




Review

# Novel Antiretroviral Structures from Marine Organisms

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**Abstract:** In spite of significant advancements and success in antiretroviral therapies directed against HIV infection, there is no cure for HIV, which can persist in a human body in its latent form and become reactivated under favorable conditions. Therefore, novel antiretroviral drugs with different modes of actions are still a major focus for researchers. In particular, novel lead structures are being sought from natural sources. So far, a number of compounds from marine organisms have been identified as promising therapeutics for HIV infection. Therefore, in this paper, we provide an overview of marine natural products that were first identified in the period between 2013 and 2018 that could be potentially used, or further optimized, as novel antiretroviral agents. This pipeline includes the systematization of antiretroviral activities for several categories of marine structures including chitosan and its derivatives, sulfated polysaccharides, lectins, bromotyrosine derivatives, peptides, alkaloids, diterpenes, phlorotannins, and xanthenes as well as adjuvants to the HAART therapy such as fish oil. We critically discuss the structures and activities of the most promising new marine anti-HIV compounds.

**Keywords:** antiretroviral agents; anti-HIV; marine metabolites; natural products; drug development

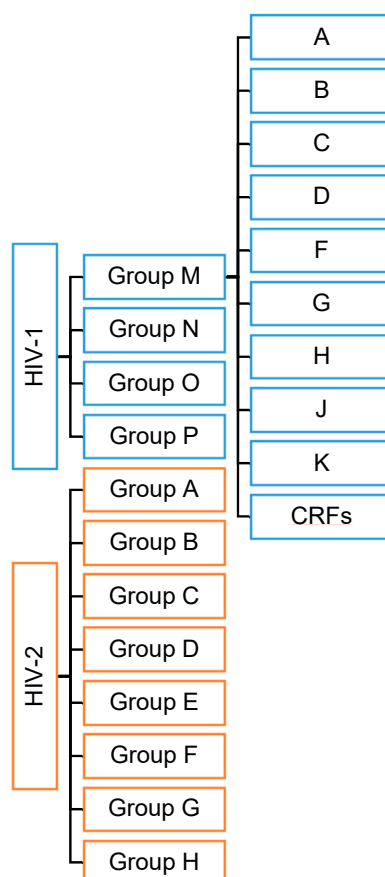
## 1. Introduction

Human immunodeficiency virus (HIV) infections pose a global challenge given that in 2017, according to the World Health Organization data, 36.9 million people were living with HIV and additional 1.8 million people were becoming newly infected globally (Table 1). HIV targets immune cells and impairs the human defense against pneumonia, tuberculosis, and shingles as well as certain types of cancer [1]. The most advanced stage of HIV infection is the Acquired Immunodeficiency Syndrome (AIDS), which can take from two to 15 years to develop, depending on the individual [2].

**Table 1.** Summary of the global human immunodeficiency virus (HIV) epidemic (2017) according to World Health Organization (WHO) data.

	People Living with HIV in 2017	People Newly Infected with HIV in 2017	HIV-Related Deaths in 2017
total	36.9 million (31.3–43.9 million)	1.8 million (1.4–2.4 million)	940,000 (670,000–1.3 million)
adults	35 million (29.6–41.7 million)	1.6 million (1.3–2.1 million)	830 000 (590,000–1.2 million)
women	18.2 million (15.6–21.4 million)		
men	16.8 million (13.9–20.4 million)		
children (<15 years)	1.8 million (1.3–2.4 million)	180,000 (110,000–260,000)	110,000 (63,000–160,000)

HIV has two viral forms: HIV-1 (the most common form that accounts for around 95% of all infections worldwide) and HIV-2 (relatively uncommon and less infectious). HIV-1 consists of groups M, N, O, and P with at least nine genetically distinct subtypes of HIV-1 within group M (A, B, C, D, F, G, H, J, and K). Additionally, different subtypes can combine genetic material to form a hybrid virus known as the ‘circulating recombinant form’ (CRFs) (Figure 1). HIV-2 consists of eight known groups (A to H). Of these, only groups A and B are pandemic. The HIV-2 mechanism is not clearly defined and neither is its difference from HIV-1. However, the transmission rate is much lower in HIV-2 than in HIV-1. HIV-2 is estimated to be more than 55% genetically distinct from HIV-1.

**Figure 1.** HIV types and strains classification.

The HIV-1 genome has reading frames coding for structural and regulatory proteins. The *gag* gene encodes the Pr55Gag precursor of inner structural proteins p24 (capsid protein, CA), p17 (matrix protein, MA), p7 (nucleoprotein, NC), and p6 involved in the virus particle release. The *pol* gene encodes the Pr160GagPol precursor of the viral enzymes p10 (protease, PR), p51 (reverse transcriptase, RT), p15 (RNase H), and p32 (integrase, IN). The *env* gene encodes the PrGp160 precursor of the gp120 (surface glycoprotein, SU) and gp41 (transmembrane protein, TM). Other genes include *tat*, encoding p14 (transactivator protein), *rev*, encoding p19 (RNA splicing regulator), *nef*, encoding p27 (negative regulating factor), *vif*, encoding p23 (viral infectivity protein), *vpr*, encoding p15 (virus protein r), *vpu*, encoding p16 (virus protein unique), *vpx* in HIV2, encoding p15 (virus protein x), and *tev*, encoding p26 (tat/rev protein) [3].

The HIV infections are extremely problematic as the virus targets the CD4+ memory T-cells population, which is essential for organism immunity. HIV can attach itself to the host cell through 1) a relatively nonspecific interaction with negatively charged cell-surface heparan sulfate proteoglycans [4], 2) specific interactions between the Env and  $\alpha 4\beta 7$  integrin [5,6], and/or 3) the interaction with pattern-recognition receptors, such as the dendritic cell-specific intercellular adhesion molecular 3-grabbing non-integrin (DC-SIGN) [7]. The attachment of HIV in any of the abovementioned ways can increase the efficacy of infection because it brings Env, a heavily glycosylated trimer of gp120 and gp41 heterodimers, into close proximity with the viral receptor CD4 and co-receptor [8]. Finally, in order for the viral entry to occur, Env needs to bind itself to the host protein CD4 [9,10].

The binding of the HIV glycoprotein gp120 to the host cell CD4 receptor causes conformational changes of the gp120 glycoprotein, which uncover additional binding sites that interact with distinct proteins on the host cell membrane, known as  $\beta$ -chemokine co-receptors (mainly CCR5 and CXCR4), which facilitate the virus entry into the cell [11].

After the infection, a progressive decline of CD4 + cells consequently leads to the failure of the immune system function and the development of opportunistic infections that usually lead to death [1]. In HIV-infected patients, immunodeficiency develops both as a result of the viral replication and the failure of the patients' homeostatic mechanisms. The continuous viral presence in the patients after the application of therapy is attributed to the CD4 + T-cell homeostasis owing to a pool of latently infected and resting CD4 + T-cells, macrophages, and follicular dendritic cells that remain in the organism. Indeed, the complex interactions of the patient's immune system with the virus, and vice versa after the viral suppression, are thought to be crucial for the control of disease progression [12,13].

Current therapeutic approaches mainly target proteins that are vital for the viral cycle. One of the prominent examples is the linear 36-amino acid synthetic peptide enfuvirtide (T20, Fuzeon), developed by Hoffmann-La Roche, and the first FDA approved fusion inhibitor for the treatment of HIV-1/AIDS acting through the binding to the gp41 subunit of the HIV-1 envelope glycoprotein. This induces a conformational change that brings the viral and cellular membranes into close enough proximity for the fusion and the subsequent viral entry into the host-cell to occur. Nevertheless, several restrictions, such as a low genetic barrier for drug resistance and a short in vivo half-life, limit its clinical use [14–17].

Other various FDA-approved antiretroviral drugs from seven mechanistic classes of inhibitors of the HIV replication are also available for the treatment of infected patients, namely, the nucleoside reverse-transcriptase inhibitors NRTIs, non-nucleoside reverse-transcriptase (RT) inhibitors NNRTIs, protein inhibitors PIs, fusion inhibitors, entry inhibitors—CCR5 co-receptor antagonists, HIV integrase strand transfer inhibitors, and multi-class combinations. None of the mentioned drug classes alone or in combination, the latter being known as the highly active antiretroviral therapy (HAART), can eradicate the HIV infection, and effective vaccines remain unavailable.

The difficulties of HIV-1 vaccine research are, in part, a result of 1) the unavailability of a model for natural immunity related to HIV; 2) the existence of genetically distinct subtypes of HIV and frequent mutations; 3) unidentified correlates of specific immune response to HIV; 4) lack of a reliable, non-human animal model for HIV infection (SIV in monkeys vs. HIV in humans).

The established latent pro-viral reservoirs in the patient's body can stochastically begin to reproduce viral particles, which makes the HIV disease practically incurable. From over 160 compounds identified so far as latency-reversing agents (LRAs), none have led to a promising cure [18].

Several rare and long-term remissions of HIV cases are described in the literature. For example, Berlin, London, and Düsseldorf's patients underwent bone marrow transplantation with stem cells from a donor with a rare genetic mutation of the CCR5. The Mississippi baby received a very early antiretroviral therapy that extended the time of the viral rebound for more than 27 months. There undoubtedly remains a lot to be learned from these cases, and further investigation of stem-cell transplantation in people living with HIV is required [19–21].

The currently used antiretroviral treatment, alone or in combination, extends the quality and life expectancy of HIV-infected individuals but does not cure them. Drug resistance, along with the emergence of drug-resistant virus strains, a high-cost of the lifetime treatment regimen, cell toxicity, and serious side effects of currently used anti-HIV drugs [2] underlie the need for a synthetic development of new drugs or the search for active anti-HIV molecules in natural sources. Mother Nature has been perfecting its chemistry for three billion years, and most of it has been done in water. Intense competition and feeding pressure as well as non-static marine environmental conditions yield compounds with chemical and structural features generally not found in terrestrial natural products.

New efficient molecules directed against HIV should demonstrate better performance in comparison with the currently approved drugs and suppress the HIV virus and/or eliminate the latent HIV reservoirs present in the human body.

Around 60% of drugs currently available on the market are derived or inspired by nature [22]. Turning to nature for drug development holds great potential, especially when it comes to marine organisms. Only few marine-derived drugs have been approved on the market so far but many are in the preclinical or clinical stage of development [23]. Marine organisms make up to two-thirds of Earth's species and produce, as a consequence of living in a highly competitive environment, unique and structurally diverse metabolites. Over the last 40 years, bioprospecting efforts have resulted in over 20,000 compounds of marine origin. The highest share of marine metabolites (up to 70%) are obtained from marine sponges, corals, and microorganisms, while mollusks, ascidians, and algae metabolites form only a minor part [24]. Oceans are, indeed, still a rather underexploited habitat, and biodiversity appears to be higher in the oceans than on land, which might be relevant when focusing on the marine environment as an untapped reservoir of novel antiretroviral candidates. In the discovery of new antiviral marine-derived drugs, researchers usually implement two strategies. They either screen the extracts from different strains (e.g., cyanobacteria, microalgae) or search directly for bioactive molecules in organisms—extract and purify them for evaluation within the drug development pipeline. It is thought that the marine environment might yield more potent anti-HIV candidates characterized by a higher efficiency (lower effective dose) and a better selectivity and which do not induce resistance development. This could, of course, only be speculation based on some of the previous success stories in the discovery of drugs from natural sources such as, e.g., lovastatin and paclitaxel. However, nature generally does create more sophisticated and perfected systems with a complex mode of action.

An excellent example is protein lectin, derived from marine red algae *Griffithsia* sp. named Griffithsin with mid-picomolar activities, which groups it among the most potent HIV entry inhibitors reported so far [25]. It inhibits the HIV infection by binding itself to high mannose glycan structures on the surface of gp120, altering the gp120 structure or its oligomeric state [26]. This interaction relies on the specific trimeric "sugar tower," including N295 and N448 [27]. Griffithsin can also prevent infections caused by other glycoprotein-enveloped viruses such as the Ebola virus, hepatitis C virus, and the severe acute respiratory syndrome coronavirus. It has been shown that the dimerization of Griffithsin is necessary for a high potency inhibition of HIV-1 [28]. However, the discrepancy between the HIV gp120 binding activity and the HIV inhibitory activity points to the presence of mechanism unrelated to a merely simple HIV gp120 binding [26]. The most promising application of Griffithsin



would be its incorporation into vaginal and rectal gels, creams, or suppositories acting as an antiviral microbicide to prevent the transmission of HIV.

Despite the vast number of structurally diverse and unique bioactive molecules from the marine environment, the global marine pharmaceutical pipeline includes only eight approved drugs: Adcetris<sup>®</sup>, Cytosar-U<sup>®</sup>, Halaven<sup>®</sup>, Yondelis<sup>®</sup>, Carragelose<sup>®</sup>, Vira-A<sup>®</sup>, Lovaza<sup>®</sup>, and Prialt<sup>®</sup> [29]. Overall, it has taken 20 to 30 years from their discovery to their entry into the market. A sustainable supply, structural complexity, optimization of formulation, and ADMET properties, and a scale-up issue have prevented further development of several highly promising marine compounds. It is by no means an easy task to identify a marine candidate that may be considered as a potential drug. Initial high costs of developing a natural product into a drug could be balanced out with careful long-term considerations (biodiversity, supply, and technical, market) [30].

This paper provides an overview of natural marine metabolites that were first identified in the period between 2013 and 2018 or the previously identified marine constituents with a recently confirmed anti-HIV activity that could be potentially used or further optimized as novel anti-HIV agents. We also comprehensively summarize anti-HIV activities for several categories of marine structures including chitosan and its derivatives, sulfated polysaccharides, lectins, bromotyrosine derivatives, peptides, alkaloids, diterpenes, phlorotannins, and xanthones as well as fish oil as an auxiliary to HAART therapy.

## 2. Marine Compounds in the Treatment of HIV/AIDS

### 2.1. Chitosan and Its Derivatives

Chitosan (2, Figure 2), a natural marine byproduct, is a poly-cationic linear polysaccharide derived from chitin (1, Figure 2) after partial deacetylation. Chitin is a structural element in the exoskeleton of mainly shrimps and crabs and is mainly composed of the randomly distributed  $\beta$ -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. It has been previously shown that this compound can exhibit a large scale of different bioactivities and can also be used as a carrier for anti-HIV drugs [31]. Chitosan is loaded with saquinavir, an anti-HIV drug with a protease inhibitory activity, which showed better cell targeting efficiency than saquinavir alone [32]. Furthermore, trimethyl chitosan has improved Atripla, an anti-HIV drug consisting of efavirenz, emtricitabine, and tenofovir disoproxil fumarate, anti-HIV 1 activity, and has allowed it to be used in lower concentrations [33]. The antiretroviral activity is manifested in the chitosan-specific cationic nature that allows the formation of electrostatic complexes or multilayer structures with other negatively charged polymers [34]. Karagozlu et al. reported about new QMW-COS and WMQ-COS oligomers with anti-HIV activities. These oligomers are conjugates of chitosan and the Gln-Met-Trp peptide, which were constructed as a continuation of the authors' previous research, in which a high potency of synthetically constructed chitosan oligomers was confirmed in anti-HIV therapy. More specifically, it was shown that these oligomers suppress syncytium formation, which occurs as a fusion of infected cells with neighboring cells, induced by HIV in a dose-dependent manner. However, the authors also noticed that after a certain period, the number of syncytia once again increased, suggesting that the cells should be re-treated with QMW-COS and WMQ-COS oligomers to maintain the primary therapeutically-relevant effect. The inhibition of the HIV-1 induced lytic effect, determined by the cell viability assay, showed that IC<sub>50</sub> for QMW-COS was 48.14  $\mu$ g/mL and was almost identical for WMQ-COS, 48.01  $\mu$ g/mL. These oligomers effectively reduced the HIV load but showed no effects on HIV-1 RT and protease in vitro. Higher dosages were also required for the reduction in the HIV-1III<sub>B</sub> p24 antigen production assessed by the ELISA assay and the HIV-1<sub>RTMDR</sub> p24 antigen production. The highest difference between the compounds was reflected in IC<sub>50</sub> values obtained from studies on the virus-induced luciferase activity in infected cells, where QMW-COS had a higher potency in comparison with WMQ-COS. Lastly, the authors determined the effects of oligomers on the interaction between gp41 and CD4 by using the CD4-gp41 ELISA assay, whereby both oligomers showed high potency. The effect of these oligomers was highest when they

were applied immediately upon the HIV-1 infection of cells, indicating that they should be used as a potential treatment in the early stages of HIV infection, probably at the entry stage [31].

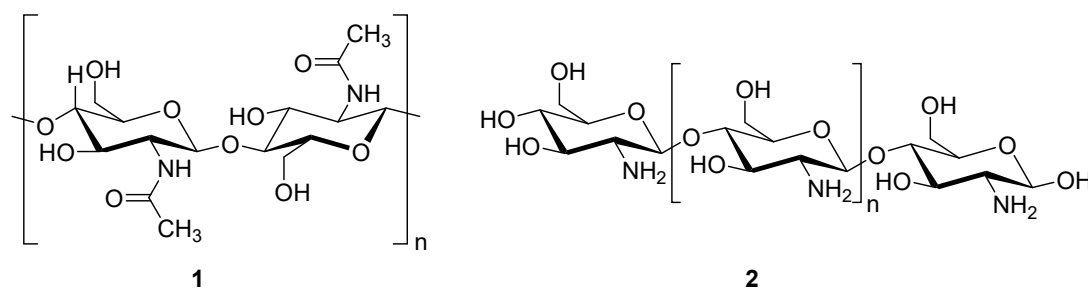


Figure 2. Chemical structures of chitin (1) and chitosan (2).

