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NATURAL MARINE ANTIVIRAL PRODUCTS

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ABSTRACT: The oceans are unique resources that provide a diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans and molluscs and from marine bacteria and cyanobacteria. Pharmacologic research with marine chemicals continue to contribute potentially novel chemical leads in the ongoing global search for therapeutic agents in the treatment of multiple diverse categories. As infectious diseases evolve and develop resistance to existing pharmaceuticals, the marine environment provides novel leads against fungal, parasitic, bacterial and viral diseases. Limitations in our current antiviral treatment options and the continuing emergence of new pathogenic viruses have contributed to a growing need for new and effective chemotherapeutic agents to treat viral diseases. The search for potent antiviral agents is urgent in view of the dramatic situation of the global human immunodeficiency virus (HIV) epidemic, a possible spread of avian influenza and of other viral diseases. Effective antiviral therapeutics are not available, and the presently approved therapy for HIV has been recognized to be toxic, unable to eradicate the causative virus, and to induce severe drug resistance. The marine environment provides a rich source of chemical diversity for the screening and identification of new compounds with desirable antiviral properties. Antiviral testing has revealed numerous compounds from structural classes including polysaccharides, terpenoids, steroids, alkaloids and peptides that potentially inhibit both RNA and DNA viruses. This review presents an account of some research directed toward the discovery of new antiviral agents from marine sources.

INTRODUCTION

The marine environment is a prolific resource for the isolation of less exploited organisms and microorganisms. There are in fact untapped habitats in the sea with unique characteristics. In addition, the potential contribution of marine sources to the discovery of new bioactive molecules has recently been recognized. These activities probably

represent a mixture of novel metabolites and products previously undiscovered from terrestrial isolates.

Since current antiviral therapies are limited by their toxicity and the tendency of transfected viruses to assume drug-resistant forms, there is a need to find new substances with antiviral activity. All possible approaches towards the development of new antiviral drugs should therefore be pursued. One potential source of these inhibitors is the marine environment [1-5]. Natural marine products have been found to be an important source of drugs and drug leads. These natural products are secondary metabolites which enhance survival fitness and may serve as chemical weapons against bacteria, fungi, viruses and small or large animals [6-8]. Most of the natural products of interest to the pharmaceutical industry are secondary metabolites, and several such compounds derived from marine organisms and microorganisms have been used in clinical trials as experimental antiviral drugs.

The inhibitory effects of marine extracts on the replication of the herpes simplex virus (HSV) and other viruses were reported almost four decades ago. However, these observations did not generate much interest, because the antiviral action of the extracts was considered to be largely non-specific. Shortly after the identification of the human immunodeficiency virus (HIV) as the causative agent of acquired immunodeficiency syndrome (AIDS) in 1984, several natural marine products and extracts were found to be potent and selective inhibitors of HIV replication in cell culture [9,10]. Their activity spectrum has been shown to extend to various enveloped and non-enveloped viruses, including those that emerge as opportunistic pathogens, e.g., HSV and cytomegalovirus (CMV) in immunosuppressed (e.g., AIDS) patients. However, the marine environment provides a never-ending set of fascinating problems for the chemists. Many of the most intriguing problems concern compounds available in only minute quantities. One solution is to focus on bioassay-guided separations.

This work reviews the recent literature (1990-early 2005) on antiviral secondary metabolites from marine organisms and microorganisms and reports a selection of results from more than one hundred bioactive compounds. Under the heading of each respective virus, this article reviews the research on the antiviral activity of extracts and compounds present in the marine environment. This review demonstrated how far the search for new bioactive metabolites from

marine organisms and microorganisms is an active sector of the chemistry of natural products.

HUMAN IMMUNODEFICIENCY VIRUS

HIV is the retrovirus that causes AIDS. The development of anti-HIV microbicides for either topical or *ex vivo* use is of considerable interest, mainly due to the difficulties in creating a vaccine that would be active against multiple clades of HIV. In this chapter we discuss the anti-HIV activity of extracts and compounds isolated from marine sources. Most reports recorded in the literature on anti-HIV activity in the marine environment include marine macro and microalgae and sponges.

The results suggest that the extracts from algae are a promising source of antiviral agents which may act on different stages of the virus replication cycle [11,12]. For example, Nakamura *et al.* [13] investigated boiling water extracts of 25 species of marine algae for their inhibitory activities on the growth of HIV in the MT-4 cell line by the preliminary microplate screening assay *in vitro*. Of the 75 samples assayed, 38 algae were found to be active. Additionally, 47 species of marine macroalgae from the coast of Korea have been screened for the presence of inhibitory compounds against human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and HIV-integrase [14,15]. One Chlorophyta, 8 out 17 Phaeophyta and 6 out 26 Rhodophyta showed inhibitory activity against HIV-1 RT. Five species (*Ecklonia cava*, *Ishige okamurae*, *Sargassum confusum*, *Sargassum hemiphyllum* and *Sargassum ringgoldianum*) belonging to Phaeophyta were shown to inhibit the 3'-processing activity of HIV-1 integrase.

The blue-green filamentous algae *Spirulina platensis* also inhibited HIV-1 replication in human T-cell lines, peripheral mononuclear blood cells and Langerhans cells [16]. The extract inactivated HIV-1 infectivity directly when preincubated with the virus before addition to human T-cell lines. These data indicate that aqueous *Spirulina platensis* extract contains antiretroviral activity that may be of potential clinical interest. Antiviral activities were also reported from the water-soluble fraction of the marine diatom *Haslea ostrearia*, which delayed HIV-1-induced sincytia formation on MT-4 cells [17].

Nicoletti *et al.* [18] investigated the antiviral activity of the green algae *Caulerpa taxifolia*. The chloroform-methanol residue showed an interesting inhibitory effect *in vitro* toward the feline immunodeficiency

virus, a valid model for studying AIDS. This extract reduced virus-induced sincytia in cultured cells, viral RT activity and viral capsin protein p24 expression.

Most of the research on marine compounds with anti-HIV activity has focused on sulphated polysaccharides (PS) and proteins. Sulphated polymannuroguluronate is a marine sulphated PS which has entered phase II clinical trials in China as the first anti-AIDS drug candidate obtained from marine brown algae. Miao *et al.* [19] investigated the binding site(s) receptors of this compound in lymphocytes mediating its anti-AIDS activities. These results indicate that the interaction of this PS and CD4 may provide a mechanistic explanation of its immunopotentiating and anti-AIDS activities in HIV-infected individuals.

