Marine Natural Products as Lead Anti-HIV Agents

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Abstract: Current anti-HIV drugs have extreme side effects and resistance to these drugs develops rapidly. The marine environment holds an unprecedented number of unusual chemical structural classes with activity against HIV. We review the literature on anti-HIV activity of marine natural products and discuss the efficacy of different structural classes.

Key Words: AIDS, HIV, marine natural products, structure-activity relationships, macrolides, modified peptides, alkaloids.

INTRODUCTION

In 1998, Acquired Immune Deficiency Syndrome (AIDS), the final stage in a pathogenic disease caused by the human immunodeficiency virus (HIV), is one of the top five most deadly diseases worldwide [1]. AIDS is an ongoing epidemic, with death tolls rising every year. In 2001, 5 million people were newly infected with HIV worldwide, causing the number of people presently living with HIV or AIDS to soar to 40 million globally [2]. Since the beginning of this epidemic a mere 20 years ago, an estimated 24.8 million have died from AIDS and/or related opportunistic infections, and there is no sign of a decrease in the global death rate, as 3 million died from AIDS in 2001 [2].

There is a light on the horizon, however, as death rates from AIDS are declining among certain populations [3]. In the industrialized nations of North America and Western Europe, new combinations of anti-HIV drugs may reduce viral loads, delay the onset of AIDS and prolong the lives of people living with AIDS. In the United States, death rates from AIDS declined markedly in 1996-1997 [3], although this decline has since leveled off [2]. Unfortunately, 96% of the HIV/AIDS population presently lives in developing nations [2], where these drug therapies may not be available or are too expensive.

Clearly, AIDS represents one of the most important and complex public health problems to face the modern world. Over the past two decades, giant strides have been made in producing drugs that provide varying levels of protection from this disease, although none of the currently developed drugs can effectively and predictably eradicate HIV from the body. As there is no evidence that this global epidemic is losing momentum, the search for novel treatments remains urgent. In this paper, we review the current literature on the search for anti-HIV leads among marine natural products. In addition, we report the results of evaluating our own library of marine natural products for anti-HIV-1 activity.

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CURRENT ANTI-HIV DRUGS AND THEIR TARGETS

Drug therapies presently in use for the treatment of HIV infection and AIDS reduce viral replication and can slow the progression of the disease, but cannot rid the body of HIV. Current and experimental drug therapies target several sites of action in the viral replication cycle. These targets were recently reviewed more thoroughly [4], and will only be briefly mentioned here. Targets of particular interest in the treatment of HIV infection include the initial recognition and binding of the viral envelope protein gp120 to the cellular receptor CD4 with subsequent cell fusion, viral RNA replication, integration into the host cell nucleus, transcription or translation, and assembly and release of new viral particles.

Several nucleosides inhibit the viral RNA-dependent DNA polymerase (reverse transcriptase), which catalyzes the transcription of HIV genetic material from RNA to DNA. These drugs act by binding to reverse transcriptase with greater selectivity than the viral DNA. Because they lack a 3'-hydroxyl group, incorporation of these compounds by reverse transcriptase causes termination of DNA chain elongation and viral replication halts. To date, most approved inhibitors of HIV reverse transcriptase are 2',3'-dideoxynucleoside analogues (nRTI), and include abacavir (**1**), didanosine (**2**), lamivudine (**3**), stavudine (**4**), zalcitabine (**5**) and zidovudine (AZT) (**6**) [5]. Several additional nRTIs are in various stages of development, including emtricitabine, which is in Phase III clinical trials [6]. A recently approved nucleotide reverse transcriptase inhibitor, tenofovir (**7**), acts in a similar manner to these nRTIs [7]. Despite their common mechanism of action, these drugs differ substantially in their pharmacologic properties, and all are subject to rapid development of viral resistance [5]. Non-nucleoside reverse transcriptase inhibitors (NNRTI), such as nevirapine (**8**), efavirenz (**9**) and delavirdine (**10**), bind to reverse transcriptase near its active site and interfere with

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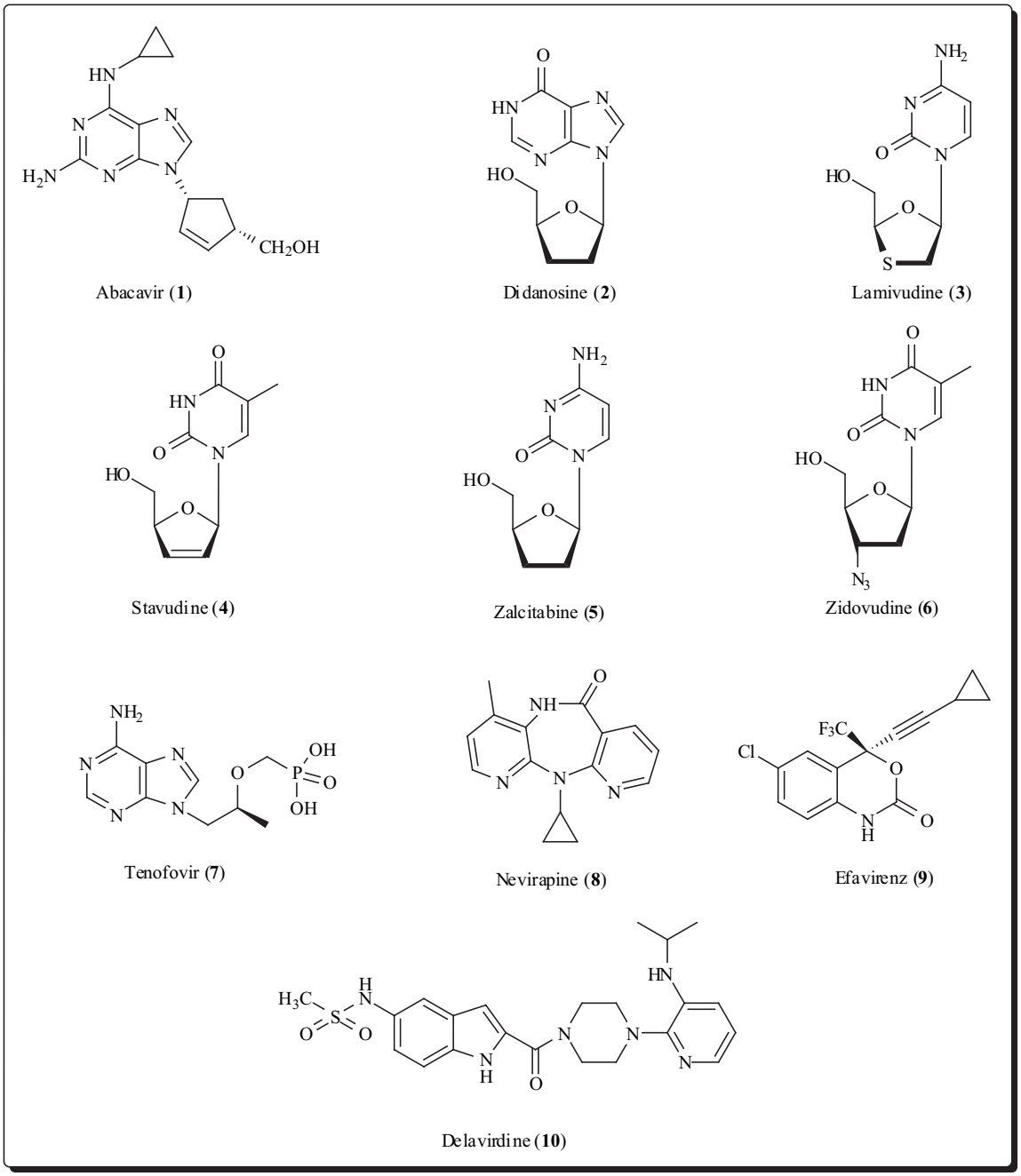
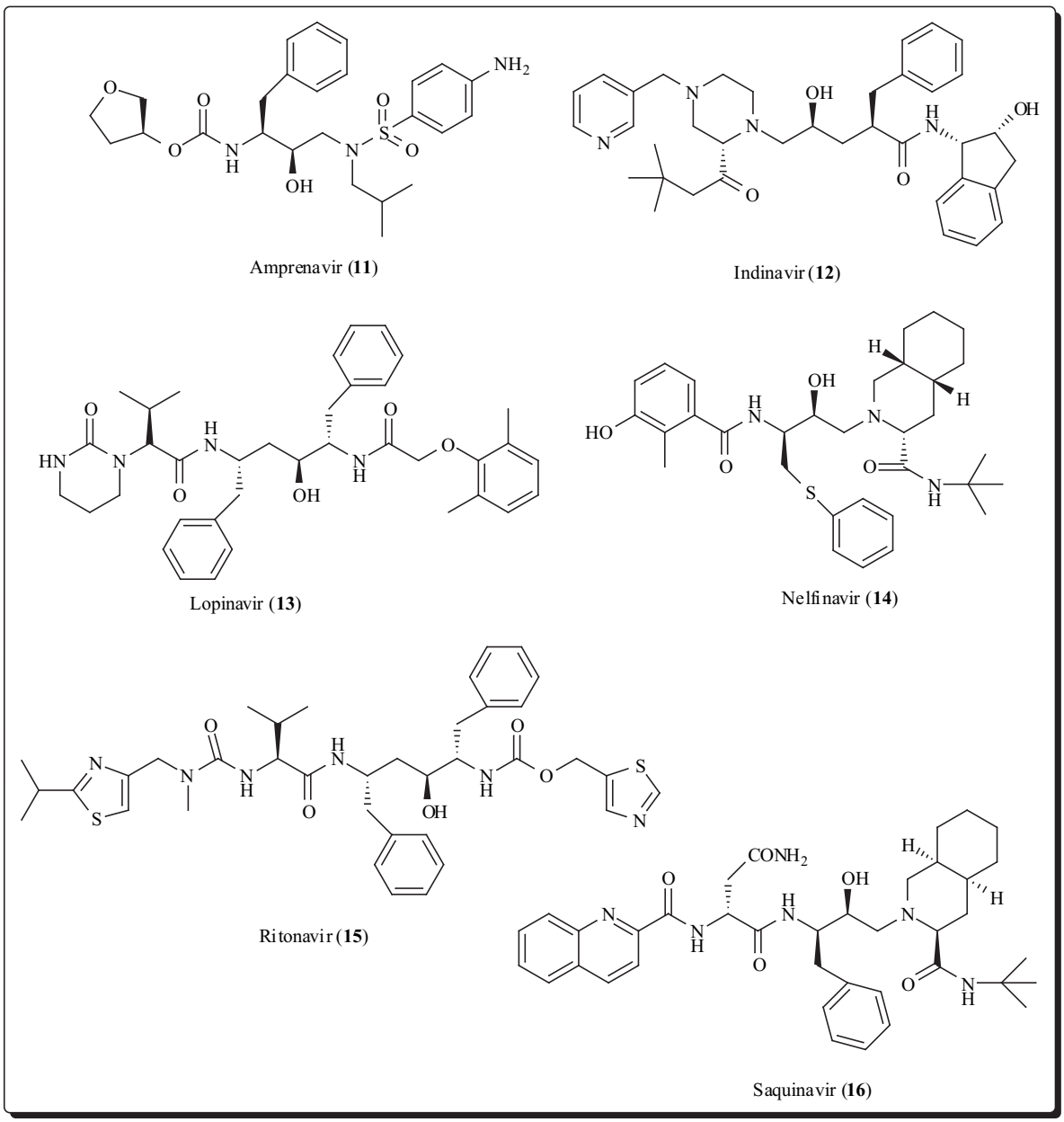


enzyme activity [5]. These compounds are particularly prone to the development of resistance, and it is hoped that several NNRTIs presently in Phase I and II clinical trials will have reduced susceptibility to resistance.

HIV protease is an enzyme that modifies structural and regulatory polyproteins into their active forms. In the absence of this protease, viral enzymes, including reverse transcriptase, integrase and the protease itself, are not produced. Protease inhibitors block the activity of this enzyme and infectivity of the virus is severely diminished [5]. FDA-approved protease inhibitors include amprenavir (**11**), indinavir (**12**), lopinavir (**13**), nelfinavir (**14**), ritonavir (**15**) and saquinavir (**16**) [5], with several additional protease inhibitors presently in clinical trials. Although highly effective, current protease inhibitors are subject to rapid development of viral resistance and treatment adherence problems caused by severe side effects. Experimental protease inhibitors, including atazanavir and tipranavir, both in Phase III clinical trials, may have the advantages of reduced side effects and/or efficacy against HIV that is already resistant to other protease inhibitors [6].

Drug therapies are costly and require medical monitoring and adherence to complex treatment schedules [7-9]. In addition, present drug therapies are not effective in all patients and side effects can be extreme. Genetic variation is a hallmark of HIV and drug resistance develops rapidly in a large percentage of patients [5]. Highly active anti-retroviral therapies (HAART) use combinations of these drugs and appear to be effective in delaying, although not preventing, the development of resistance [7-9]. HAART may provide additional benefits, including the targeting of different stages of the viral replication cycle and possible synergistic effects



of different drugs [10]. For example, there is evidence that drugs which block reverse transcriptase are more effective in reducing viral load when taken in combination with a protease inhibitor [5, 7, 9], although recent data indicate that a protease-sparing regimen (2 nRTIs + 1 NNRTI) is also highly effective [8].

Treatment with combination antiretroviral therapy can reduce viral load in plasma to undetectable levels, however, there has not been a therapy shown to completely eliminate HIV from the body. Even following antiretroviral therapy, latent, replication-competent virus remains in resting memory CD4 lymphocytes and in other reservoirs that may persist after two years of seemingly effective antiretroviral therapy [11-13]. Recent evidence suggests that even in patients whose treatment suppressed viral replication below detection limits, it would take a lifetime for this latent reservoir of HIV to be extinguished [14]. This long-lived viral reservoir represents a major obstacle to virus eradication [14, 15]. Since HIV infection cannot be entirely eradicated with current treatments, there is clearly a need for novel anti-HIV strategies.

