Marine Organism: Lead Compounds and as a Source of New Antiviral Agents

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INTRODUCTION

The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products. Because of the physical and chemical conditions in the marine environment, almost every class of marine organism exhibits a variety of molecules with unique structural features. [1,2]

The marine environment represents approximately half of the global biodiversity and could provide unlimited biological resources for the production of therapeutic drugs. [3-5] Almost all forms of life in the marine environment (e.g. algae, sponges, corals, ascidians) have been investigated for their natural product content. [6]

To date, researchers have isolated approximately 7000 marine natural products, 25 percent of which are from algae, 33 percent from sponges, 18 percent from coelenterates (sea whips, sea fans and soft corals) and 24 percent from representatives of other invertebrate phyla such as ascidians (also called tunicates), molluscs (nudibranchs, sea hares etc) and bryozoans (moss animals). A simplistic analysis of these data reveals that as the search for “Drugs from the Sea” progresses at the rate of a 10 percent increase in new compounds per year, researchers are concentrating their efforts on slow-moving or sessile invertebrate phyla that have soft bodies, and lack of spines or a shell, i.e. animals that require a chemical defence mechanism. [7]

MARINE ORGANISMS AS A POTENTIAL SUPPLY FOR DRUG FINDING

Marine chemicals have novel structures with pronounced biological activity and pharmacology. The study of such chemicals therefore is promising. [8] New trends in drug discovery from natural sources emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities. These entities are the sources of new leads for treatment of many diseases such as cancer, AIDS, inflammatory conditions and a large variety of viral, bacterial and fungal diseases. [9]

Structurally unique secondary metabolites have been isolated and identified from marine organisms and consequently a compound based on new chemical template has been developed and launched in 2004, while numerous other candidates are in clinical trials. [10-12] The first serious work on marine organisms started only 50 years ago. In the following 50 years, marine organisms (algae, invertebrates and microbes) 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. [13]

The First notable discovery of biologically active compounds from marine sources was the serendipitous isolation of C-nucleosides, spongouridine and spongothamidine, from Carribean sponge, Cryptotheca crypta in early 1950’s. These compounds were found to possess antiviral activity & synthetic analog studies eventually led to the development of cytosine

...
arabinoside (Ara-C) and together with Ara-A as an antiviral agent.\cite{14} Currently, these are the only marine related compounds in clinical use.\cite{15}

**ANTIVIRAL LEAD COMPOUNDS FROM MARINE ORGANISM**

Viruses cause many important diseases in humans, with viral-induced emerging and re-emerging infectious diseases representing a major health threat to the public. In addition, viruses can also infect livestock and marine species, causing huge losses of many vertebrate food species. Effective control of viral infection and disease has remained an unachieved goal, due to virus intracellular replicative nature and readily mutating genome, as well as the limited availability of anti-viral drugs and measures.\cite{13} The Herpes Simplex Viruses (HSV) are responsible for a broad range of human infectious diseases. Moreover, HSV infections were recognized as a risk factor for HIV infection. Most of the drugs which have been discovered in this century, at least half of them are used for the treatment of human immunodeficiency virus (HIV) infection. Taking this situation into account, the importance of developing new antiviral agents in order to increase the number of these available drugs becomes clear.\cite{16}

Some of the Antiviral lead compounds from the various marine sources such as Sponges, Algae, Sea weed, Tunicate and Mussel have been discussed here:

**SPONGES**

The search for new antiviral agents from marine sources particularly sponges yielded several promising therapeutic leads.\cite{17}

**Vidarabine or Ara-A**

In 1950, Bergmann et al.\cite{18} isolated from the Caribbean sponge *Tethya crypta* (Fig.1) the nucleosides spongothyminidine and spongouridine, which contained an arabinose sugar rather than the more common ribose sugar found in these nucleosides. Vidarabine or Ara-A is a synthetic analogue of spongouridine, which contained an arabinose sugar rather than sponge