The presence of sulphate groups in PS is necessary for anti-HIV activity, and potency increases with the degree of sulphation [20,21]. One of these compounds, calcium spirulan (Ca-SP) has been isolated from the sea alga *Spirulina platensis* as an antiviral component [22,23]. Anti-HIV-1 activity of this compound was measured by three different assays: viability of acutely infected CD4-positive cells, determination of HIV-1 p24 antigen released into culture supernatants, and inhibition of HIV-induced syncytium formation. Ca-SP may be a candidate agent for anti-HIV therapeutics that might overcome the disadvantages observed in many sulphated PS. When the role of chelation of calcium ion with sulphate groups was examined by removing the calcium, or replacing it with sodium the presence of the calcium ion in the molecule was also shown to be essential for the dose-dependent inhibition of the cytopathic effect (CPE) and syncytium formation induced by HIV-1.

Examples of sulphated PS with anti-HIV activity also included sulphated α-D(1→3)-linked mannans from *Nothogenia fastigiata* [24], from *Schizymenia dubyi* [25], anti-HIV PS from the brown seaweed *Fucus vesiculosus* [26] and from the marine microalga *Cochlodinium polykrikoides* [27]. Sulphated PS displaying antiviral activities against HIV-1 and human immunodeficiency virus type 2 (HIV-2) were also isolated from marine *Pseudomonas* and *Dinoflagellata* [28].

Reports on anti-HIV compounds isolated from marine algae also included proteins. Cyanovirin N, an 11-kDa protein, was identified in the search for antiviral agents [29]. Boyd *et al.* [30] isolated and sequenced this protein from cultures of the cyanobacterium *Nostoc ellipsosporum*. Cyanovirin N irreversibly inactivated diverse laboratory strains and

primary isolates of HIV-1, as well as strains of HIV-2 and simian immunodeficiency virus [31-34]. In addition, cyanovirin N aborts cell-to-cell fusion and transmission of HIV-1 infection. The antiviral activity of cyanovirin N is due, at least in part, to unique high-affinity interactions of this protein with the viral surface envelope glycoprotein gp120. Cyanovirin N contains four cysteines which form two intrachain disulfide bonds [35]. The positions of the disulfide linkages were established by fast atom bombardment mass spectral studies of peptide fragments generated by a tryptic digestion of the native protein. Reductive cleavage of these crosslinks resulted in loss of anti-HIV activity. More recently, Barrientos and Gronenborn [36] confirmed that cyanovirin N's antiviral activity appears to involve unique recognition of N-linked high-mannose oligosaccharides on the viral surface glycoproteins.

Another anti-HIV protein of marine origin was griffthisin. Griffthisin was isolated from the red alga *Griffithsia* spp. [37]. This protein displayed potent antiviral activity against laboratory strains and primary isolates of HIV-1. Griffthisin also aborted cell-to-cell fusion and transmission of HIV infection, blocking CD4-dependent gp120 binding receptor-expressing cells and binding to viral coat glycoproteins (gp120, gp41 and gp160) in a glycosylation-dependent manner. Taken together, these data suggest that griffthisin is a new type of protein that binds to various viral glycoproteins in a monosaccharide-dependent manner. This compound could be a potential candidate microbicide to prevent the sexual transmission of HIV and AIDS.

Besides algae, marine sponges had a prolific source of anti-HIV proteins. For example, anti-HIV bioassay-guided fractionation of aqueous extracts of the Caribbean sponge *Niphates erecta*, led to the isolation of a novel anti-HIV protein named niphatevirin [38]. Niphatevirin potently inhibited the CPE of HIV-infection in cultured human lymphoblastoid cells. Niphatevirin bound to CD4 in a manner that prevented the binding of gp120, but did not directly bind gp120. Other anti-HIV proteins were isolated from the purple fluid of the sea hare *Bursatella leachii* [39], and from aqueous extracts of the cultured cyanobacterium *Scytonema varium*, which yielded scytovirin, a protein with potent anticytophatic activity against laboratory strains and primary isolates of HIV-1 [40]. Scytovirin binds to viral coat proteins gp120, gp160 and gp41, but not to cellular receptor CD4 or other tested proteins.

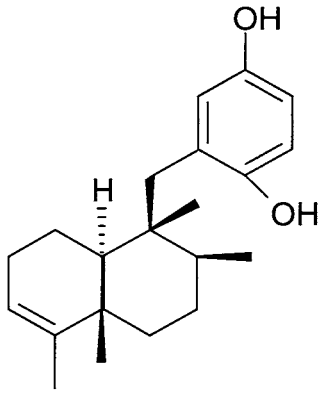

Fig. (1). Structure of avarol

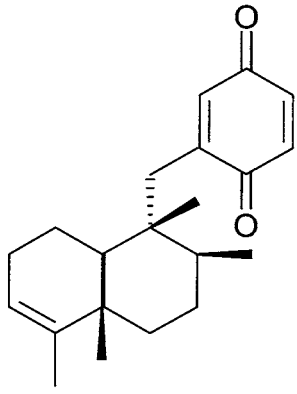

Fig. (2). Structure of avarone

Anti-HIV compounds from marine sources also included terpenoids, steroids, peptides and alkaloids. Avarol, Fig. **(1)** and avarone, Fig. **(2)**, sesquiterpenoid hydroquinones from the marine sponge *Dysidea cinerea*, are promising anti-HIV compounds [41,42]. Three new

sesquiterpene hydroquinones, rietone, 8'-acetoxyrietone and 8'-desoxyrietone, were also isolated from the soft coral *Alcyonium fauri* [43]. Rietone exhibited moderate activity in the National Cancer Institute's *in vitro* anti-HIV bioassays.

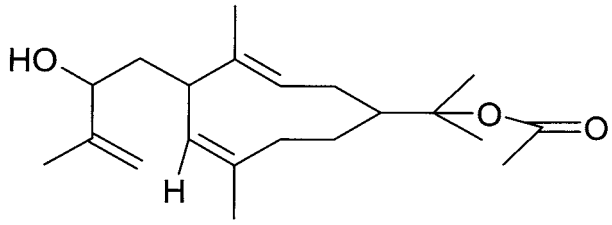

Fig. (3). Structure of cembranoid diterpenes

Bioassay-guided fractionation of an aqueous extract from a Philippine Islands collection of the soft coral *Lobophytum* spp. yielded cembranoid diterpenes, Fig. **(3),** which exhibited moderate HIV-inhibitory activity in a cell-based *in vitro* anti-HIV assay [44], while new isomalabaricane triterpenes, Fig. **(4),** have been isolated from the sponge *Stelletta* spp. [45]. Other anti-HIV diterpenes also included the dolabellane diterpenes isolated from the Brazilian brown algae *Dictyota pfaffi* [46] and *Dictyota menstrualis* [47]. To investigate the effect of these diterpenes in the reverse transcription of the viral genomic RNA, the recombinant HIV-1 RT was assayed *in vitro* in the presence of each compound. All compounds inhibited the RNA-dependent DNA-polymerase activity of HIV-1 RT and consequently virus replication.