Several experimental drugs that target different stages in the HIV replication cycle are currently in the pipeline. Initial binding of HIV gp120 to CD4 on the host cell and entry of the virus into the host cell are the targets of several experimental treatments. Of these, one is a natural product. Cyanovirin-N, a protein isolated from the terrestrial cyanobacterium *Nostoc ellipsisporum*, binds to gp120 and gp41 and thereby inhibits binding to CD4. Cyanovirin-N is undergoing consideration as a topical vaginal microbicide to inhibit sexually transmitted HIV [16]. T-20, a synthetic peptide, inhibits cell fusion by mimicking the activity of the HR-2 region of gp41. After gp120 has attached to gp41, T-20 mimics HR-2 and binds to gp120, thereby blocking the conformational changes required for viral/cell membrane fusion [16]. T-20 has been approved by the US FDA in 2003. Other attachment and fusion inhibitors include PRO 2000, another topical microbicide in Phase II clinical trials,

and PRO 542, which uses recombinant soluble CD4 to bind to and neutralize gp120 before binding can occur [16], as well as several chemokine receptor stimulators. Integrase inhibitors interfere with the gene that integrates proviral HIV-DNA into the host cell genome. The development of integrase inhibitors has been plagued with difficulties, but S-1360, an experimental integrase inhibitor with potent activity is now in clinical trials [6]. Additional experimental drugs are designed to stimulate the body's own immune system. These include cytokines (e.g. interleukin-2 and multikine, a mixture of several cytokines), genetically engineered HIV antibodies, and the natural product resveratrol, an antimicrobial and immunostimulatory compound from the skin of red grapes [17]. Resveratrol is presently in Phase I clinical trials. In addition, a large number of vaccines are also undergoing development and are in various stages of clinical trials [18]. Although vaccines may represent the best hope for widespread protection from HIV, setbacks in the development of vaccines, including the possibility of superinfection with a second strain of HIV, suggest that development of an effective vaccine is still many years away [14]. Therefore, the search for effective drugs against a variety of HIV targets is clearly a priority.

MARINE NATURAL PRODUCTS AS ANTI-HIV COMPOUNDS

Extensive screening of natural products has identified numerous compounds with confirmed activity against HIV [19-21]. In 1988, the National Cancer Institute (NCI) screened more than 20,000 samples in a primary microculture XTT antiviral assay, and approximately 1% inhibited cytopathicity of HIV-1 [22]. More recently, extracts from 694 strains (334 species) of cultured cyanobacteria were screened in the NCI primary assay for anti-HIV-1 activity and yielded positive results in 10.4% of species [23]. As of 2001, the NCI Developmental Therapeutics Program had identified 90 natural products with significant activity against HIV-1 [4]. Two natural products, cyanovirin-N and resveratrol, are presently undergoing clinical testing (see above).

The marine environment is home to incredible biodiversity in both flora and fauna, which produce metabolites of unprecedented chemical diversity [24]. Natural products from marine organisms have served as important models for the development of antiviral drugs, and these are discussed more fully in earlier reviews [4, 25]. As early as the 1950's, a series of arabinosides were identified from the sponge *Cryptotethia crypta*, and these compounds, including spongothymidine (ara-T; **17**) [26, 27] provided the inspiration to modify not only the bases but also the sugar moieties of nucleosides [4]. Ultimately, this discovery led to the synthesis of other bioactive nucleosides, including 9-β-D-arabinofuranosyladenine (ara-A; **18**) and 1-β-D-arabinosylcytosine (ara-C; **19**), potent antiviral and anticancer agents, respectively [28]. Ara-A was later identified from a natural source, the gorgonian *Eunicella cavolini* [29]. The development of 3'-azido-3'-deoxythymidine (AZT), the first anti-HIV drug, was part of these research efforts to modify nucleosides [25]. Recently, screening of marine organisms for anti-HIV activity has been gaining momentum. For example, extracts from 39 species of red algae from California were screened for reverse transcriptase activity and all but three extracts (92%) were active [30], extracts from 49 species of algae and cyanobacteria were screened in two types of assays (inhibition of HIV killing of CD4 cells and inhibition of HIV-induced cell fusion) and 12% of extracts were active in both assays [31], and 15.9% of 88 extracts from cultured marine cyanobacteria were active in the NCI's primary anti-HIV assays [23]. Natural products isolated from marine organisms are emerging as excellent resources for novel anti-HIV leads [32].

In this paper, we review the literature on marine natural products exhibiting anti-HIV activity. Anti-HIV activity of particular marine natural product structural classes (e.g. sulfated polysaccharides [33]) and marine taxonomic groups (e.g. cyanobacteria [23, 34], algae [30, 34]) has been the subject of several more specific reviews, and the more active compounds from these groups are included here, along with more recent published reports**.** In addition, we report the results of screening 93 chemically diverse marine-derived secondary metabolites for activity against HIV-1 using human peripheral blood mononuclear cells (PBM) with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a measure of cytotoxicity. Details of this assay have been described previously [35]. These 93 compounds included marine natural products, semisynthetics and bioconversion products using a marine natural product scaffold. This small library of compounds was assembled using bioassays (antimicrobial or cytotoxicity) or spectroscopy to guide their isolation and characterization. Even without the use of HIV activity to guide the isolation, 26 compounds (28%) showed activity when tested against HIV-1, with EC_{50} values <20 μM, and 12 compounds (13%) were active at EC_{50} values <5 μM (Table 1). Here we report the results of these active compounds as well as their less active analogs.

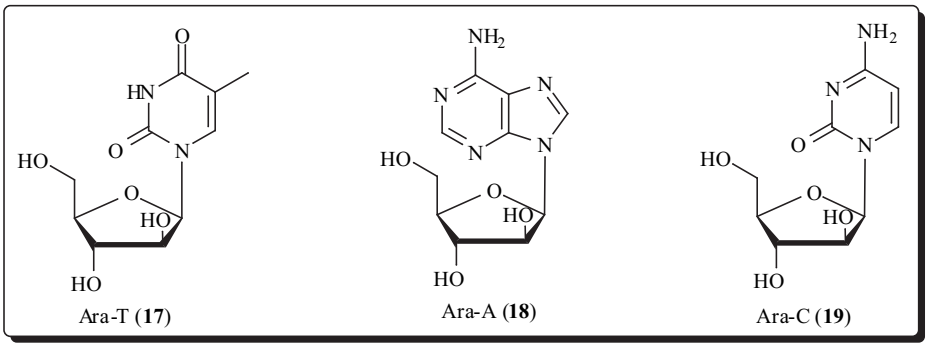


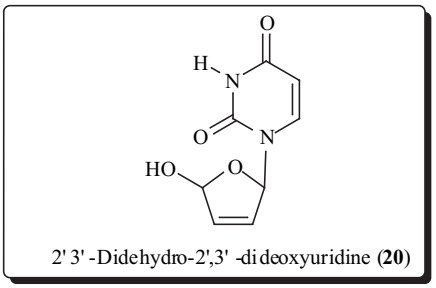
Table 1. Anti-HIV-1 Activity of Marine-Derived Compounds in Human Peripheral Blood Mononuclear (PBM) Cells. These data were generated using a previously described assay [35]. Briefly, stock solutions (20 to 40 mM) of the compounds were prepared in sterile DMSO and diluted to the desired concentration in growth medium. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantitated on day 6 after infection by a reverse transcriptase assay using $(rA)_n \bullet (dT)_{12-18}$ as template-primer. The DMSO present in the diluted solution (< 0.1%) had no effect on the virus yield. Toxicity of the compounds was also assessed in human PBM cells. The antiviral EC_{50} and cytotoxicity IC_{50} were obtained from the concentration-response curve using the median effective method. After incubation, actively metabolizing cells were quantified using the Cell Titer 96 Cell Proliferation Assay (MTT, Promega, Madison, WI), as described by the manufacturer. Compounds in this table are listed in order of greatest to least bioactivity. References in the table describe isolation and structural identification of the compounds.

Compound	Structural Class	HIV-1 in human PBM cells	Cytotoxicity in human PBM cells	
		EC_{50}, μM	IC_{50}, μM	References
AZT	Nucleoside	0.004	>100	35
Jaspamide (**24**)	Modified peptide	0.019	< 1.0 (85)[a]	41
Halichondramide (**117**)	Macrolide	0.058	1.30	120
Swinholide A (**113**)	Polyketide dimeric macrolide	0.16	< 1.0 (80.7) [a]	123
Demethyl(oxy)aaptamine (**94**)	Alkaloid	0.34	1.20	98
Swinholide B (**114**)	Polyketide dimeric macrolide	0.52	1.77	123
Misakinolide A (**115**)	Polyketide dimeric macrolide	0.58	37.5	124
Isoaaptamine (**95**)	Alkaloid	0.58	< 9.10	98
Aaptamine (**96**)	Alkaloid	1.30	< 0.10	97
20-*O*-Acetylpuupehenone (**58**)	Semisynthetic sesquiterpene	0.70	59.1	63
(+)-Aeroplysinin-1 (**108**)	Dibrominated cyclohexadiene	2.50	3.76	110
Puupehedione (**59**)	Shikimate-sesquiterpene	3.01	65.9	62
15α,19,20-Tri-*O*-acetylpuupehenol (**60**)	Semisynthetic sesquiterpene	4.48	4.60	63
3,5-Dibromoverongiaquinol (**109**)	Dibrominated cyclohexadiene	6.10	4.90	125
Ilimaquinone (**69**)	Shikimate-sesquiterpene	6.60	< 1.0 (78.7) [a]	70
Fistularin-3 (**110**)	Dibrominated cyclohexadiene	6.9	10.3	111, 112
Prenylhydroquinone (**73**)	Prenylated quinone	9.7	65.7	126
Isoswinholide A (**116**)	Polyketide dimeric macrolide	10.3	23.4	123
Cordiachromene (**74**)	Prenylated chromene	11.3	62.4	127
15α-Cyano-19,20-di-*O*-acetylpuupehenol (**61**)	Semisynthetic sesquiterpene	11.4	38.6	63
15-Cyanopuupehenone (**62**)	Shikimate-sesquiterpene	11.6	64.0	63
Manzamine A (**102**)	β-Carboline alkaloid	12.9	5.1	128
Kahalalide F (**28**)	Depsipeptide	14.2	> 100	45
Latrunculin B (**111**)	Macrolide	16.4	> 100	129
Sarasinoside C1 (**50**)	Norlanostane oligoglycoside	16.5	~ 147	58
Manzamine E (**103**)	β-Carboline alkaloid	18.7	35.5	130
Puupehenone (**63**)	Shikimate-sesquiterpene	18.7	14.5	62
(-)-12,34-Oxamanzamine F (**107**)	β-Carboline alkaloid	20.4	75.2	107
15α-Nitromethyl-19,20-di-*O*-acetylpuupehenol (**64**)	Semisynthetic sesquiterpene	25.1	15.6	63
15α-(1-Nitroethyl)-19,20-di-*O*-acetylpuupehenol (**65**)	Semisynthetic sesquiterpene	30.8	34.9	63
15α-Methoxy-19,20-di-*O*-acetylpuupehenol (**66**)	Semisynthetic sesquiterpene	36.5	7.4	63
(-)-8-Hydroxymanzamine A (**105**)	β-Carboline alkaloid	44.2	4.9	105
Ircinal A (**106**)	β-Carboline alkaloid	45.2	34.2	106
(-)-Manzamine F (**104**)	β-Carboline alkaloid	94.8	40.8	105
Kahalalide G (**29**)	Linear peptide	> 100	> 100	44
Kahalalide A (**30**)	Depsipeptide	> 100	> 100	44

[a] Values in parentheses indicate percent inhibition at 1.0 μM.

Nucleosides

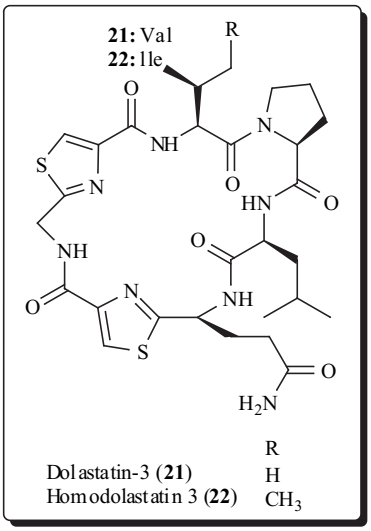
The nucleoside 2',3'-didehydro-2',3'-dideoxyuridine (**20**) was isolated from the Okinawan marine sponge *Aplysina* sp. [36]. This is the second isolation of this class of nucleosides from a natural source (including ara-A, described above), although synthetic analogs of this type and their 5'-triphosphate form are important inhibitors of HIV-1 reverse transcriptase and are presently in use as antiviral drugs. This same nucleoside was later isolated from an association of two Korean sponges, *Poecillastra* sp. with *Jaspis* sp. [37].



2' 3' -Didehydro-2',3' -dideoxyuridine (**20**)

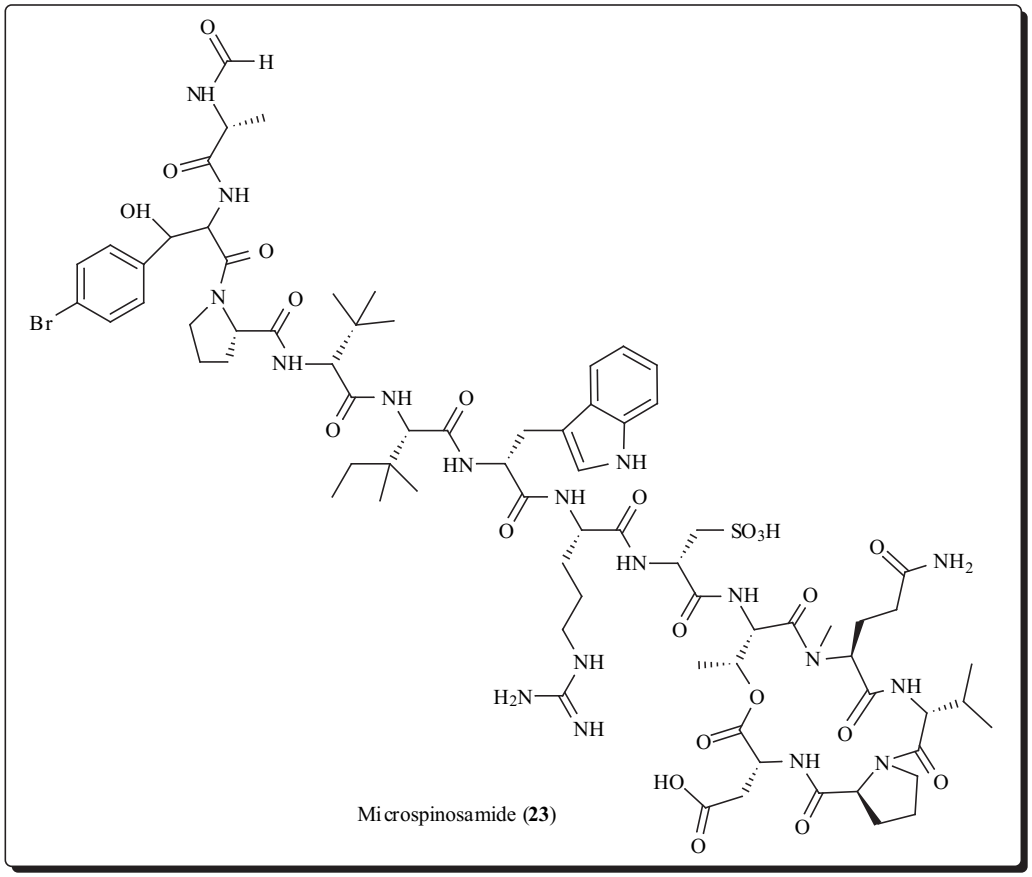
Peptides

Cyclic depsipeptides exhibit a wide range of activity against various stages of the HIV life cycle. Dolastatin 3 (**21**), a peptide isolated from a Palauan sample of the circumtropical cyanobacterium *Lyngbya majuscula* [38] and previously known from the sea hare *Dolabella auricularia* [39], inhibited HIV-1 integrase at relatively high concentrations (IC_{50} values of 5 mM for the terminal-cleavage and 4.1 mM for the strand-transfer reactions) [38]. In the same assay, homodolastatin 3 (**22**), which differs from dolastatin 3 by a single amino acid replacement, exhibited no anti-HIV-1 integrase activity. Even this minor activity of dolastatin 3 was lost after several months in the laboratory, and difficulties in handling, along with known cytotoxicity, have excluded this compound from further investigation [38].