## 2.2. Sulfated Polysaccharides

Sulfated polysaccharides (SP) are the most studied class of antiviral polysaccharides that are structural components of the alga cell wall where they play both the storage and structural role. They are an important source of galactans, commercially known as agar and carrageenan in red alga (Rhodophyta), fucans (fucoidan, sargassan, ascophyllan, and glucuronyloxyfucan) in brown alga (Phaeophyta), and ulvans-sulfated heteropolysaccharides that contain galactose, xylose, arabinose, mannose, glucuronic acid, or glucose [35–37]. Many studies indicate that, in marine algae, sulfated polysaccharides facilitate water and ion retention in extracellular matrices, which is an important mechanism for coping with desiccation and osmotic stress in a highly salted environment [38–40]. The antiviral activity of this group of compounds is mainly connected to the degree of sulfation, constituent sugars, molecular weight, conformation, and dynamic stereochemistry [41,42]. The effect of counter cation should also be considered as an important factor in observed biological activity.

The antagonizing effect of the negatively charged sulfated polysaccharides on the HIV-1 entry into cells may be due to 1) their binding onto the positively charged V3 domain of gp120, thereby preventing the virus attachment to the cell surface [43–45] or 2) the masking of the docking sites of gp120 for sCD4 on the surface of T lymphocytes, thereby disrupting the CD4-gp120 interaction [46–48] and subsequently inhibiting the expression of the viral antigen and the activity of the viral reverse transcriptase [49,50].

### 2.2.1. Heparan Sulfate

Heparinoid polysaccharides can interact with the positive-charge regions of cell-surface glycoproteins, leading to a shielding effect on these regions, which prevents the binding of viruses to the cell surface [51]. The sulfated polysaccharides content in marine mollusks is high in comparison with the bovine mucosal heparin (73.5%) and the porcine mucosal heparin (72.8%) [52]. The acidic sulfate groups on heparin (3, Figure 3), or heparin-like compounds, can inhibit HIV through electrostatic interactions with basic amino-acid residues of the transcriptional activator Tat protein [53].

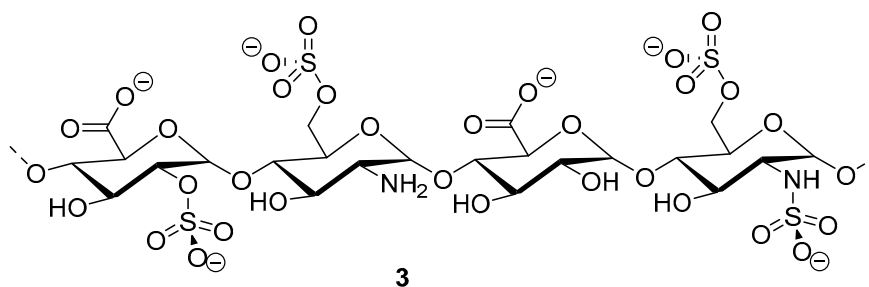


Figure 3. Structure of heparan sulfate (3).

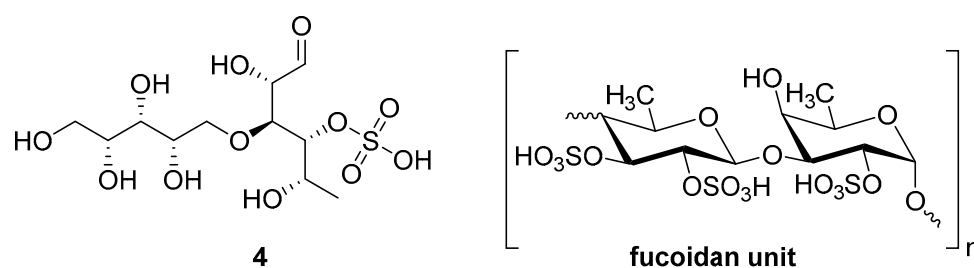
### 2.2.2. Fucose Containing SP

So far, the main anti-infectious activities documented for the fucose-containing SP are those against viruses [54]. More importantly, these polysaccharides are selective inhibitors of various enveloped viruses, including HIV [54–56]. FCSP acts during the early phase of infection by blocking the virus attachment and entry into the host cells, but may also inhibit subsequent replication stages in vitro [57].

### 2.2.3. Fucoidans

Three fucoidans extracted from three brown seaweeds (*Sargassum mclurei*, *Sargassum polycystum*, *Turbinara ornata*) inhibit the early stages of HIV-1 entry into target cells, with  $IC_{50}$  ranging from 0.33 to 0.7  $\mu$ M. Neither the sulfate content nor the position of sulfate groups are related to the anti-HIV activity of fucoidans, suggesting the involvement of other structural parameters such as the molecular weight, the type of glycosidic linkage, or even a unique fucoidan sequence [56]. Although the presence of sulfo-groups seems to be necessary for anti-HIV activity [58], these data do not support random sulfation as the main antiviral factor.

Sulfated fucan polysaccharides, ascophyllan (4, Figure 4), and two fucoidans (S and A) (5 and 6, Table 2), derived from different sources, significantly inhibit ( $IC_{50}$  1.3; 0.3; 0.6  $\mu$ g/mL) the early step of HIV-1 (R9 and JR-Fl) infection. They also inhibit the VSV-G-pseudotype HIV-1 infection in HeLa cells [59].



**Figure 4.** Structure of ascophyllan (4) and fucoidan unit.

**Table 2.** Chemical composition of polysaccharides (Fuc, Fucose; Xyl, Xylose; Glu, Glucose; Man, Mannose; Gal, Galactose) in ascophyllan, S- and A-fucoidan.

	Neutral Sugars						$SO_3^-$
	Fuc	Xyl	Glu	Man	Gal	Uronic Acid	
ascophyllan (4)	15.5	13.4	0.3	3.4	0.6	21.4	9.6
S-fucoidan (5)	24.8	1.9	0.8	1	3.1	9.6	22.6
A-fucoidan (6)	28.4	4.3	2.0	0.8	5.1	5.8	19.4

Chondroitin sulfate with fucosylated branches (FuCS) (7, Figure 5) has also attracted attention as an HIV antiviral compound. Depolymerized fucosylated CS, extracted from the sea cucumber, has shown in vitro activity against a range of viral strains, including the resistant ones [60]. FuCS is effective in blocking the laboratory strain HIV-1IIIB entry and replication by inhibiting the p24 antigen production (4.26 and 0.73  $\mu$ g/mL, respectively) and the infection of the clinic isolate HIV-1KM018 and HIV-1TC-2 (23.75 and 31.86  $\mu$ g/mL, respectively) as well as suppressing the HIV-1 drug-resistant virus. Additionally, FuCS is also effective in T-20-resistant strains ( $EC_{50}$  values ranging from 0.76 to 1.13  $\mu$ g/mL). The depolymerized fragments seem to maintain a similar anti-HIV action at the early stages of infection, apparently through interaction with an HIV envelope glycoprotein gp120. The sulfated fucose branches appear necessary for antiviral activity, which is also affected by molecular weight and carboxylation [61]. While the in vitro results of the fucosylated CS against HIV are promising, it is questionable whether the antiviral activity would be maintained in vivo. Other polyanionic HIV entry

inhibitors, which advanced into clinical trials, failed to prove effective against the heterosexual HIV-1 transmission. This was related to factors not considered in previous development stages, such as the presence of seminal plasma and the concentration and retention of polyanionic inhibitors [62].

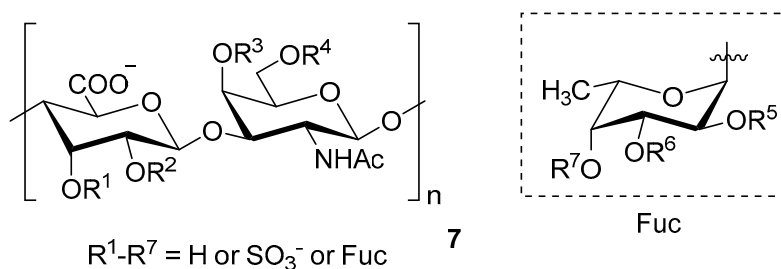


Figure 5. General chemical structure of fucosylated chondroitin sulfate (7).

The complex chemical architecture and the sulfate patterning of marine polysaccharides depends on numerous factors (species, tidal cycles, environmental variations (e.g., salinity), harvesting season, plant age, geographical location etc.) [39,63–69], making isolation, purification, and comprehensive chemical characterization a highly challenging task [70]. The development of many polysaccharides into clinical application is hindered by the still limited view of their sophisticated and diverse nature. Despite having good antiviral effects, the use of carbohydrate drugs is still in its infancy, and intensive structure-activity and in vivo studies are needed in the future.

A relatively new strategy in inducing immunity and developing an HIV vaccine is to use carbohydrates. The major difficulty of such an approach lies in mimicking the specific glycan protective epitope. Gp120 of HIV is a highly glycosylated envelope surface glycoprotein responsible for the receptor and co-receptor binding, which, together with gp41, comprises the heterodimeric envelope trimer spikes of HIV. *N*-linked glycans, mainly mannose and complex-type, cover much of the gp120 surface-accessible face of the HIV envelope spike forming the glycan shield. Inadequate mimicry of the glycan shield, tolerance mechanisms, and/or the inability to induce a domain-exchange are reflecting difficulties in creating the proper specificity of Abs [71]. Most of the vaccines for HIV-1 in preclinical trials are based on a Man $\alpha$ 1-2Man oligomannosyl epitope (various conjugates, engineered yeast strains, and modified glycoproteins) [72–79]. Better specificity could potentially be gained using carbohydrates of marine origin.

### 2.3. Lectins

Lectins are a group of proteins that specifically, but reversibly, bind glycosylated molecules on the cell surface. Precisely, this group of molecules can affect cell-cell interactions, protect cells from pathogens, influence cell adhesion, and affect the intracellular glycoprotein translocation [80]. Recently, lectins have become promising agents for antiretroviral therapy, and different researches have confirmed their anti-HIV properties. Their antiretroviral activity is manifested through an alteration of the interaction between HIV gp120 or gp41 and the corresponding receptors [81], which, in the end, inhibit the HIV cell function, HIV infectivity, and the formation of the syncytium, multi-nucleated cells [82–84].

Several published review papers describe the previously found marine lectins with antiretroviral action [85,86]. For example, Gogineni et al. reported about some new, unusual lectins, such as the  $\beta$ -galactose specific lectin (CVL), CGL, DTL, DTL-A, SVL-1, and SVL-2 [86]. Additionally, Akkouh et al. reported about some new algal lectins, such as *Boodlea Coacta* Lectin, Griffithsin and *Oscillatoria Agardhii* Agglutinin (OAA), and some cyanobacterial lectins, such as Cyanovirin-N, Scytovirin, Microcystis Viridis Lectin, and Microvirin.

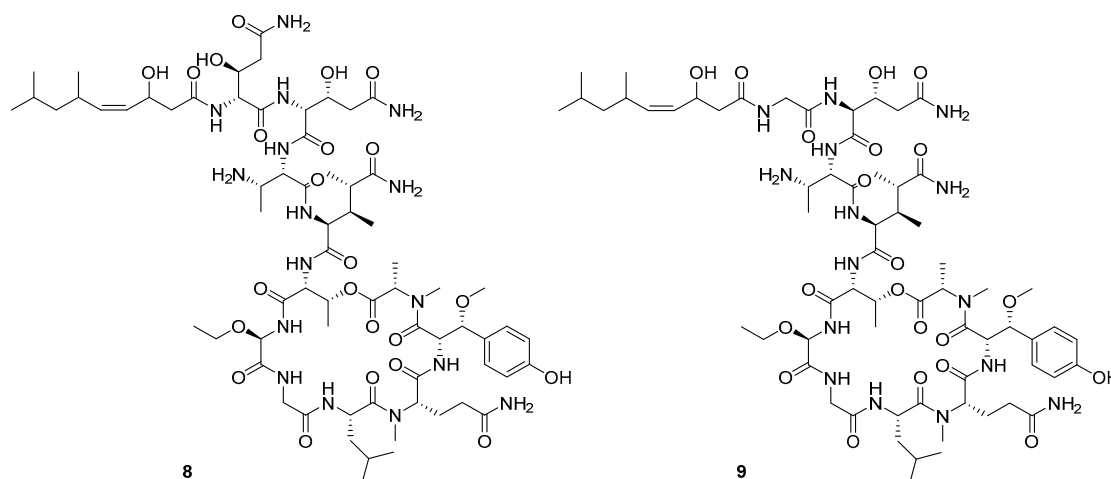
However, in the last few years, there has not been as much research focused on anti-HIV lectins from marine sources. Only Hirayama et al. (2016) reported about the new high-mannose specific lectin and its recombinants that possess anti-HIV activity [87]. In their research performed on the red

alga *Kappaphycus alvarezii*, authors confirmed KAA-1 and KAA-2, two KAA mannose-binding lectin isomers, as potent anti-HIV agents. The anti-HIV role of action of these two compounds includes a strong binding to the virus envelope glycoprotein gp120 and, consequently, the inhibition of HIV entry into the host cells. These KAA recombinants, as well as the native one, inhibited the HIV-1 entry at IC<sub>50</sub>s (neutralization assay in Jurkat cells) of 7.3–12.9 nM. Authors concluded in the end that KAAs, besides their strong inhibitory effect on HIV entry into the cells, have a potential as agents in treatments against other viruses possessing high mannose glycans on their envelope as well.

#### 2.4. Peptides

It has been shown that the majority of marine peptides have strong anti-HIV activity. They are usually isolated from marine organisms through the process of enzymatic hydrolysis [88]. The most common source of such constituents is marine sponges that are known for their unique metabolome [89] and are a source of more than 36% of all marine bioactive compounds [90]. Their bioactive peptides can be found in cyclic or linear forms and contain unusual amino acids that form unique structures rarely found in other species. Antiretroviral activity of such structures works on several different levels: blocking of virus entry, inhibition of the cytopathic viral activity, neutralization of viral particles, or inhibition of viral fusion and entry [89,91].

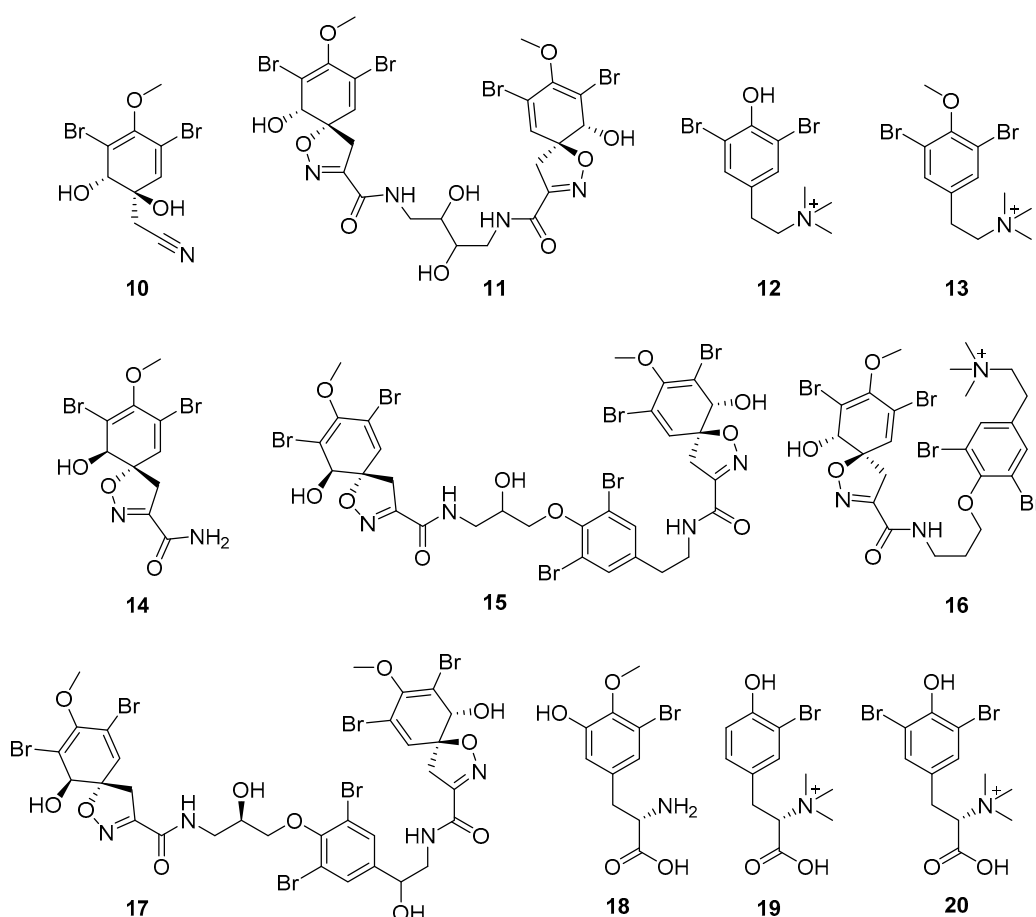
Recently, Shin et al. discovered two new depsipeptides from marine sponges *Stelletta sp.*, stelletapeptin A (8, Figure 6), and stelletapeptin B (9, Figure 6), with the inhibition of the cytopathic effect of HIV-1 infection [92]. Confirming the mentioned theory about the unique metabolome of marine sponges, the authors revealed that these two compounds have previously undescribed nonproteinogenic amino-acid parts on peptides that are rarely found in nature. Namely, stelletapeptin A and stelletapeptin B have an unexpected polyketide subunit, 3-hydroxy-6,8-dimethylnon-4-enoic acid, 3-OHGln, and 3-OHAsn residues. Their high potency is witnessed through low EC<sub>50</sub> values (inhibition of the cytotoxic effect upon HIV infection)—values of 23 nM for stelletapeptin A and 27 nM for stelletapeptin B.