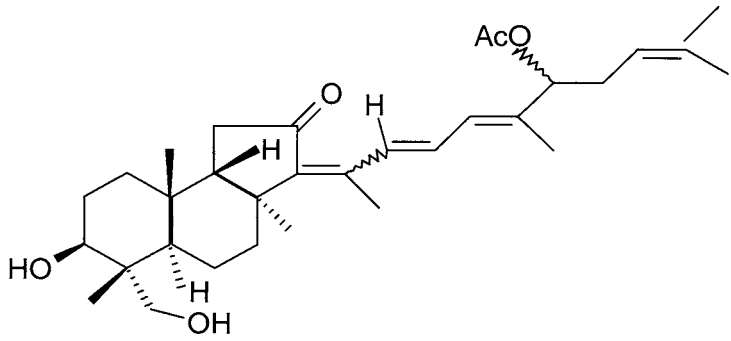

Fig. (4). Structure of isomalabaricane triterpenes

McKee *et al.* [48] evaluated a total of 22 sulphated sterols isolated from marine sponges for their antiviral activity against HIV-1 and HIV-2. In general, sterols with sulphate groups at positions 2, 3 or 6 were the most active. However, those compounds which were sulphated on the sterol D ring were completely inactive against both HIV-1 and HIV-2. New steroid sulphates, which have proved to be cytoprotective against HIV, were also isolated from the marine sponge *Pseudoaxinissa digitata* [49].

Among anti-HIV marine peptides, microspinosamide is a new cyclic depsipeptide incorporating 13 aminoacid residues which was isolated from extracts of an Indonesian collection of the marine sponge *Sidonops microspinosa* [50]. Microspinosamide inhibited the CPE of HIV-1 infection in an *in vitro* assay. Another HIV-inhibitory depsipeptide, neamphamide A, was isolated from the Papua New Guinea marine sponge *Neamphius huxlegi* [51]. Kahalalides are bioactive peptides isolated from the marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* spp., which possess activity against AIDS-opportunistic infections [52].

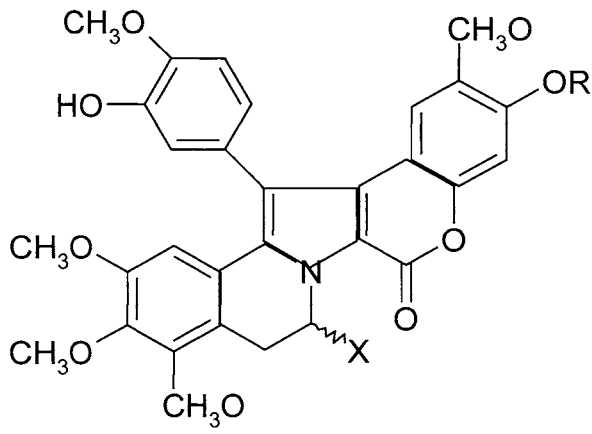

Fig. (5). Structure of lamellarins

Marine alkaloids were also reported to possess activity *in vitro* against AIDS-opportunistic infectious diseases such as tuberculosis and toxoplasmosis [53,54]. Examples of these alkaloids included the ascidian alkaloids lamellarins, Fig. **(5)** [55], and dragmacidin F, Fig. **(6)**, a new

antiviral bromoindole alkaloid from the Mediterranean sponge *Halicortex* spp. [56]. The lamellarins form a group of more than 30 polyaromatic pyrrole alkaloids isolated from diverse marine organisms, mainly but not exclusively, ascidians and sponges. Inhibition of HIV-1 integrase by lamellarin-α-20-sulphate, along with other effects on nuclear proteins, provide an experimental basis indicating that DNA manipulating enzymes are important targets for lamellarins [57].

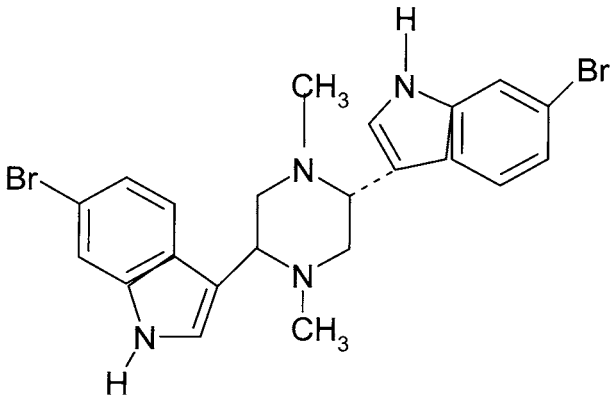

Fig. (6). Structure of dragmacidin F

Two novel alkaloids named manadomanzamines A and B were isolated from the Indonesian sponge *Acanthostrongylophora* spp. [58]. The compounds exhibited activities against HIV-1 and AIDS-opportunistic fungal infections. Oral and intravenous pharmacokinetic studies indicated that the compounds have low metabolic clearance, a reasonably long pharmacokinetic half-life, which supports the value of these compounds as potential leads for further preclinical assessment and possible development [59]. Another marine sponge, *Petrosia similis*, afforded two compounds belonging to bis-quinolizidine alkaloids, namely petrosin and petrosin A [60]. Cell assays indicated that these compounds inhibited the early steps of HIV replication. In the extracellular HIV-1 RT inhibition assay, the compounds inhibited HIV-1 RT.

Two new polycyclic guanidine alkaloids, cambrescidins 826 and dehydroaranobine A, were isolated from the marine sponge *Monanchora* spp. [61]. The pentacyclic guanidine alkaloids inhibit HIV-1 envelope-

mediated fusion *in vitro*. Sorbicillactone A is another alkaloid isolated from a strain of *Penicillium chrysogenum* cultured from a sample of the Mediterranean sponge *Ircinia fasciculata* [62]. It possesses a unique bicyclic lactone structure. The compound exhibits promising activities in several viral test systems, in particular the ability to protect human T cells against the CPE of HIV-1.

Loya *et al.* [63] investigated the activity against HIV-1 RT in the organic extract of the red sea sponge *Toxiclona toxius*. Five novel natural compounds, namely toxiusol, Fig. **(7)**, shaagrockol B and C, toxicol A, all of which are sulphated hexaprenoid hydroquinones, and toxicol E, the *p*-hydroquinone derivative of toxicol A, were isolated. All compounds exhibited inhibitory activity of both DNA polymerizing functions of HIV-1 RT, but failed to inhibit RT-associated ribonuclease H activity. Prenylhydroquinone sulphates, which inhibit HIV-integrase enzymes, have been isolated from the marine sponge *Ircinia* spp. collected from New Caledonia [64].

