data. Databases containing millions of chemical compounds tested in various biological assays are increasingly becoming available as online collections. Online compound resources can be classified into two groups: (1) clinically oriented drug "encyclopedias" and (2) chemically oriented small molecule databases. Clinically oriented drug resources include the Pharmacogenomics Knowledge Base (PharmGKB) and the RxList, which is a drug index resource on the Internet (Hatfield, May, and Markoff 1999; Hodge, Altman, and Klein 2007). These knowledge bases tend to offer very detailed clinical information about selected drugs (their pharmacology, metabolism, and indications), with their data content being targeted toward pharmacists, physicians, and consumers.

Chemically oriented small molecule databases include PubChem, ChEBI, ChemSpider, TTD (Therapeutic Target Database), and KEGG (Kyoto Encyclopedia of Genes and Genomes) (Chen, Ji, and Chen 2002; Kanehisa et al. 2006; Degtyarenko et al. 2008; Wang et al. 2009; Pence 2010). These excellent databases provide information about the nomenclature, structure, and/or physical properties of large numbers of small-molecule drugs and, in some cases, their drug targets. These databases are typically oriented toward medicinal chemists, biochemists, and molecular biologists.

It is noted that PubChem (http://pubchem.ncbi.nlm.nih.gov) is a public repository for biological properties of small molecules hosted by the U.S. National Institutes of Health (NIH). The website PubChem is primarily intended to serve as a repository for HTS data from federally funded screening centers and academic research laboratories; the major advantage of PubChem resides in its ability to serve as a chemical gateway to biomedical databases such as PubMed (Wang et al. 2009). The PubChem BioAssay database currently contains biological test results for more than 700,000 compounds (Wang et al. 2012).

The DrugBank (http://www.drugbank.ca) is a richly annotated resource that combines detailed drug data with comprehensive drug target and drug action information (Wishart et al. 2008). Each DrugCard entry now contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half being devoted to pharmacological, pharmacogenomic, and molecular biological data. Recently, the Seaweed Metabolite Database (http://www.swmd.co.in) was developed to share organized information about marine algal compounds and their biological activity available in the literature. Apart from this prior information, the database also contains the geographical origin of a seaweed, its method of compound extraction, and a chemical description of its compounds (Davis and Vasanthi 2011).

The study of protein–small molecule interactions is vital for understanding protein function and for practical applications in drug discovery. Protein crystal structures, nearly 80,000 in the RCSB Protein Data Bank currently, provide crucial insights into protein function and enable researchers to study interactions in atomic detail (Berman et al. 2007). CancerResource addresses the complexity of cancer by not only covering a large, but specific, set of compound–target interactions, experimental data, and supporting information but also by allowing individual data to be processed for advanced analyses (http://bioinformatics.charite.de/cancerresource/; Ahmed et al. 2011). Homologous proteins have similar functions and often interact with their small molecules in a similar manner. Thus, it is possible to infer protein–small molecule interactions even if there are no crystal structures available for a particular protein of interest as long as there are structures of sufficiently close homologs. The resource BindingDB (http://www.bindingdb.org) has experimentally determined the binding affinities of protein–ligand complexes, for protein targets including isoforms and mutational variants, and small-molecule ligands (Liu et al. 2007). Potential applications of these databases are if a naturally occurring compound inhibits cellular proliferation, a search of the database for chemically similar compounds may reveal that a similar compound binds a protein known to be involved in regulation of the cell cycle thus elucidation of the mechanism of a biological effector molecule.

Virtual screening has been widely used to discover new leads by computationally identifying compounds with higher probability of strong binding affinity to a target protein. Screening methods can be classified into structure-based and ligand-based approaches based on the amount of structural and bioactivity data available. In the structure-based approach, where the 3D structure of the receptor is known, high-throughput docking is employed (Sousa et al. 2006). Docking involves a complex optimization task of finding the most favourable 3D binding conformation of the ligand to the receptor molecule. Being computationally intensive, docking is not suitable for very large virtual screening experiments. If the information on the receptor is scanty, the ligand-based method is used, which is efficient and robust in screening chemical databases and virtual libraries against molecules with known activities or properties (Geppert, Vogt, and Bajorath 2010). On the assumption that structurally similar molecules exhibit more similar biological activity than dissimilar or less similar molecules, quantitative structure–activity relationship (QSAR) modeling provides an effective means for both exploring and exploiting the relationship between chemical structure and its biological action toward the development of novel drug candidates (Tropsha 2010).

The concept of QSAR was introduced by Corwin Hansch and coworkers in the 1960s. The QSAR approach can be generally described as an application of data analysis methods and statistics to develop models that can accurately predict biological activities or properties of compounds based on their structures. The most fundamental goal is to predict whether a given molecule will bind to a target and, if so, how strongly it will bind to the target. It is noted that QSAR attempts to find a consistent relationship between biological activity and molecular properties, so that these "rules" can be used to evaluate the activity of new compounds.

The general form of a QSAR equation is $P(i) = f(\mathrm{SD}_i)$, where $P(i)$ is a physical, chemical, or biological property of the compound i; SD_i is a vector of structural descriptors of i; and f is a mathematical function such as linear regression, partial least squares (PLS), artificial neural networks, or support vector machines. The two main objectives for the development of a QSAR are as follows: (1) A predictive and robust QSAR with a specified chemical domain for prediction of activity of untested molecules is developed. (2) The QSAR acts as an informative tool by extracting a significant pattern in the descriptor related to the measured biological activity and allows one to understand the mechanism of the given biological activity, which could help in designing novel molecules with improved activity profiles.