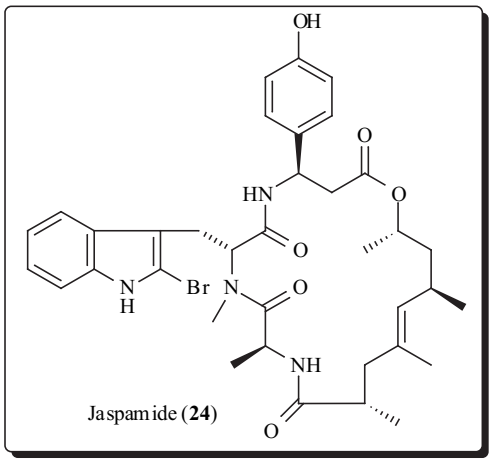
	R
Dolastatin-3 (**21**)	H
Homodolastatin 3 (**22**)	CH_3

Fortunately, other cyclic depsipeptides appear considerably more promising. Extracts from the Indonesian sponge *Sidonops microspinosa* exhibited activity in the NCI's primary anti-HIV screen, and the cyclic depsipeptide microspinosamide (**23**) was determined to be the active component [40]. Microspinosamide inhibited the cytopathic



Microspinosamide (**23**)

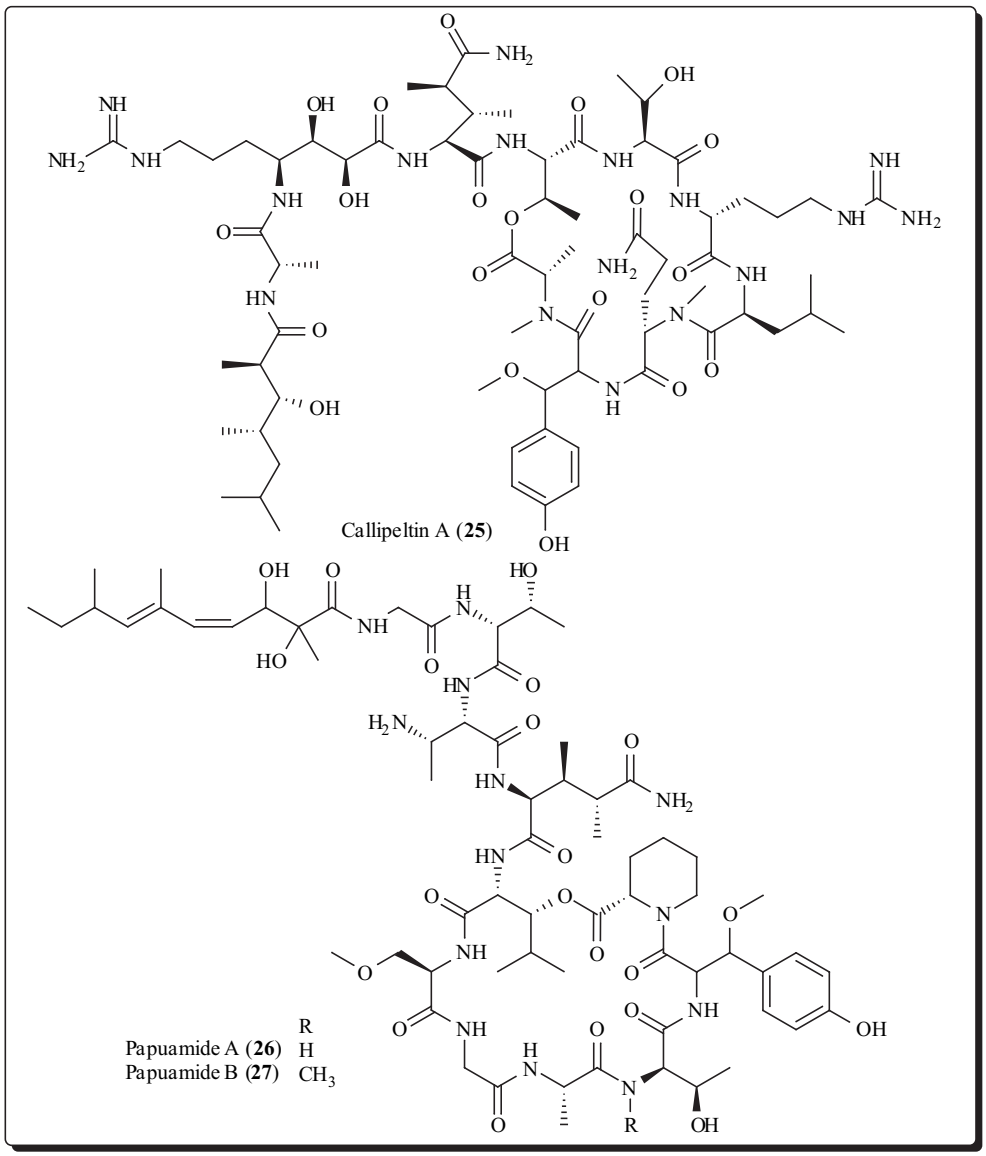
effect of HIV-1 in a cell-based assay with an EC_{50} of 0.2 μg/ml, but was cytotoxic to host cells at 3.0 μg/ml [40].

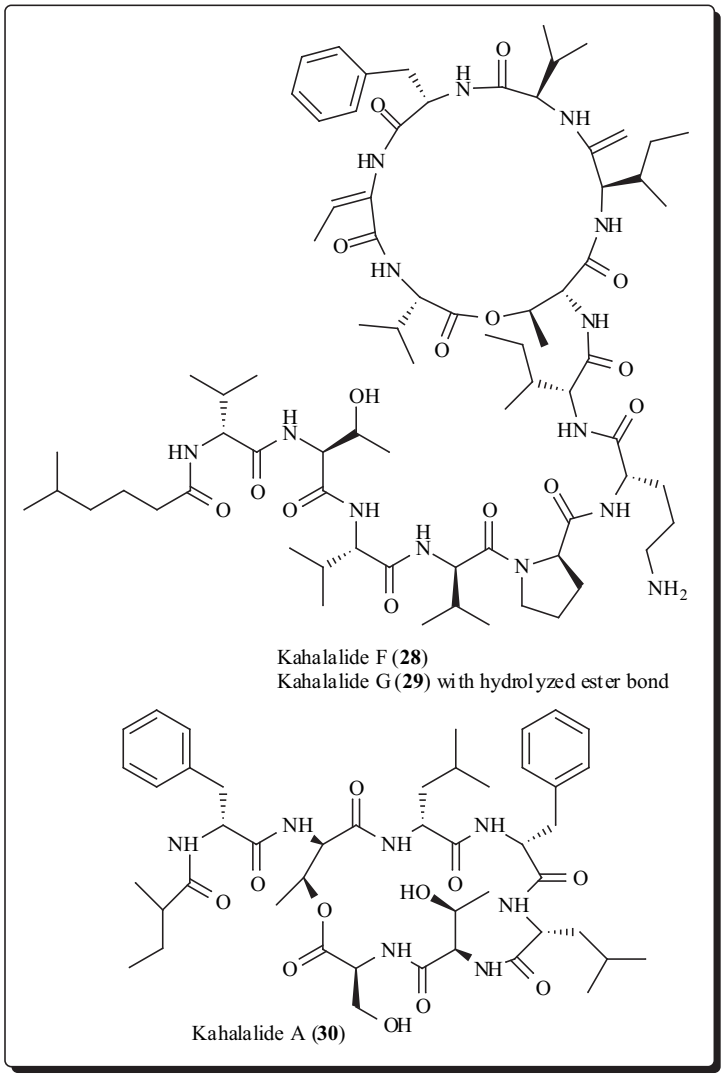


Jaspamide (**24**), an insecticidal and antimicrobial cyclic depsipeptide, was first isolated from the Indo-Pacific marine sponge *Jaspis* sp. [41]. With an EC_{50} of 0.019 μM, jaspamide represents the most active of the marine natural products assayed in the present study (Table 1), although this activity is an order of magnitude lower than that of the AZT control (EC_{50}, 0.004 μM) in the same assay [35]. Jaspamide's high cytotoxicity is a major limiting factor for its application as an HIV-1 lead. Clearly, structural modification of this molecule may improve activity and reduce cytotoxicity.

Other highly active cyclic depsipeptides include callipeltin A (**25**), isolated from anti-HIV extracts of the lithistid sponge *Callipelta* sp. collected in New Caledonia [42]. At six days post-infection, callipeltin A inhibited HIV-induced cytopathic effects with an ED_{50} of 0.01 μg/ml [42]. The cyclic depsipeptides papuamides A (**26**) and B (**27**), isolated from the sponges *Theonella mirabilis* and *T. swinhoei* from Papua New Guinea [43], are among the most promising marine natural products with anti-HIV activity to date. At six days post-infection, both compounds inhibited HIV-1 infection in human T-cells with an EC_{50} of 3.6 ng/ml, well below cytotoxic doses [43].

Kahalalides F (**28**), G (**29**) and A (**30**) are polypeptides isolated from the sacoglossan mollusk *Elysia rufescens* [44].





Kahalalide F (**28**)
Kahalalide G (**29**) with hydrolyzed ester bond

Kahalalide A (**30**)

Previous studies indicate activity against Herpes Simplex Virus II as well as antifungal and antibacterial activity for kahalalide F, but not for kahalalides A or G [44, 45]. Kahalalide F exhibited moderate activity against HIV-1 in the present study, with an EC_{50} of 14.2 μM, and with no cytotoxicity against PBM cells. Neither kahalalides G nor A exhibited any activity against HIV-1 in the present study (Table 1).

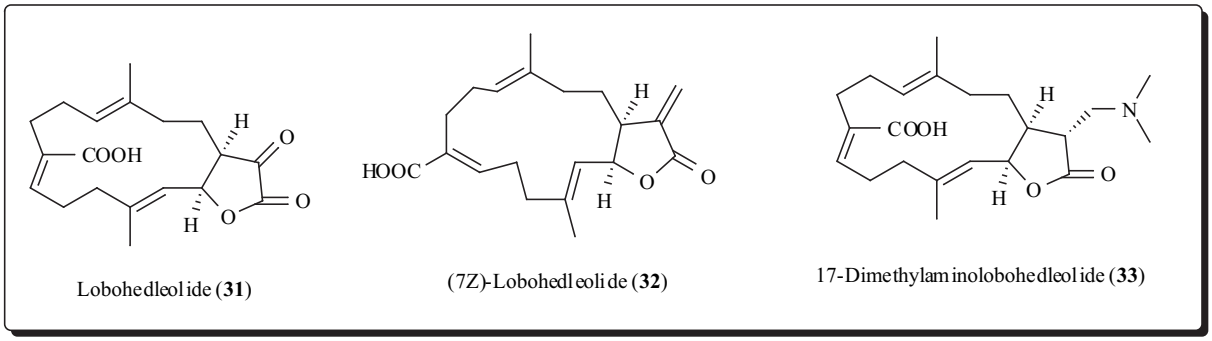
Proteins

The protein niphatevirin, isolated from the Bahamian sponge *Niphates erecta* [46], also had very impressive anti-HIV activity. Niphatevirin inhibited cytopathicity of HIV-1 in cultured human lymphoblastoid (CEM-SS) cells with an IC_{50} of 10 nM. Niphatevirin binds to CD4 and thereby interferes with binding of CD4 to gp120.