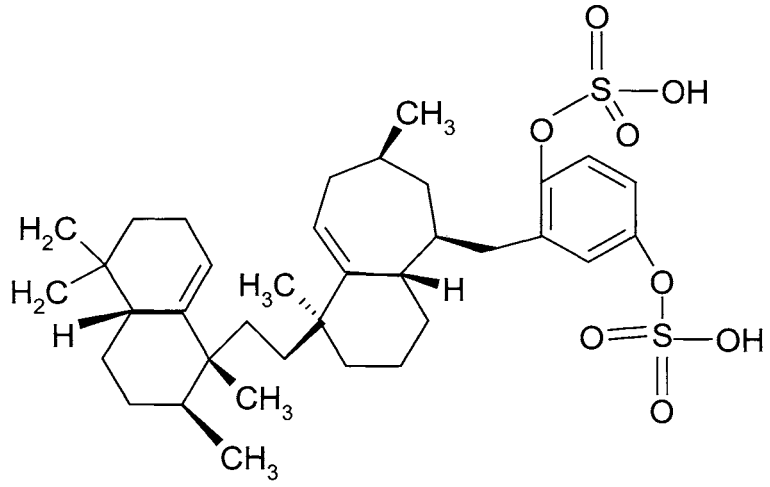

Fig. (7). Structure of toxiusol

Examples of other anti-HIV compounds also included four phlorotannin derivatives, eckol, 8,8'-dieckol, 8,4'''-dieckol and phlorofucofuro-eckol A, isolated from the brown alga *Ecklonia cava* [65]. Among these compounds, 8,8'-dieckol and 8,4'''-dieckol exhibited an inhibitory effect on HIV-1 RT and protease. An enzyme kinetic assay

showed that these compounds non-competitively inhibited RNA-dependent DNA synthesis activity of HIV- RT.

HERPES SIMPLEX VIRUS

Infections with HSV range from simple cold sores and fever blisters to severe central nervous system diseases. Approximately 16-35%, 40-80% and >90% of the population is sero-positive for or infected by herpes simplex virus type I (HSV-1), herpes simplex virus type II (HSV-2) and varicella zoster virus (VZV), respectively. More alarmingly, over the past decade, the incidence and severity of infections caused by HSV have increased due to the growth in the number of immunocompromised patients produced by aggressive chemotherapy regimes, expanded organ transplantation and a greater occurrence of HIV infections. Unfortunately, prolonged therapies with acyclovir, the most successful antiherpetic drug, have resulted in some undesirable complications and also induced the emergence of drug-resistant viruses. There is thus an urgent need for novel anti-HSV agents, especially those with a different mode of action from acyclovir.

In order to find new sources of antiviral agents with different mechanisms of action, extracts of marine algae from all over the world were assayed for anti-HSV activity. The first screening of 89 types of seaweed collected from British Columbia, Canada and Korea for antiviral activity was reported by Kim *et al.* [66]. *Analipus japonicus* was the most potent anti-herpes algae. Extracts from 13 types of Korean seaweed previously shown to contain antiviral activity were investigated in more detail in order to learn their mechanism of action [14]. Four species, *Enteromorpha linza*, *Colpomenia bullosa*, *Scytosiphon lomentaria* and *Undaria pinnatifida* were active against HSV. In experiments to determine the site of action of these antiviral extracts, the predominant activity was virucidal (i.e., direct inactivation of virus particles) rather than inhibition of virus replication.

Ohta *et al.* [67] screened 106 microalgae for CPE on Vero cells of HSV-1. The green alga *Dunnliella primolecta* had the highest anti-HSV-1 activity, since 10 μg/ml of extract from this alga completely inhibited the CPE. The antiviral activity was apparently excited during HSV adsorption and invasion of the cells. Examples of anti-HSV marine algae also included *Laminaria abyssalis*, a Brazilian marine alga [68], and the red marine alga *Polysiphonia denudata* from the Bulgarian Black Sea coast,

which selectively inhibited the reproduction of HSV-1 and HSV-2 in cell cultures [69]. An extract from another red marine alga, *Ceramium rubrum* from the Bulgarian Black Sea coast also inhibited the reproduction of HSV-1 and HSV-2 in cell cultures [70].

Spirulina (Arthospira), a filamentous unicellular alga, is one of the most extensively studied from the chemical, pharmacological and toxicological points of view [71]. A sulphated PS, named Ca-SP, has been isolated from *Spirulina platensis* as an antiviral component [22,72]. The inhibitory effect of polyanionic substances, such as sulphated PS, on the replication of HSV and other viruses was reported almost four decades ago. Since 1988, the activity spectrum of sulphated PS has been shown to extend to various enveloped and non-enveloped viruses [21].

The anti-HSV-1 activity of Ca-SP was assessed by plaque yield reduction and compared with those of dextran sulphate as a representative sulphated PS. These data indicate that Ca-SP is a potent antiviral agent against HSV-1, as even at low concentrations of Ca-SP, no enhancement of virus-induced syncytium formation was observed, as occurred in dextran sulphate-treated cultures. Recently, Lee *et al.* [73] investigated the effects of structural modifications of Ca-SP on antiviral activity. Calcium ion binding with the anionic part of the molecule was replaced with various metal cations, and their inhibitory effects on the replication of HSV-1 were evaluated. Replacement of calcium ion with sodium and potassium ions maintained the antiviral activity, while divalent and trivalent metal cations reduced the activity. Depolymerization of sodium spirulan with hydrogen peroxide decreased the antiviral activity as its molecular weight decreased.

The cell-wall sulphated PS of the red microalga *Porphyridium* spp. also appears to be a good candidate for the development of an anti-HSV drug [74,75]. Treatment of cells with 1 μg/ml PS resulted in 50% inhibition of HSV infection as measured by the plaque assay. Inhibition of the production of new viral particles was also shown when pre-infected cell cultures were treated with the PS. It seems therefore that the PS is able to inhibit viral infection by preventing adsorption of the virus into the host cells and/or by inhibiting the production of new viral particles inside the host cells. The cell-wall sulphated PS of this red microalga *Porphyridium* spp. also had impressive antiviral activity against VZV [74].

Zhu *et al.* [76] isolated another PS from the brown alga *Sargassum patents* as an antiviral component against HSV-1 and HSV-2. The gas

chromatography assay showed that the PS consisted of fucose, galactose, mannose, xylose, glucose and galactosamine. Fucose is the major constituent sugar (35.3%) followed by galactose (18.4%). This PS inhibited the replication of HSV-2, and significantly inhibited the virus attachment to its host cells [77]. All the results from this study suggested that the antiviral mode of action of this compound could be ascribed to the inhibition of virus adsorption, which is different from that of the current drug of choice acyclovir. A sulphated PS was isolated from another *Sargassum* spp., the brown alga *Sargassum horneri* [78,79]. Fucose was also detected as the main component sugar of this PS. This compound showed potent antiviral activity against HSV-1. Time-of-addition experiments suggested that it inhibited not only the initial stages of viral infections, such as attachment to and penetration into host cells, but also later replication stages after virus penetration.