Table 1. Antiviral Substances from Marine Sponges \cite{17}

<table>
<thead>
<tr>
<th>SUBSTANCES</th>
<th>CLASS</th>
<th>SOURCE</th>
<th>ACTION SPECTRUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara-A</td>
<td>Nucleoside</td>
<td>Cryptotethya crypta</td>
<td>HSV-1, HSV-2, VZV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dysidea avara</td>
<td>HIV-1</td>
</tr>
<tr>
<td>Avarol</td>
<td>Sesquiterpene</td>
<td>Aaptos aaptos</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td>hydroquinone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-methyl</td>
<td>Alkaloid</td>
<td>Hamigeran sp.</td>
<td>HSV-1</td>
</tr>
<tr>
<td>aaptamine</td>
<td></td>
<td>Hamigera tarangaensis</td>
<td>herpes and polio viruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sidonops</td>
<td>HIV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microspinosas</td>
<td></td>
</tr>
<tr>
<td>Microspinosamide</td>
<td>Cyclic</td>
<td>Mycale sp.</td>
<td>A59 coronavirus,</td>
</tr>
<tr>
<td></td>
<td>depsipeptide</td>
<td></td>
<td>HSV-1</td>
</tr>
<tr>
<td>Mycalamide A-B</td>
<td>Nucleosides</td>
<td>Theonella sp.</td>
<td>HIV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papuamides A–D</td>
<td>Cyclic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>depsipeptides</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

arabinoside (vidarabine, Ara-A) was first described by Privat de Garilhe and De Rudder in 1964\cite{19}. It was the first nucleoside antiviral to be licensed for the treatment of systemic herpes virus infection and one of the three marine-derived drugs currently approved by the FDA in the United States\cite{20}, however the marketing of the drug has been discontinued because the availability of newer and better antiviral agents on the market. Vidarabine was also reported to be capable of inhibiting acyclovir-resistant HSV and VZV (varicella-zoster virus)\cite{21,22}.

![Figure 1. Tethya crypta](image)

Vidarabine is an inhibitor of viral DNA synthesis\cite{23}. Adenine arabinoside (vidarabine) is converted into adenine arabinoside triphosphate (ara-ATP) in *vivo*\cite{24} by kinases encoded by viruses, which in turn inhibit viral DNA polymerase and hence DNA synthesis of herpes, vaccinia and varicella zoster viruses\cite{20,25}.

**Mycalamide A, B**

Perry et al\cite{26} first reported the isolation and *in vitro* antiviral activity of mycalamide A and mycalamide B from a New Zealand sponge of the genus *Mycale* in 1988 and 1990, respectively. The crude extract containing 2% mycalamide A was found to be active against A59 corona virus. Mycalamide A also inhibited the Herpes simplex type I and Polio type I viruses. The property of protein synthesis inhibition may be attributed to their biological activity as antiviral agents.\cite{27}

Four analogues of mycalamide A have recently been reported to bind the nucleoprotein (NP) of influenza virus and inhibit its multiplication. It has also shown experimentally that these compounds might bind to the N-terminal 13-amino acid region of NP which mediates the nuclear transport of NP and its binding to viral RNA, and hence may inhibit viral replication.\cite{28}

**Avarol**

Avarol, a sesquiterpenoid hydroquinone with a rearranged drimane skeleton, was first isolated from the marine sponge *Disidea avara* (Fig.2) in 1974.\cite{29} The chemical structure of avarol was established by standard analytical methods and chemical degradation and by its stereocontrolled total synthesis.\cite{30,31}

![Figure 2. Disidea avara](image)

The compound showed a dose-dependent inhibitory effect on the replication of the etiologic agent of Acquired Immune Deficiency Syndrome (AIDS) and human T-lymphotropic
reovirus/lymphadenopathy-associated virus in human H9 cells. Studies dating back to 1988 showed that the antiviral effects of avarol were due to an increase in intracellular levels of superoxide radicals such as superoxide dismutases and of glutathione peroxidise. The effects of avarol were further elucidated and it was found that it completely blocks the synthesis of glutamine transfer tRNA, which is crucial for synthesis of a viral protease required for viral proliferation. Other important biological targets inhibited by avarol or its derivatives include reverse transcriptase which plays a key role in early stages of viral infection. The anti-HIV and cytotoxic cyclic depsipeptides, papuamides, were isolated from the sponges Theonella mirabilis and Theonella swinhoei. Two groups from the National Cancer Institute and the University of British Columbia independently reported the isolation of papuamides A and B from T. mirabilis and papuamides A, B, C, and D from T. swinhoei, respectively. Papuamides A and B have been evaluated for their antijHIV activity in cell based assays in CEM-SS T-cell cultures, and found to be highly potent. Activities for both compounds (Papuamides A and B) were found to be virtually identical. Papuamides C and D were found to be less potent with 30% and 55% inhibition at a concentration of 40 and 20 fold higher than papuamides A and B. In a recent study Xie et al. reported the total synthesis of papuamide B. The mechanism of a direct interaction of papuamide A with the virus has been proposed with a membrane targeting mechanism believed to be responsible for the virucidal activity of the compound.