Terpenes

Cembrane Diterpenes

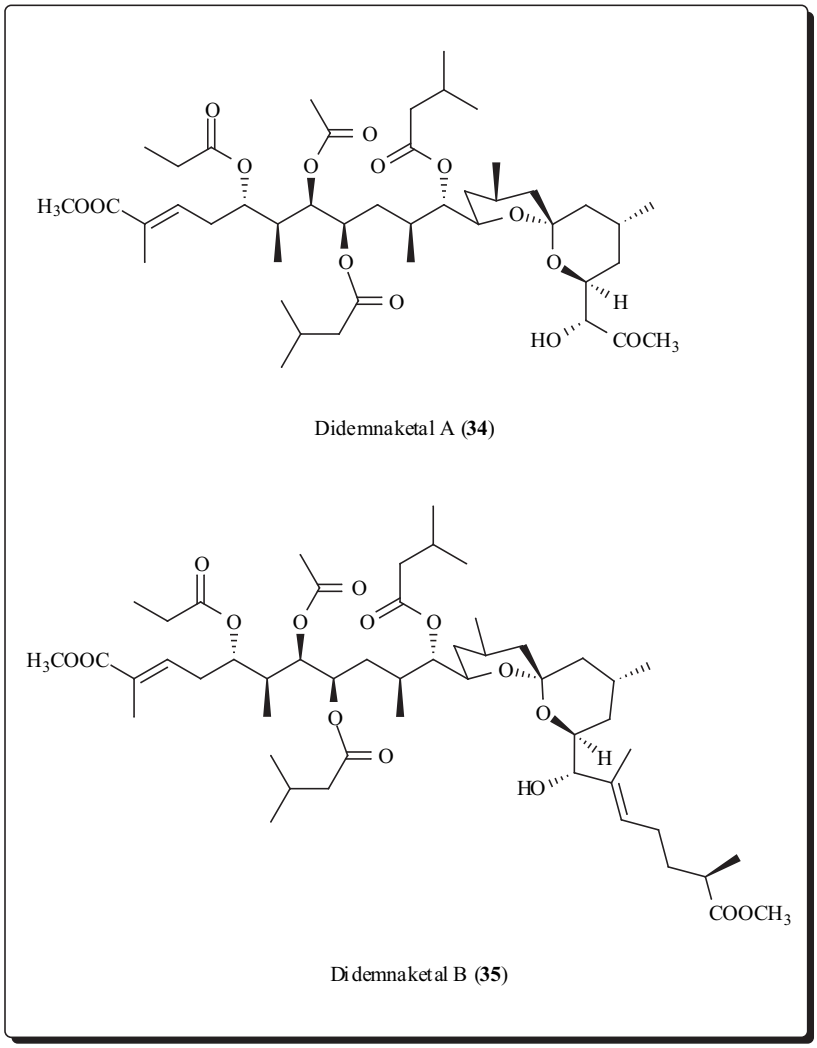
The cembranoid diterpenes lobohedleolide (**31**), (7*Z*)-lobohedleolide (**32**) and 17-dimethylaminolobohedleolide (**33**) were isolated from an anti-HIV extract of a Philippine



Lobohedleolide (**31**)

(7Z)-Lobohedleolide (**32**)

17-Dimethylaminolobohedleolide (**33**)



Didemnaketal A (**34**)

Didemnaketal B (**35**)

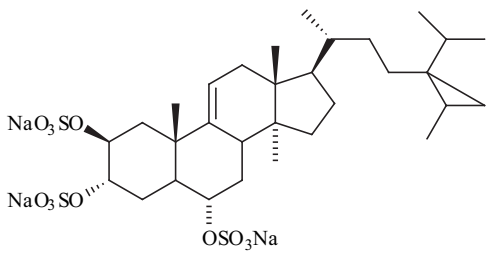
soft coral, *Lobophytum* sp. [47]. These compounds inhibited HIV-1 infection in a cell-based assay at EC_{50} 3.3-4.6 μg/ml; however, cytoprotection was only 55-70%.

Linear Heptaprenoid

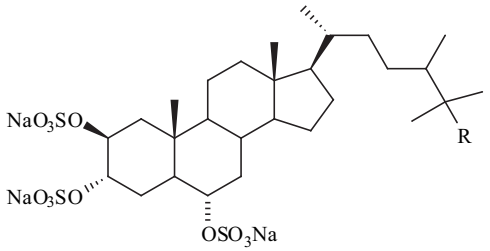
The crude extract from the ascidian *Didemnum* sp., collected in Palau, was active in an HIV-1 protease inhibition assay. The inhibition was traced to the compounds didemnaketal A (**34**) and B (**35**), which inhibit HIV-1 protease with IC_{50} values of 2 and 10 μM, respectively [48]. Didemnaketal A is believed to be an oxidation product of didemnaketal B, a linear heptaprenoid. In spite of promising activity in assays, these compounds were not pursued for further drug development due to the lability of esters under physiological conditions [48]. In an attempt to determine the minimum structure necessary for HIV-1 protease inhibition, analogs of didemnaketal A were synthesized [49]. Whereas most of the derivatives were less active than the natural product, the derivative showing activity comparable to that of the natural product itself, did not contain the free hydroxy group believed to be necessary for protease inhibition. Unlike most protease inhibitors, which block the active site of the protease and prevent binding to the substrate, this compound appears to prevent dimerization of HIV-1 protease itself, an unusual mechanism of action for protease inhibitors [49].

Sulfated Sterols

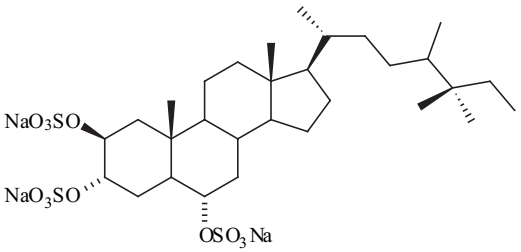
Anti-HIV screening of 22 marine sulfated sterols identified 15 compounds which were cytoprotective against HIV-1 infection in the NCI primary screen [50]. Sulfated sterols with anti-HIV activity are common to numerous genera of sponges [51], and four sponge sterols, ibisterol sulfate (**36**), halistanol sulfate (**37**), 26-methylhalistanol sulfate (**38**), and 25-demethylhalistanol sulfate (**39**), showed complete cytoprotection against HIV-1 infection with EC_{50} values of 13, 6, 3 and 6 μM, respectively [50]. In addition, 26-methylhalistanol sulfate inhibited viral replication at IC_{50} 12.2-24.4 μM. Halistanol sulfate prevented cytopathic effects of HIV-1 in target cells at EC_{50} 1.1-5.4 μM, but was cytotoxic to target cells at higher concentrations (IC_{50} 54-61 μM). Similar patterns of activity were seen with the other three compounds. In other studies, halistanol sulfates F (**40**) and G (**41**), isolated from the Mediterranean sponge *Pseudoaxinissa digitata*, were cytoprotective against HIV-1 in the NCI primary assays at EC_{50} values of 3 and 6 μg/ml, respectively [52]. Ibisterol sulfate, isolated from the Bahamian sponge *Topsentia* sp., was cytoprotective against HIV-1 with an EC_{50} value of 10 μg/ml [53]. Halistanol sulfate and ibisterol sulfates B (**42**) and C (**43**), isolated from a Philippine *Xestospongia* sp., inhibited HIV-1 integrase at IC_{50} 0.4, 2.3 and 1.8 μg/ml, respectively [54].



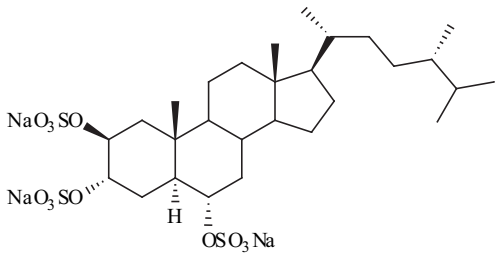
Ibisterol sulfate (**36**)



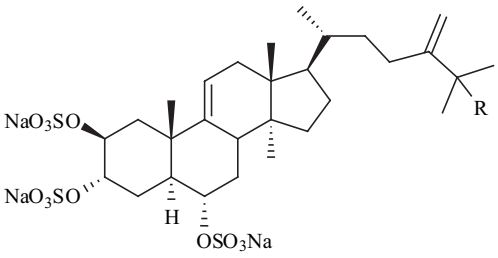
	R
Halistanol sulfate (**37**)	CH_3
25-Demethylhalistanol sulfate (**39**)	H
Halistanol sulfate F (**40**)	CH_2CH_3



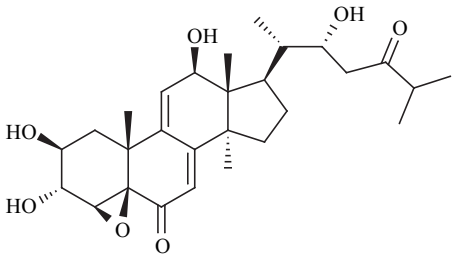
26-Methylhalistanol sulfate (**38**)



Halistanol sulfate G (**41**)



	R
Ibisterol sulfate B (**42**)	H
Ibisterol sulfate C (**43**)	CH_3

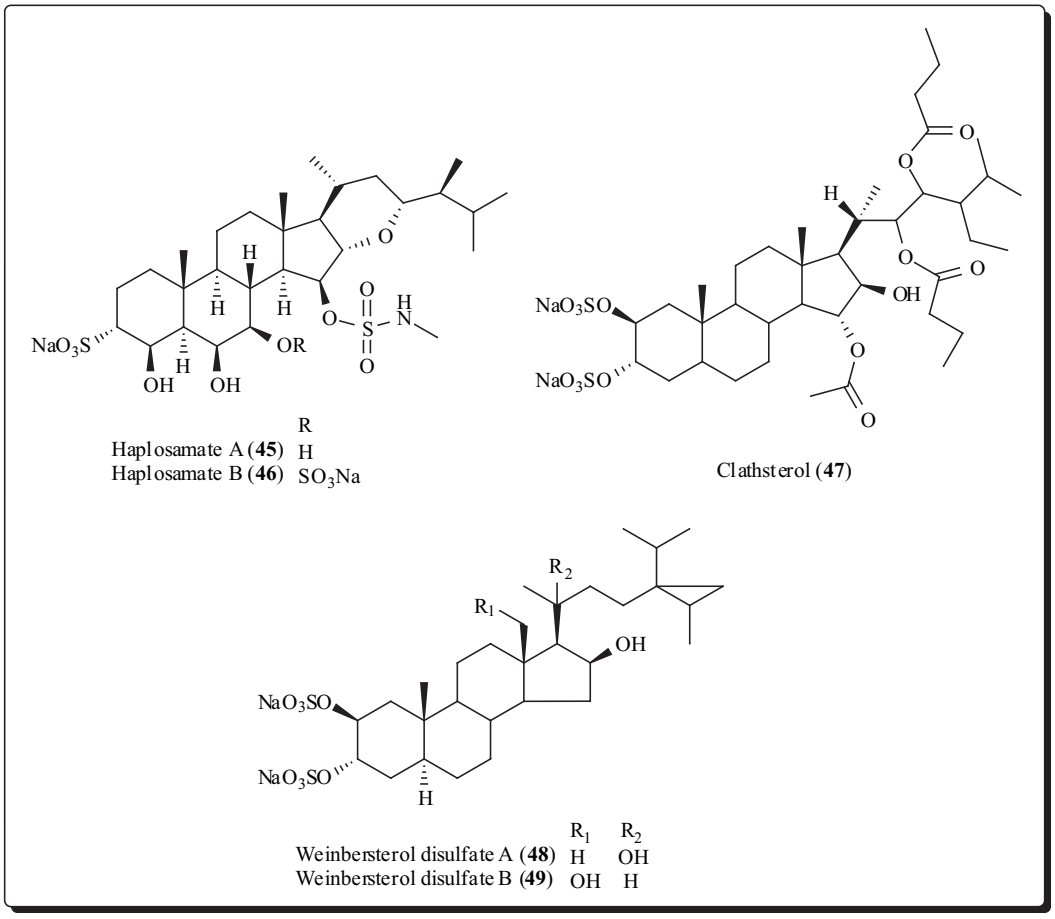


44β, 5β-epoxy-2β,3α,12β,22*S*-tetrahydroxy-14α-methylcholest 7,9(11)-dien-6,24-dione (**44**)

A non-sulfated sterol epoxide (**44**) from the same sponge was considerably less active (IC_{50} 26 μg/ml) [54].

In addition, four echinoderm sterols with sulfates on the A and B rings inhibited cytopathicity of HIV-1 at EC_{50} values ranging from 13-48 μM and aborted HIV-1 replication at slightly higher concentrations [50]. Seven sulfated sterols from ophiuroids also inhibited cytopathic effects of HIV-1 infection at considerably higher concentrations (EC_{50} 86-241 μM). Although some of these sulfated sterols were cytotoxic to host cells, cytoprotection and inhibition of viral replication occurred at concentrations below cytotoxic levels [50].

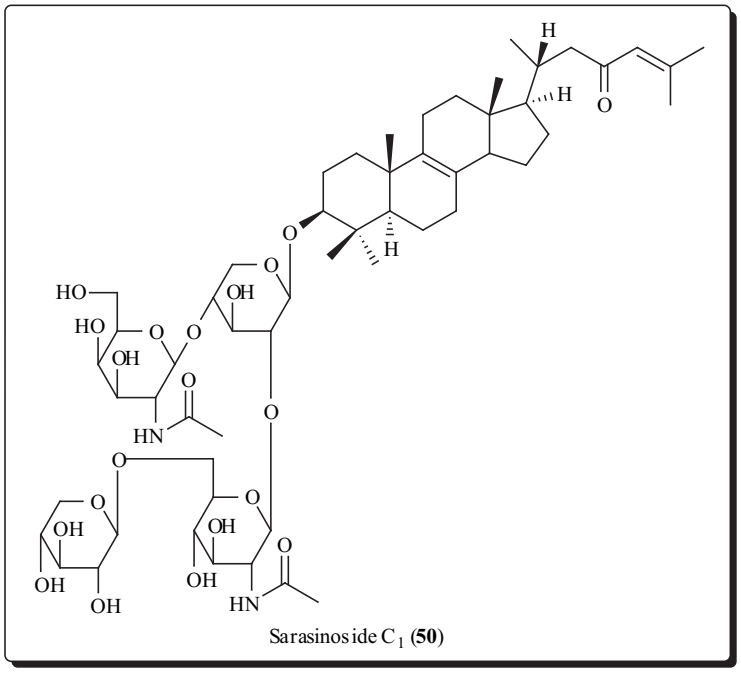
Haplosamates A (**45**) and B (**46**), steroidal sulfamate esters from two Philippine sponges, inhibit HIV-1 integrase with IC_{50} values of 50 and 15 μg/ml, respectively [55]. Clathsterol (**47**), a sulfated sterol isolated from the Red Sea

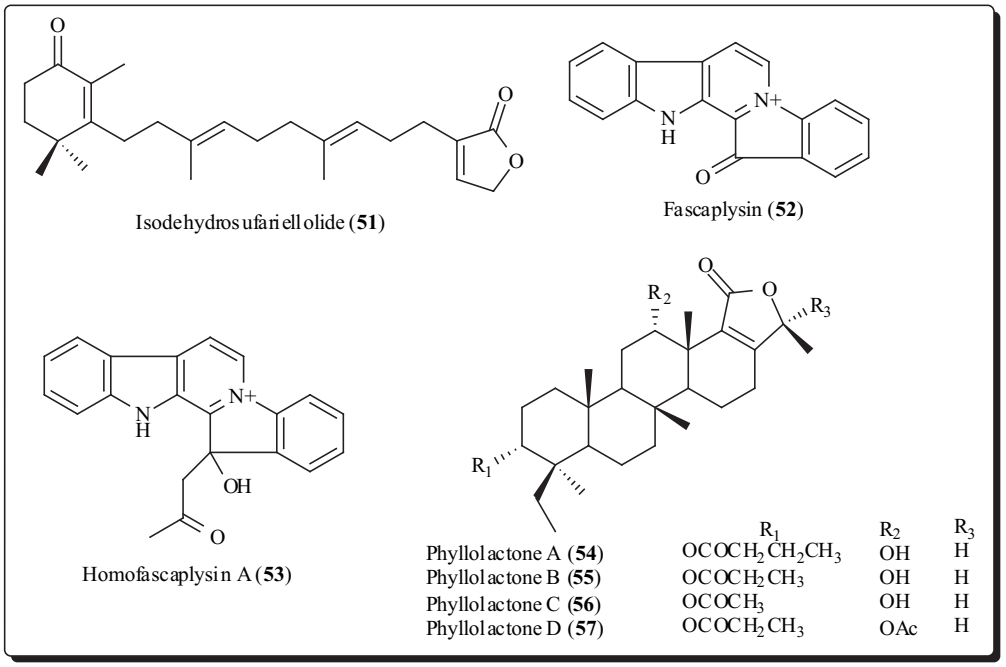


sponge *Clathria* sp., inhibited HIV-1 reverse transcriptase at 10 μM [56]. Two sulfated tetrahydroxy sterols, weinbersterol disulfates A (**48**) and B (**49**), isolated from the Bahamian sponge *Petrosia weinbergi*, showed significantly greater anti-HIV activity, with EC_{50} values of 1.0 μg/ml [57].

Steroidal Glycosides

In the present study, the norlanostane oligoglycoside sarasinoside C1 (**50**), isolated from the Palauan sponge *Asteropus sarasinosum* [58], exhibited an EC_{50} of 16.5 μM against HIV-1 and low cytotoxicity.



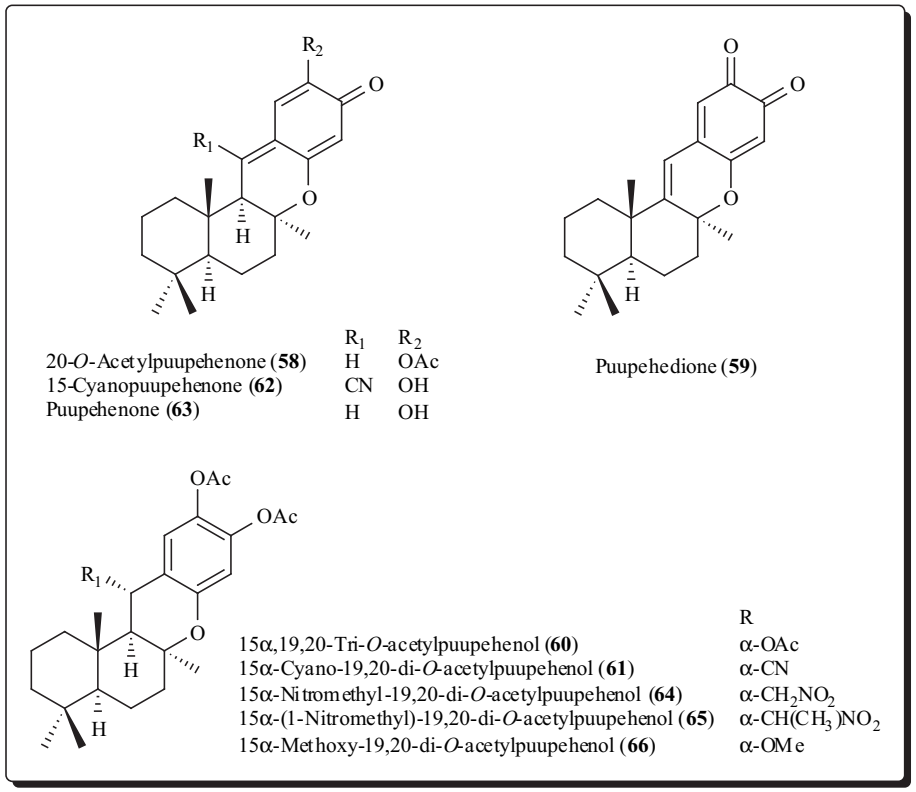


Sesterterpenes

A crude extract from the Fijian sponge *Fascaplysinopsis reticulata* exhibited inhibition against HIV reverse transcriptase with an IC_{50} of 0.4 μg/ml, and was further fractionated to determine the active constituents [59]. At 1 mg/ml, the sesterterpene isodehydrosuffariellolide (**51**) showed 81% inhibition of reverse transcriptase, and the alkaloid salts fascaplysin (**52**) and homofascaplysin A (**53**) showed inhibition of 58 and 94%, respectively [59]. A more recent study found that fascaplysin isolated from another Fijian sponge, *Hyrtios* cf. *erecta*, inhibited HIV-1 reverse transcriptase activity by 90% at 0.12 mM, although similar activity was not observed for homofascaplysin A [60]. In addtion, the novel bishomoscalarane sesterterpenes phyllolactones A-D (**54-57**), isolated from the Indo-Pacific sponge *Phyllospongia lamellose*, inhibited HIV-1 envelope-mediated fusion *in vitro* with IC_{50} values of 1.5-2.2 μM [61].

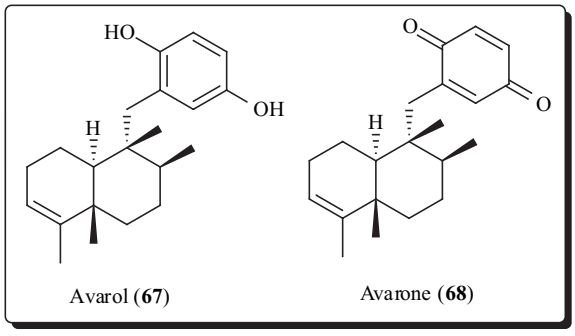
Hydroquinone/Quinone Terpenes

20-*O*-Acetylpuupehenone (**58**), puupehedione (**59**), 15α,19,20-tri-*O*-acetylpuupehenol (**60**), 15α-cyano-19,20-di-



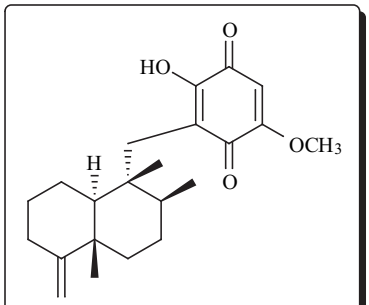
O-acetylpuupehenol (**61**), 15-cyanopuupehenone (**62**), puupehenone (**63**), 15α-nitromethyl-19,20-di-*O*-acetylpuupehenol (**64**), 15α-(1-nitroethyl)-19,20-di-*O*-acetylpuupehenol (**65**) and 15α-methoxy-19,20-di-*O*-acetylpuupehenol (**66**) are natural shikimate-sesquiterpene derived metabolites [62] or semisynthetic derivatives of puupehenone [63], isolated from sponges of the orders Verongida and Dictyoceratida. Among this group of compounds, activity against HIV ranged from an EC_{50} of 0.70 μM for 20-*O*-acetylpuupehenone (**58**) to an EC_{50} of 36.5 μM for 15α-methoxy-19,20-di-*O*-acetylpuupehenol (**66**). 20-*O*-Acetylpuupehenone (**58**) and puupehedione (**59**) exhibited the greatest activity against HIV-1, along with relatively low cytotoxicity, suggesting that the C-20 acetate or ketone is essential for optimal activity. 15α,19,20-tri-*O*-acetylpuupehenol (**60**), the next most active of this group, was considerably more cytotoxic. Compounds **61-66** had EC_{50} values ranging from 11.4-30.8 μM, but were significantly less toxic. Further synthetic modification of this class of compounds may prove useful in increasing activity against HIV-1.

Much excitement was generated over the sesquiterpene hydroquinone avarol (**67**) and its corresponding quinone avarone (**68**), originally isolated from the Mediterranean sponge, *Dysidea avara* [64], when it was later demonstrated that these compounds exert a cytoprotective effect on HIV-infected cells [65]. Avarol and avarone inhibited HIV reverse transcriptase and expression of p24 and p17 gag proteins at concentrations as low as 0.1 μg/ml (0.3 μM), well below cytotoxic doses [65]. It has been suggested that instead of inhibiting reverse transcriptase, avarol may effect its cytoprotection of HIV-infected cells by inhibiting the UAG suppressor glutamine tRNA, which is believed to be required for synthesis of the viral protease [66]. When applied at a concentration of 1 μg/ml, avarol not only suppressed synthesis of this tRNA almost completely, but expression of the gene encoding this tRNA was also inhibited. In spite of the early enthusiasm for avarol and avarone, later studies were unable to substantiate these claims of anti-HIV activity [67]. Numerous derivatives of avarol and avarone have been subsequently isolated from the Red Sea sponge *Dysidea cinerea*, and several of these compounds have been demonstrated to inhibit HIV-1 reverse transcriptase [68, 69].



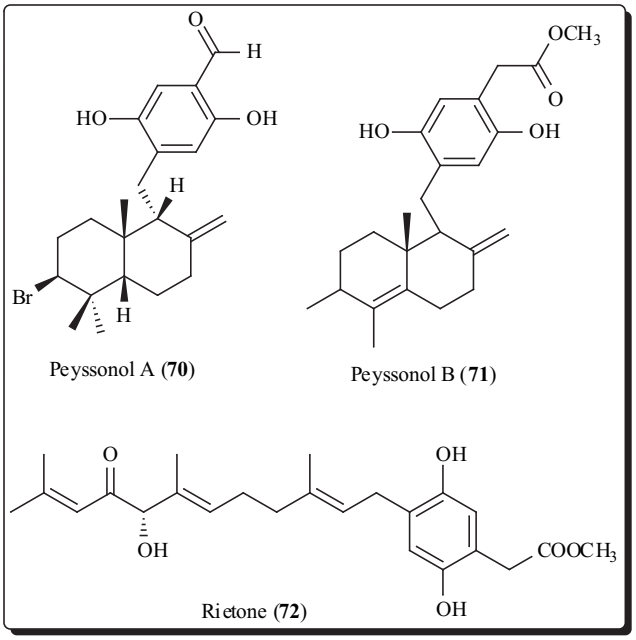
Avarol (**67**) Avarone (**68**)

Another sesquiterpene hydroquinone with anti-HIV activity is ilimaquinone (**69**), which was initially isolated from the Hawaiian sponge *Hippiospongia metachromia* [70]. Ilimaquinone was shown to selectively inhibit the RNase H function of HIV reverse transcriptase, an enzyme which degrades viral RNA after its corresponding DNA has been synthesized, thereby allowing formation of the second DNA strand [71]. Inhibition of RNase H function by ilimaquinone occurred at an IC_{50} value of 5.4 μg/ml [71]. In the present study, ilimaquinone exhibited an EC_{50} of 6.60 μM, significantly greater than that of other shikimate-sesquiterpenes that we tested (**58-60**), but had greater cytotoxicity.



Ilimaquinone (**69**)

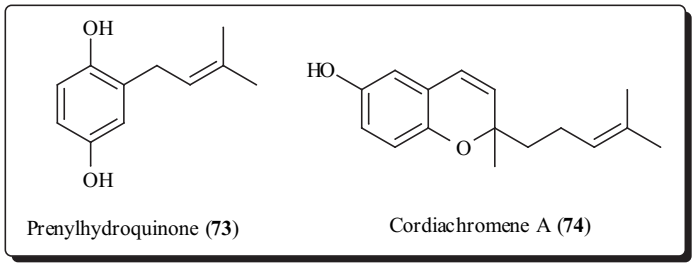
Two additional sesquiterpene hydroquinones, peyssonols A (**70**) and B (**71**) from the Red Sea alga *Peyssonnelia* sp., show potent inhibitory activity against the RNA-directed synthesis of both HIV-1 and HIV-2 reverse transcriptases [72]. Rietone (**72**), a sesquiterpene hydroquinone isolated from the South African soft coral *Alcyonium fauri*, also showed moderate inhibition (EC_{50} 1.23 μM and IC_{50} 9.32 μM) of HIV replication, although the assay did not determine at which stage in the viral replication cycle rietone was active [73].



Peyssonol A (**70**) Peyssonol B (**71**)

Rietone (**72**)

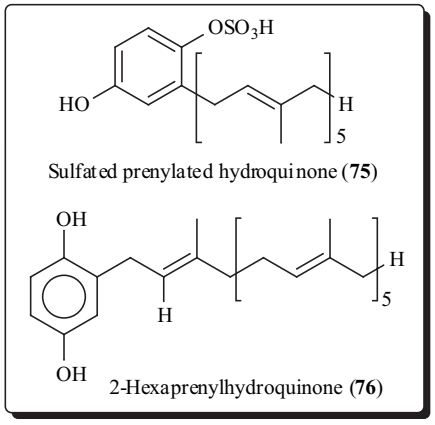
In the present study, prenylhydroquinone (**73**), an ascidian-derived prenylated quinone, was active against HIV-1 at an EC_{50} of 9.7 μM, well below cytotoxic doses. The 1,4-hydroquinone portion of this molecule clearly plays a critical role in its activity, as other sesquiterpene hydroquinones, such as avarone, were highly active against HIV with reasonable cytotoxicity. Cordiachromene A (**74**), another closely related ascidian-derived prenylated chromene with the C-4 blocked phenol functionality, was less active. These data, combined with the previously observed activity



Prenylhydroquinone (**73**) Cordiachromene A (**74**)

of avarol and avarone, suggest that studies focused on hydroquinone-derived libraries would be valuable.

A sulfated prenylated hydroquinone (**75**) isolated from the Australian sponge *Sarcotrogus* sp., inhibited both HIV-1 and HIV-2 reverse transcriptases at an IC_{50} of 5.7 μM [74]. This lack of specificity is relatively unusual for nonnucleoside reverse transcriptase inhibitors, although another prenylated quinone, 2-hexaprenylhydroquinone (**76**), isolated from the Red Sea sponge *Ircinia* sp., is also a general inhibitor of retroviral reverse transcriptases, including HIV-1 and HIV-2 reverse transcriptases [75].



Sulfated prenylated hydroquinone (**75**)

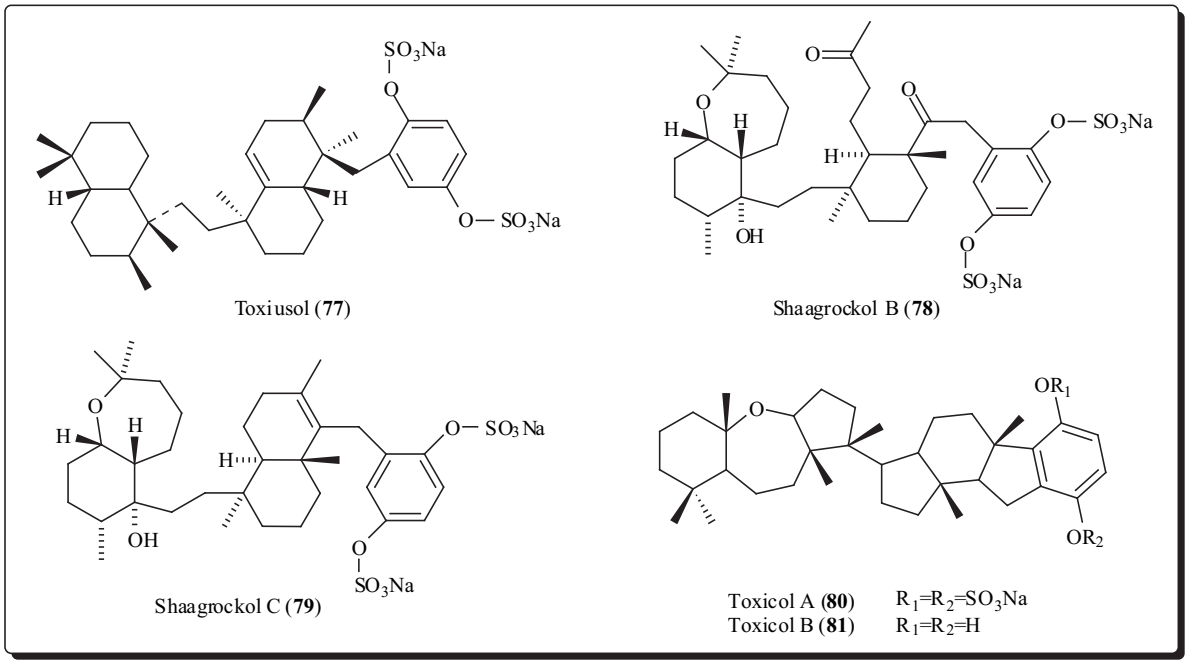
2-Hexaprenylhydroquinone (**76**)

Five sulfated hexaprenoid hydroquinones from the anti-HIV extract of the Red Sea sponge *Toxiclona toxius* exhibited potent inhibition of the RNA- and DNA-dependent DNA polymerase functions of HIV-1 reverse transcriptase, but with no inhibitory effect on RNase-H activity [76]. These compounds, toxiusol (**77**), shaagrockol B (**78**) and C (**79**), and toxicol A (**80**) and B (**81**), caused 50% inhibition of RNA-dependent DNA polymerase with IC_{50} values of 1.5-8.6 μM and of DNA-dependent DNA polymerase with IC_{50} values of 0.8-6.6 μM.

Despite promising anti-HIV activity in a variety of members of this class of compounds, no naturally occurring marine quinone or hydroquinone terpenes has advanced as a serious candidate for anti-HIV drug development [67]. One obstacle to this further development is the limited availability of large quantities of the compounds for follow-up studies. Chemical synthesis is the traditionally preferred route for obtaining large quantities of compounds, yet recent advances in molecular and cellular biology provide promising alternatives. Although these methods are not yet widely used, cell cultures of the sponge *Dysidea avara* were demonstrated to produce avarol at levels comparable to those of field-collected specimens [77].

Sulfated/Nonsulfated Polysaccharides, Polyphenols

Sulfated polysaccharides are believed to inhibit HIV infection by inhibiting virus binding to host cells [21], but there is some evidence that their antiviral activity may be independent of the effect of these compounds on the gp120-



Toxiusol (**77**)

Shaagrockol B (**78**)

Shaagrockol C (**79**)

Toxicol A (**80**) $R_1=R_2=SO_3Na$

Toxicol B (**81**) $R_1=R_2=H$

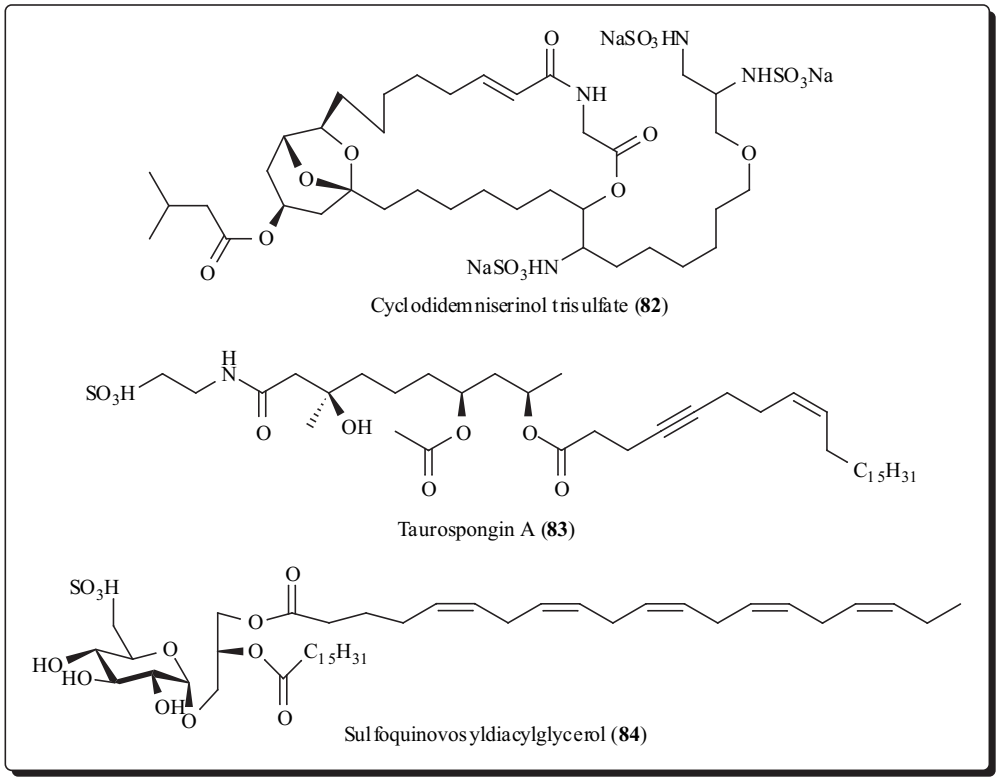
CD4 interaction, as sulfated polysaccharides appear to block HIV infection subsequent to viral adsorption and prior to viral entry into the host cell [78]. The seaweed compound fucoidan was effective at reducing viral load *in vitro* by four fold at a concentration of 10 μg/ml [78], activity comparable to that of dextran sulfate and its synthetic analog dextrin sulfate. Fucoidan appears to bind to a host cell membrane component other than CD4 and is an effective antiviral agent with low toxicity [78]. By contrast, although highly active, dextran sulfate is poorly absorbed by the body and produces toxic side effects, while dextrin sulfate is less toxic but also less effective. Additionally, a synergistic effect was demonstrated between fucoidan and AZT at low concentrations (e.g. 0.1 μg/ml fucoidan with 0.014 μM AZT) [79], and this synergism may permit the use of reduced AZT doses in AIDS patients.

Sulfated polysaccharides clearly have other mechanisms of action as well. Extracts from the Californian red alga *Schizymenia pacifica* contained a sulfated polysaccharide in the λ-carrageenan family, which selectively inhibited HIV reverse transcriptase [80]. Inhibition of reverse transcriptase occurred immediately upon addition of this algal extract [80]. Sulfated polysaccharides with anti-HIV activity may be widespread within the genus *Schizymenia.* A related species, *S. dubyi*, collected in Sicily, contains sulfated glucuronogalactan, which inhibits syncytium formation as well as HIV reverse transcriptase activity at 5 μg/ml [81, 82]. Interestingly, this alga exhibits annual variation in anti-HIV activity. Extracts from the alga collected during the spring/summer showed complete suppression of syncytium formation and reverse transcriptase activity at a concentration of 5 μg/ml, without corresponding cytotoxicity, whereas at other times of the year, similar suppression occurred at much higher concentrations [83].

Other examples of the anti-HIV activity of this class of compounds come from the aqueous extract of the brown seaweed *Fucus vesiculosus*, which inhibited both HIV reverse transcriptase activity and HIV-induced syncytium formation [84]. Further fractionation of the algal extract indicated that inhibition was due to a variety of polyphenols and both sulfated and non-sulfated polysaccharides, including fucoidan, which inhibited syncytium formation at 1-2.5 μg/ml and reverse transcriptase at 50 μg/ml [84]. Several other fractions were inhibitory in both reverse transcriptase and syncytium formation assays [84], as was carageenan, a common cell wall polysaccharide from red algae [30].

Although more widely reported from algae, sulfated polysaccharides from marine invertebrates also exhibit anti-HIV activity. Extracts of the tunicate *Didemnum molle* collected in Pohnpei and Indonesia, exhibited anti-HIV activity in preliminary screening. This activity was traced to the mucus exuded by the animals during collection, from which the sulfated mannose homopolysaccharide kakelokelose was identified as the active component [85]. A sulfated β-galactan isolated from the Korean clam *Meretrix petechialis* inhibited syncytium formation by 33% at 100 μg/ml and by 56% at 200 μg/ml, although dextran sulfate was considerably more active, with 65 and 95% inhibition at the same concentrations [86]. Rosacelose, a sulfated polysaccharide isolated from the aqueous extract of the Mediterranean sponge *Mixylla rosacea*, inhibited HIV-1 *in vitro* at an IC_{50} of 5 μg/ml [87].

Despite impressive anti-HIV activity of sulfated polysaccharides, this class of compounds has not been considered promising for drug development due to poor absorption in the body following oral administration and to their anticoagulant activity [33]. However, the use of these



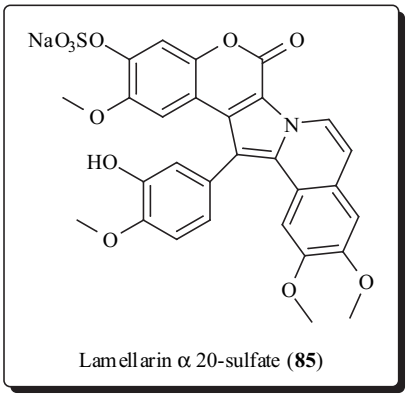
compounds in topical form (i.e., as prevention of sexually-transmitted HIV) may be a viable alternative to oral delivery [21]. In addition, anti-HIV activity of several sulfated polysaccharides, including the seaweed compounds fucoidan and carageenan, occurs at concentrations in the range of 0.5 to 12 μg/ml, which is 100-fold lower than their anticoagulant threshold [88]. Chemical modification of nonsulfated polysaccharides into effective anti-HIV compounds by addition of sulfate residues has met with some success [80].

Sulfolipids

The sulfated serinolipid cyclodidemniserinol trisulfate (**82**), isolated from the Palauan ascidian *Didemnum guttatum*, inhibited purified HIV integrase at IC_{50} 60 μg/ml, however, this compound behaved similarly toward a control enzyme (Molluscum contagiosum virus topoisomerase) and therefore the activity was nonselective [89]. The sulfolipid taurospongin A (**83**), isolated from the Okinawan sponge *Hippospongia* sp., inhibited HIV reverse transcriptase with an IC_{50} of 6.5 μM and low cytotoxicity [90]. Even more promising is sulfoquinovosyldiacylglycerol (**84**), a sulfolipid isolated from the Japanese red alga *Gigartina tenella*, which strongly and selectively inhibited DNA polymerases α and β and HIV-1 reverse transcriptase with IC_{50} values of 0.25, 3.6 and 11.2 μM, respectively [91]. Both the sulfo group and the unsaturated fatty acid chain components of this molecule were considered important for its inhibitory activity.

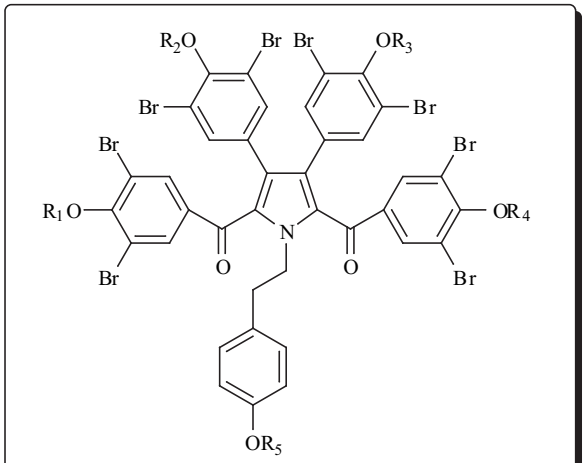
Alkaloids

Lamellarin α 20-sulfate (**85**) is a sulfated alkaloid isolated from didemnid ascidians [92]. Lamellarin α 20-sulfate inhibits terminal cleavage by HIV-1 integrase with an IC_{50} of 16 μM and strand transfer activity with an IC_{50} of 22 μM. In cell culture, this alkaloid also inhibited formation of preintegration complexes at an IC_{50} of 88 μM, provirus formation at an IC_{50} of 62 μM, and HIV replication (from initial fusion through integration and gene expression) at an IC_{50} of 8 μM, with relatively low cytotoxicity (LD_{50} 274 μM) [92].



Lamellarin α 20-sulfate (**85**)

The aromatic alkaloid polycitone A (**86**), isolated from the South African ascidian *Polycitor* sp., is a potent inhibitor of HIV-1 reverse transcriptase associated DNA polymerase and RNase H activities [93]. Polycitone A inhibited DNA polymerase at an IC_{50} of 0.25 μM, and RNase H at an IC_{50} of 30 μM, whereas its methylated derivatives (**87**, **88**) were less active (IC_{50} 3.7 and 9.3 μM against DNA polymerase and > 100 μM against RNase H). Unfortunately, polycitone A is a general inhibitor of DNA polymerases and therefore not sufficiently selective to serve as an anti-HIV drug, however, structural modification of polycitone A may improve its selectivity towards HIV enzymes [93].