In order to evaluate the potency of novel antiviral drugs, 11 natural sulphated PS from 10 green algae (*Enteromorpa compressa, Monostroma nitidum, Caulerpa brachypus, Caulerpa okamurai, Caulerpa scapelliformis, Chaetomorpha crassa, Chaetomorpha spiralis, Codium adhaerens, Codium fragile* and *Codium latum*) were assayed for anti-HSV-1 activity [80]. Except for one from *Enteromorpha compressa*, all PS showed potent anti-HSV-1 activity while having low cytotoxicities. These experiments demonstrated that some sulphated PS not only inhibited the early stages of HSV-1 replication such as virus binding to and penetration into host cells, but also interfered with later steps of virus replication. A sulphated PS fraction was isolated from another green alga *Caulerpa racemosa* [81]. The polymer contained galactose, glucose, arabinose and xylose as the major component sugars, and contained 9% sulphate hemiester groups. This compound was a selective inhibitor of reference strains of HSV-1 and HSV-2 in Vero cells, and lacked any cytotoxic effects.

Other sulphated PS with known antiviral activity are carrageenans. Natural carrageenans of diverse structural types isolated from the red seaweed *Gigartina skottsbergii* were recently identified as potent and selective inhibitors of HSV-1 and HSV-2 [82,83]. Time-of-addition and attachment studies suggested that the main target for antiviral action of the carrageenans was virus adsorption, whereas no effect on virus internalization or early or later protein synthesis was detected [84]. However, the λ-carrageenan was still significantly inhibitory when added

any time after adsorption. Carrageenans were also extracted from Chilean samples of *Stenogramme interrupta*, with promising antiherpetic activity [85]. This carrageenan is composed predominantly of 0.5 M KCl-insoluble and 1 M KCl-soluble fractions. The insoluble fraction contained γ-carrageenan as the major component, with α-carrageenan and pyruvated carrageenan as minor components.

Other sulphated PS with known antiviral activity are galactans and agarans. Talarico *et al.* [86] presented the chemical composition and antiviral activity against HSV-1 and HSV-2 of sulphated galactans obtained from two red seaweeds collected in Brazil, *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*. The galactans lacked cytotoxic effects and showed a broad spectrum of antiviral activity against HSV-1 and HSV-2. No direct virus inactivation was observed after virion treatment with the galactans. The mode of action of these compounds can be mainly ascribed to an inhibitory effect on virus adsorption. Most importantly, significant protection against murine vaginal infection with HSV-2 was afforded by topical treatment with the sulphated galactans. Another sulphated galactans was isolated as the major component of an aqueous extract of the seaweed *Undaria pinnatifida* [87]. This PS was evaluated for antiviral activity against 32 clinical strains of HSV: 14 strains of HSV-1 and 18 strains of HSV-2. The mode of action of the compound was shown to be the inhibition of viral binding and entry into the host cells. Additionally, some agarans sulphates isolated from the red seaweed *Acantophora spicifera* showed very selective and potent antiviral activity against both HSV-1 and HSV-2 [88]. These sulphated agarans are made up of A-units highly substituted with sulphate groups.

Other anti-HSV sulphated PS included sulphated galactans from the marine alga *Bostrychia montagnei* [89], the red seaweed *Pterocladia capillacea* [90], extracts of *Cryptopleura ramosa* and *Nothogenia fastigiata*, two red seaweeds from the South American coast [91,92], and fucoidans from the brown seaweed *Adenocystis utricularis* [93].

Besides sulphated PS, reports on anti-HSV compounds isolated from marine algae are recorded in the literature. Specimens of the brown alga *Dictyota pfaffii* from Atol das Rocas, northeast Brazil, afforded the rare dolabellane diterpene 10,18-diacetoxy-8-hydroxy-2,6-dolabella-diene and the new 10-acetoxy-8,18-dihydroxy-2,6-dolabella-diene [46]. These substances showed strong anti-HSV-1 activity *in vitro*. Several antiviral diterpenes were isolated from other brown algae of the *Dictyota* genus,

Dictyota dichotoma and *Dictyota linearis* [94]. The diterpenes isopachydictyolal from *Dictyota dichotoma*, and 4α-acetyldictyodial, Fig. **(8)** from *Dictyota linearis* are new natural products which showed potent antiviral activity against HSV-1 using Vero cells as hosts.

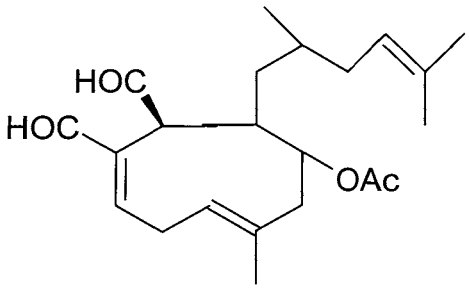

Fig. (8). Structure of 4α-acetyldictyodial

A 6-cyano-5-methoxy-12-methylindolo[2,3-α]carbazole and 6-cyano-5-methoxyindolo[3-α]carbazole were isolated from the blue-green alga *Nostoc sphaericum* [95]. These compounds are active against HSV-1. Larsen *et al.* [96] isolated three new chlorine-containing β-carbolines, bauerines A-C, from the blue-green alga *Dichothrix baueriana*. These alkaloids show activity against HSV-2.

Other marine organisms and microorganisms in addition to marine algae have been reported to produce anti-HSV compounds, including terpenoids, steroids, alkaloids, peptides and sulphated PS.

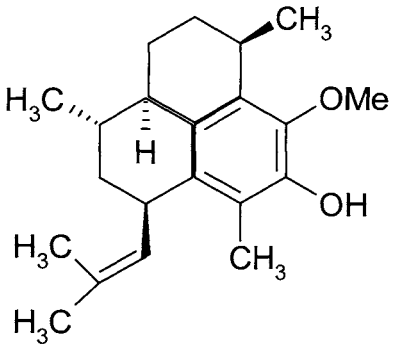

Fig. (9). Structure of pseudopterosins

Betancour *et al.* [97] reported for the first time the antiherpetic activity of a series of sponge-derived diterpenes. A family of 13 new diterpene glycosides, pseudopterosins P-Z, Fig. **(9)**, and seco-pseudopterosins H and I have been isolated from two collections of the marine sponge *Pseudopterogorgia elisabethae* from the Southwestern Caribbean sea in Colombia [98]. Pseudopterosin P displayed strong antiviral activity against HSV-1, HSV-2 and VZV. Seven new diterpenes, helioporins A-G, Fig. **(10)** have also been isolated from the blue coral *Heliopora coerulea* [99]. Helioporins A and B showed antiviral activity against HSV-1. Anti-HSV terpenoids of marine origin also included norsesterterpene peroxide acids from the red sea sponge *Diacarmus erythraeanus*, which showed *in vitro* antiviral activity against HSV-1 [100], and two new trisulphated triterpene glycosides, liouvillosides A and B, from the Antarctic sea cucumber *Staurocucumis liouvillei*, which were found to be virucidal against HSV-1 [101]. Comin *et al.* [102] isolated disulphated polyhydroxysteroids with inhibitory effect on the replication of HSV-2 from the Antarctic ophiuroid *Astrotoma agassizii*.