**Figure 6.** Structures of stelletapeptin (8) A and stelletapeptin B (9).

Furthermore, newly discovered anti-HIV constituents derived from marine sponges *Verongula rigida* and *Aiolochoxia crassa* with amino-acid structure were published by Gomez-Archila et al. (2014) [93]. In their paper, they evaluated and confirmed the anti-HIV effect of 11 bromotyrosine derivatives (Table 3), whereby aeroplysinin-1 (10), 19-deoxyfistularin 3 (15), purealidin B (16), fistularin 3 (17) and 3-bromo-5-hydroxy-O-methyltyrosine (18, Figure 7) were the most potent in their anti-HIV activity. Aeroplysinin 1 (15) and purealidin B (16), compounds found in *V. rigida* species inhibited the HIV-1 replication in a dose-dependent manner by more than 50%. Specifically, for aeroplysinin 1, HIV-a replication was inhibited by 74% at a concentration of 20 μM, whereas purealidin was less

potent with inhibitory power of 57% at a concentration of 80  $\mu\text{M}$ . These two compounds had been previously isolated; however, their anti-HIV activity was proven in this research. The same was with 3-bromo-5-hydroxy-*O*-methyltyrosine (**18**) that has a relatively high percentage of inhibition of HIV activity (47%) in a dose-dependent manner. However, the exact mechanism of action remains unclear. In the same study, additional tests with these compounds on the HIV RT inhibition (qPCR of the early and late transcripts), nuclear import (qPCR analysis of 2-LTR transcript), and HIV entry inhibition (viral infectivity assay) were performed. The results showed that aeroplysinin-1 (**10**), 19-deoxyfistularin 3 (**15**), purealidin B (**16**), fistularin 3 (**17**), and 3-bromo-5-hydroxy-*O*-methyltyrosine (**18**) influenced the nuclear import of the HIV virus with around or more than 50% of inhibition: aeroplysinin-1 (**10**) showed 67% of inhibition at 10  $\mu\text{M}$ , 19-deoxyfistularin 3 62% inhibition at 20  $\mu\text{M}$ , purealidin B 66% of inhibition at 20  $\mu\text{M}$ , fistularin 3 47% of inhibition at 10  $\mu\text{M}$ , and 3-bromo-5-hydroxy-*O*-methyltyrosine 73% of inhibition at 80  $\mu\text{M}$ . Viral RT inhibition was not high for all compounds, whereby the highest results were around 50% of inhibition. For example, purealidin B had 58% of inhibition at 20  $\mu\text{M}$  in the qPCR analysis of early transcripts. As for the HIV entry inhibition, all compounds were active in a dose-dependent manner, with the highest results of inhibition obtained for 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium (**13**), from 14% to 30%. Finally, the authors stressed the structural similarity of these compounds with the HIV integrase and protease inhibitors, suggesting that these compounds can have a broader mode of antiviral action.



**Figure 7.** Structures of aeroplysinin-1 (**10**), dihydroxyaerotionin (**11**), 3,4-dibromo-*N,N,N*-trimethyltyraminium (**12**), 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium (**13**), purealidin R (**14**), 19-deoxyfistularin 3 (**15**), purealidin B (**16**), fistularin-3 (**17**), 3-bromo-5-hydroxy-*O*-methyltyrosine (**18**), 3-bromo-*N,N,N*-trimethyltyrosinium (**19**), and 3,5-dibromo-*N,N,N*-trimethyltyrosinium (**20**).



Table 3. Summary of anti-HIV compounds from marine organisms.

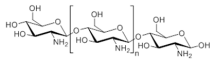
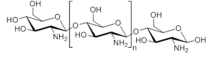
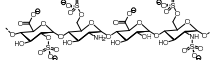
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
Peptide + chitosan oligomer	QMW-COS	not disclosed <sup>a</sup>	marine byproduct	IC <sub>50</sub> —inhibition of HIV-1 induced lytic effects (cell viability assay)	48.14 µg/mL 67.35 µg/mL 81.03 µg/mL 68.13 µg/mL 39.13 µg/mL	<b>anti-HIV-1</b> ; inhibition of the HIV entry at an early stage, blocking the fusion of HIV-1 infected cells, interference of gp41-CD4 binding		[31]
				IC <sub>50</sub> —inhibition of HIV-1 <sub>IIIIB</sub> p24 antigen production (ELISA)				
IC <sub>50</sub> —inhibition of HIV-1 <sub>RTMDR</sub> p24 antigen production (ELISA)								
IC <sub>50</sub> —inhibition of virus-induced luciferase activity in infected TZM-bl cells								
IC <sub>50</sub> —inhibition of the interaction between gp41 and CD4 (CD4-gp41 ELISA)								
Sulfated polysaccharides	heparan sulfate (3)	not disclosed <sup>a</sup>	-	IC <sub>50</sub> —Inhibition of HIV-1 induced lytic effects (cell viability assay)	48.01 µg/mL 98.73 µg/mL 144.02 µg/mL 250 µg/mL 51.48 µg/ml	<b>anti-HIV-1</b> ; inhibition of the HIV entry at an early stage		[31]
				IC <sub>50</sub> —inhibition of HIV-1 <sub>IIIIB</sub> p24 antigen production (ELISA)				
				IC <sub>50</sub> —inhibition of HIV-1 <sub>RTMDR</sub> p24 antigen production (ELISA)				
				IC <sub>50</sub> —inhibition of virus-induced luciferase activity in infected TZM-bl cells				
				IC <sub>50</sub> —inhibition of interaction between gp41 and CD4 (CD4-gp41 ELISA)				
Sulfated polysaccharides	heparan sulfate (3)	not disclosed	-	EC <sub>50</sub> —inhibition of HIV-1 <sub>IIIIB</sub> strain (syncytia assay)	0.24 µg/mL 0.73 µg/mL 4.26 µg/mL 1.14 µg/mL 23.75 µg/mL 31.86 µg/mL 1.09 µg/mL 0.95 µg/mL 1.12 µg/mL 71.76 µg/mL 97.63 µg/ml	<b>anti-HIV-1</b> ; electrostatic interactions with basic amino acid residues of Tat		[53]
				EC <sub>50</sub> —inhibition of HIV-1 <sub>IIIIB</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>IIIIB</sub> /H9 strain (co-cultivation assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>RF</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>KM018</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>TC-2</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>A17</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>RF/V82F/184V</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>L10R/M461/L63P/V82T/184V</sub> strain (p24 assay)				
				EC <sub>50</sub> —inhibition of HIV-1 <sub>CBL-20</sub> strain (syncytia assay)				
EC <sub>50</sub> —inhibition of HIV-1 <sub>ROD</sub> strain (syncytia assay)								

Table 3. Cont.

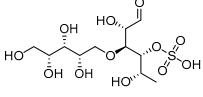
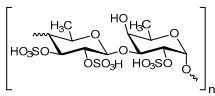
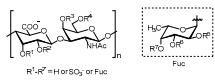
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	fucose containing	Nha Trang bay, Vietnam	<i>Sargassum mcclurei</i> , <i>Sargassum polycystum</i> , and <i>Turbinara Ornate</i> brown seaweeds	U373-CD4-CXCR4 cells 211 infected with pseudotype viral IC <sub>50</sub> —inhibition (F <sub>SP</sub> crude extract)-(p24 ELISA) IC <sub>50</sub> —inhibition (F <sub>TO</sub> crude extract)-(p24 ELISA) IC <sub>50</sub> —inhibition (F <sub>SM</sub> crude extract)-(p24 ELISA)	0.34 µg/mL 0.39 µg/mL 0.96 µg/mL	<b>anti-HIV-1</b> ; inhibition of the early phase of infection, by blocking the virus attachment and entry into the host cells		[56]
	ascophyllan (4)	not disclosed	different sources	IC <sub>50</sub> —inhibition of HIV-1 <sub>R9</sub> -real-time PCR	1.3 µg/mL	<b>anti-HIV-1</b> ; early step of HIV-1 (R9 and JR-FL) infection; inhibition of VSV-G-pseudotyped HIV-1 infection in HeLa cells		[59]
	fucoidan S (5) fucoidan A (6)	not disclosed	different sources	IC <sub>50</sub> —inhibition of HIV-1 <sub>R9</sub> -real-time PCR (fucoidan S) IC <sub>50</sub> —inhibition of HIV-1 <sub>R9</sub> -real-time PCR (fucoidan A)	0.3 µg/mL 0.6 µg/ml	<b>anti-HIV-1</b> ; early step of HIV-1 (R9 and JR-FL) infection; inhibition of VSV-G-pseudotyped HIV-1 infection in HeLa cells		[59]
	chondroitin sulfate (7)	not disclosed		EC <sub>50</sub> —HIV-1 p24 detection-PBMC assay-inhibition of HIV-1 <sub>IIIIB</sub> , HIV-1 <sub>L10R/M46I/L63P/V82T/I84V</sub> , HIV-1 <sub>A17</sub> , HIV-1 <sub>RF</sub> , and HIV-1 <sub>RF/V82F/184V</sub> strains	0.01–0.08 µM	<b>anti-HIV-1</b> ; inhibition of HIV-1 replication; inhibition of the HIV-1 entry		[61]
Lectins	KAA-1	not disclosed	red alga <i>Kappaphycus alvarezii</i>	IC <sub>50</sub> —neutralization assay in Jurkat cells (median tissue culture infectious dose (TCID50) method using Jurkat cells)	9.2 nM	<b>anti-HIV-1</b> ; inhibition of the HIV-1 entry		[87]
	KAA-2	not disclosed	red alga <i>Kappaphycus alvarezii</i>	IC <sub>50</sub> —neutralization assay in Jurkat cells (median tissue culture infectious dose (TCID50) method using Jurkat cells)	7.3 nM	<b>anti-HIV-1</b> ; inhibition of the HIV-1 entry		[87]

Table 3. Cont.

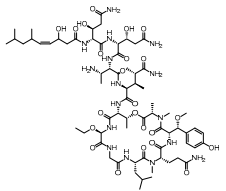
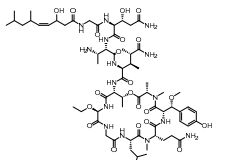
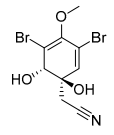
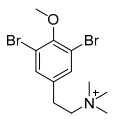
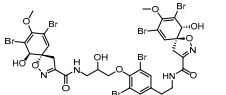
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
Peptides	stellettapeptin A (8)	north-western Australia	marine sponge <i>Stelletta sp.</i>	EC <sub>50</sub> —inhibition of the cytotoxic effect upon HIV-1 infection	23 nm	anti-HIV-1; cytopathic effect of HIV-1 infection		[92]
	stellettapeptin B (9)	north-western Australia	marine sponge <i>Stelletta sp.</i>	EC <sub>50</sub> —inhibition of the cytotoxic effect upon HIV-1 infection	27 nm	anti-HIV-1; cytopathic effect of HIV-1 infection		[92]
Bromotyrosine derivatives	aeropylsinin-1 (10)	Colombia	marine sponge <i>Verongula rigida</i>	% of inhibition of HIV-1 replication by flow cytometry % of reverse transcription inhibition (qPCR analysis of late transcripts) % of nuclear import inhibition (qPCR analysis of 2-LTR transcript) % of HIV entry inhibition (viral infectivity assay)	74% of inhibition at 20 μM 48% of inhibition at 10 μM 67% of inhibition at 10 μM dose dependent manner 2–20%	anti-HIV-1; inhibition of HIV-1 replication, RT, nuclear import and entry		[93]
	3,5-dibromo-N,N,N,O-tetramethyl Tyraminium (13)	Colombia	marine sponge <i>Verongula rigida</i>	% of HIV entry inhibition (viral infectivity assay)	dose depended manner 14–30%	anti-HIV-1; inhibition of HIV-1 entry		[93]
	19-deoxy fistularin 3 (15)	Colombia	marine sponge <i>Verongula rigida</i>	% of reverse transcription inhibition (qPCR analysis of early transcripts) % of reverse transcription inhibition (qPCR analysis of late transcripts) % of nuclear import inhibition (qPCR analysis of 2-LTR transcript)	35% inhibition at 20 μM 11% inhibition at 20 μM 62% inhibition at 20 μM	anti-HIV-1; inhibition of HIV-1 replication, RT, nuclear import		[93]

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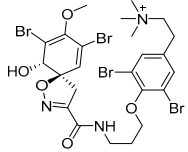
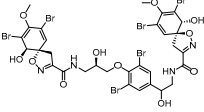
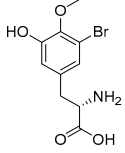
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	purealidin B (16)	Colombia	marine sponge <i>Verongula rigida</i>	% of inhibition of HIV-1 replication by flow cytometry % of reverse transcription inhibition (qPCR analysis of early transcripts) % of reverse transcription inhibition (qPCR analysis of late transcripts) % of nuclear import inhibition (qPCR analysis of 2-LTR transcript) % of HIV entry inhibition (viral infectivity assay)	57% of inhibition at 80 $\mu$ M 58% of inhibition at 20 $\mu$ M 34% of inhibition at 20 $\mu$ M 66% of inhibition at 20 $\mu$ M dose depended manner 2–11%	<b>anti-HIV-1</b> ; inhibition of HIV-1 replication, RT, nuclear import and entry		[93]
	fistularin 3 (17)	Colombia	marine sponge <i>Verongula rigida</i>	% of reverse transcription inhibition (qPCR analysis of late transcripts) % of nuclear import inhibition (qPCR analysis of 2-LTR transcript) % of HIV entry inhibition (viral infectivity assay)	24% of inhibition at 5 $\mu$ M, 47% of inhibition at 10 $\mu$ M, dose depended manner 11–13%	<b>anti-HIV-1</b> ; inhibition of, HIV-1 RT, nuclear import and HIV-1 entry		[93]
	3-bromo-5-hydroxy-O-methyltyrosine (18)	Colombia	marine sponge <i>Aiolochoira crassa</i>	% of inhibition of HIV-1 replication by flow cytometry % of reverse transcription inhibition (qPCR analysis of early transcripts) % of reverse transcription inhibition (qPCR analysis of late transcripts) % of nuclear import inhibition (qPCR analysis of 2-LTR transcript) % of HIV entry inhibition (viral infectivity assay)	47% of inhibition at 80 $\mu$ M, 54% of inhibition at 160 $\mu$ M, 50% of inhibition at 40 $\mu$ M, 73% of inhibition at 80 $\mu$ M, dose depended manner 2–12%	<b>anti-HIV-1</b> ; inhibition of HIV-1 replication, RT, nuclear import and entry		[93]

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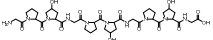
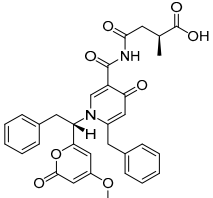
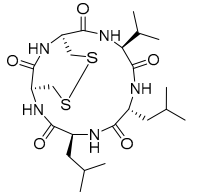
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
Peptides	APCHP (21)	not disclosed	Alaska pollack	EC <sub>50</sub> —against anti-HIV-1 induced cell lysis (MTT assay) EC <sub>50</sub> —HIV-1-induced RT activation in MT-4 cells EC <sub>50</sub> —against p24 production (western blot)	459 µM (0.403 mg/mL) 374 µM (0.327 mg/mL) 405 µM (0.356 mg/mL)	<b>anti-HIV-1</b> ; inhibition of induced syncytia formation by interference of HIV fusion inhibition of cell lysis, RT activity and production of p24 antigen		[94]
	SM-peptide	not disclosed	<i>Spirulina maxima</i>	IC <sub>50</sub> —protective activity on HIV-1-induced cell lysis-MTT assay % of RT Inhibition in HIV-1-infected cells (reverse transcriptase assay kit) % of HIV-1 p24 antigen production (p24 antigen production assay)	0.691 mM (0.475 mg/mL) 90% inhibition at 1.093 mM (0.75 mg/mL) 95% of inhibition at 1.093 mM (0.75 mg/mL)	<b>anti-HIV-1</b> ; inhibition of the HIV-1 RT activity and p24 antigen production	Leu-Asp-Ala-Val-Asn-Arg	[95]
Alkaloids	aspermigrin C (22)	Yongxing Island, South China Sea Yongxing Island, South China Sea	marine fungus <i>Aspergillus niger</i> SCSIO Jcw6F30 isolated from marine alga <i>Sargassum sp.</i>	IC <sub>50</sub> —inhibitory effects on infection by CCR5-tropic HIV-1 SF162 in TZM-bl cells	4.7 µM	<b>anti-HIV-1</b>		[96]
	malformin C (23)	Yongxing Island, South China Sea Yongxing Island, South China Sea	marine fungus <i>Aspergillus niger</i> SCSIO Jcw6F30 isolated from marine alga <i>Sargassum sp.</i>	IC <sub>50</sub> —inhibitory effects on infection by CCR5-tropic HIV-1 SF162 in TZM-bl cells	1.4 µM	<b>anti-HIV-1</b>		[96]