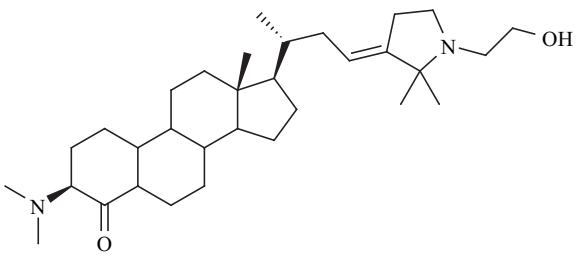
	R_1	R_2	R_3	R_4	R_5
Polycitone A (**86**)	H	H	H	H	H
Pentamethoxy derivative (**87**)	CH_3	CH_3	CH_3	CH_3	CH_3
Tetramethoxy derivative (**88**)	CH_3	CH_3	CH_3	CH_3	H

Plakinamine C (**89**) and two other steroidal alkaloids (**90**, **91**) from the Pacific sponge *Corticium* sp. exhibited minor inhibition of syncytia formation in MT_4 cells [94]. These compounds caused a slight delay of infection at concentrations ranging from 0.05-0.1 μg/ml. The bromoindole alkaloid dragmacidin F (**92**), isolated from the Mediterranean sponge *Halicortex* sp., inhibited syncytium formation in MT_4 cells at EC_{50} of 0.91 μM [95]. Trikendiol (**93**) is a red pigment from the African sponge *Trikentrion loeve* [96]. This alkaloid inhibited cytopathicity of HIV-1 infection in a CEM-4 assay at an IC_{50} of 2 μg/ml.

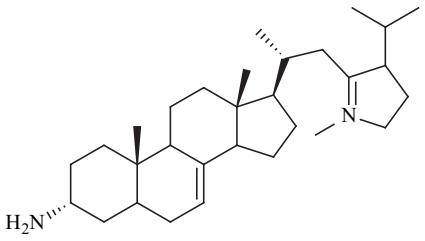
Other highly active alkaloids include the aaptamines. In the present study, we tested demethyl(oxy)aaptamine (**94**), isoaaptamine (**95**) and aaptamine (**96**), all isolated from the Indonesian sponge *Aaptos aaptos* [97, 98]. The oxidized demethyl(oxy)aaptamine represents the most active of these three alkaloids, with an EC_{50} of 0.34 μM, while aaptamine was least active, having an EC_{50} of 1.3 μM. Although this activity is promising, these compounds are also among the most cytotoxic in this study, with IC_{50} values of < 0.1 to 1.2 μM against PBM cells (Table 1). The greater activity of the oxidation product **94** than the principal metabolite **96** suggests that additional semisynthetics using **96** as a starting substrate may yield improved activity against HIV and reduced cytotoxicity.

Batzelladine Alkaloids

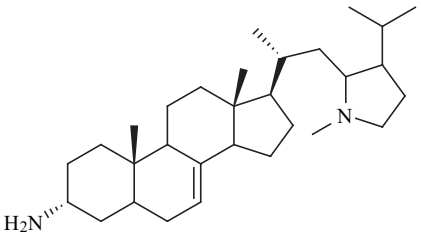
SmithKline Beecham screened 5000 extracts from terrestrial plants and marine organisms, and only one extract, from the Caribbean sponge *Batzella* sp., inhibited binding of HIV gp120 to human CD4 receptor [99]. The alkaloids batzelladines A (**97**) and B (**98**) were the active components



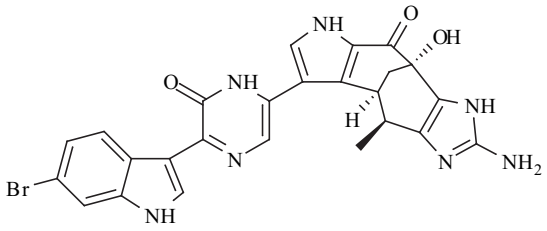
Plakinamine C (**89**)



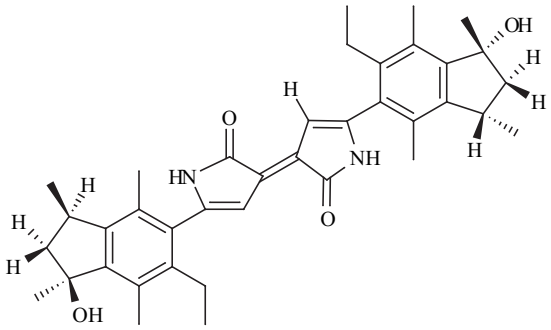
Steroidal alkaloid (**90**)



Steroidal alkaloid (**91**)



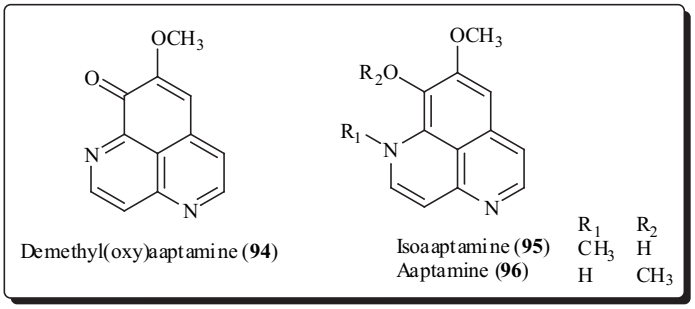
Dragmacidin F (**92**)



Trikendiol (**93**)

of this extract, with IC_{50} values of 30 μM in a primary assay that measures association between gp120 and CD4 [99]. Both compounds were also active in a secondary cell-based assay, however, in a 7-day cell-based assay, the compounds were toxic to host cells and therefore antiviral activity could not be assessed. It was suggested that for these compounds to be further considered as potential leads for HIV, their inhibitory activity must be separated from their cytotoxicity [99], although more recent interest in the development of toxins to attack reservoirs of latently infected cells [100] suggests that cytotoxicity in itself, may not be sufficient cause for rejection of anti-HIV compounds as potential drug leads.

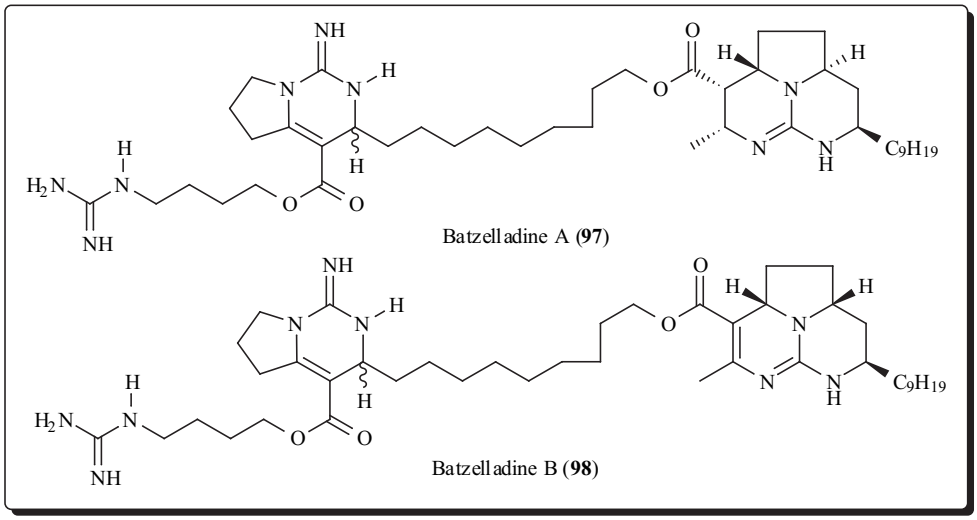
The pyrroloquinoline alkaloids isobatzelline C (**99**), initially isolated from the deep water Bahamian sponge *Batzella* sp. [101], and makaluvamines A (**100**; from a Fijian sponge in the genus *Zyzzya* [102]) and H (**101**; from a Pohnpei sponge *Z. fuliginosa* [103]), were re-isolated from a Papua New Guinean sample of *Z. fuliginosa*, and inhibit



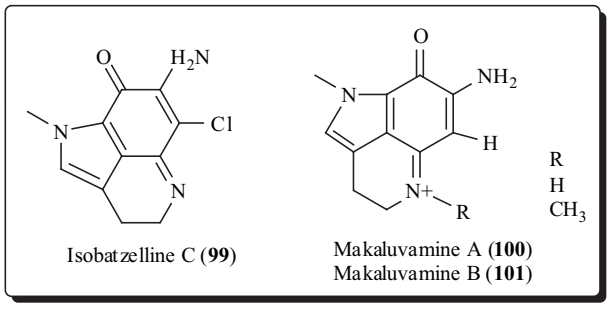
Demethyl(oxy)aaptamine (**94**)

Isoaaptamine (**95**)
Aaptamine (**96**)

R_1	R_2
CH_3	H
H	CH_3

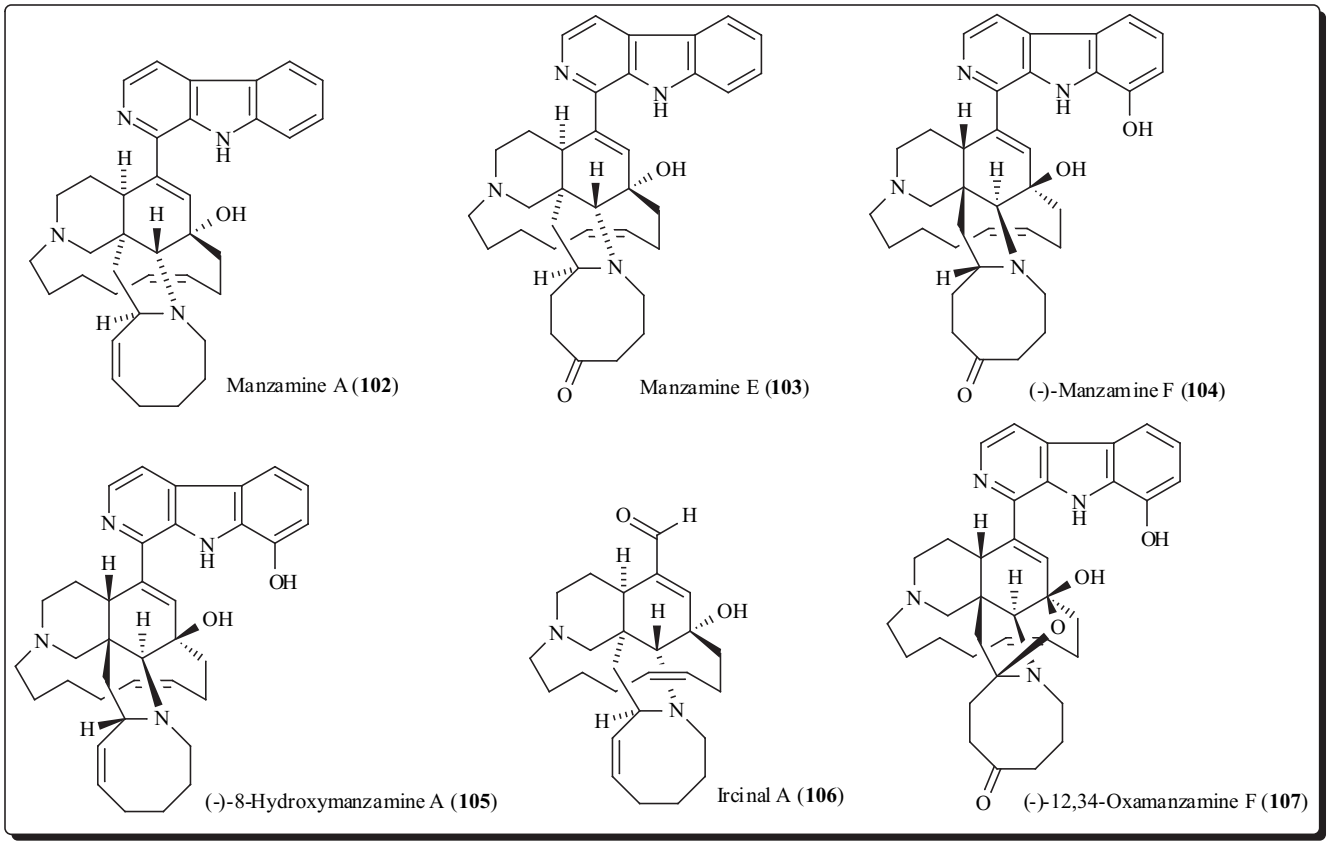


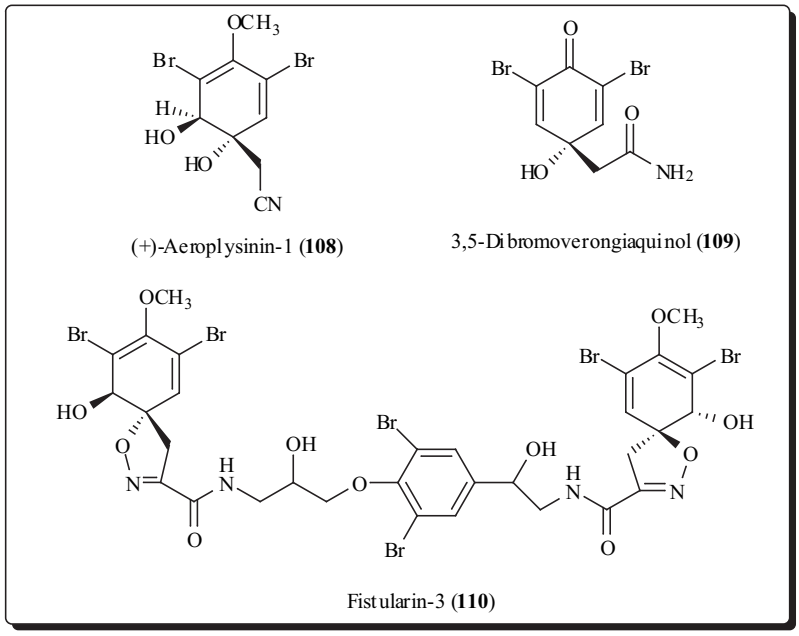
HIV-1 envelope-mediated cell fusion at IC_{50}'s of ~200 nM for isobatzelline C and ~5 μM for the makaluvamines [104].



Manzamine Alkaloids

Manzamines A (**102**), E (**103**), F (**104**) and (-)-8-hydroxymanzamine A (**105**) are biologically active polycyclic β-carboline alkaloids isolated from a variety of Indo-Pacific sponges [105]. Manzamines A and E had only moderate activity against HIV-1, with EC_{50} values of 12.9 and 18.7 μM, respectively; however, this activity was considerably greater than that of (-)-8-hydroxymanzamine A or (-)-manzamine F. Ircinal A (**106**), another alkaloid from the Okinawan sponge *Ircinia* sp., is considered a potential biogenic precursor of the manzamines [106]. Ircinal A was active against HIV-1 at a concentration similar to that of compound **105**, although it was considerably less cytotoxic.





(-)-12,34-Oxamanzamine F (**107**), the microbial transformation product of **105** [107], was active against HIV-1 at half the concentration, and had an order of magnitude lower cytotoxicity, than the parent compound. This improvement in bioactivity is encouraging from the perspective of using these natural products as starting materials for semisynthetic or biocatalytically-derived libraries. In addition, manzamines are potent antimalarial agents [105] and may represent important drugs in the fight against this deadly disease in immunocompromised patients in developing tropical countries.

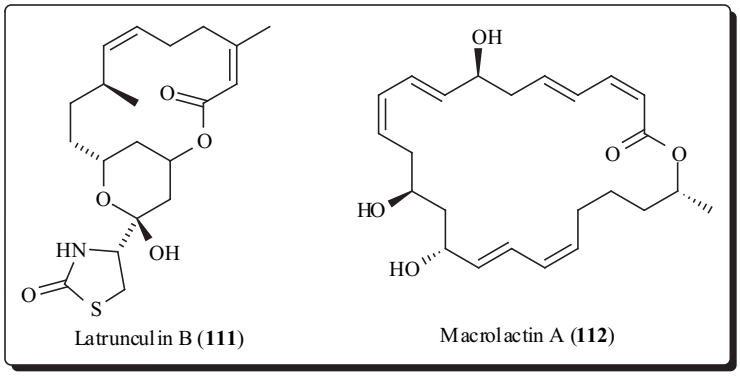
Dibrominated Spiroisoxazolines

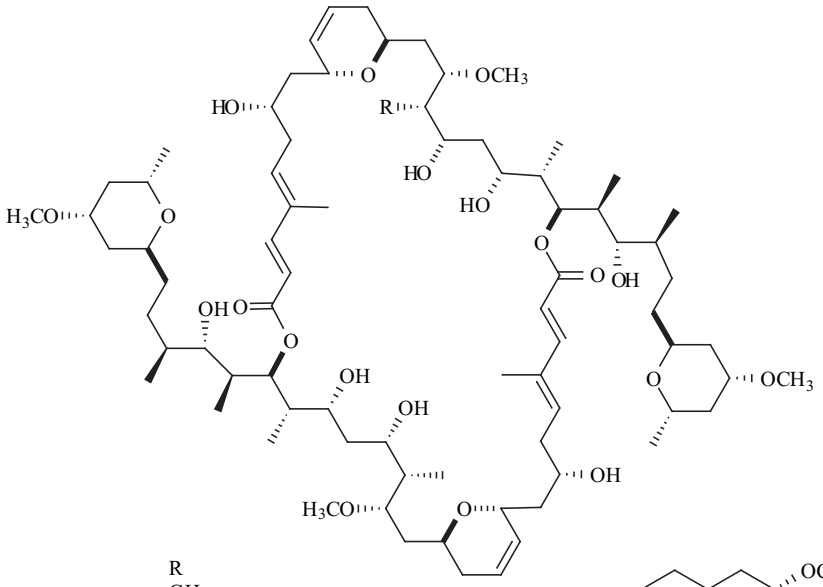
(+)-Aeroplysinin-1 (**108**), an antibacterial and cytotoxic dibrominated spirohexadiene isolated from verongid sponges [108-110] had an EC_{50} of 2.5 μM against HIV-1 and an IC_{50} of 3.8 μM against PBM cells. The related 3,5-dibromoverogiaquinol (**109**) and fistularin-3 (**110**), from the Mediterranean verongid sponge *Aplysina cavernicola* [111] and the Caribbean sponge *Aiolochroia crassa*, respectively [112], were slightly less active (EC_{50} values of 6.1 and 6.9 μM, respectively). These data suggest that dibrominated spirohexadiene systems represent a novel group of lead compounds and warrant further investigation for their activity against HIV-1.

Lactones

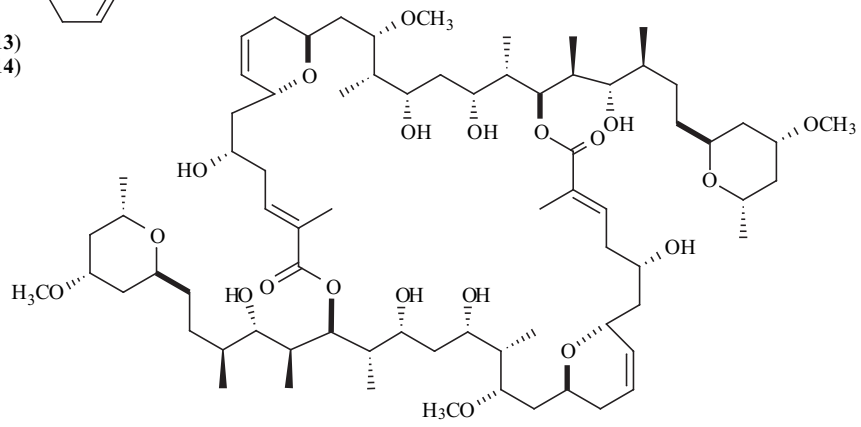
Latrunculin B (**111**), a 2-thiazolidinone macrolide isolated from the Red Sea sponge *Latrunculia magnifica* [113], exhibited moderate activity against HIV-1 (EC_{50} 16.4 μM) in the present study, and was not cytotoxic. Macrolactin A (**112**), a 24-membered ring lactone, isolated from an unidentified gram-positive deep sea bacterium, inhibited HIV replication with maximum protection occurring at 10 μg/ml [114]. Further study of the mechanism of action of macrolactin A was precluded by a lack of material, although recent reports of the synthesis this molecule may prove a promising source for additional studies [115, 116].

The swinholides and misakinolide are dimeric lactone macrolides ranging from 40- to 46-membered rings. These compounds have been isolated from Indo-Pacific sponges in the genus *Theonella* [117-119], and have been previously reported to exhibit antitumor, antiviral and antifungal activities. In the present study, these macrolides also exhibited potent anti-HIV-1 activity with EC_{50} of 0.16 μM and 0.52 μM for swinholides A (**113**) and B (**114**), respectively. Misakinolide A (**115**) does not differ significantly in activity from swinholide B, with an EC_{50} of 0.58 μM. The least active of the swinholides was the artifact isoswinholide A (**116**) with an EC_{50} of 10.3 μM. The only

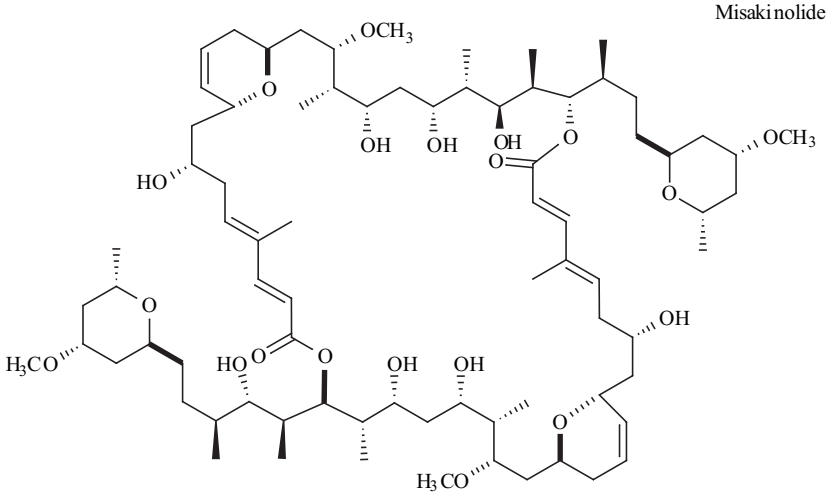




R
CH_3 Swinholide A (**113**)
H Swinholide B (**114**)



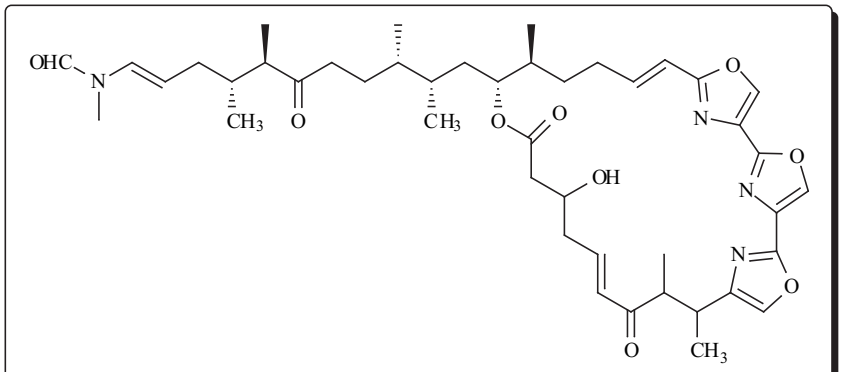
Misakinolide A (**115**)



Isoswinholide A (**116**)

change in structure between swinholide A and isoswinholide A is the lactonization position at C-23 instead of C-21 in swinholide A. This results in a 46-membered macrolide ring in isoswinholide A, as compared with a 44-membered ring in swinholide A. This change in structure reduces the HIV activity of this molecule by two orders of magnitude and indicates that an increase in the macrocycle above 44, lactonization at C-23, or creation of a free hydroxyl at C-21 reduces activity. In contrast, the reduction of ring size in misakinolide A to a 38-membered macrolide did not significantly alter HIV activity.

Even more active than the swinholides, the antifungal macrolide halichondramide (**117**) was first isolated from the

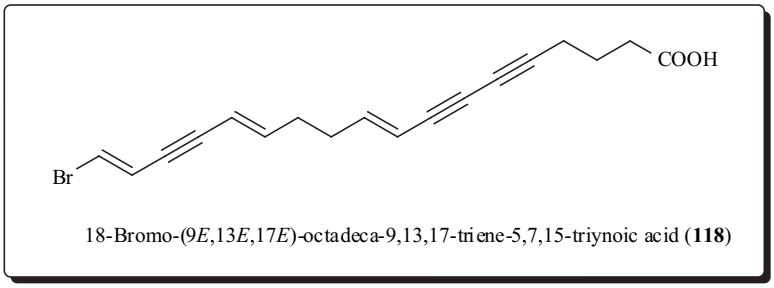


Halichondramide (**117**)

sponge *Halichondria* sp. from Kwajelein [120]. In the present study, halichondramide showed an EC_{50} of 0.058 μM, only slightly less active than the most active cyclic depsipeptide jaspamide (**24**). Unfortunately, like jaspamide, halichondramide was also cytotoxic (Table 1).

Polyacetylenes

Several polyacetylenic brominated acids isolated from the Bahamian sponge *Xestospongia muta* inhibited HIV-1 protease with IC_{50} values ranging from 6 to 12 μM [121]. These compounds, the most active of which was compound **118**, were also inhibitory to enzymes from other unrelated systems. However, this lack of specificity for the viral enzyme precluded further study of these compounds.



18-Bromo-(9*E*,13*E*,17*E*)-octadeca-9,13,17-triene-5,7,15-triynoic acid (**118**)

Two additional polyacetylenes, petrosynol (**119**) and petrosolic acid (**120**), isolated from sponges of the genus *Petrosia*, inhibited activity of the DNA polymerase function of HIV-1 reverse transcriptase, with virtually no inhibition of the associated RNAse H [122]. Petrosolic acid inhibited RNA-dependent DNA polymerase at an IC_{50} of 1.2 μM and DNA-dependent DNA polymerase at IC_{50} of 6.2 μM, whereas the less active petrosynol exhibited IC_{50} values of 15.8 μM and 36 μM, respectively [122].

CONCLUSION

A review of the literature on anti-HIV activity of marine natural products indicates that promising activity occurs among multiple structural classes and against multiple targets in the viral replication cycle. Within structural classes, the limited data available support the need for more extensive structure-activity relationship studies in order to develop lead anti-HIV compounds. Among the anti-HIV compounds tested in the present study, the major limitation is associated with their cytotoxicity. However, the reasonable yields of these natural products from relatively common sponges and ascidians makes them intriguing targets for microbial metabolism and/or semisynthetic modifications utilizing combinatorial techniques aimed at the reduction of toxicity while enhancing the activity against HIV. In addition, cytotoxicity in itself may not negate the potentially valuable effects of anti-HIV compounds. It has been proposed that hybrid toxins be used to target and kill the long-lived reservoirs of latently infected cells [100]. Studies of cytotoxic anti-HIV marine compounds may prove useful in identifying potential toxins for these latently infected reservoirs and a major dividend of this type of research may be the discovery of compounds that selectively kill HIV-infected cells or cancer cells.

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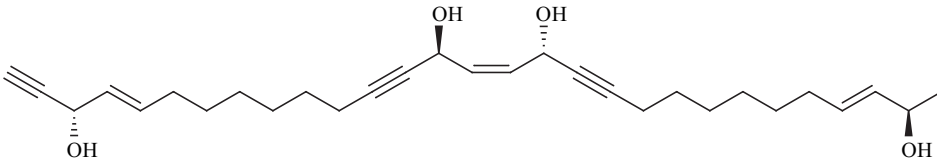
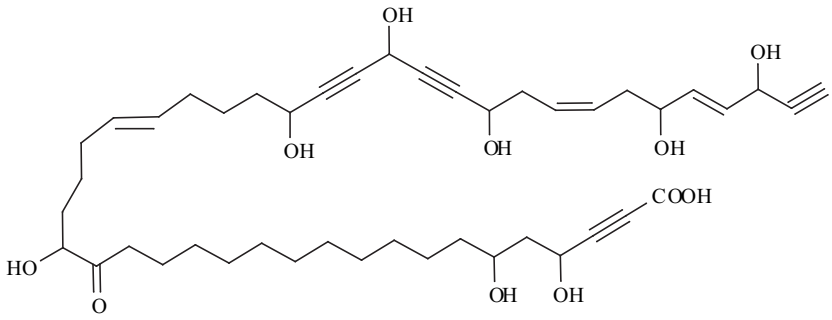


Petrosynol (**119**)



Petrosolic acid (**120**)

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