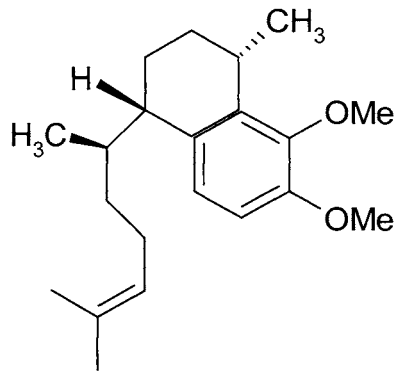

Fig. (10). Structure of helioporins

Marine sponges have been shown to be a prolific source of anti-HSV alkaloids. Dragmacidin F, Fig. **(6)** is a new antiviral bromoindole alkaloid isolated from the Mediterranean sponge *Halicortex* spp. collected from the south coast of Ustica Island, Italy [56]. This compound,

containing an unprecedented carbon skeleton that is very likely derived from cyclization of a partially oxidized form of dragmacidin D, showed *in vitro* antiviral activity against HSV-1, thus proving itself responsible for the antiviral property exhibited by *Halicortex* extracts. The crude extract from the marine sponge *Aaptos* spp. collected in Abrolhos, Bahia, Brazil, afforded a new alkaloid 8,9-dimethoxy-4-methyl-4H-benzo[*D*][1,6]naphthyridine and the known demethyloxyaaptamine [103]. Both compounds showed potent antiviral activity against HSV-1 and low toxicity to Vero cells, suggesting that they may be selectively targets for the inhibition of virus replication. Ichiba *et al.* [104] reported the isolation of a β-carboline alkaloid, 8-hydroxymanzamine A, Fig. **(11)** from the sponge *Pachypellina* spp., which exhibited moderate anti-HSV-2 activity.

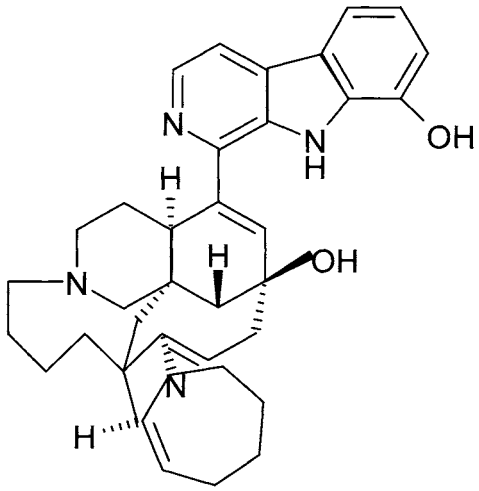

Fig. (11). Structure of 8-hydroxymanzamine A

A marine *Pseudomonas* species WAK-1 strain simultaneously produced extracellular glycosaminoglycan and sulphated PS which showed anti-HSV-1 activity in RPMI 8226 cells [105], while the water-soluble fraction of the marine diatom *Haslea ostrearia* delayed HSV-1-induced syncitia formation [17]. A novel acid PS, nostoflan, was isolated from the cyanobacterium *Nostoc flagelliforme*, which had a broad antiviral spectrum against enveloped viruses, including HSV-1 and HSV-2, whose cellular receptors are carbohydrates [106]. Two new

caprolactans with antiviral activity towards HSV-2 have been isolated from another marine bacterium [107].

As we can see above, marine microorganisms represent an under-explored resource for the discovery of novel antiviral agents. Rowley *et al.* [108] described a series of peptides designated halovirs A-E, Fig. **(12)** that are produced from a marine-derived fungus of the genus *Scytalidium*. These lipophilic and linear peptides are potent *in vitro* inhibitors of HSV-1 and HSV-2. Evidence is presented that the halovirs directly inactivate HSV, a mechanism of action that could be applicable in the prevention of HSV transmission. Recently, Rowley *et al.* [109] presented structure-activity relationships defining key structural elements for optimal viral inhibition. Results demonstrate that an N(α)-acyl chain of at least 14 carbons and a dipeptide are critical for maintaining the antiviral activity.

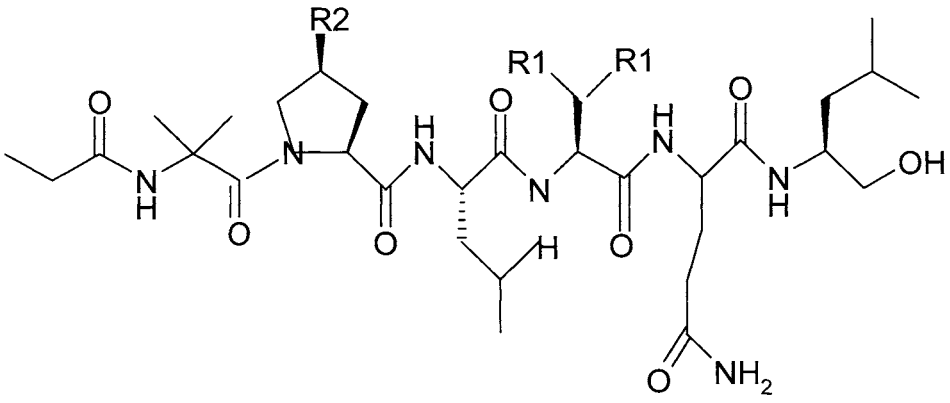

Fig. (12). Structure of halovirs

Examples of other antiviral compounds of marine origin also included two new saponins isolated from the holothurian *Thyone aurea* which showed interesting activity against HSV-1 [110], and fatty acids from the soft coral *Nephthea* spp. with antiviral activity against VZV [111].

CYTOMEGALOVIRUS

In terms of their biological and pathogenic properties, HSV fall naturally into several subfamily groupings, including CMV, although detailed classification is at present premature. Nevertheless CMV clearly constitute a group of their own with internal consistency. Human CMV is, together with HSV-1, one of the agents responsible for opportunistic infections in HIV-infected people.

As we can see in the present review, sulphated PS show antiviral activity against enveloped viruses, including human pathogens such as HIV, HSV and human CMV [112]. Examples included Ca-SP from the blue-green alga *Spirulina platensis* which inhibited, among others, human CMV [23]. This alga also yielded sulfolipids which have been found to be active against CMV [113].

A few reports on anti-CMV compounds of marine origin belonging to other structural types have also been found in the literature. Examples included two plastoquinones isolated from the brown alga *Sargassum micracanthum* which was found to have potent antiviral activity against human CMV [114], and pseudopterosin P, Fig. **(9)** from the sea whip *Pseudopterogorgia elisabethae* [98].