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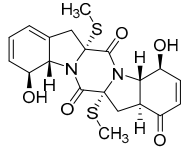
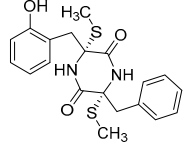
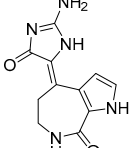
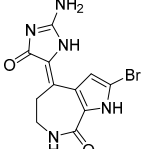
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	eutypellazine E (24)	South Atlantic Ocean	deep-sea sediment fungus <i>Eutypella</i> sp. MCCC 3A00281	IC <sub>50</sub> —anti-HIV bioassay-pNL4.3.Env-.Luc co-transfected 293T cells	3.2 μM	<b>anti-HIV-1</b> ; inhibitory effects against HIV-1 replication		[97]
	eutypellazine J (25)	South Atlantic Ocean	deep-sea sediment fungus <i>Eutypella</i> sp. MCCC 3A00281	IC <sub>50</sub> —anti-HIV bioassay-pNL4.3.Env-.Luc reactivation activity-In vitro latent HIV reactivating assay-flow cytometry-based screening	4.9 μM 80 μM	<b>anti-HIV-1</b> ; inhibitory effects against HIV-1 replication, latency reactivating agent		[97]
	debromo-hymenialdisine (26)	Coral reefs in the Red Sea	<i>S. carteri</i> sponge extract	% of reduction of HIV-1 replication-cell-based assay	30% of inhibition at 13 μM	<b>anti-HIV 1</b> ; decrease the transcription of the HIV-1, abrogate the G2-checkpoint of the cell cycle		[98]
	Hymenialdisine (27)	Coral reefs in the Red Sea	<i>S. carteri</i> sponge extract	% of reduction of HIV-1 replication-cell-based assay	<40% of inhibition at 3.1 μM	<b>anti-HIV 1</b> ; decrease the transcription of the HIV-1, abrogate the G2-checkpoint of the cell cycle		[98]



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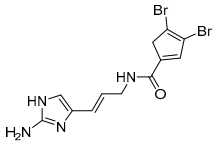
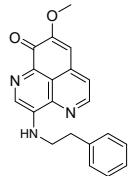
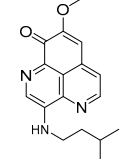
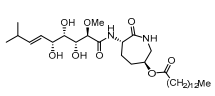
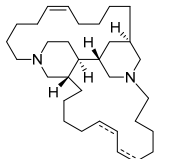
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	Oroidin (28)	Coral reefs in the Red Sea	<i>S. carteri</i> sponge extract	% of inhibition - HIV-1 RT biochemical assay % of reduction of HIV-1 replication-cell-based assay	90% of inhibition at >25 $\mu$ M 50% of inhibition at 50 $\mu$ M	<b>anti-HIV-1;</b> inhibition of HIV-1 RT, reduction of HIV-1 replication		[98]
	3-(phenethylamino)demethyl(oxy)aaptamine (29)	Woody Island (Yongxing, Hainan, China) and Seven Connected Islets in the South China Sea	<i>A. aptos</i> sponge extract	% of inhibition against HIV-1 replication-anti-HIV-1 activity assay-cell-based VSVG/HIV-1 pseudotyping system	88% of inhibition at 10 $\mu$ M	<b>anti-HIV-1;</b> inhibitory effects against HIV-1 replication		[99]
	3-(isopentylamino)demethyl(oxy)aaptamine (30)	Woody Island (Yongxing, Hainan, China) and Seven Connected Islets in the South China Sea	<i>A. aptos</i> sponge extract	% of inhibition against HIV-1 replication-anti-HIV-1 activity assay-cell-based VSVG/HIV-1 pseudotyping system	72.3% of inhibition at 10 $\mu$ M	<b>anti-HIV-1;</b> inhibitory effects against HIV-1 replication		[99]
	bengamide A (31)	not disclosed	screening of previously isolated compounds (originally isolated from the sponge <i>Jaspis cf. coriacea</i> )	EC <sub>50</sub> —multi-cycle viral replication assay % inhibition of p24 <sup>Gag</sup> production-of PBMC assay-p24 <sup>Gag</sup> was quantified by ELISA EC <sub>50</sub> —inhibition of LTR promoter-driven gene expression-LTR-based reporter assays	0.015 $\mu$ M >90% of inhibition at 0.3 $\mu$ M 0.17 $\mu$ M	<b>anti-HIV-1;</b> inhibition of NF- $\kappa$ B-mediated retroviral gene expression		[100]
	haliclonycyclamine A + B (32)	not disclosed	screening of previously isolated compounds	EC <sub>50</sub> —multi-cycle viral replication assay	3.8 $\mu$ M	<b>anti-HIV-1;</b> inhibitory effects against HIV-1 replication		[100]

Table 3. Cont.

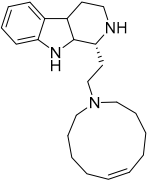
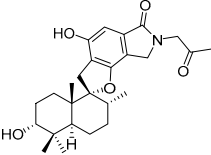
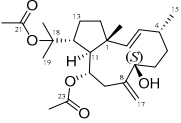
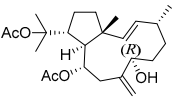
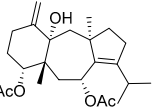
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	keramamine C (33)	not disclosed	screening of previously isolated compounds	EC <sub>50</sub> —multi-cycle viral replication assay	3.4 μM	anti-HIV-1; inhibitory effects against HIV-1 replication		[100]
	stachybotrin D (34)	Xisha Island, China	sponge <i>Xestospongia testudinaria</i>	EC <sub>50</sub> —inhibitory Effects on Wild-Type and NNRTI-Resistant HIV-1 Replication: EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>wt</sub> EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>RT-K103N</sub> EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>RT-L100I,K103N</sub> EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>RT-K103N,V108I</sub> EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>RT-K103N,G190A</sub> EC <sub>50</sub> —inhibition of VSVG/HIV-1 <sub>RT-K103N/P225H</sub>	8.4 μM 7.0 μM 23.8 μM 13.3 μM 14.2 μM 6.2 μM	anti-HIV-1; HIV-1 RT inhibition (inhibitory effects on wild type and five NNRTI-resistant HIV-1 strains)		[101]
Diterpenes	dolabelladienol A (35)	Atol das Rocas, in Northeast Brazil	brown alga <i>Dictyota pfaffii</i>	EC <sub>50</sub> —inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	2.9 μM	anti-HIV-1; -inhibition of the cytopathic effect of HIV-1		[102]
	dolabelladienol B (36)	Atol das Rocas, in Northeast Brazil	brown alga <i>Dictyota pfaffii</i>	EC <sub>50</sub> —inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	4.1 μM	anti-HIV-1; -inhibition of the cytopathic effect of HIV-1		[102]
	dolastane (38)	Praia do Velho, Angra dos Reis, in the south of Rio de Janeiro State, Brazil	brown alga <i>Canistrocarpus cervicornis</i>	EC <sub>50</sub> —inhibition of HIV-1 replication-CXCR4-tropic HIV-1—MTT method	0.35 μM	anti-HIV-1; inhibition of HIV-1 replication, potent effect on HIV-1 infectivity		[103]

Table 3. Cont.

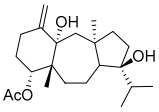
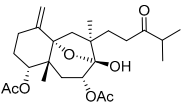
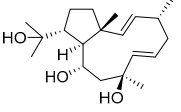
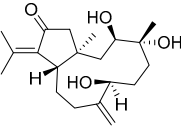
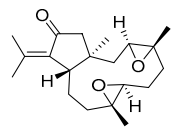
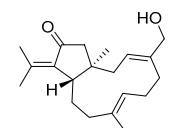
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	dolastane (39)	Praia do Velho, Angra dos Reis, in the south of Rio de Janeiro State, Brazil	brown alga <i>Canistrocarpus cervicornis</i>	EC <sub>50</sub> —inhibition of HIV-1 replication-CXCR4-tropic HIV-1-MTT method	0.794 μM	anti-HIV-1; inhibition of HIV-1 replication, potent effect on HIV-1 infectivity		[103]
	secodolastane diterpene (40)	Praia do Velho, Angra dos Reis, in the south of Rio de Janeiro State, Brazil	brown alga <i>Canistrocarpus cervicornis</i>	EC <sub>50</sub> —inhibition of HIV-1 replication-CXCR4-tropic HIV-1-MTT method	3.67 μM	anti-HIV-1; inhibition of HIV-1 replication		[103]
	8,10,18-trihydroxy-2,6-dolabelladiene (41)	Atol das Rocas reef, Brazil	brown alga <i>Dictyota friabilis</i>	EC <sub>50</sub> —inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	6.16 μM	anti-HIV-1; inhibition of the cytopathic effect of HIV-1		[104,105]
	oxygenated dolabellane (42)	Santa Marta Bay (Colombian Caribbean Sea)	octocoral <i>Eunicea laciniata</i>	EC <sub>50</sub> —inhibition of HIV-1-Inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	3.9 μM	anti-HIV-1; inhibition of the cytopathic effect of HIV-1		[106]
	oxygenated dolabellane (43)	Santa Marta Bay (Colombian Caribbean Sea)	octocoral <i>Eunicea laciniata</i>	EC <sub>50</sub> —inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	0.73 μM	anti-HIV-1; inhibition of the cytopathic effect of HIV-1		[106]
	oxygenated dolabellane (44)	Santa Marta Bay (Colombian Caribbean Sea)	octocoral <i>Eunicea laciniata</i>	EC <sub>50</sub> —inhibition of HIV-1-Inhibition of the cytopathic effect of HIV-1-MT-2 cells—MTT method	0.69 μM	anti-HIV-1; inhibition of the cytopathic effect of HIV-1		[106]

Table 3. Cont.

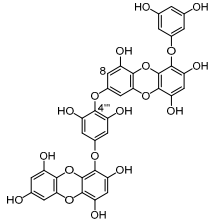
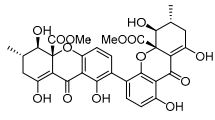
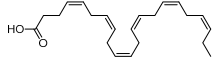
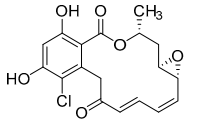
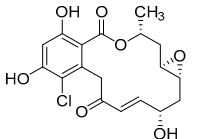
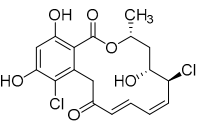
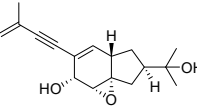
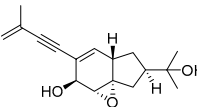
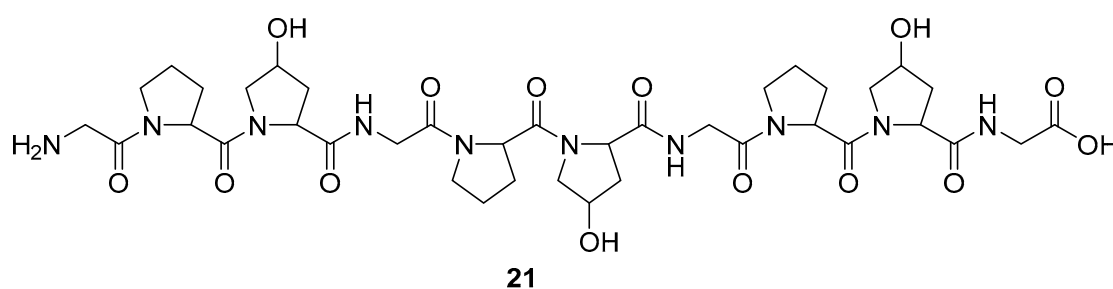
Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference	
	8,4'''-dieckol (45)	not disclosed	brown alga, <i>Ecklonia cava</i>	Inhibition of syncytia formation on C8166 cells (HIV-1 <sub>IIIb</sub> , HIV-1 <sub>RF</sub> and HIV-1 <sub>LAI</sub> )-inverted microscope Inhibition of the cytopathic effect of HIV-1-C8166 cells—MTT method  Effect on p24 antigen production-p24 antigen capture ELISA and immunoblast analysis RT activity assay—commercial fluorescence RT assay kit		Inhibition in dose-dependent manner * Cell viability was more than 90% dose-dependent inhibition Inhibited 91% activity of HIV-1 <sub>IIIb</sub> RT and approximately 80% for rest of the HIV-1 strains tested, HIV-1 <sub>RTMDR1</sub> strain was inhibited at a ratio of 76.1% At the highest concentration, inhibition was more than 80% for all viral strains except for RTMDR1 (76.33%)	<b>anti-HIV-1</b> ; inhibition of the cytopathic effects of HIV-1: inhibition of syncytia formation, lytic effects, inhibition of viral p24 antigen production, HIV-1 entry inhibition and RT inhibition		[107]
	penicillixanthone A (46)	not disclosed	from the jellyfish-derived fungus <i>Aspergillus fumigatus</i>	IC <sub>50</sub> —inhibition of PXA on infection by CCR5-tropic HIV-1 in TZM-bl cells IC <sub>50</sub> —inhibition of PXA on infection by CXCR4-tropic HIV-1 in TZM-bl cells	0.36 μM 0.26 μM	<b>anti-HIV-1</b> ; inhibition of infection against CCR5-tropic HIV-1 SF162 and CXCR4-tropic HIV-1 NL4-3		[108]	
	docosahexanoic acid (48)	not disclosed		In vivo study on male rat models-Male F344 (control) and HIV-1Tg rats		<b>anti-HIV-1</b> ; neuroprotective effect on neuroinflammations induced by ethanol (in the presence of HIV viral proteins)		[109]	

Table 3. Cont.

Group	Compound	Location	Organism	Assay	Dose	Activity	Structure	Reference
	radicol (49)	Tutuila, American Samoa	<i>H. fuscoatra</i>	EC <sub>50</sub> —In Vitro Model of HIV-1 Latency-high-throughput primary cell-based HIV-1 latency assay	9.1 μM	<b>anti-HIV-1</b> ; reactivation of latent viral loads in CD4+ T-cells		[110]
Phlorotannins and xanthenes	pochonin B (50)	Tutuila, American Samoa	<i>H. fuscoatra</i>	EC <sub>50</sub> —In Vitro Model of HIV-1 Latency-high-throughput primary cell-based HIV-1 latency assay	39.6 μM	<b>anti-HIV-1</b> ; reactivation of latent viral loads in CD4+ T-cells		[110]
Auxiliary therapy to HAART therapy—fish oil	pochonin C (51)	Tutuila, American Samoa	<i>H. fuscoatra</i>	EC <sub>50</sub> —In Vitro Model of HIV-1 Latency-high-throughput primary cell-based HIV-1 latency assay	6.3 μM	<b>anti-HIV-1</b> ; reactivation of latent viral loads in CD4+ T-cells		[110]
Others	truncateol O (52)	Yongxing Island, Hainan Province of China	sponge-associated fungus <i>Truncatella angustata</i>	IC <sub>50</sub> —Anti-HIV bioassays-VSV-G pseudotyped HIV-1—Luciferase assay system	39 μM	<b>anti-HIV-1</b> ; inhibition of the HIV replication		[111]
	truncateol P (53)	Yongxing Island, Hainan Province of China	sponge-associated fungus <i>Truncatella angustata</i>	IC <sub>50</sub> —Anti-HIV bioassays-VSV-G pseudotyped HIV-1—Luciferase assay system	16.1 μM	<b>anti-HIV-1</b> ; inhibition of the HIV replication		[111]

<sup>a</sup> Tripeptide conjugates of chitosan (a natural marine byproduct), prepared in the laboratory.

Marine sponges are not the sole source of bioactive proteins. For example, Jang et al. reported about a new small hydroxyproline-rich peptide from Alaska Pollack collagen (APHCP, **21**, Figure 8) that exhibits a unique antiviral activity [94]. This peptide is a Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly peptide, and the authors showed that the most important part of a peptide for anti-HIV activity is the hydroxyl group at hydroxyproline, whereas a peptide with only prolines does not exhibit antiviral activity. Its anti-HIV 1 mode of action is manifested through the inhibition of the induced syncytia formation by the interference of an HIV fusion, inhibition of cell lysis, RT activity, and the production of the p24 antigen. It was shown that APHCP can decrease the HIV-1 induced cell lysis at a potency around  $EC_{50}$  of 459  $\mu\text{M}$  ( $EC_{50}$  against anti-HIV-1 induced cell lysis—MTT assay). Additionally, through the inhibition of the viral RT at  $EC_{50}$  at 374  $\mu\text{M}$ , this peptide's crucial role in the inhibition of the conversion of viral RNA to DNA was also confirmed. With  $EC_{50}$  of 405  $\mu\text{M}$ , this compound effectively suppressed the p24 production in viral cells, as determined by the Western blot analysis.



