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Review

Marine compounds and their antiviral activities

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ABSTRACT

Available treatments for many infectious diseases are limited. In particular, diseases caused by viral pathogens have demonstrated the need for new medicines, due to the increasing appearance of resistance to these available treatments. Thousands of novel compounds have been isolated from various marine organisms and tested for pharmacological properties, many of which are commercially available. The screening of natural products derived from marine species for antiviral activity has yielded a considerable number of active crude aqueous and organic solvent extracts. Today, over 40 compounds are commercially available in pharmacological markets, including alternative antiviral medicines or those being tested as potential antiviral drugs. Many more are being tested as potential antiviral drugs at the preclinical and clinical stages. The growing interest in marine-derived antiviral compounds, along with the development of new technology in marine cultures and extraction, will significantly expedite the current exploration of the marine environment for compounds with significant pharmacological applications, which will continue to be a promising strategy and new trend for modern medicine.

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Contents

1. Introduction

Mankind has known for the last several thousand years that marine organisms contain substances capable of potent biological activity. However, the first serious investigation of marine organisms started only half a century ago. Since then, almost all forms of life in the marine environment (e.g. bacteria, algae, sponges, fungi, corals, ascidians, etc.) have been investigated for their natural product content. Currently, important pharmacological and therapeutic products are being obtained and actively sought from the ocean (Bhadury et al., 2006; da Silva et al., 2006; Magarvey et al., 2004; Mayer and Hamann, 2005; Mayer et al., 2007, 2009; Pereira et al., 2004; Prudhomme et al., 2008; Schaeffer and Krylov, 2000; Sipkema et al., 2005; Tziveleka et al., 2003).

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Table 1
Marine-derive antiviral agents.

Compound	Organism	Use	Status[a]	Reference
Acyclovir	Synthetic derivative of arabinosyl nucleosides from sponge, *Tethya cripta*	Antiviral – HSV	OM	Elion et al. (1977)
Ara-A (vidarabine)	Synthetic derivative of arabinosyl nucleosides from sponge, *Tethya cripta*	Antiviral – HSV	OM	Privat de Garilhe and de Rudder (1964)
Ara-C (cytarabine)	Sponge, *Tethya cripta*	Antiviral – HSV	OM	Privat de Garilhe and de Rudder (1964)
Avarol	Sponge, *Disidea avara*	Antiviral – HIV	PCD	Muller et al. (1985, 1987)
Azidothymidine (zidovudine)	Synthetic derivative of arabinosyl nucleosides from sponge, *Tethya cripta*	Antiviral – HSV, HIV	OM	Horwitz et al. (1964)
Cyanovirin-N	Cyanobacteria, *Nostoc ellipsosporum*	Antiviral – HIV-1, HIV-2, SIV	PCD	Boyd et al. (1996)

[a] OM: on the market; PCD: preclinical development.

1.1. Organisms of interest

Over the past 50 years, marine organisms have provided key structures and compounds that proved their potential for industrial development as cosmetics, nutritional supplements, fine chemicals, agrochemicals and therapeutic agents for a variety of diseases (Tziveleka et al., 2003). During the past 30 years, thousands of novel compounds and their metabolites, with diverse biological activities ranging from anticancer to antiviral, have been isolated from various marine sources, some of which are currently in use (Table 1) (Arif et al., 2004).

1.1.1. Bacteria

Currently, it appears that there have been only a few studies focused on finding bioactive compounds derived from marine bacteria to be used as antitumor agents, as well as agents against infectious organisms. Structurally and functionally diverse bioactive compounds have been isolated from prokaryotes, including members of the myxobacteria (e.g., *Sorangium*) and cyanobacteria (e.g., *Nostoc*) (Magarvey et al., 2004). Most studies aimed at identifying bioactive compounds from marine bacteria have centered on bacteria of the order Actinomycetales, as well as on bacterial production of exopolysaccharides, with cyanobacteria being considered a eukaryotoic algae (Magarvey et al., 2004; Arena et al., 2006, 2009).

The order *Actinomycetales* is composed of approximately 80 genera. The diversity of actinomycete secondary metabolites is unrivaled and unmatched in medical significance, accounting for more than 50% of the antibiotics identified to date (Magarvey et al., 2004; Prudhomme et al., 2008). Pure active compounds extracted from the marine actinomycete *Salinispora tropica* have shown inhibitory effects in many malignant cell types (Prudhomme et al., 2008). In particular, Salinosporamide A, related to the known proteasome inhibitor omuralide, was shown to be a potent inhibitor of human multiple meyloma (Feling et al., 2003). Salinosporamide A has begun phase I clinical trials as a potent inhibitor of dividing melanoma cells. It possesses the unique ability to inhibit the proteolytic activity of the 20S proteasome subunit, without affecting any other proteases.

Prudhomme et al. (2008) tested Salinosporamide A for its utility as an antimalarial drug. Salinosporamide A was shown to have inhibitory activity against parasite development in vitro (*Plasmodium falciparum*) and in vivo (*P. yoelii*). The exact mode by which salinosporamide A inhibits *Plasmodium* erythrocytic development is unknown, however, it is likely due to the inhibition of the proteasome complex. It is interesting to note that chloroquine resistant strains are still sensitive to Salinosporamide A. Targeting the proteasome system has a huge therapeutic implication as it can restrain growth and survival of most cell types (Prudhomme et al., 2008). These attributes, taken with the fact that it is already in phase I clinical trials as an antitumor agent, makes it an excellent candidate to test as alternative therapies, such as antibacterial, antiparasitic, antifungal or antiviral agents.

1.1.2. Algae

Micro- and macroalgae were one of the first sources of natural compounds showing in vitro anti-HIV activity (Schaeffer and Krylov, 2000). Algae include a wide variety of plants that range from diatoms, which are microscopic, unicellular organisms, to seaweeds extending over 30 m. Many are of economic importance as food, fertilizer, agar, potash, or sources of iodine. The antibacterial activity of an aquatic microalgae was first reported for *Chlorella vulgaris* (Pratt and Fong, 1940). An early report of the antimicrobial properties of seaweed extracts was published a decade later (Pratt et al., 1951), and several other papers appeared in the next two decades (Burkholder and Sharma, 1969).

1.1.3. Sponges

Pharmaceutical interest in sponges arose in the early 1950s with the discovery of the nucleosides spongothymidine and spongouridine in the marine sponge *Cryptotethia crypta* (Bergmann and Feeney, 1950, 1951). These nucleosides were the basis for the synthesis of Ara-C, the first marine-derived anticancer agent, and the antiviral drug Ara-A (Privat de Garilhe and de Rudder, 1964). Ara-C is currently used in the routine treatment of patients with leukemia and lymphoma. One of its fluorinated derivatives has also been approved for use in patients with pancreatic, breast, bladder, and lung cancer (Sipkema et al., 2005). With respect to the diversity of their secondary metabolites, marine sponges have since been considered a "gold mine," with more than 15,000 marine products described for sponges, which are responsible for more than 5300 different products, and hundreds of new compounds being discovered every year (Sipkema et al., 2005).

The chemical diversity of sponge products is remarkable. In addition to the unusual nucleosides, bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and amino acid derivatives (which are frequently halogenated) have been described from sponges (Sipkema et al., 2005; Tziveleka et al., 2003). Their early appearance in evolution has given them a lot of time for the development of an advanced chemical defense system. It is interesting to note that the synthesis of secondary metabolites is regulated, depending on conditions that the sponge experiences. The huge number of different secondary metabolites discovered in marine sponges and the complexity of the compounds and their biosynthetic pathways (and corresponding kilobases of DNA for the programming of their synthesis) can be regarded as an indication of their importance for survival (Sipkema et al., 2005).

In sponges the role of the chemical constituents is clouded by the complexity of the sponge-symbiont relationship. Many different bacterial species permanently inhabit sponges and contribute

Table 2
Antivirals derived from marine bacteria.

Compound	Organism	Activity	Reference
EPS-1	*Bacillus licheniformis*	HSV-2 replication; Thl cytokine up-regulation	Arena et al. (2006)
EPS-2	*Geobacillus thermodenitrificans*	HSV-2 replication; Thl cytokine up-regulation	Arena et al. (2009)
Macrolactin A	Deep-sea bacterium	HIV replication, HSV	Gustafson et al. (1989a,b)

considerably to the total sponge biomass. It has been suggested that the growth of "useful" microorganisms may be under control of the sponge host and serve as source of food or supply other metabolic products. However, it has also been found that associated bacteria might be the actual producers of a number of compounds that have been isolated from sponges (Sipkema et al., 2005). *Oscillatoria spongelia*, a cyanobacterial symbiont that can constitute up to 40% of *Dysidea herbacea*, is the producer of antimicrobial polybrominated biphenyl ethers and might keep the sponge free of other bacteria (Unson et al., 1994).

1.1.4. Fungi

As interests have turned to marine microorganisms, marine fungi have proved to be a rich and promising source of novel bioactive natural products. Most of these microorganisms grow in a unique and extreme habitat and therefore have the capability to produce unique and unusual secondary metabolites. It is believed that the metabolites possibly act as a chemical defense adaptation of fungi competing for substrates. The production of these unique secondary metabolites by marine fungi is most likely due to their adaptation to a very distinct set of environmental pressures (Bhadury et al., 2006).

To date, more than 275 new compounds have been isolated from marine fungi and the number of compounds is on the increase (Bugni and Ireland, 2004). Most of these metabolites are analogues of those discovered previously from terrestrial fungi. Some of these metabolites with potential clinical importance could be produced in bulk by total or semi-synthetic pathways, through implementation of fermentation technologies and using (post) genomic technologies in which biosynthetic gene clusters are cloned and expressed in vector systems (Bhadury et al., 2006). Marine fungal-derived compounds such as sargassamide, halimide and avrainvillamide have shown selective inhibition of cancer cell lines, and show in vivo activity in preclinical models (P-388 lymphocytic leukemia) (http://www.cancer.ucsd.edu/summaries/wfenical.asp). Two of the above potential drugs have been licensed to the pharmaceutical industry and are in preclinical development. The diversity of the natural products from marine fungi clearly demonstrates that there are potentials for transferring some of these compounds into clinical trials for future development of anti-infective drugs (Bhadury et al., 2006).

1.2. Marine antiviral compounds

Marine antiviral agents (MAVAs) represent a significantly unique natural marine resource whose multi-potential uses include the following applications: (1) The biological control of human enteropathogenic virus contamination and disease transmission in sewage-polluted waters. This application would be particularly important to communities that utilize their coastal waters for recreational activities and for food industries (e.g. fish, shellfish), as well as to those regions of the country, such as Hawaii, where the loss of these marine resources would have a devastating effect on the lifestyle and economy of the people. (2) The chemotherapy of viral diseases of humans and lower animals.

To be of practical use, it is imperative that MAVAs be isolated in pure culture, identified, characterized, and their spectrum and mechanisms of antiviral activity be clearly established. Also the active principle(s) and moieties should be identified and chemically characterized in order to facilitate application of biotechnological methods for increased yields and cost effective production. (3) The biological control of viral diseases of marine animals. Under natural conditions, there are only a few practical ways to prevent viral transmission to marine mammals. This is especially troublesome when marine mammals are kept in captivity for various uses. The seeding of MAVAs to these special environments could control viral disease transmission.

1.2.1. Bacteria-derived antivirals

Currently, it appears that there have been only a few compounds derived from marine bacteria with antiviral activity (Table 2), keeping in mind that cyanobacteria are traditionally grouped as blue–green algae. Studies using marine bacteria as sources for antiviral compounds have focused mainly on marine bacterial exopolysaccharides (Arena et al., 2006, 2009). Many marine bacteria produce exopolysaccharides (EPS) as a strategy for growth, adherence to solid surfaces and survival in adverse conditions. There is a growing interest in isolating new exopolysaccharide-producing bacteria from marine environments, particularly those that exist in extreme marine environments characterized by extreme pressure and temperature, as well as high concentrations of H_2S and heavy metals (Vincent et al., 1994). Bacteria from deep-sea hydrothermal vents, such as *Alteromonas macleodii* subsp. *fijiensis*, *Vibrio diabolicus*, *Alteromonas infernus* and strain HYD721, produce EPS with original structures that enable them to survive in their extreme environments (Arena et al., 2009). Bacterial EPS produced in these environments provide novel chemical compositions, properties and structures that can have potential applications in different industrial fields. More recently, the hydrothermophilic *Thermotoga maritima* and *Thermococcus litoralis* have been reported as producers of extra polysacchardes (Rinker and Kelly, 2000).

1.2.1.1. Exopolysaccharides. Sulphated exopolysaccharides (EPS) are known to interfere with the adsorption and penetration of viruses into host cells, as well as inhibit various retroviral reverse transcriptases. Two new exopolysaccharides, EPS-1 and EPS-2, were isolated from *Bacillus licheniformis* and *Geobacillus thermodenitrificans*, respectively, two bacteria isolated from a shallow marine hot spring of Vulcano Island, Italy (Arena et al., 2006, 2009). These two EPS were shown to be noncytotoxic to PBMC's and WISH cells at concentrations $\leq$300 μg/ml. The antiviral effects produced by EPS-1 and EPS-2 at concentrations of 200 and 300 μg/ml were shown to significantly reduce HSV-2 viral titer. It was determined that both EPS-1 and EPS-2 are able to inhibit HSV-2 replication in PBMCs by up-regulating the expression of proinflammatory cytokines, particularly triggering polarization in favor of the Th1 subset (Arena et al., 2006, 2009).

1.2.1.2. Macrolactin A. Macrolactin A is another marine bacteria-derived antiviral compound that includes 24-membered ring lactones, related glucose β-pyranosides and open chain acids, and it shows the protection to human T-lymphoblast cells against human HIV replication, with maximum protection at a concentration of 10 μg/ml (Gustafson et al., 1989b).

Table 3
Antivirals derived from marine algae.

Compound	Organism	Activity	Reference
Al	Microalgae, *Cochlodinium polykrikoides*	Influenza virus A and B; RSV A and B; HSV-1	Hasui et al. (1995)
A2	Microalgae, *Cochlodinium polykrikoides*	Influenza virus A and B; RSV A and B; parainfluenza type 2	Hasui et al. (1995)
AcDa-1	*Dictyota menstrualis*	HIV-1 replication and RNA-dependent DNA polymerase activity of the viral RT	Pereira et al. (2004)
Calcium spirulan	Cyanobacteria, *Arthrospira platensis* (previously called *Spirulina platensis*)	HSV-1 replication; Measles replication; Mumps replication; Influenza replication; Polio replication; Coxsackie replication; HIV-1 replication; HCMV replication; Selectively inhibition of penetration into host cells	Hayashi et al. (1996)
Cyanovirin-N	Cyanobacteria, *Nostoc ellipsosporum*	HIV-1 and HIV-2 and SIV fusion, replication and CPE	Boyd et al. (1996)
Da-1		HIV-1 replication and RNA-dependent DNA polymerase activity of the viral RT	Pereira et al. (2004)
Fucoidan	Brown seaweed, *Fucus vesiculosus*	HSV-1 and HSV-2; HCMV; VSV; Sinbis virus; HIV-1 RT	Béress et al. (1993), Moen and Clark (1993)
Galactan Sulfate	Red seaweed, *Agardhiella tenera*	HIV-1 and HIV-1 CPE and syncytia formation; HIV-1 binding to host cells; Binding of anti-gpl20 mAb to HIV-1 gpl20; Other enveloped viruses (herpes viruses, togaviruses, arenaviruses, etc.)	Witvrouw et al. (1994)
Griffithsin	Red alga, *Griffithsia sp.*	HIV-1 glycoproteins (e.g., gpl20, gp41 and gpl60)	Mori et al. (2005)
Naviculan	Diatom, *Navicula directa*	HSV-1 and HSV-2 adhesion, penetration and replication	Lee et al. (2006)
SAE	Red alga, *Schizymenia pacifca*	HIV RT; AMV RT; RMLV RT	Nakashima et al. (1987a,b)

1.2.2. Algae-derived antivirals

Compounds extracted from algae have in vitro or in vivo activity against a wide range of viruses, including herpes viruses (HSV-1, HSV-2, HCMV), togaviruses (Sindbis virus, Semliki Forest virus), paramyxoviruses (RSV), rhabdoviruses (VSV), and both human and simian immune deficiency viruses (HIV and SIV) (Table 3). The antiviral effects of polysaccharides from marine algae towards the mumps and influenza B virus were reported over 50 years ago by Gerber et al. (1958). Subsequently, polysaccharide fractions from extracts of red algae were found to inhibit HSV and other viruses (Burkholder and Sharma, 1969; Dieg, 1974; Ehresmann et al., 1977; Richards et al., 1978). However, these observations did not generate much interest because the antiviral action of these compounds was considered to be largely nonspecific (Witvrouw and De Clercq, 1997). The isolation from algae of sulfated polysaccharides and other compounds with antiviral activity against enveloped viruses increased the interest in algae as a source of antiviral compounds.

1.2.2.1. Galactan sulfate. Galactan sulfate (GS), a polysaccharide isolated from the red seaweed *Agardhiella tenera*, shows activity against HIV-1 and HIV-2 (Witvrouw et al., 1994). Viral-induced CPE of HIV-1 and HIV-2 in MT-4 cells is inhibited with IC_{50} values of 0.5 and 0.05 μg/L, respectively. Viral-induced syncytia formation in Molt-4 cells and HIV-1- or HIV-2-infected HUT-78 cells is inhibited at GS concentrations >5 μg/L. GS inhibits the binding of HIV-1 to cells, as well as the binding of anti-gp120 mAb to HIV-1 gp120. GS is also active against other enveloped viruses, including herpes viruses, togaviruses, arenaviruses and others (Witvrouw et al., 1994).

1.2.2.2. A1 and A2. Extracellular sulfated polysaccharides A1 and A2 were isolated and purified from *Cochlodinium polykrikoides*, a marine microalgae (Hasui et al., 1995). A1 and A2 inhibit the cytopathic effects of influenza virus types A and B grown on MDCK cells, and RSV types A and B grown on Hep-2 cells. In MT-4 cells, both A1 and A2 have an IC_{50} value of 1.7 μg/ml against HIV-1. A1 is also active against HSV-1, while A2 is active against parainfluenza virus type 2, both in HMV-2 cells. A1 and A2 are noncytotoxic at 100 μg/ml and produce weak (10%) inhibitory effects on blood coagulation at concentrations inhibitory to virus (Hasui et al., 1995).

1.2.2.3. Fucoidan. Fucoidan is a sulfated polysaccharide isolated from the brown seaweed *Fucus vesiculosus* (Béress et al., 1993) and it shows inhibitory effect on the replication of DNA viruses: herpes virus (HSV-1, HSV-2) and HCMV (Baba et al., 1988; Béress et al., 1993; Moen and Clark, 1993). This compounds is also active against RNA viruses: VSV, Sinbis virus, and HIV-1 (Baba et al., 1988). However, this compound is not active against coxsackievirus, poliovirus, and parainfluenza virus. A water-soluble, noncarbohydrate component of fucoidan isolated from *F. vesiculosus* is able to inhibit HIV RT in vitro at 50 μg/ml (Moen and Clark, 1993). Preincubation of cell-free virus to 200 μg/ml causes 100% reduction in the amount of HIV-1 p24 antigen released. Tests show that these effects are not due to the killing of target cells. In fact, fucoidan produced no adverse effects on cell proliferation and protein metabolism. The preincubation of target cells with fucoidan actually protects them from HIV-1 infection (Moen and Clark, 1993). In addition to its antiviral activity, fucoidan possesses low anticoagulation properties (Baba et al., 1988; Béress et al., 1993; Moen and Clark, 1993).

1.2.2.4. Sea algal extract. Nakashima et al. (1987a) prepared a citrate buffer extract of the marine red alga *Schizymenia pacifca*. A cell-free system was used to test the effect of the extract on reverse transcriptase (RT) from avian myelblastosis virus (AMV) and Rauscher murine leukemia virus. Nakashima et al. (1987b) purified this new RT inhibitor from *S. pacifca*. The compound, termed "sea algal extract" (SAE), is a sulfated polysaccharide composed of galactose (73%), sulfonate (20%) and 3,6-anhydrogalactose (0.65%). SAE was determined to be a specific inhibitor for HIV RT and HIV replication in vitro, and produces no adverse effects on cell growth. The sulfate residues are hypothesized to play a key role in RT inhibition. This hypothesis is supported by inducing activity in polysaccharides (Nakashima et al., 1987b).

1.2.2.5. Calcium spirulan. Like other algae, a number of cyanobacteria species have been found to produce highly anti-HIV active sulfoglycolipids (Gustafson et al., 1989a; Loya et al., 1995; Reshef et al., 1997). In addition to their activity, sulfoglycolipids constitute part of the chloroplast membrane and are therefore abundant (Harwood, 1987). Calcium spirulan (Ca-SP), a sulfated polysaccharide, was isolated from a marine blue–green alga, *Arthrospira platensis* (previously called *Spirulina platensis*) (Hayashi et al., 1996). Hayashi et al. (1996) demonstrated Ca-Sp to be a potent antiviral agent against HIV-1 in MT-4 cells. The IC_{50} value for cytotoxicity of Ca-Sp against MT-4 cells is 2900 μg/ml. Viral replication is reduced at ED_{50} values of 11.4 and 2.3 μg/ml when Ca-Sp is added to cell medium immediately and 3 h prior to infection, respectively. Ca-SP was subsequently found to inhibit replication of several

Table 4
Antivirals derived from marine sponges.

Compound	Organism	Activity	Reference
Acyclovir	Synthetic derivative of arabinosyl nucleosides from *Tethya cripta*	HSV	Elion et al. (1977)
Ara-A/Ara-C (vidarabine/cytarabine)	Synthetic derivative of arabinosyl nucleosides from *Tethya cripta*	HSV	Privat de Garilhe and de Rudder (1964)
Avarol/Avarone	*Disidea avara*	HIV-1 synthesis of the natural UAG suppressor glutamine transfer tRNA; HIV-1 crossing the blood-brain barrier	Muller et al. (1985, 1987)
Azidothymidine (zidovudine)	Synthetic derivative of arabinosyl nucleosides from *Tethya cripta*	HSV, HIV	Horwitz et al. (1964)
Calyceramide A-C	*Discodermia calyx*	Influenza Neuraminidase	Nakao et al. (2001)
Clathsterol	*Clathria* sp.	HIV-1 RT	Rudi et al. (2001)
Crambescidin 826	*Monanchora* sp.	HIV-1 envelope-mediated fusion	Chang et al. (2003)
Dehydrofurodendin	Madagascan *Lendenfeldia* sponge	HIV-1 reverse transcriptase-associated RNA- and DNA-directed DNA polymerase	Chill et al. (2004)
Hamigeran B	*Hamigera tarangaensis*	Herpes Virus; Polio Virus	Wellington et al. (2000)
Ilimaquinone	*Hippiospongia metachromia*	RNase H function of the reverse transcriptase	Loya and Hizi (1993)
Microspinosamide	*Sidonops microspinosa*	HIV-1 CPE	Rashid et al. (2001)
Neamphamide A	*Neamphius huxleyi*	HIV-1 CPE	Oku et al. (2004)
Petrosin	*Petrosia similes*	HIV-1 replication, giant cell formation and recombinant reverse transcriptase	Goud et al. (2003)
Polyacetylenetriol	*Petrosia* sp.	HIV-1 RNA- and DNA-dependent DNA polymerase activities of retro viral RT	Loya et al. (2002)
Weinbersterol A, B	*Petrosia weinberg*	Feline leukemia virus; Mouse influenza virus; Mouse corona virus	Sun et al. (1991)

other viruses, including HCMV (in HEL cells), HSV-1 (in HeLa cells), measles (in Vero cells), mumps (in Vero cells), influenza A (in MDCK cells), polio (in Vero cells), and coxsackie virus (in Vero cells), by selectively blocking the penetration of virus into host cells. Ca-Sp also has a low anticoagulant activity (Hayashi et al., 1996).

1.2.2.6. Cyanovirin-N. Cyanovirin-N (CV-N) was initially isolated from an aqueous cellular extract of the cyanobacterium *Nostoc ellipsosporum* (Boyd et al., 1996, 1997) using bioassay-guided fractionation (Parish et al., 1990). Cyanovirin-N is an 11-kDa, 101 amino acid antiviral peptide (NSC 682999; C467H737N133O164S4) (Boyd et al., 1996, 1997; Gustafson et al., 1997) and is unlike any other protein thus far characterized. Low nanomolar concentrations of CV-N prevent the in vitro replication and cytopathic effects of primate retroviruses, including SIV and diverse laboratory strains and clinical isolates of HIV-1 and HIV-2 (Bewley et al., 1998; Gustafson et al., 1997). CV-N mediates these antiviral effects through apparently conserved interactions with the viral envelope glycoprotein (Boyd et al., 1996, 1997). CN-V effectively prevents cell-to-cell fusion and transmission of HIV from infected to uninfected cells. Pretreatment of HIV virions irreversibly neutralizes virus infectivity with no toxicity to host cells. The U.S. National Cancer Institute (NCI) selected this novel anti-HIV agent for preclinical development as a potential prophylactic viricide (Boyd et al., 1996, 1997). It was first proposed that cyanovirin inactivates HIV by interacting with the virus' surface envelope glycoprotein gp120. Mariner et al. (1998) found that CV-N does not block the binding of sCD4-receptor to HIV-1 lysates, nor the attachment of intact HIV-1 virions to several target T-cell lines. They concluded that the virucidal effects of CV-N result from interference with a step(s) in the fusion process subsequent to the initial binding of the virus to target cells.

1.2.2.7. Diterpenes. More recently, Pereira et al. (2004) reported an extensive study on the mechanism of action of two diterpenes, Da-1 and AcDa-1, isolated from the marine alga *Dictyota menstrualis*, which inhibit HIV-1 virus replication in the PM-1 cell line. The EC_{50} values for Da-1 and AcDa-1 are 12.7 μg/ml (40 μM) and 24.1 μg/ml (70 μM), respectively. At 100 μM, virus production is inhibited by 97% and 70% for Da-1 and AcDa-1, respectively. Although both diterpenes did not affect viral attachment or internalization of the virus into PM-1 cells, they inhibit the RNA-dependent DNA polymerase activity of the viral reverse transcriptase enzyme. The IC_{50} values for Da-1 and AcDa-1 inhibition of HIV RT are 10 and 35 μM, respectively. These compounds do not affect cell viability and proliferation (Pereira et al., 2004).

1.2.2.8. Naviculan. A sulfated polysaccharide, naviculan, was isolated from *Navicula directa*, a diatom collected from deep-sea water in Toyama Bay, Japan (Lee et al., 2006). This compound was shown to inhibit HSV-1 and HSV-2 (IC_{50} = 7–14 μg/ml) by interfering with the early stages of viral replication, most likely affecting viral adhesion and penetration into host cells.

1.2.2.9. Griffithsin. A potent HIV-inactivating protein, griffithsin, was isolated from the red alga *Griffithsia* sp. Griffithsin is a new type of lectin that displays potent antiviral activity against laboratory strains and primary isolates of HIV-1 (IC_{50} = 0.043–0.63 nM) (Mori et al., 2005). This activity requires binding to viral glycoproteins (e.g., gp120, gp41 and gp160) in a monosaccharide-dependent manner.

1.2.3. Sponge-derived antivirals

Sponges are also a rich source of compounds with antiviral properties (Table 4). The high number of HIV-inhibiting compounds isolated from sponges does not reflect an increased potential to fight AIDS in comparison to other viral diseases, but rather shows the interests of many researchers. The strong focus on screening sponges for anti-HIV activity has led to discovery of numerous compounds, however the mechanisms of inhibition are still poorly characterized. Papuamides C and D (Ford et al., 1999), haplosamates A and B (Qureshi and Faulkner, 1999), and avarol (Muller et al., 1985, 1987; Muller and Schroder, 1991), which has also been patented as an antipsoriasis drug, are examples of HIV-inhibiting compounds from different sponges.

1.2.3.1. Avarol and derivatives. Avarol is one of the few compounds for which the mechanism by which it inhibits the progression of HIV infection is more or less known (Muller et al., 1985, 1987). In vitro and animal data indicate that avarol combines useful properties of an increased humoral immune response, as IgG and IgM production is significantly increased, and interference with the post-transcriptional processes of viral infection.

Table 5
Antivirals derived from marine fungi.

Compound	Organism	Activity	Reference
Equisetin	*Fusarium heterosporum*	HIV-1 integrase	Singh et al. (1998)
Halovir A–E	*Scytidium* sp.	HSV-1 and HSV-2 membrane destabilization	Rowley et al. (2003)
Phomasetin	*Phoma* sp.	HIV-1 integrase	Singh et al. (1998)
Sansalvamide A	*Fusarium* sp.	MCV topoisomerase-catalyzed DNA relaxation, DNA-binding and covalent complex formation	Hwang et al. (1999)
Stachyflin	*Stachybotrys* sp. RF-7260	Influenza A virus (H1N1) fusion	Minagawa et al. (2002a,b)

Avarol inhibits HIV by almost completely blocking the synthesis of the natural UAG suppressor glutamine transfer tRNA (Muller and Schroder, 1991). Synthesis of this tRNA is upregulated after viral infection, and it is important for the synthesis of a viral protease, which is necessary for viral proliferation. Low concentrations of only 0.9 and 0.3 μM of avarol result in 80% and 50% inhibition of viral release from infected cells, respectively. Uninfected cells are highly resistant to avarol (Muller et al., 1985, 1987; Muller and Schroder, 1991). Sponge metabolites such as avarol, avarone, the avarol derivatives, 6′-hydroxy avarol and 3′-hydroxy avarone, and several phloroglucinol derivatives (Muller et al., 1985, 1987; Muller and Schroder, 1991) have been reported to interact with reverse transcriptase, along with illimaquinone (Loya and Hizi, 1993) which targets the RNase H function of the reverse transcriptase.

1.2.3.2. Arabinosyl nucleoside derivatives. The most important contribution of the marine environment to this area was the isolation and characterization of arabinosyl nucleosides from the sponge *Tethya cripta*, which were used as models for the synthesis of Ara-A (vidarabine), which has been used therapeutically against herpetic encephalitis since the late 1970s (Privat de Garilhe and de Rudder, 1964). Other examples of products of semi-synthetic modifications of the arabinosyl nucleosides are Ara-C (cytarabine) (Privat de Garilhe and de Rudder, 1964), acyclovir (Elion et al., 1977) and azidothymidine (zidovudine) (Horwitz et al., 1964), which are, nowadays, in clinical use (Table 1).

1.2.3.3. Calyceramides A–C. As part of a search for novel influenza virus neuraminidase inhibitors, three active sulfated calyceramides A–C were isolated from the marine sponge *Discodermia calyx* (Nakao et al., 2001). Interestingly, calyceramides A–C inhibits neuraminidase from bacterium *Clostridium perfringens* with IC_{50} values of 0.4, 0.2 and 0.8 μg/ml respectively. These values were all slightly more potent than 4-acetyl neuraminic acid (IC_{50} = 1.5 mg/ml). It remains to be determined if these compounds will also inhibit influenza virus neuraminidase with similar potency (Nakao et al., 2001).

1.2.3.4. Anti-HIV compounds. Over the years, much attention has turned toward HIV research. Clathsterol, a novel and active sulfated sterol from the Red Sea sponge *Clathria* sp., was shown to be active against HIV-1 RT at 10 μM (Rudi et al., 2001). An HIV-inhibitory cyclic depsipeptide, microspinosamide, was isolated from the marine sponge *Sidonops microspinosa* (Rashid et al., 2001). Microspinosamide inhibits the cytopathic effect of HIV-1 infection in cell-based in vitro assays with an EC_{50} value of 0.2 μg/ml. However, this compound is only cytoprotective over a modest concentration range due to cytotoxic effects towards host cells (IC_{50} value of approximately 3 μg/ml) (Rashid et al., 2001). An extensive study was performed on the mechanism of action of polyacetylenetriol, isolated from the marine sponge *Petrosia* sp. (Loya et al., 2002). Polyacetylenetriol shows selective inhibition of the RNA- and DNA-dependent DNA polymerase activities of retroviral RT (IC_{50} = 0.95 μM), as compared to cellular DNA polymerases (IC_{50} = 2.6 μM). Furthermore, a reversible non-competitive mechanism involving a putative hydrophobic interaction was shown to play a critical role in the inhibition of the HIV-1 RT enzyme. Although polyacetylenetriol lacked sufficient specificity and thus could probably not be used as an anti-HIV agent, it was concluded that structural modifications of the side chains of the lead polyacetylenic molecule might produce new potent and selective anti-AIDS drugs (Loya et al., 2002). A new polycyclic guanidine alkaloid, crambescidin 826, was reported from the marine sponge *Monanchora* sp. (Chang et al., 2003). Crambescidin 826 inhibits HIV-1 envelope-mediated fusion in vitro (IC_{50} = 1–3 μM), thus suggesting that this class of compound might ultimately aid in the rational design of small molecule HIV-1 fusion inhibitors (Chang et al., 2003). A new C22 furanoterpene was isolated from a Madagascan *Lendenfeldia* sponge and designated dehydrofurodendin, which is active against HIV-1 RT-associated RNA- and DNA-directed DNA polymerase (IC_{50} = 3.2–5.6 μM) (Chill et al., 2004).

A new HIV-inhibitory depsiundecapeptide, neamphamide A, was isolated from the Papua New Guinea marine sponge *Neamphius huxleyi* (Oku et al., 2004). Neamphamide A potently inhibits the cytopathic effect of HIV-1 infection in cell-based in vitro assays (EC_{50} = 28 nM). HIV inhibition was reported for two bisquinolizidine alkaloids, petrosins, which were isolated from the Indian marine sponge *Petrosia similes* (Goud et al., 2003). The extensive investigation determined that both petrosins inhibit HIV-1 replication (IC_{50} = 41.3–86.8 μM), formation of giant cells (IC_{50} = 21.2–36.1 μM) and recombinant reverse transcriptase in vitro (IC_{50} = 10.6–14.8 μM) (Goud et al., 2003).

1.2.3.5. Hamigeran B and Weinbersterols. Other compounds such as Hamigeran B from *Hamigera tarangaensis*, shows 100% in vitro inhibition against both the herpes and polio viruses (Wellington et al., 2000). Weinbersterols A and B from *Petrosia weinbergi* exhibits in vitro activity against feline leukemia virus, mouse influenza virus, and mouse corona virus (Sun et al., 1991).