INFLUENZA VIRUS

Influenza continues to have a significant impact on public health. Airborne transmission, facile mutation, vaccine shortages and actual and perceived side effects and limitations of both vaccines and prophylactic drugs contribute to the search for new therapies and preventive medicines. For the past decades, besides a variety of synthetic antiviral drugs with different molecular targets, a number of natural marine products and extracts, mainly with a marine algae origin, have been recognized to control infections caused by the influenza virus.

Reports on the anti-influenza virus effects of extracts from marine algae from all over the world have been found in the literature [115]. These results show that the blue-green algae (cyanobacterium) are able to produce compounds with anti-influenza activity that may be of potential clinical interest. For example, aqueous and methanolic extracts of cultured cyanobacteria of several genera, *Microcystis*, *Nodularia*, *Oscillatoria*, *Scytonema*, *Lyngbya* and *Calothrix* were evaluated for their *in vitro* antiviral activity against the influenza A virus in Madin Darby kidney cells [116,117]. The further analysis of methanolic extracts of cultured strains of genus *Microcystis* revealed a remarkable antiviral

activity against the influenza A virus for *Microcystis aeruginosa*, *Microcystis ichthyoblabe* and *Microcystis wesenbergii*. The antiviral activity observed was associated with protease inhibitory activity of approximately 90%, which may be the agent responsible for reducing virus replication.

Nowotny *et al.* [118] investigated in more detail the antiviral activity of one of these species, *Microcystis aeruginosa*. Virus-specific protein synthesis decreased if the extract was present throughout the whole replication time. The antiviral effect was verified in the Allantois on shell systems using different subtypes of influenza virus A and B [119]. Virus replication was inhibited for 90% with only 10-20 μg/ml of extract. An extract of the red marine alga *Ceramium rubrum* from the Bulgarian Black Sea coast considerably inhibited the reproduction of influenza virus type A and B *in vitro* and *in ovo* [70]. The inhibition affected adsorption as well as the intracellular stages of viral replication. Extracts from other marine organisms besides algae have been shown to possess anti-influenza virus activity. Extracts prepared from economically important marine bivalves were found to possess high antiviral activity when tested against influenza virus type A and B [120].

As a result of these investigations, reports on the isolation of anti-influenza active compounds from marine sources have been found in the literature. Structures of pure compounds have been determined mainly as sulphated PS. A novel acid PS, nostoflan, was isolated from the cyanobacterium *Nostoc flagelliforme* [106]. Nostoflan showed potent antiviral activity against the influenza A virus. Sulphated PS with antiviral activity *in vitro* against a number of human and avian influenza viruses have been isolated from the green marine alga *Ulva lactuca* [121], the marine microalga *Cochlodinium polykrikoides* [27], marine *Pseudomonas* spp. [122] and the blue-green alga *Spirulina platensis* [23]. However, sulfolipids isolated from *Spirulina platensis* have also been found active against the influenza virus [113].

Another anti-influenza compound from marine origin included a new sesquiterpene hydroquinone, named strongylin A, which was isolated from the marine sponge *Strongylophora hartmani* [123].

OTHER VIRUSES

Reports on the antiviral activity found in the marine environment against other viruses have also been found in the literature. Screening of

lipophilic and hydrophilic extracts from marine algae for antiviral activity in different *in vitro* systems revealed some species with interesting effects. For example, Hudson *et al.* [14] and Kim *et al.* [66] screened 89 types of seaweed collected from British Columbia, Canada, and Korea for antiviral activity against the Sindbis virus in Vero cell monolayers. The antiviral activities were proportionately more frequent in the Korean extracts (56% compared with 27% of the Canadian extracts), but in general the more potent extracts were of Canadian origin.

Examples of antiviral activity of extracts from marine algae also included *Sargassum wightii*, a seaweed which showed highest activity against the vaccinia virus [124]. Nakano and Kamei [125] examined the antiviral efficacy of an extract from another *Sargassum* species, *Sargassum hemiphyllum*, that markedly promotes production of interferon-β in MG-63 cells in culture. Antiviral tests revealed the therapeutic efficacy of this alga in mice infected with the Aujeszky's disease virus. These results suggest that this extract manifests its antiviral activity by modulating the host's immunodefense systems.

Aqueous extracts from the marine microalgae *Porphyridium cruentum*, *Chlorella antotrophica* and *Ellipsoidon* spp. produced a significant inhibition of the *in vitro* replication of the haemorrhagic septicaemia virus and African swine fever virus [126], while extracts of the seaweeds *Cheilosporum spectabile* and *Rhizophora mucronata* were found to be effective in protecting mice from the lethal Semliki forest virus infection [127]. Extracts from four species of Brazilian marine algae collected from the coast of Rio of Janeiro State, were active against human T-cell lymphotropic virus type 1- (HTLV-1)- induced syncytium formation *in vitro* [128].

Other marine organisms and microorganisms besides algae have also been found to be active against several viruses, such as marine *Vibrio* spp. which was active against infections caused by the hematopoietic necrosis virus and Oncorhynchus masou virus [129], and the aqueous extracts of marine cephalopods, which inhibited the Moloney murine leukaemia virus RT activity [130].

Several studies were undertaken in order to investigate in more detail the nature of the antiviral compounds and their mechanisms of action. Structures of active pure compounds have been determined as PS, steroids, aminoacid derivatives, diterpenes, lipids and alkaloids.

As can be seen in the present review, most of the research on marine compounds with antiviral activity has focused on sulphated PS. Several sulphated seaweed PS show high antiviral activity against enveloped viruses, including important human pathogens such as HIV, HSV, human CMV, the dengue virus and respiratory syncytial virus [112]. Examples included the cell-wall sulphated PS of the red microalga *Porphyridium* spp. which was highly inhibitory for malignant cell transformation by the Moloney murine sarcoma virus [131] and the marine microalga *Cochlodinium polykrikoides* which produced extracellular sulphated PS that inhibited the CPE of the respiratory syncytial virus type A and B [27].

Another sulphated PS is produced by the marine microalga *Gyrodinium impudicum* strain KG03 from Korea exhibited impressive antiviral activity *in vitro* against the encephalomyocarditis virus [132]. This is the first reported marine source of antiviral sulphated PS against this virus. This PS may be useful in the development of marine bioactive PS for biotechnological and pharmaceutical products. Examples of other antiviral PS of marine origin also included a PS alginate isolated from the marine alga *Fucus gardneri* which showed activity against the potato virus [133], and κ/β-carrageenan obtained from the red marine alga *Tichocarpus crinitus*, which possesses antiviral effect against the tobacco mosaic virus in the early stages [134].