**Figure 8.** Structure of the Alaska Pollack collagen hydroxyl proline (APHCP) peptide (**21**).

Similarly, one new anti-HIV peptide was isolated from *Spirulina maxima* (SM-peptide) [95]—the Leu-Asp-Ala-Val-Asn-Arg peptide, and the authors showed its HIV-1 infection inhibition in a human T cell line MT4. The peptide inhibited cell lysis, p24 antigen production, and HIV-1 RT. Specifically,  $IC_{50}$  (obtained by a cell viability assay) against an anti-HIV 1 infection was determined as 0.691 mM, the inhibition of the HIV-1-induced RT activation (RT assay kit) in MT4 cells was at a high 90% at a concentration of 1.093 mM, and the p24 production (p24 antigen production assay) was inhibited at 95% at a concentration of 1.093 mM.

### 2.5. Alkaloids

Marine organisms are well-established sources of natural alkaloids. Although the term ‘alkaloid’ seems puzzling and is prone to scientific controversy, alkaloids are generally defined as nitrogen-containing compounds derived from plants and animals. Relatively few alkaloids from marine sources have been found to possess antiretroviral properties and, so far, none have found their clinical use.

Aspernigrin C (**22**, Figure 9) and malformin C (**23**, Figure 9) have been isolated from marine-derived black aspergilli, *Aspergillus niger* SCSIO Jcw6F30, and their inhibitory activity against the chemokine receptor subtype 5 (CCR5) tropic HIV-1 SF162 has been evaluated. They show potent inhibition of infection with  $IC_{50}$  values  $4.7 \pm 0.4 \mu\text{M}$  and  $1.4 \pm 0.06 \mu\text{M}$ , which is comparable to the nucleoside reverse transcriptase inhibitor—abacavir ( $IC_{50} = 0.8 \pm 0.1 \mu\text{M}$ ) and the HIV-1 entry inhibitor ADS-J1 ( $IC_{50} = 0.8 \pm 0.1 \mu\text{M}$ ). In comparison to other aspernigrins, it has been suggested that the 2-methylsuccinic moiety is responsible for the potency of aspernigrin C [96].



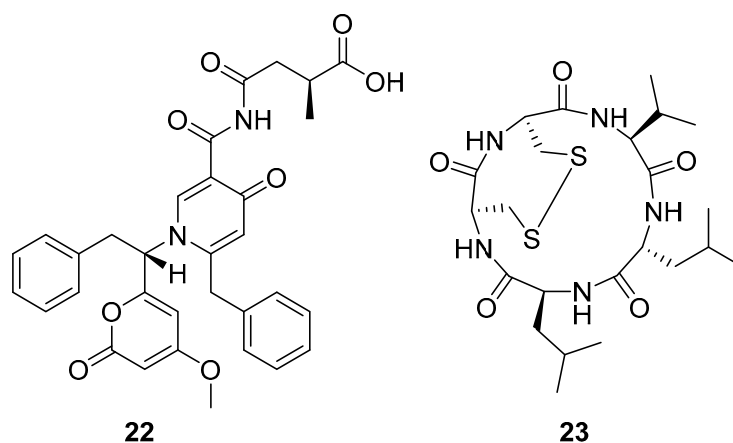


Figure 9. Structures of aspernigrin C (22) and malformin C (23).

Thiodiketopiperazine-type alkaloids, eutypellazines A-M, isolated from the EtOAc extract of the fermentation broth of deep-sea sediment fungus *Eutypella sp.* shows potent inhibitory effects against pNL4.3.Env-.Luc co-transfected 293T HIV model cells. Eutypellazine E (24, Figure 10) exerts activity in a low micromolar range ( $IC_{50} = 3.2 \pm 0.4 \mu M$ ), while eutypellazine J (25, Figure 10) shows a reactivating effect toward latent HIV-1 in J-Lat A2 cells. This could be used as a promising strategy to expunge the HIV-1 infection by activating latent virus cellular reservoirs in combination with HAART [97].

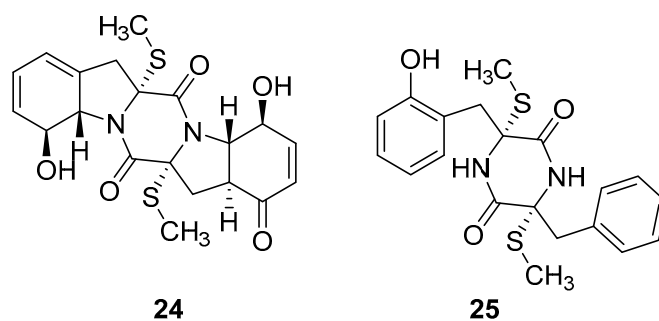


Figure 10. Structures of eutypellazine E (24) and eutypellazine J (25).

The *S. carteri* Red Sea sponge extract yields three previously characterized compounds: debromohymenialdisine (DBH) (26, Figure 11), hymenialdisine (HD) (27, Figure 11), and oroidin (28, Figure 11). DBH and HD exhibited a 30–40% inhibition of HIV-1 at 3.1  $\mu M$  and 13  $\mu M$  but with associated cytotoxicity. Conversely, oroidin displayed a 50% inhibition of viral replication at 50  $\mu M$  without observed cytotoxicity. Also, it showed inhibition of HIV-1 reverse transcriptase up to 90% at 25  $\mu M$  [98].

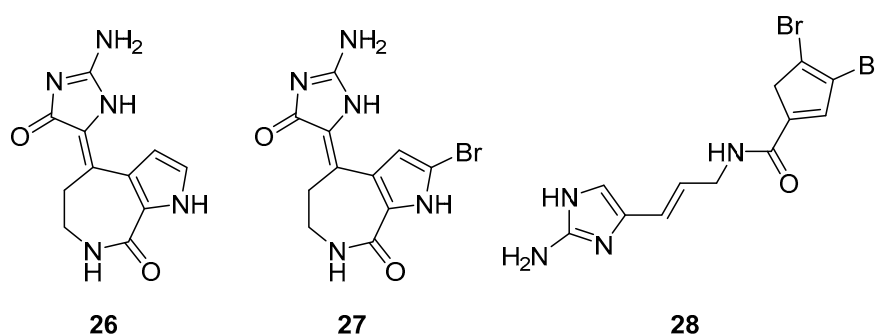
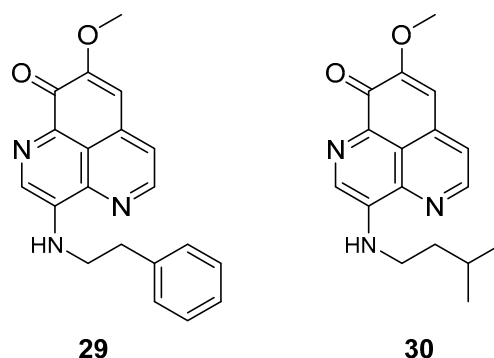


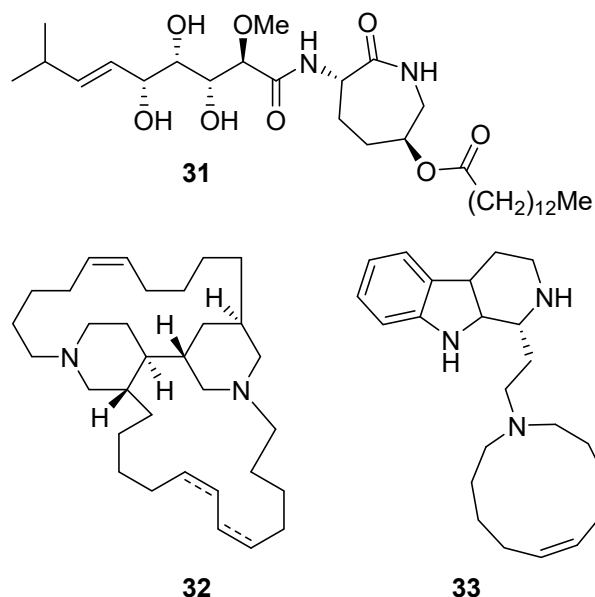
Figure 11. Structures of debromohymenialdisine (26), 10Z-hymenialdisine (27), and oroidin (28).

The two known alkaloids of the aptamine family containing 1*H*-benz[*de*]-1,6-naphthyridine skeleton, namely 3-(phenethylamino)demethyl(oxy)aptamine (29, Figure 12) and 3-(isopentylamino)demethyl(oxy)aptamine (30, Figure 11), were isolated from the sponge *A. aptos*. They exhibited anti-HIV activity, with inhibitory rates of 88.0% and 72.3%, respectively, at a concentration of 10  $\mu$ M [99].



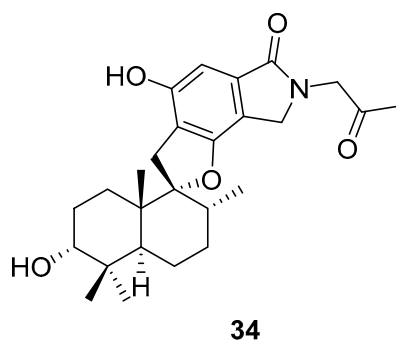
**Figure 12.** Structures of 3-(phenethylamino)dimethyl(oxy)aptamine (29) and 3-(isopentylamino)dimethyl(oxy)aptamine (30).

Bengamide A (31, Figure 13), haliclonycyclamine A+B (32, Figure 13) and keramamine C (33, Figure 13) inhibit HIV-1 with a 50% effective concentration of 3.8  $\mu$ M or less. The most potent among them, bengamide A, blocked HIV-1 in a T cell line with an  $EC_{50}$  of 0.015  $\mu$ M (which was comparable to control antiretrovirals indinavir 0.029  $\mu$ M, efavirenz 0.0024  $\mu$ M, and raltegravir 0.011  $\mu$ M) and in peripheral blood mononuclear cells with  $EC_{50}$  of 0.032  $\mu$ M. It was concluded that HIV-1 LTR NF- $\kappa$ B response elements are required for a bengamide A-mediated inhibition of LTR-dependent gene expression [100].



**Figure 13.** Structures of bengamide A (31), haliclonycyclamines A + B (32), keramamine C (33).

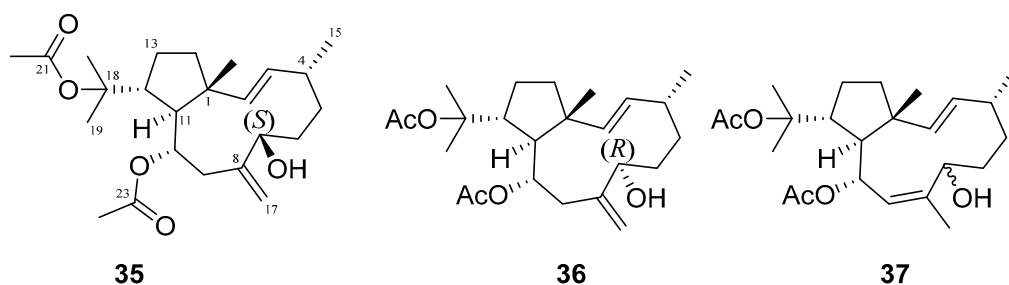
Phenylspirodrimane, stachybotrin D (34, Figure 14) isolated from the sponge-derived fungus *Stachybotrys chartarum* MXH-X73, was discovered to be a HIV-1 RT inhibitor, which showed inhibitory effects on the wild type ( $EC_{50}$  8.4  $\mu$ M) and five NNRTI-resistant HIV-1 strains ( $EC_{50}$  7.0; 23.8; 13.3; 14.2; 6.2  $\mu$ M) [101].



**Figure 14.** Structure of stachybotrin D (34).

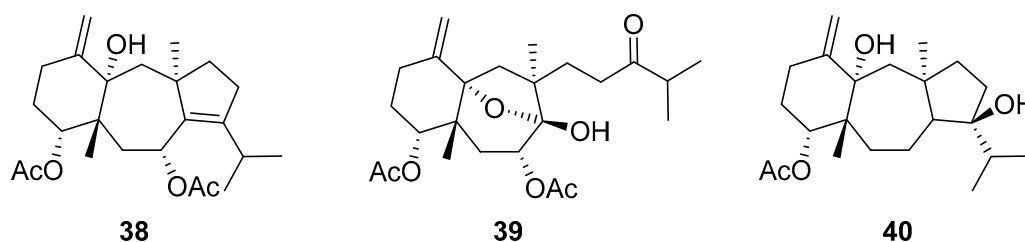
### 2.6. Diterpenes

Many terpenes from marine natural products demonstrated anti-HIV properties. Mechanisms of action involve blocking of different steps of the HIV-1 replicative cycle as reverse transcriptase inhibitors, protease inhibitors, or entry inhibitors. Among them, diterpenes from marine algae are nowadays in the spotlight due to their promising anti-HIV activities [102]. Dolabellane diterpenes are compounds from the diterpene group that have recently been extensively studied for their anti-HIV activity. Pardo-Vargas et al. characterized three new dolabellane diterpenes isolated from the marine brown alga *Dictyota pfaffii* from Northeast Brazil: (1*R*\*,2*E*,4*R*\*,7*S*,10*S*\*,11*S*\*,12*R*\*)10,18-diacetoxy-7-hydroxy-2,8(17)-dolabelladiene, (1*R*\*,2*E*,4*R*\*,7*R*\*,10*S*\*,11*S*\*,12*R*\*)10,18-diacetoxy-7-hydroxy-2,8(17)-dolabelladiene, (1*R*\*,2*E*,4*R*\*,8*E*,10*S*\*,11*S*,12*R*\*)10,18-diacetoxy-7-hydroxy-2,8-dolabelladiene, named dolabelladienols A–C (35–37, Figure 15), respectively [102]. In particular, the new compounds, dolabelladienols A and B, showed potent anti-HIV-1 activities that can be confirmed with their low IC<sub>50</sub> values of 2.9 and 4.1 μM and low cytotoxic activity against MT-2 lymphocyte tumor cells. These promising anti-HIV-1 agents were even more active than previously known 2,6-dolabelladienes series.



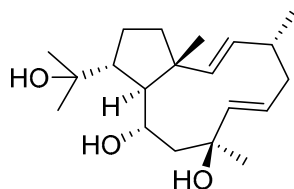
**Figure 15.** Structures of the new dolabellane diterpenoids dolabelladienols A–C (35–37).

De Souza Barros et al. tested marine dolastanes (38, 40, Figure 16) and secodolastane diterpenes (39, Figure 16) isolated from the brown alga *Canistrocarpus cervicornis* for anti-HIV-1 activity [103]. They observed that the marine diterpenes 38–40 inhibit the HIV-1 replication in a dose-dependent manner (EC<sub>50</sub> values of 0.35, 3.67, and 0.794 μM) without a cytotoxic effect (CC<sub>50</sub> values ranging from 935 to 1910 μM). Additionally, they investigated the virucidal effect of these diterpenes and their potential use as microbicides. Dolastane-diterpenes 38 and 40 showed a potent effect on HIV-1 infectivity, whereas no virucidal effect was observed for secodolastane diterpene 39, demonstrating another mechanism of antiretroviral activity. Therefore, the authors suggested a potential use of marine dolastanes 38 and 40 as microbicides that could directly inhibit virus infectivity and possibly act before the virus penetrates the target cells [103].



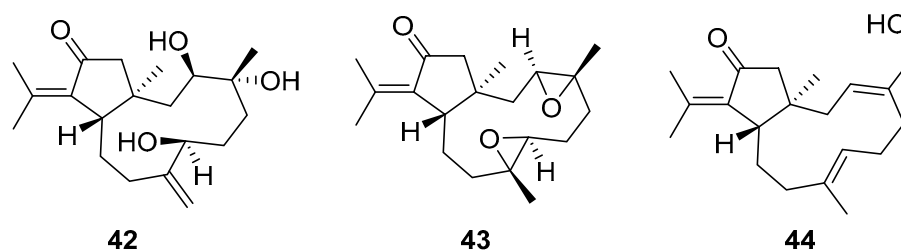
**Figure 16.** Structures of marine dolastanes (38 and 40) and secodolastane diterpene (39) derived from *Canistrocarpus cervicornis*.

Dolabelladienetriol from brown alga *Dictyota* spp has also been evaluated as a potential microbicide against HIV-1 in tissue explants. Namely, Stephens et al. examined the 8,10,18-trihydroxy-2,6-dolabelladiene (41, Figure 17) in pretreated peripheral blood cells (PBMC) and macrophages along with their protective effect in the ex vivo explant model of the uterine cervix [104]. Pre-treatment of peripheral PBMC and macrophages with dolabelladienetriol showed inhibitory effects on HIV-1 replication. Furthermore, in the explant model dolabelladienetriol inhibited viral replication in a dose-dependent manner from 20 to 99% in concentrations of 0.15 and 14.4  $\mu$ M without a loss in the viability of the tissue. The authors concluded that this compound has great potential as a possible microbicide. The same compound was also theoretically analyzed as an inhibitor of the wild-type and mutants' HIV-1 reverse transcriptase [105]. Firstly, the structure-activity relationship studies revealed that a low dipole moment and high HOMO (highest occupied molecular orbital)-LUMO (lowest unoccupied molecular orbital) gap values are related to the antiviral activity. Secondly, molecular docking studies with RT wild-type and mutants showed a seahorse-like conformation of 8,10,18-trihydroxy-2,6-dolabelladiene, hydrophobic interactions, and hydrogen bonds with important residues of the binding pocket. Finally, the authors suggested a new derivative of the 8,10,18-trihydroxy-2,6-dolabelladiene with an aromatic moiety in the double bond to improve its biological activity.



**Figure 17.** Structure of (1R,2E,4R,6E,8S,10S,11S,12R)-8,10,18-trihydroxy-2,6-dolabelladiene (41).

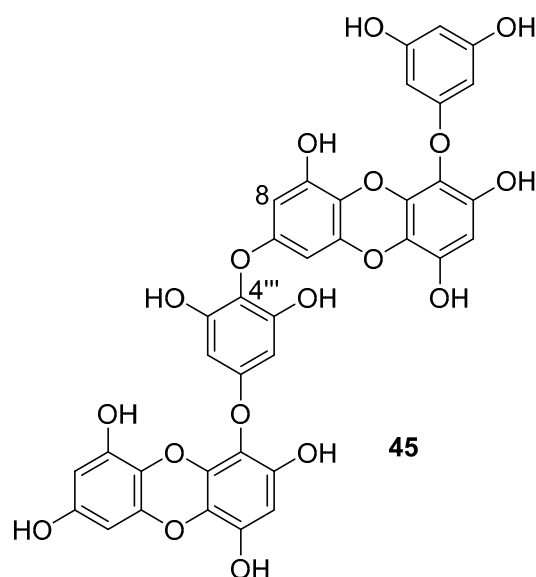
Although dolabellane diterpenes of brown alga *Dictyota* spp showed a strong anti-HIV-1 activity, this was not confirmed for dolabellane diterpenes isolated from octocorals. Therefore, some chemical transformations have been conducted to improve the anti-HIV-1 potency of the main dolabellane 13-keto-1(R),11(S)-dolabella-3(E),7(E),12(18)-triene from Caribbean octocoral *Eunicea laciniata* [106]. Oxygenated dolabellanes derivatives (42–44, Figure 18), obtained by epoxidation, epoxide opening, and allylic oxidation of ketodolbellatriene have shown significantly improved antiviral activities and a low cytotoxicity to MT-2 cells, which makes them promising antiviral compounds.



**Figure 18.** Structures of semi-synthesized oxygenated dolabellanes (42–44) originally isolated from the Caribbean octocoral *Eunicea laciniata*.

### 2.7. Phlorotannins and Xanthones

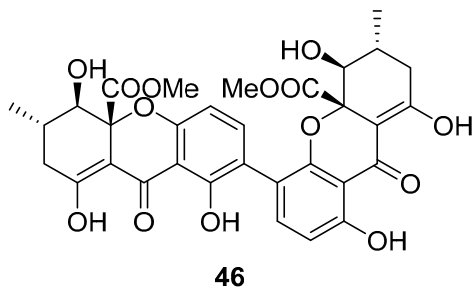
Phlorotannins are tannin derivatives made from several phloroglucinol units linked to each other in different ways. Phlorotannins contain phenyl linkage (fucols), ether linkage (fuhalols and phlorethols), phenyl and ether linkage (fucophloroethols), and dibenzodioxin linkage (eckols) [86,107]. So far, a series of phlorotannins have been identified with potent anti-HIV activity. For example, 8,8'-bieckol and 6,6'-bieckol from marine brown alga *Ecklonia cava* has shown an enhanced HIV-1 inhibitory effect [112,113]. Karadeniz et al. reported that 8,4'''-dieckol (45, Figure 19) is another phlorotannin derivative isolated from the same brown alga that could be used as a drug candidate for the development of new generation anti-HIV therapeutic agents [107]. The compound showed HIV-1 inhibitory activity at nontoxic concentrations. More precisely, the results indicated that 8,4'''-dieckol inhibited the cytopathic effects of HIV-1, including HIV-1 induced syncytia formation, lytic effects, and viral p24 antigen production. Furthermore, 8,4'''-dieckol inhibited an HIV-1 entry and RT enzyme with the inhibition ratio of 91% at a concentration of 50  $\mu$ M.



**Figure 19.** Chemical structure of 8,4'''-dieckol (45) from *E. cava*.

Recently, for the first time, xanthone dimer was identified as a potential anti-HIV-1 agent [108]. Xanthones are secondary metabolites from higher plant families, fungi, and lichen [114,115]. Although structurally related to flavonoids, xanthones are not as frequently encountered in nature [9]. Penicillixanthone A (PXA) (46, Figure 20), a natural xanthone dimer, has been isolated from the jellyfish-derived fungus *Aspergillus fumigatus* with fourteen other natural products [108]. However, only penicillixanthone A showed inhibitory activities in an HIV infection. Marine-derived PXA displayed potent anti-HIV-1 activity against CCR5-tropic HIV-1 SF162 and CXCR4-tropic HIV-1 NL4-3, with  $IC_{50}$  of 0.36 and 0.26  $\mu$ M, respectively. A molecular docking study confirmed that PXA might bind

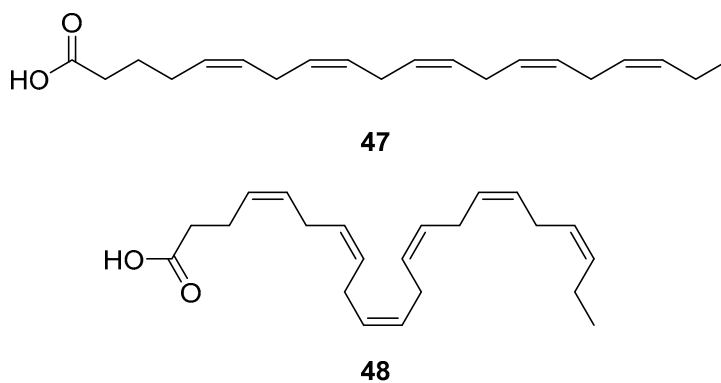
to either CCR5 or CXCR4 to prevent HIV entry into target cells. Therefore, PXA, as a CCR5/CXCR4 dual-coreceptor antagonist, may be seen as a new potential lead product type for the development of anti-HIV therapeutics.



**Figure 20.** Structure of penicillixanthone A (46).

### 2.8. Fish Oil as an Adjuvant to HAART Therapy

HAART therapy can cause severe side effects, e.g., insulin resistance, lipoatrophy, dyslipidemia, and abnormalities of fat distribution. Therefore, finding an adequate diet and supplementation to lower the negative effects of the HAART combination therapy is desirable [116]. Fish oil contains omega-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic (EPA, 20:5n-3) (47, Figure 21) and docosahexaenoic (DHA, 22:6n-3) (48, Figure 21) acids, which may have beneficial effects for HIV-infected patients. It has been shown that the addition of fish oil to the diet of HIV-infected individuals receiving usual antiretroviral therapy can significantly lower serum triglycerides levels [117], which is highly relevant knowing that HIV dyslipidemia is a serious problem related to an increased frequency of cardiovascular disease.



**Figure 21.** Structures of eicosapentaenoic (47) and docosahexaenoic acid (48).

Recently, He et al. analyzed the influence of DHA on the locomotor activity in ethanol-treated HIV-1 transgenic rats [109]. The prevalence of alcohol use and alcohol abuse in infected individuals is much higher, and numerous ethanol and HIV-1 viral proteins have synergistic effects on inflammation in the central nervous system [118–120]. HIV remains in the body in its latent form after HAART therapy and, as such, can induce neuroinflammation. DHA depletion has been found to be associated with various neurological abnormalities, and its administration can have a neuroprotective effect. DHA taken daily could reverse the effects of the ethanol negative effect on the locomotor activity in the presence of HIV viral proteins. An *in vivo* study, using real-time quantitative PCR, showed that the addition of DHA can reduce elevated levels of IL-6, IL-18, and increase the expression of NF- $\kappa$ B in the striatum. This proved the potential of this fish oil constituent as an adjuvant in HIV patients' treatment that can help in lowering the interactive effects of ethanol consumption during HIV infection.



### 2.9. Others

Resorcylic acid lactones, namely radicicol (**49**, Figure 22) pochonin B (**50**, Figure 22) and C (**51**, Figure 22) isolated from *H. fuscoatra* exhibited a 92–98% reactivation efficiency of the latent HIV-1 relative to SAHA (suberoylanilide hydroxamic acid, vorinostat, HDAC inhibitor) and EC<sub>50</sub> of 9.1, 39.6 and 6.3 μM [110]. The reactivation strategy is, indeed, a promising strategy to expunge the HIV-1 infection by reactivating latent viral loads, mainly in CD4 + T-cells, which quickly rebound when antiviral treatment is interrupted. It was noted that all active compounds contain Michael acceptor functionality. The PKC-independent mechanism of reactivation of the latent HIV-1 remains to be elucidated.

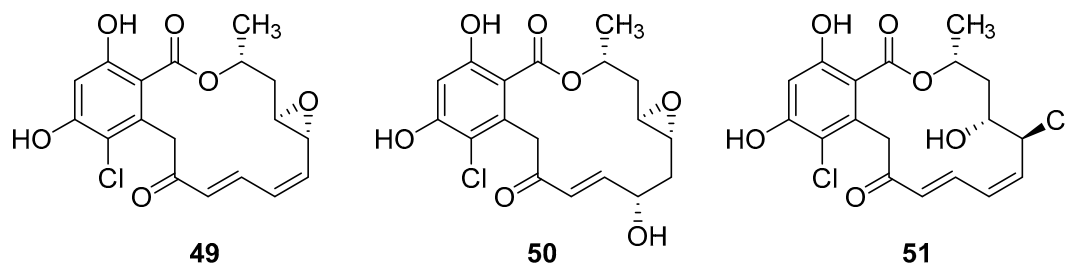


Figure 22. Structures of radicicol (**49**), pochonin B (**50**), pochonin C (**51**).

A team of researchers led by Zhao isolated new isoprenylated cyclohexanols from the sponge-associated fungus *Truncatella angustata* named truncateols O-V [111]. In vitro testing showed that truncateols O and P (**52** and **53**, Figure 23), analogues bearing the alkynyl group in the side chain, exhibit a significant inhibition toward the HIV-1 virus with IC<sub>50</sub> values of 39.0 μM and 16.1 μM, respectively. These compounds could be considered as new anti-HIV lead compounds due to lower cytotoxicity (CC<sub>50</sub> > 100 μM) in comparison with the positive control efavirenz (CC<sub>50</sub> = 40.6 μM).

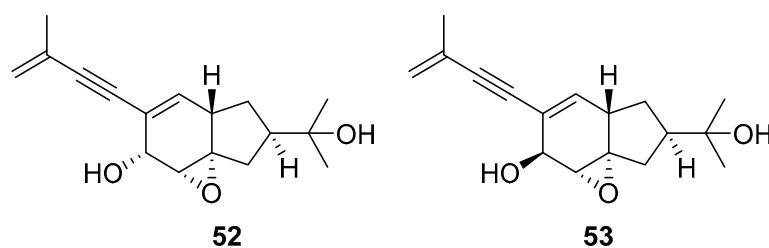


Figure 23. Structures of truncateols O (**52**) and P (**53**).

### 3. Future Directions in the Anti-HIV Marine Drug Development

Marine organisms have been acknowledged as a precious source of bioactive compounds that may provide novel anti-HIV structures or lead structures for structural optimization. A large amount of evidence from scientific research confirmed a high biological potential of these compounds to treat serious diseases, including infective ones. Some of the marine-derived bioactive compounds discovered much earlier have emerged with novel properties and potential applications after a decade or two. Isolation and structural elucidation of compounds from marine organisms is not an easy task and still carries challenges. Identification of all the compounds is a daunting task, especially with regards to complex structural motifs that may be present in a single marine extract. Taxonomic knowledge is still insufficient to enable unambiguous species classification that can result in the false prediction of chemical constituents and hamper structural analysis. Furthermore, a temporal lag between the discovery, chemical characterization, and associated pharmacological activities is quite common, and the majority of marine metabolites are usually tested for anticancer activity, whereas anti-HIV and other possible biological effects are neglected or mostly not performed due to a lack of

funding. Targeted assays and in vivo analyses are similarly performed only for some of the potential candidates, while the translation into clinical trials remains very limited. Thus, the financial gap is certainly a relevant factor contributing to the slow drug development process in this area. In particular, the development of anti-HIV compounds, which act by mechanisms that differ from existing antivirals, requires a well-designed and focused approach to studying the mode of action. Libraries should be created for specifically defined crude extracts, their corresponding simplified fractions as well as for pure compounds for a well-balanced natural product discovery program. Additionally, there exist but few publications in which scientists have tried to modify known compounds of marine origin to improve their bioactivity. We are, however, continuously witnessing advancements in the deep-sea exploration technology, sampling strategies, genome sequencing, genome mining, genetic engineering, chemo-enzymatic synthesis, nanoscale NMR structure determination, and development and optimization of suitable fermentation strategies to ensure a continued supply of unique bioactive compounds from the oceans. Therefore, the grounds have been met for a broad, international effort based on scientific collaboration that would rely on well-equipped infrastructure and human resources as a prerequisite for a full advancement in the field and development of new drug candidates for the pharmaceutical market in the future.

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## References

1. AVERT. Symptoms and Stages of HIV Infection. Available online: <https://www.avert.org/about-hiv-aids/symptoms-stages> (accessed on 12 April 2019).
2. HIV/AIDS. Available online: <https://www.who.int/news-room/fact-sheets/detail/hiv-aids> (accessed on 12 April 2019).
3. Li, G.; Piampongsant, S.; Faria, N.R.; Voet, A.; Pineda-Peña, A.-C.; Khouri, R.; Lemey, P.; Vandamme, A.-M.; Theys, K. An integrated map of HIV genome-wide variation from a population perspective. *Retrovirology* **2015**, *12*, 18. [[CrossRef](#)] [[PubMed](#)]
4. Saphire, A.C.S.; Bobardt, M.D.; Zhang, Z.; David, G.; Gallay, P.A. Syndecans Serve as Attachment Receptors for Human Immunodeficiency Virus Type 1 on Macrophages. *J. Virol.* **2001**, *75*, 9187–9200. [[CrossRef](#)] [[PubMed](#)]
5. Arthos, J.; Cicala, C.; Martinelli, E.; Macleod, K.; Van Ryk, D.; Wei, D.; Xiao, Z.; Veenstra, T.D.; Conrad, T.P.; Lempicki, R.A.; et al. HIV-1 envelope protein binds to and signals through integrin  $\alpha 4\beta 7$ , the gut mucosal homing receptor for peripheral T cells. *Nat. Immunol.* **2008**, *9*, 301–309. [[CrossRef](#)] [[PubMed](#)]
6. Cicala, C.; Martinelli, E.; McNally, J.P.; Goode, D.J.; Gopaul, R.; Hiatt, J.; Jelicic, K.; Kottlilil, S.; Macleod, K.; O’Shea, A.; et al. The integrin  $\alpha 4\beta 7$  forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. *PNAS* **2009**, *106*, 20877–20882. [[CrossRef](#)] [[PubMed](#)]
7. Geijtenbeek, T.B.; Kwon, D.S.; Torensma, R.; van Vliet, S.J.; van Duijnhoven, G.C.; Middel, J.; Cornelissen, I.L.; Nottet, H.S.; KewalRamani, V.N.; Littman, D.R.; et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **2000**, *100*, 587–597. [[CrossRef](#)]
8. Orloff, G.M.; Orloff, S.L.; Kennedy, M.S.; Maddon, P.J.; McDougal, J.S. Penetration of CD4 T cells by HIV-1. The CD4 receptor does not internalize with HIV, and CD4-related signal transduction events are not required for entry. *J. Immunol.* **1991**, *146*, 2578–2587. [[PubMed](#)]

9. Maddon, P.J.; Dalglish, A.G.; McDougal, J.S.; Clapham, P.R.; Weiss, R.A.; Axel, R. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **1986**, *47*, 333–348. [[CrossRef](#)]
10. McDougal, J.; Kennedy, M.; Sligh, J.; Cort, S.; Mawle, A.; Nicholson, J. Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. *Science* **1986**, *231*, 382–385. [[CrossRef](#)]
11. Alkhatib, G. The biology of CCR5 and CXCR4. *Curr. Opin. HIV AIDS* **2009**, *4*, 96–103. [[CrossRef](#)]
12. Okoye, A.A.; Picker, L.J. CD4(+) T-cell depletion in HIV infection: Mechanisms of immunological failure. *Immunol. Rev.* **2013**, *254*, 54–64. [[CrossRef](#)]
13. Schragger, L.K.; D'Souza, M.P. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. *JAMA* **1998**, *280*, 67–71. [[CrossRef](#)] [[PubMed](#)]
14. Berkhout, B.; Eggink, D.; Sanders, R.W. Is there a future for antiviral fusion inhibitors? *Curr. Opin. Virol.* **2012**, *2*, 50–59. [[CrossRef](#)] [[PubMed](#)]
15. Greenberg, M.L.; Cammack, N. Resistance to enfuvirtide, the first HIV fusion inhibitor. *J. Antimicrob. Chemother.* **2004**, *54*, 333–340. [[CrossRef](#)] [[PubMed](#)]
16. Baldwin, C.E.; Sanders, R.W.; Deng, Y.; Jurriaans, S.; Lange, J.M.; Lu, M.; Berkhout, B. Emergence of a drug-dependent human immunodeficiency virus type 1 variant during therapy with the T20 fusion inhibitor. *J. Virol.* **2004**, *78*, 12428–12437. [[CrossRef](#)] [[PubMed](#)]
17. Rimsky, L.T.; Shugars, D.C.; Matthews, T.J. Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. *J. Virol.* **1998**, *72*, 986–993. [[PubMed](#)]
18. Abner, E.; Jordan, A. HIV “shock and kill” therapy: In need of revision. *Antiviral Res.* **2019**, *166*, 19–34. [[CrossRef](#)]
19. Hütter, G.; Nowak, D.; Mossner, M.; Ganepola, S.; Müßig, A.; Allers, K.; Schneider, T.; Hofmann, J.; Kücherer, C.; Blau, O.; et al. Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation. *N. Engl. J. Med.* **2009**, *360*, 692–698. [[CrossRef](#)] [[PubMed](#)]
20. Ananworanich, J.; Robb, M.L. The transient HIV remission in the Mississippi baby: Why is this good news? *J. Int. AIDS Soc.* **2014**, *17*, 19859. [[CrossRef](#)]
21. The Lancet HIV. Like London buses, two putative cure cases arrive at once. *Lancet HIV* **2019**, *6*, e205. [[CrossRef](#)]
22. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs from 1981 to 2014. *J. Nat. Prod.* **2016**, *79*, 629–661. [[CrossRef](#)]
23. Erakovic Haber, V.; Spaventi, R. Discovery and Development of Novel Drugs. In *Progress in Molecular and Subcellular Biology*; Springer: Cham, Switzerland, 2017; Volume 55, pp. 91–104.
24. Blunt, J.W.; Copp, B.R.; Hu, W.-P.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2009**, *26*, 170. [[CrossRef](#)] [[PubMed](#)]
25. Mori, T.; O'Keefe, B.R.; Sowder, R.C.; Bringans, S.; Gardella, R.; Berg, S.; Cochran, P.; Turpin, J.A.; Buckheit, R.W.; McMahon, J.B.; et al. Isolation and Characterization of Griffithsin, a Novel HIV-inactivating Protein, from the Red Alga *Griffithsia* sp. *J. Biol. Chem.* **2005**, *280*, 9345–9353. [[CrossRef](#)] [[PubMed](#)]
26. Xue, J.; Gao, Y.; Hoorelbeke, B.; Kagiampakis, I.; Zhao, B.; Demeler, B.; Balzarini, J.; Liwang, P.J. The role of individual carbohydrate-binding sites in the function of the potent anti-HIV lectin griffithsin. *Mol. Pharm.* **2012**, *9*, 2613–2625. [[CrossRef](#)] [[PubMed](#)]
27. Huang, X.; Jin, W.; Griffin, G.E.; Shattock, R.J.; Hu, Q. Removal of two high-mannose N-linked glycans on gp120 renders human immunodeficiency virus 1 largely resistant to the carbohydrate-binding agent griffithsin. *J. Gen. Virol.* **2011**, *92*, 2367–2373. [[CrossRef](#)] [[PubMed](#)]
28. Xue, J.; Hoorelbeke, B.; Kagiampakis, I.; Demeler, B.; Balzarini, J.; Liwang, P.J. The griffithsin dimer is required for high-potency inhibition of HIV-1: Evidence for manipulation of the structure of gp120 as part of the griffithsin dimer mechanism. *Antimicrob. Agents Chemother.* **2013**, *57*, 3976–3989. [[CrossRef](#)] [[PubMed](#)]
29. Gerwick, W.H.; Moore, B.S. Lessons from the Past and Charting the Future of Marine Natural Products Drug Discovery and Chemical Biology. *Chem. Biol.* **2012**, *19*, 85–98. [[CrossRef](#)] [[PubMed](#)]
30. Martins, A.; Vieira, H.; Gaspar, H.; Santos, S.; Martins, A.; Vieira, H.; Gaspar, H.; Santos, S. Marketed Marine Natural Products in the Pharmaceutical and Cosmeceutical Industries: Tips for Success. *Mar. Drugs* **2014**, *12*, 1066–1101. [[CrossRef](#)] [[PubMed](#)]
31. Karagozlu, M.Z.; Karadeniz, F.; Kim, S.-K. Anti-HIV activities of novel synthetic peptide conjugated chitosan oligomers. *Int. J. Biol. Macromol.* **2014**, *66*, 260–266. [[CrossRef](#)] [[PubMed](#)]

32. Ramana, L.N.; Sharma, S.; Sethuraman, S.; Ranga, U.; Krishnan, U.M. Evaluation of chitosan nanoformulations as potent anti-HIV therapeutic systems. *Biochim. Biophys. Acta* **2014**, *1840*, 476–484. [[CrossRef](#)]
33. Shohani, S.; Mondanizadeh, M.; Abdoli, A.; Khansarinejad, B.; Salimi-Asl, M.; Ardestani, M.; Ghanbari, M.; Haj, M.; Zabihollahi, R. Trimethyl Chitosan Improves Anti-HIV Effects of Atripla as a New Nanoformulated Drug. *Curr. HIV Res.* **2017**, *15*, 56–65. [[CrossRef](#)]
34. Cheung, R.C.F.; Ng, T.B.; Wong, J.H.; Chan, W.Y. Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. *Mar. Drugs* **2015**, *13*, 5156–5186. [[CrossRef](#)] [[PubMed](#)]
35. Wijesekara, I.; Pangestuti, R.; Kim, S.-K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.* **2011**, *84*, 14–21. [[CrossRef](#)]
36. Jiao, G.; Yu, G.; Zhang, J.; Ewart, H.S. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Mar. Drugs* **2011**, *9*, 196–223. [[CrossRef](#)] [[PubMed](#)]
37. Gupta, S.; Abu-Ghannam, N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innov. Food Sci. Emerg. Technol.* **2011**, *12*, 600–609. [[CrossRef](#)]
38. Aquino, R.S.; Grativol, C.; Mourão, P.A.S. Rising from the Sea: Correlations between Sulfated Polysaccharides and Salinity in Plants. *PLoS ONE* **2011**, *6*, e18862. [[CrossRef](#)]
39. Torode, T.A.; Marcus, S.E.; Jam, M.; Tonon, T.; Blackburn, R.S.; Hervé, C.; Knox, J.P. Monoclonal Antibodies Directed to Fucoidan Preparations from Brown Algae. *PLoS One* **2015**, *10*, e0118366. [[CrossRef](#)] [[PubMed](#)]
40. Deniaud-Bouët, E.; Kervarec, N.; Michel, G.; Tonon, T.; Kloareg, B.; Hervé, C. Chemical and enzymatic fractionation of cell walls from Fucales: Insights into the structure of the extracellular matrix of brown algae. *Ann. Bot.* **2014**, *114*, 1203–1216. [[CrossRef](#)]
41. Adhikari, U.; Mateu, C.G.; Chattopadhyay, K.; Pujol, C.A.; Damonte, E.B.; Ray, B. Structure and antiviral activity of sulfated fucans from *Stoechospermum marginatum*. *Phytochemistry* **2006**, *67*, 2474–2482. [[CrossRef](#)]
42. Zhang, H.; Wang, J.-Q.; Nie, S.-P.; Wang, Y.-X.; Cui, S.W.; Xie, M.-Y. Sulfated modification, characterization and property of a water-insoluble polysaccharide from *Ganoderma atrum*. *Int. J. Biol. Macromol.* **2015**, *79*, 248–255. [[CrossRef](#)]
43. Witvrouw, M.; De Clercq, E. Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen. Pharmacol.* **1997**, *29*, 497–511. [[CrossRef](#)]
44. Meiyu, G.; Fuchuan, L.; Xianliang, X.; Jing, L.; Zuowei, Y.; Huashi, G. The potential molecular targets of marine sulfated polymannuroguluronate interfering with HIV-1 entry. Interaction between SPMG and HIV-1 rgp120 and CD4 molecule. *Antiviral Res.* **2003**, *59*, 127–135. [[CrossRef](#)]
45. Damonte, E.B.; Matulewicz, M.C.; Cerezo, A.S. Sulfated seaweed polysaccharides as antiviral agents. *Curr. Med. Chem.* **2004**, *11*, 2399–2419. [[CrossRef](#)] [[PubMed](#)]
46. Witvrouw, M.; Schols, D.; Andrei, G.; Snoeck, R.; Ikeda, S.; Pauwels, R.; Van Schepdael, A.; Arnout, J.; Claes, P.; Desmyter, J.; et al. New Polyacetal Polysulphate Active against Human Immunodeficiency Virus and other Enveloped Viruses. *Antivir. Chem. Chemother.* **1992**, *3*, 351–360. [[CrossRef](#)]
47. Parish, C.R.; Jakobsen, K.B.; Coombe, D.R.; Bacic, A. Isolation and characterization of cell adhesion molecules from the marine sponge, *Ophlitaspongia tenuis*. *Biochim. Biophys. Acta* **1991**, *1073*, 56–64. [[CrossRef](#)]
48. Lynch, G.; Low, L.; Li, S.; Sloane, A.; Adams, S.; Parish, C.; Kemp, B.; Cunningham, A.L. Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD4-gp120 interaction, but do not inhibit monocyte infection. *J. Leukoc. Biol.* **1994**, *56*, 266–272. [[CrossRef](#)] [[PubMed](#)]
49. Talyshinsky, M.M.; Souprun, Y.Y.; Huleihel, M.M. Anti-viral activity of red microalgal polysaccharides against retroviruses. *Cancer Cell Int.* **2002**, *2*, 8. [[CrossRef](#)] [[PubMed](#)]
50. Gerenčar, M.; Turecek, P.L.; Kistner, O.; Mitterer, A.; Savidis-Dacho, H.; Barrett, N.P. In vitro and in vivo anti-retroviral activity of the substance purified from the aqueous extract of *Chelidonium majus* L. *Antiviral Res.* **2006**, *72*, 153–156. [[CrossRef](#)]
51. Neyts, J.; Snoeck, R.; Schols, D.; Balzarini, J.; Esko, J.D.; Van Schepdael, A.; De Clercq, E. Sulfated polymers inhibit the interaction of human cytomegalovirus with cell surface heparan sulfate. *Virology* **1992**, *189*, 48–58. [[CrossRef](#)]
52. Vidhyanandhini, R.; Saravanan, R.; Vairamani, S.; Shanmugam, A. The anticoagulant activity and structural characterization of fractionated and purified glycosaminoglycans from venerid clam *Meretrix casta* (Chemnitz). *J. Liq. Chromatogr. Relat. Technol.* **2014**, *37*, 917–929. [[CrossRef](#)]

53. Li, P.; Sheng, J.; Liu, Y.; Li, J.; Liu, J.; Wang, F. Heparosan-Derived Heparan Sulfate/Heparin-Like Compounds: One Kind of Potential Therapeutic Agents. *Med. Res. Rev.* **2013**, *33*, 665–692. [[CrossRef](#)]
54. Ahmadi, A.; Zorofchian Moghadamtousi, S.; Abubakar, S.; Zandi, K. Antiviral Potential of Algae Polysaccharides Isolated from Marine Sources: A Review. *Biomed. Res. Int.* **2015**, *2015*, 1–10. [[CrossRef](#)] [[PubMed](#)]
55. Rabanal, M.; Ponce, N.M.A.; Navarro, D.A.; Gómez, R.M.; Stortz, C.A. The system of fucoidans from the brown seaweed *Dictyota dichotoma*: Chemical analysis and antiviral activity. *Carbohydr. Polym.* **2014**, *101*, 804–811. [[CrossRef](#)] [[PubMed](#)]
56. Thuy, T.T.T.; Ly, B.M.; Van, T.T.T.; Van Quang, N.; Tu, H.C.; Zheng, Y.; Seguin-Devaux, C.; Mi, B.; Ai, U. Anti-HIV activity of fucoidans from three brown seaweed species. *Carbohydr. Polym.* **2015**, *115*, 122–128. [[CrossRef](#)] [[PubMed](#)]
57. Hoshino, T.; Hayashi, T.; Hayashi, K.; Hamada, J.; Lee, J.B.; Sankawa, U. An antivirally active sulfated polysaccharide from *Sargassum horneri* (TURNER) C. AGARDH. *Biol. Pharm. Bull.* **1998**, *21*, 730–734. [[CrossRef](#)] [[PubMed](#)]
58. Schaeffer, D.J.; Krylov, V.S. Anti-HIV Activity of Extracts and Compounds from Algae and Cyanobacteria. *Ecotoxicol. Environ. Saf.* **2000**, *45*, 208–227. [[CrossRef](#)] [[PubMed](#)]
59. Ueno, M.; Nogawa, M.; Siddiqui, R.; Watashi, K.; Wakita, T.; Kato, N.; Ikeda, M.; Okimura, T.; Isaka, S.; Oda, T.; et al. Acidic polysaccharides isolated from marine algae inhibit the early step of viral infection. *Int. J. Biol. Macromol.* **2019**, *124*, 282–290. [[CrossRef](#)]
60. Huang, N.; Wu, M.-Y.; Zheng, C.-B.; Zhu, L.; Zhao, J.-H.; Zheng, Y.-T. The depolymerized fucosylated chondroitin sulfate from sea cucumber potently inhibits HIV replication via interfering with virus entry. *Carbohydr. Res.* **2013**, *380*, 64–69. [[CrossRef](#)] [[PubMed](#)]
61. Lian, W.; Wu, M.; Huang, N.; Gao, N.; Xiao, C.; Li, Z.; Zhang, Z.; Zheng, Y.; Peng, W.; Zhao, J. Anti-HIV-1 activity and structure–activity-relationship study of a fucosylated glycosaminoglycan from an echinoderm by targeting the conserved CD4 induced epitope. *Biochim. Biophys. Acta* **2013**, *1830*, 4681–4691. [[CrossRef](#)]
62. Pirrone, V.; Wigdahl, B.; Krebs, F.C. The rise and fall of polyanionic inhibitors of the human immunodeficiency virus type 1. *Antiviral Res.* **2011**, *90*, 168–182. [[CrossRef](#)]
63. Torode, T.A.; Siméon, A.; Marcus, S.E.; Jam, M.; Le Moigne, M.-A.; Duffieux, D.; Knox, J.P.; Hervé, C. Dynamics of cell wall assembly during early embryogenesis in the brown alga *Fucus*. *J. Exp. Bot.* **2016**, *67*, 6089–6100. [[CrossRef](#)]
64. Raimundo, S.C.; Avci, U.; Hopper, C.; Pattathil, S.; Hahn, M.G.; Popper, Z.A. Immunolocalization of cell wall carbohydrate epitopes in seaweeds: Presence of land plant epitopes in *Fucus vesiculosus* L. (Phaeophyceae). *Planta* **2016**, *243*, 337–354. [[CrossRef](#)] [[PubMed](#)]
65. Andrade, L.R.; Leal, R.N.; Nosedá, M.; Duarte, M.E.R.; Pereira, M.S.; Mourão, P.A.S.; Farina, M.; Amado Filho, G.M. Brown algae overproduce cell wall polysaccharides as a protection mechanism against the heavy metal toxicity. *Mar. Pollut. Bull.* **2010**, *60*, 1482–1488. [[CrossRef](#)]
66. Zvyagintseva, T.N.; Shevchenko, N.M.; Chizhov, A.O.; Krupnova, T.N.; Sundukova, E.V.; Isakov, V.V. Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the developmental conditions. *J. Exp. Mar. Bio. Ecol.* **2003**, *294*, 1–13. [[CrossRef](#)]
67. Mak, W.; Hamid, N.; Liu, T.; Lu, J.; White, W.L. Fucoidan from New Zealand *Undaria pinnatifida*: Monthly variations and determination of antioxidant activities. *Carbohydr. Polym.* **2013**, *95*, 606–614. [[CrossRef](#)] [[PubMed](#)]
68. Fletcher, H.R.; Biller, P.; Ross, A.B.; Adams, J.M.M. The seasonal variation of fucoidan within three species of brown macroalgae. *Algal Res.* **2017**, *22*, 79–86. [[CrossRef](#)]
69. Stengel, D.B.; Connan, S.; Popper, Z.A. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnol. Adv.* **2011**, *29*, 483–501. [[CrossRef](#)]
70. Fitton, J.H.; Stringer, D.N.; Karpinić, S.S. Therapies from Fucoidan: An Update. *Mar. Drugs* **2015**, *13*, 5920–5946. [[CrossRef](#)]
71. Astronomo, R.D.; Burton, D.R. Carbohydrate vaccines: Developing sweet solutions to sticky situations? *Nat. Rev. Drug Discov.* **2010**, *9*, 308–324. [[CrossRef](#)]
72. Wang, Y.; Ye, X.-S.; Zhang, L.-H. Oligosaccharide assembly by one-pot multi-step strategy. *Org. Biomol. Chem.* **2007**, *5*, 2189. [[CrossRef](#)]