Cheminformatics study entails the calculation of chemical descriptors that are expected to accurately reflect intricate details of underlying chemical structures (Tropsha 2010). A molecular descriptor can be defined as a numerical representation of chemical information encoded within a molecular structure via a mathematical procedure. Types of QSAR are based on the dimensionality of molecular descriptor used. In 0D-QSAR, the descriptors are derived from the molecular formula, for example, molecular weight, number, and type of the atoms. A substructure list representation of a molecule can be considered a one-dimensional (1D) molecule representation, and it consists of a list of molecule fragments.

A molecular graph contains topological or two-dimensional (2D) QSAR information of how the atoms are bounded in a molecule, as well as information on both the type of bonding and the interaction of particular atoms. Molecular hydrophobicity (lipophilicity) is normally quantified as log P where P is the partition coefficient, a measure of differential solubility of a compound in two immiscible solvents. The octanol/water coefficient, P, is the ratio of concentration of a neutral molecule in 1-octanol to its concentration in water when the phases are at equilibrium (Kujawski et al. 2012). In toxicology, partitioning is critical to understanding the tendency of chemicals to cross biological membranes, and the properties of 1-octanol are similar to those of natural membranes. Other descriptors are the ones related to steric effects, such as the molar refraction (MR) index, various parameters accounting for the shape of a compound, and descriptors indicating the presence or absence of certain structural features.

The 3D-QSAR descriptors include molecular surface, molecular volume, and other geometrical properties. Popular 3D-QSAR methods are comparative molecular field analysis (CoMFA), comparative

molecular similarity indices analysis (CoMSIA), and GRID (Bordas, Komives, and Lopata 2003). The basic idea behind CoMFA is that the biological activity of molecules is related to their electrostatic and steric interactions. The molecules (ligands) being studied are aligned structurally on a 3D grid. Using a probe atom, electrostatic and steric fields are determined at every point in the grid. It is noted that CoMSIA, on the other hand, also takes into account hydrophobic parameters. The GRID is similar to CoMFA and can also be used to determine the interaction energies between the probe and the ligand. In addition, GRID can be used to calculate hydrogen-bonding energies (Duch et al. 2007).

In four-dimensional QSAR (4D-QSAR), the fourth dimension represents an ensemble of conformations, orientations, or protonation states for each molecule (Vedani et al. 2000). This reduces the bias that may come from the ligand alignment, but it requires identification of the most likely bioactive conformation and orientation (or protonation state), which is frequently obtained using evolutionary algorithms. Five-dimensional QSAR (5D-QSAR) carries this one step further, allowing for changes in receptor-binding pocket and ligand topology (Vedani and Dobler 2002). Adding solvation effects to 5D-QSAR results in six-dimensional QSAR (6D-QSAR), which allows, in combination with flexible docking, relatively accurate identification of the endocrine-disrupting potential associated with a drug candidate (Vedani, Dobler, and Lill 2005). Software tools are used for calculation of molecular descriptors; most of them are publicly available and free for academic use (from ChemAxon, OpenEye, and OpenBabel), but some are commercial tools (from VLifeMDS).

Success of QSAR modeling depends on the appropriate selection of a data set for QSAR studies. The number of compounds in the data set for QSAR studies should not be too small or, for practical reasons, too large. In model validation schemes, the data set is divided into three subsets: (1) training, (2) test, and (3) external evaluation sets. Training sets are used in model development, and if they are too small chance correlation and overfitting become major problems not allowing one to build truly predictive models. In the case of continuous response activity, the number of compounds in the training set should be at least 20 and about 10 compounds should be in each of the test and external evaluation sets; so, the total minimum number of compounds should not be less than 40. In the case of classification or category response activity, the training set should contain at least about 10 compounds of each class, and test and external evaluation sets should contain no less than 5 compounds for each class. Outliers in a data set can be errors due to structure representation or biological activity, and they should be removed before proceeding with model development (Tropsha 2010). Statistical and machine learning techniques, such as multiple linear regression (MLR), principal component analysis (PCA), and PLS, are then used to solve the problem. It should be mentioned that MLR is still one of the most widely used artificial intelligence techniques in QSAR studies.

33.6 CONCLUSION

Drug discovery and development in this millennium is armed with not only new and efficient techniques for producing and screening new entities but also computational techniques, both hardware and software, that were unimaginable a decade ago. It is now possible to design algorithms and empirical screens to predict a priori absorption and distribution properties of lead molecules in silico. Although computational methods are well established in drug discovery and molecular design, their application in the field of natural products is still in its infancy and more specifically to marine-derived drugs. Computer-assisted approaches, such as docking, pharmacophore modeling, and virtual screening, have to be carried out in the field of bioactive natural products to assess their druggability. This can potentially save research from pursuing wrong leads. The investment of time and resources that can be directed to more promising novel agents will allow the lead-to-market time to shorten considerably in the near future. Combined with experimentation and informatics, computer modeling is expected to accelerate drug discovery, more specifically drug discovery from marine-derived products, to find solutions to many problems such as cancer in the near future.

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