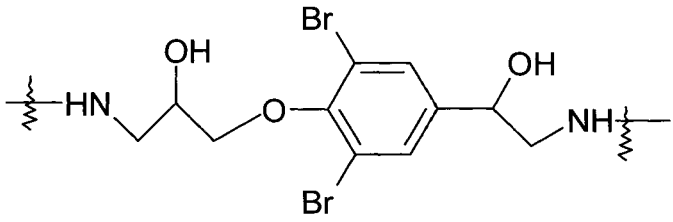

Fig. (13). Structure of fistularin 3

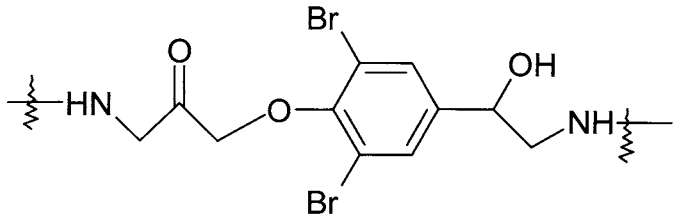

Fig. (14). Structure of 11-ketofistularin 3

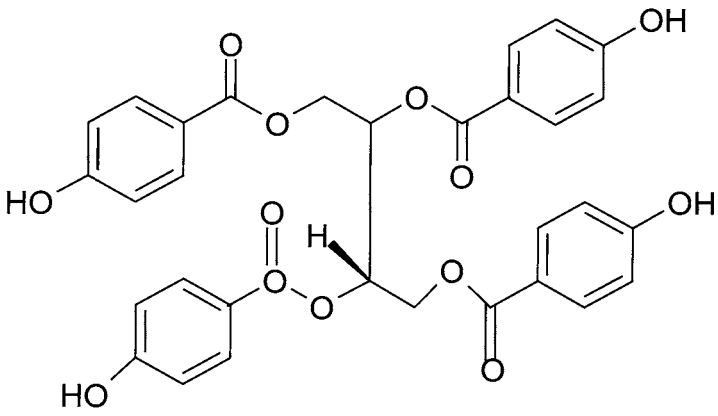

Fig. (15). Structure of kelletinin A

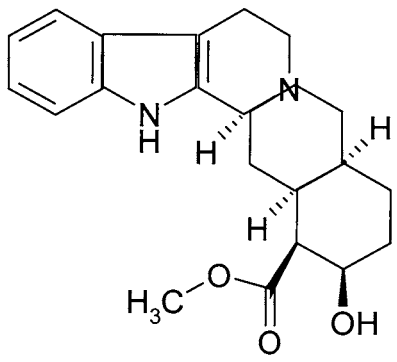

Fig. (16). Structure of halitunal

Gauvin *et al.* [135] isolated 5-α,8-α, epidioxy sterols from the marine sponge *Luffariella variabilis*, which showed inhibitory activity against the HTLV-1. Anti-HTLV-1 activity were also found in two brominated tyrosine metabolites, fistularin 3, Fig. **(13)** and 11-ketofistularin 3, Fig. **(14)** from the marine sponge *Aplysina archeri* [136], and kelletinin A, Fig. **(15)**, an ester of *p*-hydroxybenzoic acid extracted from the marine gastropod *Buccinulum corneum* [137]. From the marine alga *Halimeda tuna*, Koehn *et al.* [138] isolated an unusual diterpene aldehyde, halitunal, Fig. **(16)** which shows antiviral activity against murine coronavirus A59 *in vitro*. Examples of antiviral marine compounds also included neofolitispates, pentacyclic guanidine alkaloids isolated from the sponge *Neofolitispa dianchora* which show antiviral activity against the hepatitis B virus [139], and clavulone, Fig. **(17)**, a prostaglandin analog found in the soft coral *Clavularia viridis* which presented antiviral activity against the vesicular stomatitis virus [140].

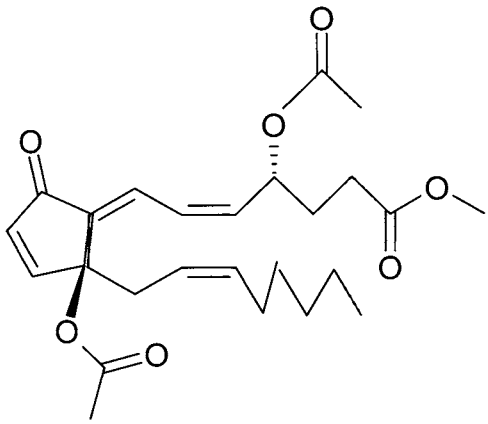

Fig. (17). Structure of clavulone

Chemical investigation of an extract of the soft coral *Nephthea* spp. showing antiviral activity against the Ranikhet disease virus *in vitro* afforded wax esters, cholesterol, 1-O-alkyl-glycerols and fatty acids [111]. Another glycolipid derived from a marine sponge, α-galactosylceramide, Fig. **(18)** is currently in human clinical trials as an

anticancer agent. However, it has also been shown to be effective in reducing the amount of hepatitis B virus DNA detected in mice [141]. It was assumed that the antiviral activities associated with α-galactosylceramide were mediated through the activation of natural killer Y cells.

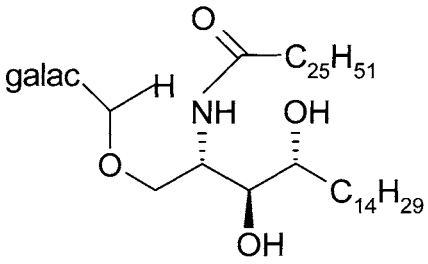

Fig. (18). Structure of α-galactosylceramide

A protein-bound pigment, allophycocyanin, purified from the blue-green alga *Spirulina platensis*, is the first compound reported to exhibit anti-enterovirus 71 activity [142]. Enterovirus 71 infection causes significant morbidity and mortality in children, yet there is no effective treatment. Allophycocyanin neutralized the enterovirus 71-induced CPE in both human rhabdomyosarcoma cells and African green monkey kidney cells. This compound was also able to delay viral RNA synthesis in the infected cells and to abate the apoptotic process in enterovirus 71-infected rhabdomyosarcoma cells, with evidence of characteristic DNA fragmentation decreasing membrane damage and declining cell sub-G1 phase.

ABBREVIATIONS

HSV = Herpes Simplex Virus
HIV = Human Immunodeficiency Virus
AIDS = Acquired Immunodeficiency Syndrome
CMV = Cytomegalovirus
HIV-1 = Human Immunodeficiency Virus type 1
RT = Reverse Transcriptase
PS = Polysaccharides

Ca-SP = Calcium Spirulan
CPE = Cytophatic Effect
HIV-2 = Human Immunodeficiency Virus type 2
HSV-1 = Herpes Simplex Virus type I
HSV-2 = Herpes Simplex Virus type II
VZV = Varicella-Zoster Virus
HTLV-1 = Human T-cell Lymphotropic Virus type 1

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