**Microspinosamide**

Isolation of microspinosamide, a cyclic depsipeptide from an Indonesian collection of the sponge Sidonops microspinosa was reported in 2001. Microspinosamide contained 13 amino acid residues including alanine, tryptophan, arginine, threonine, aspartate, valine, two prolines, tert-leucine, β-methylisoleucine, N-methylglutamine, cysteic acid and a new residue, β-hydroxy-p-bromo-phenylalanine. The Anti-HIV activity of crude extract of S. microspinosa was first discovered during the National Cancer Institute’s primary anti-HIV screening.

**Figure 3. Aaptos aapta**

4-Methylaaptamine

Isolation of the alkaloid 4-methylaaptamine from the marine sponge Aaptos sp. (fig. 3) and the preliminary activity of its crude extract to inhibit 76% of HSV-1 replication in Vero cells at a concentration of 2.4 µg/mL was first reported by Coutinho et al. Another study confirmed the anti-HSV-1 activity of 4-methyla-aptamine with an EC50 of 2.4 µM, which is even more potent than acyclovir. 4-Methyldaaptamine was found to inhibit HSV-1-infection in Vero cells suggesting the inhibition of initial events during HSV-1 replication.

**Dragmacidin F**

Cutignano et al. reported the isolation of a new bromoindole alkaloid, dragmacidin F from a marine sponge of the genus Halicortex. The compound demonstrated in vitro antiviral activity against HSV-1 and HIV-1 and hence is most likely responsible for the antiviral property exhibited by Halicortex extracts.

**ALGAE**

**Sphingosine derivative**

The Ulva fasiata (fig.4) has a global distribution and the potential pharmaceutical application of its compounds have been investigated. Organic components have been identified in algae of Ulva genus. Simple bromophenols, especially 2,4,6-tribromo-phenol, lipid components dimethylsulfoniopropionate (DMSP) have been largely described for this genus. The extract obtained by maceration from the algae presented the most significant antiviral activity, possibly because two different mechanisms were involved: virucidal and inhibition of cell entry. Another polysaccharide from the green marine algae Ulva lactuca has been isolated. The substance has been investigated after acid hydrolysis by thin-layer and gas chromatography. The following carbohydrate components have been found: arabinose-xylose-rhamnose-galactose-mannose-glucose in ratio 1:1:9:5:2.5:16 respectively. One unidentified sugar has been demonstrated too. The polysaccharide has been studied for antiviral activity in vitro against a number of human and avian influenza viruses. A considerable inhibition of the viral reproduction was found. The effect was dose-dependent, strain-specific and selective. Two species of marine algae, Constantinea simplex (fig. 5) and Farlowia mollis, were tested for antiviral activity in tissue culture and in experimental infections of mice. Treatment of
mouse embryo fibroblast cell monolayers with either compound before viral inoculation was effective in inhibiting the replication of herpes simplex virus type 1 and type 2, vaccinia virus, and vesicular stomatitis virus, but not encephalomyocarditis virus, Semliki Forest virus, or murine cytomegalovirus. Prophylactic administration of these extracts was effective in reducing final mortality or prolonging the mean day of death of animals inoculated by the intraperitoneal, intracerebral, or intranasal routes with herpes simplex virus type 2.