1.2.4. Fungi-derived antivirals

The search for antiviral compounds from marine fungi has yielded some promising results (Table 5). Compounds like equisetin and phomasetin are isolated from marine fungi *Fusarium heterosporum* and a *Phoma* sp. respectively, and have shown significant HIV-1 integrase inhibition in bioassay-based experiments (IC_{50} = 7–20 μM) (Singh et al., 1998). Sansalvamide A, a cyclic depsipeptide isolated from the marine fungus *Fusarium* sp., was found to inhibit the topoisomerase of the pathogenic poxvirus *Molluscum contagiosum* (MCV) by preventing topoisomerase catalyzed DNA relaxation, DNA-binding and covalent complex formation (IC_{50} = 124 μM) (Hwang et al., 1999). The isolation and identification of this metabolite is particularly significant, because MCV can cause severe lesions in AIDS patients (Bhadury et al., 2006). A series of novel linear peptides, Halovirs A–E, isolated from the marine fungus *Scytidium* sp., have shown potent antiviral activity against HSV-1 and HSV-2 (Rowley et al., 2003). The ED_{50} values (1-h duration) for Halovirs A, B, C, D, E are 1.1, 3.5, 2.2, 2.0 and 3.1 μM, respectively. In a standard plaque reduction assay, Halovir A can equally inhibit the replication of HSV-1 and HSV-2 with an ED_{50} value of 280 nm. The mode of action is still not clear, however it

Table 6
Antivirals derived from marine macroorganisms.

Compound	Organism	Activity	Reference
Cyclodidemniserinol Trisulfate	Ascidian, *Didemnum guttatum*	HIV-1 integrase	Mitchell et al. (2000)
Didemnaketal A, B	Ascidian, *Didemnum* sp.	HIV-1 protease	Potts et al. (1991)
Didemnins	Tunicate, *Tridemnum solidum*	HSV-1; Rift Valley fever; Venezuelan equine encephalomyelitis; Yellow fever	Rinehart et al. (1988)
Eudistomin	Tunicate, *Eudistoma olivaceum*	HSV-1 and HSV-2	Hudson et al. (1988)
Lamellarin	Prosobranch mollusk, *Lamellaria;* didemnid ascidians	HIV-1 integrase	Reddy et al. (1999)
Polycitone A	Ascidian, *Polycitor* sp.	RNA- and DNA-directed DNA polymerases	Loya et al. (1999)
Polyphemusin I, II	Horseshoe crab, *Limulus polyphemus*	HIV fusion	Morimoto et al. (1991)
Tachyplesin I-III	Horseshoe crab, *Tachypleus tridentatus*	HIV fusion	Miyata et al. (1989)
Thalassiolin A-C	Sea grass, *Thalassia testudinum*	HIV integrase-catalyzed strand transfer and replication	Rowley et al. (2002)

is presumed that halovirs render HSV non-infectious by possible membrane destabilization (Rowley et al., 2003).

Stachyflin, a novel terpenoid isolated from the fungus *Stachybotrys* sp. RF-7260, shows in vitro antiviral activity against influenza A virus (H1N1) with an IC_{50} value of 0.003 μM, which is significantly better than other anti-H1N1 drugs such as amantadine and zanamivir (Minagawa et al., 2002a, 2002b). Stachyflin, with a pentacyclic moiety, includes a novel *cis*-fused decalin and its antiviral activity is mediated through the inhibition of fusion between the viral envelope and the host cell membrane. Such activity is thought to be unique among antiviral compounds (Minagawa et al., 2002a,b).

1.2.5. Antivirals derived from miscellaneous marine organisms.

In addition to marine microorganisms, several marine macroorganisms have been examined for the presence of compounds with anti-HIV activities (Table 6).

1.2.5.1. Tachypesins and polyphemusins. The peptides Tachyplesins I–III and polyphemusins I and II, which are highly abundant in hemocyte debris of the horseshoe crabs *Tachypleus tridentatus* and *Limulus polyphemus*, respectively, were found to be HIV-cell fusion inhibitors (Miyata et al., 1989; Morimoto et al., 1991). More than 100 synthetic peptide analogues of the above mentioned metabolites have been synthesized and tested. The synthetic peptide T22 analog of Polyphemusin II (*Limulus polyphemus*) shows strong anti-HIV activity and relatively low in vitro cytotoxicity (EC_{50} = 2.6 μM; comparable to 5.2 μM for AZT) (Morimoto et al., 1991).

1.2.5.2. Thalassiolins A–C. Thalassiolins A–C are sulfated-flavone glycosides, isolated from the Caribbean sea grass *Thalassia testudinum* (Rowley et al., 2002). Thalassiolin A, the most active compound of this series, inhibits HIV replication in cell culture (IC_{50} = 30 μM) by targeting integrase-catalyzed strand transfer (IC_{50} = 0.4 μM). Interestingly, the presence of sulfated glucose functionality increased its potency against the HIV integrase, while molecular modeling studies indicated that the probable binding site of the molecule was the catalytic core domain of the HIV-1 integrase (Rowley et al., 2002).

1.2.5.3. Inhibitors of HIV-1 integrase. Didemnaketals A and B were isolated from the ascidian *Didemnum* sp., and found to be inhibitors of HIV-1 protease (IC_{50} values of 2 and 10 μM, respectively) (Potts et al., 1991). Bioassay-guided fractionation of extracts of the Palauan ascidian *Didemnum guttatum* led to the isolation of cyclodidemniserinol trisulfate as an inhibitor of HIV-1 integrase (IC_{50} = 60 μg/ml) (Mitchell et al., 2000). This compound also inhibits MCV topoisomerase (IC_{50} = 72 μg/ml), showing no selectivity for integrase inhibition. Lamellarin, a 20-sulfate isolated from an Arabian Sea ascidian (Reddy et al., 1999). Lamellarins were first isolated from prosobranch mollusks of the genus *Lamellaria* and subsequently obtained from didemnid ascidians (Andersen et al., 1985). Lamellarin is another inhibitor of HIV-1 integrase. This compound inhibits the integrase terminal cleavage activity (IC_{50} = 16 μM) and strand transfer activity (IC_{50} = 22 μM) (Reddy et al., 1999). Polycitone A (Loya et al., 1999), an aromatic alkaloid isolated from the ascidian *Polycitor* sp. exhibits potent inhibitory impact on both RNA- (IC_{50} = 245 ± 15 nM) and DNA-directed DNA polymerases (IC_{50} = 470 ± 22 nM). Polycitone A interferes with the DNA primer extension, as well as the formation of the RT-DNA complex (Loya et al., 1999).

1.2.5.4. Anti-HSV compounds. The β-carboline alkaloid eudistomin, was isolated from a tunicate *Eudistoma olivaceum*, which, along with other related β-carbolines, exhibits anti-HSV-1 and 2 activity (Hudson et al., 1988). Other marine antiviral compounds, called didemnins, are cyclic depsipeptides isolated from *Tridemnum* species of tunicates that show in vitro and in vivo antiviral properties against HSV-1, along with the viruses causing Rift Valley fever, Venezuelan equine encephalomyelitis and yellow fever (Rinehart et al., 1988).

2. Significance

The use of natural products in the manufacturing of drugs is an ancient and well-established practice that has yielded such familiar products as morphine, digitalis, penicillin, and aspirin (Roussis, 2005). The marine environment represents approximately half of the global biodiversity and is estimated to contain between 3 and 500 million different species, offering an almost infinite resource for novel compounds (Tziveleka et al., 2003). It has been reported that oceans contain 34 of the 36 global phyla and possess nearly 300,000 described species of plants and animals, which represent only a small percentage of the total number of species that have yet to be discovered (Bhadury et al., 2006; da Silva et al., 2006). Marine microorganisms are known producers of pharmacological and anti-viral agents and may provide unlimited biological resources for the production of therapeutic drugs for the treatment and control of viral diseases in humans, livestock and marine farming species, as well as ever more novel compounds with potential as pharmaceuticals, nutritional supplements, cosmetics, agrichemicals, and enzymes, all of which have strong potential market value (Table 1) (Bhadury et al., 2006). In addition, ecological pressures, such as competition for space, predation, symbiosis and tide variations, throughout thousand of years, originated the biosynthesis of complex secondary metabolites by these organisms, which in turn, allowed their adaptation to a competitive and hostile environment (da Silva et al., 2006).

The current antiviral drug armamentarium comprises nearly 40 compounds that have been officially approved for clinical use. Most of these drugs date back the last 9 years, and at least half of them are used for the treatment of HIV infection (da Silva et al., 2006). Currently, more than 200 natural products with promising levels

of anti-HIV activity have been isolated from aqueous or organic extracts of marine organisms, following bioassay-guided protocols. Continuous searching for and testing of natural compounds for their antiviral potential will likely lead to the discovery and development of a new generation of antiviral agents that can effectively control viral diseases in humans, as well as in other valuable animal species.

3. Future of marine antiviral drugs

Insufficient knowledge of the conditions and specific parameters for the growth and cultivation of marine micro- or macro-organisms have discouraged pharmaceutical industries from pursuing commercialization of marine-derived bioactive molecules. Difficulties inherent to the marine environment initially allowed research to focus only on near-shore, easily accessible species, collected by routine methods such as scuba diving. However, advances in marine technology and biotechnology, along with the development of specialized tools such as remote platforms and manned submersibles, have and will continue to allow for more efficient and systematic explorations of extreme environments, in addition to the maintenance and cultivation of rare marine species (Tziveleka et al., 2003).

One of the challenges in the future will be the large-scale production of these compounds to meet the demand for clinical trials and drug development. It is believed that some form of combinatorial genetic and metabolic engineering will be the future solution for commercial production of these compounds. Integration between combinatorial biochemistry and computer-based molecular modeling designs, along with post-genomic technologies, could be used for sustainable production of these metabolites. Already, some of the marine metabolites being tested clinically are being produced either through aquaculture (e.g., compounds like Bryostatin, ET-743), chemical synthesis (compounds like Dolastatin, Ziconotide, Halichondrin B derivatives, etc.) or by fermentation processes (Thiocoraline) (Munro et al., 1999). One successful example is the chemical synthesis of Corollosporine, an antibacterial metabolite from the marine fungus *Corollospora maritime* (Ohzeki and Mori, 2001).

Chemical synthesis may be a solution for some compounds, but it could be economically nonviable for others (Bhadury et al., 2006). Large-scale cultivation of marine fungi using bioreactor technology or other means will also be essential for the steady supply of natural products into the marine-based drug market. Fermentation processes have gained considerable importance in the last few years for commercial production of these metabolites. Solid-state fermentation (SSF) has been used widely for the production of biologically active secondary metabolites from fungi. Combinatorial biosynthesis involving introduction of novel biosynthesis genes into microorganisms will result in the synthesis of the novel metabolites due to the effect of new enzymes on the metabolic pathways (Bhadury et al., 2006).

Another important challenge, as with any antiviral drugs, will be the development of resistance by various viruses. However, when considering the numerous undiscovered organisms in the marine environment, along with their unique metabolites, it is plausible that increasing numbers of novel drugs will be discovered that viruses have not yet developed resistance to. It is also important to note that many organisms of different species produce similar classes of compounds, each suited for their unique composition and living environment. The fact that varying derivatives of a common class of compound are being produced by multiple organisms may be a solution. A virus that has developed resistance to a particular drug may not be resistant to other naturally occurring derivatives, which have the potential to possess similar, if not identical, antiviral activities. Should no alternatives be found occurring in nature, the application of biochemical technologies will allow the manipulation of naturally occurring compounds to produce chemical derivatives that are far superior to the original (Bhadury et al., 2006). This will allow the creation of compounds with reduced cytotoxicities and increased specificities.

The discovery of new drugs, as well as the adaptation of current drugs, will play a key role in the war against viral resistance. All in all, taking into consideration all of the discoveries made over the last 50 years, along with the advances in technology, it is clear that the marine environment will play a vital role in the future development and trials of anti-infective drugs.

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