73. Astronomo, R.D.; Lee, H.-K.; Scanlan, C.N.; Pantophlet, R.; Huang, C.-Y.; Wilson, I.A.; Blixt, O.; Dwek, R.A.; Wong, C.-H.; Burton, D.R. A Glycoconjugate Antigen Based on the Recognition Motif of a Broadly Neutralizing Human Immunodeficiency Virus Antibody, 2G12, Is Immunogenic but Elicits Antibodies Unable To Bind to the Self Glycans of gp120. *J. Virol.* **2008**, *82*, 6359–6368. [[CrossRef](#)]
74. Luallen, R.J.; Lin, J.; Fu, H.; Cai, K.K.; Agrawal, C.; Mboudjeka, I.; Lee, F.-H.; Montefiori, D.; Smith, D.F.; Doms, R.W.; et al. An Engineered *Saccharomyces cerevisiae* Strain Binds the Broadly Neutralizing Human Immunodeficiency Virus Type 1 Antibody 2G12 and Elicits Mannose-Specific gp120-Binding Antibodies. *J. Virol.* **2008**, *82*, 6447–6457. [[CrossRef](#)] [[PubMed](#)]
75. Dunlop, D.C.; Ulrich, A.; Appelmelk, B.J.; Burton, D.R.; Dwek, R.A.; Zitzmann, N.; Scanlan, C.N. Antigenic mimicry of the HIV envelope by AIDS-associated pathogens. *AIDS* **2008**, *22*, 2214–2217. [[CrossRef](#)] [[PubMed](#)]
76. Joyce, J.G.; Krauss, I.J.; Song, H.C.; Opalka, D.W.; Grimm, K.M.; Nahas, D.D.; Esser, M.T.; Hrin, R.; Feng, M.; Dudkin, V.Y.; et al. An oligosaccharide-based HIV-1 2G12 mimotope vaccine induces carbohydrate-specific antibodies that fail to neutralize HIV-1 virions. *PNAS* **2008**, *105*, 15684–15689. [[CrossRef](#)] [[PubMed](#)]
77. Astronomo, R.D.; Kaltgrad, E.; Udit, A.K.; Wang, S.-K.; Doores, K.J.; Huang, C.-Y.; Pantophlet, R.; Paulson, J.C.; Wong, C.-H.; Finn, M.G.; et al. Defining Criteria for Oligomannose Immunogens for HIV Using Icosahedral Virus Capsid Scaffolds. *Chem. Biol.* **2010**, *17*, 357–370. [[CrossRef](#)] [[PubMed](#)]
78. Wang, S.-K.; Liang, P.-H.; Astronomo, R.D.; Hsu, T.-L.; Hsieh, S.-L.; Burton, D.R.; Wong, C.-H. Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. *PNAS* **2008**, *105*, 3690–3695. [[CrossRef](#)] [[PubMed](#)]
79. Wang, L.-X. Toward oligosaccharide- and glycopeptide-based HIV vaccines. *Curr. Opin. Drug Discov. Devel.* **2006**, *9*, 194–206. [[PubMed](#)]
80. Kumar, K.K.; Reddy, G.S.; Reddy, B.; Shekar, P.C.; Sumanthi, J.; Chandra, K.I.P. Biological role of lectins: A review. *J. Orofac. Sci.* **2012**, *4*, 20. [[CrossRef](#)]
81. Balzarini, J. Inhibition of HIV entry by carbohydrate-binding proteins. *Antiviral Res.* **2006**, *71*, 237–247. [[CrossRef](#)]
82. Balzarini, J.; Neyts, J.; Schols, D.; Hosoya, M.; Van Damme, E.; Peumans, W.; De Clercq, E. The mannose-specific plant lectins from *Cymbidium* hybrid and *Epipactis helleborine* and the (N-acetylglucosamine)n-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication. *Antiviral Res.* **1992**, *18*, 191–207. [[CrossRef](#)]
83. Hansen, J.E.; Nielsen, C.M.; Nielsen, C.; Heegaard, P.; Mathiesen, L.R.; Nielsen, J.O. Correlation between carbohydrate structures on the envelope glycoprotein gp120 of HIV-1 and HIV-2 and syncytium inhibition with lectins. *AIDS* **1989**, *3*, 635–641. [[CrossRef](#)]
84. Sato, T.; Hori, K. Cloning, expression, and characterization of a novel anti-HIV lectin from the cultured cyanobacterium, *Oscillatoria agardhii*. *Fish. Sci.* **2009**, *75*, 743–753. [[CrossRef](#)]
85. Akkouh, O.; Ng, T.B.; Singh, S.S.; Yin, C.; Dan, X.; Chan, Y.S.; Pan, W.; Cheung, R.C.F. Lectins with anti-HIV activity: A review. *Molecules* **2015**, *20*, 648–668. [[CrossRef](#)] [[PubMed](#)]
86. Gogineni, V.; Schinazi, R.F.; Hamann, M.T. Role of Marine Natural Products in the Genesis of Antiviral Agents. *Chem. Rev.* **2015**, *115*, 9655–9706. [[CrossRef](#)] [[PubMed](#)]
87. Hirayama, M.; Shibata, H.; Imamura, K.; Sakaguchi, T.; Hori, K. High-Mannose Specific Lectin and Its Recombinants from a Carrageenophyta *Kappaphycus alvarezii* Represent a Potent Anti-HIV Activity Through High-Affinity Binding to the Viral Envelope Glycoprotein gp120. *Mar. Biotechnol.* **2016**, *18*, 144–160. [[CrossRef](#)] [[PubMed](#)]
88. Vo, T.-S.; Kim, S.-K. Potential Anti-HIV Agents from Marine Resources: An Overview. *Mar. Drugs* **2010**, *8*, 2871–2892. [[CrossRef](#)] [[PubMed](#)]
89. Cheung, R.C.F.; Ng, T.B.; Wong, J.H. Marine Peptides: Bioactivities and Applications. *Mar. Drugs* **2015**, *13*, 4006–4043. [[CrossRef](#)] [[PubMed](#)]
90. Qaralleh, H. Chemical and bioactive diversities of marine sponge *Neopetrosia*. *Bangladesh J. Pharmacol.* **2016**, *11*, 433. [[CrossRef](#)]
91. ANEIRO, A.; GARATEIX, A. Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. *J. Chromatogr. B* **2004**, *803*, 41–53. [[CrossRef](#)]
92. Shin, H.J.; Rashid, M.A.; Cartner, L.K.; Bokesch, H.R.; Wilson, J.A.; McMahon, J.B.; Gustafson, K.R. Stelletapeptins A and B, HIV-inhibitory cyclic depsipeptides from the marine sponge *Stelletta* sp. *Tetrahedron Lett.* **2015**, *56*, 4215–4219. [[CrossRef](#)]

93. Wildeman; Gomez-Archila, L.G.; Galeano, E.; Martínez, A.; Castrillón, F.J.D.; Rugeles, M.T. Bromotyrosine Derivatives from Marine Sponges Inhibit the HIV-1 Replication in Vitro. *Vitae* **2014**, *21*, 114–125.
94. Jang, I.S.; Park, S.J. Hydroxyproline-containing collagen peptide derived from the skin of the Alaska pollack inhibits HIV-1 infection. *Mol. Med. Rep.* **2016**, *14*, 5489–5494. [[CrossRef](#)] [[PubMed](#)]
95. Jang, I.-S.; Park, S.J. A Spirulina maxima-derived peptide inhibits HIV-1 infection in a human T cell line MT4. *Fish. Aquat. Sci.* **2016**, *19*, 37. [[CrossRef](#)]
96. Zhou, X.; Fang, W.; Tan, S.; Lin, X.; Xun, T.; Yang, B.; Liu, S.; Liu, Y. Aspernigrins with anti-HIV-1 activities from the marine-derived fungus *Aspergillus niger* SCSIO Jcsw6F30. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 361–365. [[CrossRef](#)] [[PubMed](#)]
97. Niu, S.; Liu, D.; Shao, Z.; Proksch, P.; Lin, W. Eutypellazines A–M, thiodiketopiperazine-type alkaloids from deep sea derived fungus *Eutypella* sp. MCCC 3A00281. *RSC Adv.* **2017**, *7*, 33580–33590. [[CrossRef](#)]
98. O'Rourke, A.; Kremb, S.; Bader, T.; Helfer, M.; Schmitt-Kopplin, P.; Gerwick, W.; Brack-Werner, R.; Voolstra, C. Alkaloids from the Sponge *Stylissa carteri* Present Prospective Scaffolds for the Inhibition of Human Immunodeficiency Virus 1 (HIV-1). *Mar. Drugs* **2016**, *14*, 28. [[CrossRef](#)] [[PubMed](#)]
99. Yu, H.-B.; Yang, F.; Sun, F.; Li, J.; Jiao, W.-H.; Gan, J.-H.; Hu, W.-Z.; Lin, H.-W. Aaptamine derivatives with antifungal and anti-HIV-1 activities from the South China Sea sponge *Aaptos aaptos*. *Mar. Drugs* **2014**, *12*, 6003–6013. [[CrossRef](#)] [[PubMed](#)]
100. Tietjen, I.; Williams, D.E.; Read, S.; Kuang, X.T.; Mwimanzi, P.; Wilhelm, E.; Markle, T.; Kinloch, N.N.; Naphen, C.N.; Tenney, K.; et al. Inhibition of NF- $\kappa$ B-dependent HIV-1 replication by the marine natural product bengamide A. *Antiviral Res.* **2018**, *152*, 94–103. [[CrossRef](#)] [[PubMed](#)]
101. Ma, X.; Li, L.; Zhu, T.; Ba, M.; Li, G.; Gu, Q.; Guo, Y.; Li, D. Phenylspirodrimanes with Anti-HIV Activity from the Sponge-Derived Fungus *Stachybotrys chartarum* MXH-X73. *J. Nat. Prod.* **2013**, *76*, 2298–2306. [[CrossRef](#)] [[PubMed](#)]
102. Pardo-Vargas, A.; de Barcelos Oliveira, I.; Stephens, P.; Cirne-Santos, C.; de Palmer Paixão, I.; Ramos, F.; Jiménez, C.; Rodríguez, J.; Resende, J.; Teixeira, V.; et al. Dolabelladienols A–C, New Diterpenes Isolated from Brazilian Brown Alga *Dictyota pfaffii*. *Mar. Drugs* **2014**, *12*, 4247–4259. [[CrossRef](#)]
103. de Souza Barros, C.; Cirne-Santos, C.C.; Garrido, V.; Barcelos, I.; Stephens, P.R.S.; Giongo, V.; Teixeira, V.L.; de Palmer Paixão, I.C.N. Anti-HIV-1 activity of compounds derived from marine alga *Canistrocarpus cervicornis*. *J. Appl. Phycol.* **2016**, *28*, 2523–2527. [[CrossRef](#)]
104. Stephens, P.R.S.; Cirne-Santos, C.C.; de Souza Barros, C.; Teixeira, V.L.; Carneiro, L.A.D.; Amorim, L.; dos, S.C.; Ocampo, J.S.P.; Castello-Branco, L.R.R.; de Palmer Paixão, I.C.N. Diterpene from marine brown alga *Dictyota friabilis* as a potential microbicide against HIV-1 in tissue explants. *J. Appl. Phycol.* **2017**, *29*, 775–780. [[CrossRef](#)]
105. Miceli, L.; Teixeira, V.; Castro, H.; Rodrigues, C.; Mello, J.; Albuquerque, M.; Cabral, L.; de Brito, M.; de Souza, A.; Miceli, L.A.; et al. Molecular Docking Studies of Marine Diterpenes as Inhibitors of Wild-Type and Mutants HIV-1 Reverse Transcriptase. *Mar. Drugs* **2013**, *11*, 4127–4143. [[CrossRef](#)] [[PubMed](#)]
106. Pardo-Vargas, A.; Ramos, F.A.; Cirne-Santos, C.C.; Stephens, P.R.; Paixão, I.C.P.; Teixeira, V.L.; Castellanos, L. Semi-synthesis of oxygenated dolabellane diterpenes with highly in vitro anti-HIV-1 activity. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4381–4383. [[CrossRef](#)] [[PubMed](#)]
107. Karadeniz, F.; Kang, K.-H.; Park, J.W.; Park, S.-J.; Kim, S.-K. Anti-HIV-1 activity of phlorotannin derivative 8,4''-dieckol from Korean brown alga *Ecklonia cava*. *Biosci. Biotechnol. Biochem.* **2014**, *78*, 1151–1158. [[CrossRef](#)] [[PubMed](#)]
108. Tan, S.; Yang, B.; Liu, J.; Xun, T.; Liu, Y.; Zhou, X. Penicillixanthone A, a marine-derived dual-coreceptor antagonist as anti-HIV-1 agent. *Nat. Prod. Res.* **2017**, 1–5. [[CrossRef](#)] [[PubMed](#)]
109. He, J.; Huang, W.; Zheng, S.; Vigorito, M.; Chang, S.L. Effects of docosahexaenoic acid on locomotor activity in ethanol-treated HIV-1 transgenic rats. *J. Neurovirol.* **2018**, *24*, 88–97. [[CrossRef](#)] [[PubMed](#)]
110. Mejia, E.J.; Loveridge, S.T.; Stepan, G.; Tsai, A.; Jones, G.S.; Barnes, T.; White, K.N.; Drašković, M.; Tenney, K.; Tsiang, M.; et al. Study of Marine Natural Products Including Resorcyclic Acid Lactones from *Humicola fuscoatra* That Reactivate Latent HIV-1 Expression in an in Vitro Model of Central Memory CD4+ T Cells. *J. Nat. Prod.* **2014**, *77*, 618–624. [[CrossRef](#)] [[PubMed](#)]
111. Zhao, Y.; Liu, D.; Proksch, P.; Zhou, D.; Lin, W. Truncateols O-V, further isoprenylated cyclohexanols from the sponge-associated fungus *Truncatella angustata* with antiviral activities. *Phytochemistry* **2018**, *155*, 61–68. [[CrossRef](#)]

112. Ahn, M.-J.; Yoon, K.-D.; Min, S.-Y.; Lee, J.S.; Kim, J.H.; Kim, T.G.; Kim, S.H.; Kim, N.-G.; Huh, H.; Kim, J. Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. *Biol. Pharm. Bull.* **2004**, *27*, 544–547. [[CrossRef](#)]
113. Artan, M.; Li, Y.; Karadeniz, F.; Lee, S.-H.; Kim, M.-M.; Kim, S.-K. Anti-HIV-1 activity of phloroglucinol derivative, 6,6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.* **2008**, *16*, 7921–7926. [[CrossRef](#)]
114. Cardona, M.L.; Fernández, I.; Pedro, J.R.; Serrano, A. Xanthones from *Hypericum reflexum*. *Phytochemistry* **1990**, *29*, 3003–3006. [[CrossRef](#)]
115. Negi, J.S.; Bisht, V.K.; Singh, P.; Rawat, M.S.M.; Joshi, G.P. Naturally Occurring Xanthones: Chemistry and Biology. *J. Appl. Chem.* **2013**, *2013*, 1–9. [[CrossRef](#)]
116. Ahmed, M.; Husain, N.E. Managing dyslipidemia in HIV/AIDS patients: Challenges and solutions. *HIV/AIDS* **2014**, *7*, 1. [[CrossRef](#)] [[PubMed](#)]
117. Vieira, A.D.S.; Silveira, G.R.M. da Effectiveness of n-3 fatty acids in the treatment of hypertriglyceridemia in HIV/AIDS patients: A meta-analysis. *Cien. Saude Colet.* **2017**, *22*, 2659–2669. [[CrossRef](#)] [[PubMed](#)]
118. Maria Jose MB, M.N.; MB, M.J.; Agudelo, M.; Yndart, A.; Vargas-Rivera, M.E. Platelets Contribute to BBB Disruption Induced by HIV and Alcohol. *J. Alcohol. Drug Depend.* **2015**, *3*, 1–6. [[CrossRef](#)]
119. Flora, G.; Pu, H.; Lee, Y.W.; Ravikumar, R.; Nath, A.; Hennig, B.; Toborek, M. Proinflammatory synergism of ethanol and HIV-1 Tat protein in brain tissue. *Exp. Neurol.* **2005**, *191*, 2–12. [[CrossRef](#)] [[PubMed](#)]
120. Acheampong, E.; Mukhtar, M.; Parveen, Z.; Ngoubilly, N.; Ahmad, N.; Patel, C.; Pomerantz, R.J. Ethanol strongly potentiates apoptosis induced by HIV-1 proteins in primary human brain microvascular endothelial cells. *Virology* **2002**, *304*, 222–234. [[CrossRef](#)]



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