Neither preparation was effective in mice inoculated intraperitoneally with encephalomyocarditis virus or murine cytomegalovirus or in animals infected intravaginally with herpes simplex virus type 2.[55]

Figure 5. Constantinia simplex

TUNICATE

Significant antiviral activity was observed for a series of indole alkaloids, Eudistomin- C from Tunicate Eudistoma olivaceum (fig. 6) being most effective against Human Simplex Virus.[56] The tetrahydro-β carbolines generally exhibited higher levels of biological activity than their fully aromatic relatives; the oxathiazepino -eudistomins, for example, exhibit the highest level of antiviral activity and were also endowed with antimicrobial activity. Eudistomin K is significantly active against Herpes simplex Type I (HSV-1) and Polio virus.[57]

Figure 6. Eudistoma olivaceum

GREEN MUSSEL

The mussels are not only a cheap source of protein for human consumption but also found to possess some complex bioactive compounds which have tremendous potential in medical science. Brown mussel hydrolysate is available for human use in Russian market with trade names Viramid and Midel as antiviral drugs. The effect of the extract from the mussel Perna viridis (fig. 7) on HIV was tested by a number of assays. The initial screening was done using the HIV-1 cytotoxicity assay. The measurement of cell viability of HIV infected cells in presence and absence of the extract was carried out by XTT-Formazan assay.

Figure 7. Perna viridis

Identification of anti-HIV activity in the Indian green mussel Perna viridis and its use in management of HIV disease, AIDS. Since marine bivalve are a natural source and available in abundance along the Indian coastline, development of anti-HIV drug will be economically viable. The extract from Indian green mussel ( Perna viridis ) has earlier been found to be active against influenza, herpes and hepatitis viral strains.[58]

SEA WEED

Sulphated fucan

Natural compounds offer interesting pharmacological perspectives for antiviral drug development. The sulphated-fucan containing fractions isolated from the brown seaweed Cystoseira indica (Fig. 8). The crude water extract and the main fraction obtained by anion exchange chromatography have been found to have potent antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) without cytotoxicity for Vero cell cultures. Furthermore, they had no direct inactivating effect on virions in a virucidal assay and lacked anticoagulant activity.

Figure 8. Cystoseira indica

The mode of action of these compounds could be mainly ascribed to an inhibitory effect on virus adsorption. Chemical, chromatographic and spectroscopic methods showed that the major polysaccharide had an apparent molecular mass of 35 kDa and contained a backbone of α-(1→3)-linked fucopyranosyl residues substituted at C-2 with fucopyranosyl and xylopyranosyl residues. This sulphated fucan, considered the active principle of the C. indica water extract, also contained variously linked xylose and galactose units and glucuronic acid residues. Sulphate groups,
HIV-1 integrase is an attractive target for anti-retroviral chemotherapy, but to date no clinically useful inhibitors have been developed. The screening of diverse marine natural products for compounds active against integrase \textit{in vitro} and \textit{in vivo} has been found to be highly selective against the lamellarins, which showed selectivity for the integrase enzyme. A new member of the family named lamellarin alpha 20-sulfate, the structure of which was determined from spectroscopic data, displayed the most favorable therapeutic index. Lamellarin alpha 20-sulfate was found to be active against \textit{HIV} replication in vitro by authentic \textit{HIV} replication intermediates isolated from infected cells. Lamellarin alpha 20-sulfate was found to be active against \textit{HIV} replication in vitro by authentic \textit{HIV} replication intermediates isolated from infected cells. Lamellarin alpha 20-sulfate was found to be active against \textit{HIV} replication in vitro by authentic \textit{HIV} replication intermediates isolated from infected cells.

**FUTURE PROSPECTS**

One possible future direction could be to perform sequence based screens in order to identify enzymes that have been shown to be involved in the synthesis of anti-viral compounds. With advancement of technologies a new generation of potent and effective antiviral agents may be obtained from these marine sources. Sequence based screens, metagenomic clonal library screening using Genome Sequence Tags (GST) and other phylogenetic approaches could provide a new future dimension in search for antiviral natural compounds. The successes in metagenomics coupled with heterologous expression and high throughput microbial cultivation techniques could pave the way for commercial production of such compounds in the future, greatly facilitating their analysis and commercialization.

**CONCLUSION**

The literature regarding antiviral compounds shows the significance of marine natural products in the drug discovery and development process. With advancement of technologies a new generation of potent and effective antiviral agents may be obtained from these sources.

**REFERENCES**

D. Dhivy et al. Marine organisms: Lead compounds and as a source of new antiviral agents


References: