Potential of small-molecule fungal metabolites in antiviral chemotherapy

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Abstract

Various viral diseases, such as acquired immunodeficiency syndrome, influenza, and hepatitis, have emerged as leading causes of human death worldwide. Scientific endeavor since invention of DNA-dependent RNA polymerase of pox virus in 1967 resulted in better understanding of virus replication and development of various novel therapeutic strategies. Despite considerable advancement in every facet of drug discovery process, development of commercially viable, safe, and effective drugs for these viruses still remains a big challenge. Decades of intense research yielded a handful of natural and synthetic therapeutic options. But emergence of new viruses and drug-resistant viral strains had made new drug development process a never-ending battle. Small-molecule fungal metabolites due to their vast diversity, stereochemical complexity, and preapproved biocompatibility always remain an attractive source for new drug discovery. Though, exploration of therapeutic importance of fungal metabolites has started early with discovery of penicillin, recent prediction asserted that only a small percentage (5–10%) of fungal species have been identified and much less have been scientifically investigated. Therefore, exploration of new fungal metabolites, their bioassay, and subsequent mechanistic study bears huge importance in new drug discovery endeavors. Though no fungal metabolites so far approved for antiviral treatment, many of these exhibited high potential against various viral diseases. This review comprehensively discussed about antiviral activities of fungal metabolites of diverse origin against some important viral diseases. This also highlighted the mechanistic details of inhibition of viral replication along with structure–activity relationship of some common and important classes of fungal metabolites.

Keywords

Fungal, metabolites, antiviral, human immunodeficiency virus, bioactivity, inhibitors

Introduction

Viral diseases: A therapeutic challenge

Disease caused by pathogenic viral infections afflicted whole human race with high mortality rates[1,2] and remained as one of the prime causes of human death worldwide.[3] Human immunodeficiency virus (HIV), influenza, and hepatitis C virus (HCV) are three most perilous viral diseases which cause maximum human death. Discovery of effective vaccines though have somewhat helped in eradication of some important viral pathogens, such as smallpox, polio, mumps, etc. but proved ineffective in combating these viral diseases with high mortality rate.[4] A virus is a RNA/DNA-containing unique pathogen which requires utilization of host cell machinery and environment for its replication. Due to this unique feature, it is very difficult to make viruses or their replication as a therapeutic target, without exerting any adverse effect to the host cells.[5] Earlier days viral replication was thought to be carried out by cellular enzymes and thus the scope for selective inhibition of viral replication was very discouraging.[6] Active research on antiviral drug development started with discovery of first viral enzyme DNA-dependent RNA polymerase of pox virus in 1967.[7] Subsequent research resulted in discovery of several other enzymes and their biochemical function in various stages of viral replication. Common viral replication cycles involve several stages, which include attachment and entry to the host cell, transcription of viral mRNA, replication of viral genome, assembly and budding as progeny virus particles. Viral reverse transcriptase (RT) or RNA-dependent RNA polymerase does not have a

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proofreading mechanism.[8] Therefore, viruses containing RNA genomes such as HIV, HCV, and influenza are genetically susceptible to easy mutation. Accumulated mutation in viral genome is one of the main reasons behind the emergence of drug-resistant strain.[9–11] Although several new antiviral drugs have been approved in last two decades, rapid emergence of drugs resistance viral strains and adverse health effects, which arises due to prolonged use of these drugs, made continuous search for new antivirals inevitable. Thus, designing or discovery of safe drugs which is less susceptible to drug resistance still remains a serious challenge to synthetic and natural product chemist.

Current therapeutic approaches and requirement for novel drug

The arsenal of present-day antiviral may be divided into two broad categories[12] according to their targets. Directly acting antivirals, that directly affect viral proteins or viral genomes, are generally more specific, and therefore, less toxic but poses a higher risk of creating resistant viruses. That is why these single point targeted drugs are frequently ineffective, unsafe, and detrimental to therapeutic response. Whereas, host-acting antivirals, which modify host cellular factors or arouse immune response components to affect virus life cycles. These drugs may possess a broader antiviral activity spectrum and less risk of developing virus resistance but may be more toxic to the host cell. Ideally, combinations of effective therapeutic agents that target multiple stages in the viral replication cycle with little or no toxicity to the host are desirable. Therefore, currently, a combined therapy (or highly active antiretroviral therapy, HAART) is used,[13,14] which employ a cocktail of antivirals which target different biological process of viral replication cycle.[12] Due to the therapeutic synergy, it effectively suppresses viral replication and significantly prolongs the life of virus-infected patients. Therefore, there remains a pressing need for developing new antivirals with novel modes of action.[15]

Fungal metabolites as source of novel drug: Past history, present status, and future potential

Small-molecule secondary metabolites from natural resources traditionally played an important role in drug discovery and proved to be great savior against various dreaded diseases.[16,17] Due to their structural diversity, stereochemical complexity,[18,19] and evolutionarily optimized biocompatibility[17,20] these compounds still remained as indomitable attraction in medicinal chemist community. Metabolites of fungal origin being source and inspiration of many blockbuster drugs and drug leads are one of the major contributors to this success story. Enthusiastic search for bioactive metabolites[20,21] triggered by the success of penicillin resulted in many other natural and semi-synthetic antimicrobials, such as griseofulvin[22] and cephalosporins,[23] etc. Cyclosporine, an immunosuppressive cyclic peptide, was first discovered from soil-derived fungus, *Tolypocladium inflatum (Beauveria nivea)* and *Cylindrocarpon lucidum*[24] has changed started a new era in organ transplantation.[25] Subsequently, cyclosporine A has been reported from a variety of different common organisms, including many strains of *T. inflatum* as well as *Acremonium, Beauvaria, Fusarium, Paecillomyces,* and *Verticillium* species.[26] Presently there are more than 15 fungal metabolites that are in use as FDA-approved drugs and some of these are still dominating the drug market.[27] For example, mevastatin, lovastatins along with some synthetic analogs, like, atorvastatin had 15.5 billion sales in 2004, antibiotics market dominated by two fungal metabolites penicillin and cephalosporin had 15 billion dollar sales only in 2002, fungi-derived oral antifungal agent griseofulvin had 31.1 million sales in 2007, and antibiotic amoxicillin and immunosuppressive fungal metabolites cyclosporine had 1.4 and 1.7 billion dollars sales, respectively, during the period of 2004–2008.[27]

In last 50 years a large number of structurally diverse metabolites were isolated from numerous fungal species. Some of these finally came out as lifesaving drug[27] and many of these are at different stages of drug development process. According to the recent estimation[28–30] so far only a small fraction of fungal species have been identified and much less have been scientifically investigated for bioactive metabolites. Thus, there is plenty of scope for finding many other potential drug lead through exploration of new fungal metabolites and their bioactivity study. Another big advantage of isolating compounds from fungal species is that those can be cultivated or cultured so that large amount of the species can be generated at a reasonable time and cost. Development of efficient and automated isolation techniques and enhanced capability of carrying out bioassay of large number of compounds (through high throughput screening) can accelerate the exploration of more and more new fungal metabolites. One of the main problems in antiviral metabolite research is the accessibility of the bioassay facility. Many countries with abundance of fungal species are not having sophisticated and comprehensive antiviral screening facilities. Therefore, major portions of the isolated metabolites

not ever been tasted against any viral strain. Recently, many countries opened their antiviral screening facility for international synthetic and natural product compounds under certain terms and conditions. Enthusiastic exploration of much more new fungal species, development of easy but effective metabolite isolation techniques, and easing the access to sophisticated and comprehensive screening facilities will surely expedite the antiviral fungal metabolite exploration process.

Fungal metabolite inhibitors for various viral diseases

Though no fungal metabolite has been approved so far for the treatment of viral drug, many of these metabolites exhibited extraordinary activity against various strains, especially against HIV and influenza viruses. This paper critically reviewed the bioactivity of different classes of fungal metabolites against various viral diseases. In many cases same fungal metabolites were obtained from various other fungal species. To maintain the focus, only the fungal species from where its bioactivity is first detected is mentioned here, though in some cases references have been given for the initial isolation of these metabolites. Many of these antiviral fungal metabolites also exhibited other important biological activities but those are not mentioned here unless their mechanisms of action influence the antiviral activity. Most of the cases the structure and stereochemistry of the fungal metabolites have been determined by using various spectroscopic techniques such as NMR, MS, UV, IR, single crystal X-ray, polarimetry, and CD spectrometry and are not mentioned individually in the text. Where structure confirmation of metabolites needed extra measures such as, synthetic modification or derivatization, only those cases are mentioned individually in the text. Bioactivities of some already reviewed[31–33]fungal metabolites is also included here for a further in-depth discussion of their important structural features and to make this review more comprehensive.

Inhibitors of HIV

Acquired immunodeficiency syndrome (AIDS), caused by HIV, is one of the major contributors to an increase in infectious disease mortality.[34,35] Since first reported official cases of AIDS in 1981 it has erupted as pandemic for last four decades. According to 2011 estimate, more than 34 million people were living with HIV infection and number of newly infected cases and number of deaths in the same year reached more

than 2.5 million and 1.7 million, respectively.[36] Due to its increasing extension and the high associated mortality rate, undoubtedly AIDS represents a global health threat. Fighting this disease remains one of the major challenges for chemotherapy in the 21st century.

The causative agent, HIV is a structurally unique member of Retroviridae family which contains two copies of positive single-stranded RNA and a conical capsid. HIV has the potential to infect pivotal cells of the human immune system such as dendritic cells, macrophages, and mainly T helper cells. Though there are two major types of this virus existing as HIV-1 and HIV-2, the former type is the major cause of the epidemic of AIDS worldwide. Four decades of intensive research from industry and academia resulted in 26 clinically approved drugs till date. These antiretroviral drugs can be classified into six different groups according to their biological targets, namely entry inhibitors (fusion inhibitors and chemokine receptors CCR5 and CXCR4), RT inhibitors (nucleoside or nonnucleoside based), integrase inhibitors, and protease inhibitors[37] (Figure 1). These drugs are mainly directed against three key viral enzymes required for replication, RT, protease and integrase, one viral envelope glycoprotein gp41, and a coreceptor (CCR5) of the host cell.[38] A major concern in antiretroviral drug therapy is rapid emergence of resistant strain.[39] That is why single point targeted drugs are frequently ineffective, unsafe, and detrimental to therapeutic response. The current combined therapy (HAART) with synergistic effect of antivirals of different target points effectively suppress viral replication and significantly prolonged the life of AIDS patients.[35] Thus, the continual emergence of drug resistance viral strains has created a pressing need for continuous search and development of potent anti-HIV drugs with new modes of action.[40] This resulted in discovery of a number of potential metabolites from various natural sources.[12] In this portion HIV inhibitory activity of various fungal metabolites is presented and their mode of inhibition has been discussed.

Entry inhibitors. Blocking of HIV entry to host human cell is also one of the most important targets for HIV therapies.[41–43] The viral spike of HIV-1 is composed of three gp120 envelope glycoproteins attached noncovalently to three gp41 transmembrane molecules. Entry of HIV to host cell begins with highly specific binding of the HIV glycoprotein gp120 envelope protein with a CD4 molecule, on the surface of most susceptible cells. Viral entry is initiated by binding to the CD4 receptor on the cell surface, which induces large conformational changes in gp120 which trigger the entry of viral materials to the host cell. Several studies demonstrated
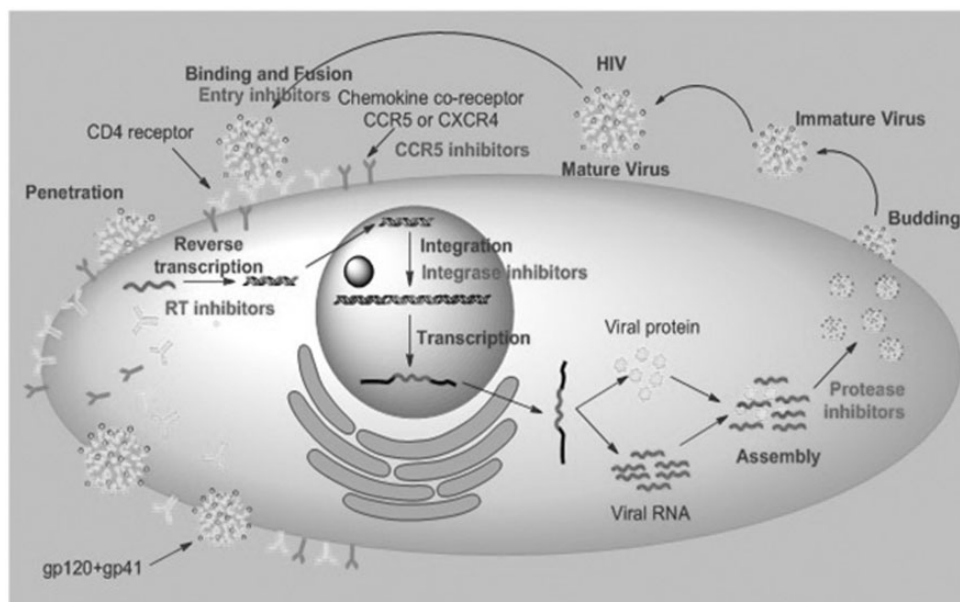
Figure 1. Various stages of HIV replication. HIV: human immunodeficiency virus.
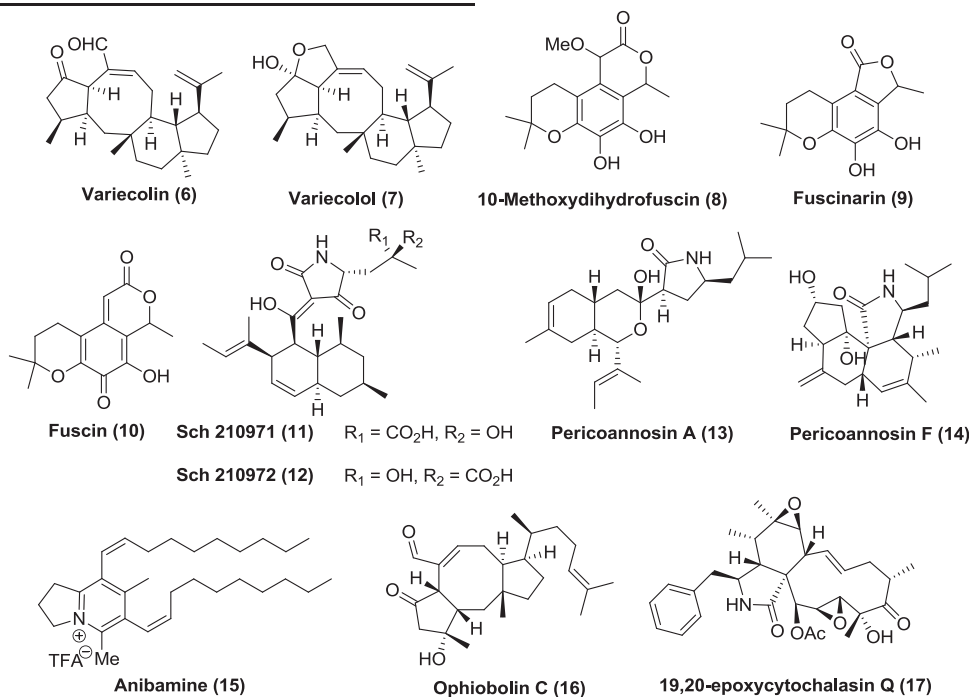
that chemokine receptors CCR5 and CXCR4 are essential as coreceptors for gp120 and CD4 binding. Inhibition of such binding can prohibit the entry of viral material to the host cell and thus can prevent further replication. This is a very effective alternative target for HIV therapeutics. The molecule which inhibits fusion of viral and host cell through specific binding with glycoprotein is called fusion inhibitor. Other types of entry inhibition take place through specific binding with the coreceptors CCR5 and CXCR4. Several fungal metabolites with viral entry inhibitory activity are discussed here.

Isochromophilones I [1a, 1 b] and II [2a, 2 b], isolated from a cultured broth of *Penicillium nicillium*[44,45] as first nonpeptide HIV-1 entry inhibitor, strongly inhibited the gp120–CD4 binding with IC$_{50}$ values of 6.6 and 3.9 μM, respectively.[44] Ochrephilone [3], sclerotiorin [4], rubrorotiorin [5], and the other structurally related compounds isolated from the same extract exhibited very weak or no inhibition even at higher concentration. Isochromophilone II exhibited anti-HIV activity at 25 μM but exhibited no effect on cell proliferation in lymphocytes except at very high concentration 250 μM.[45]

Isochromophilone I (1a & 1b)

Isochromophilone II (2a & 2b)

Ochrephilone (3)

Sclerotiorin (4)

Rubrorotiorin (5)
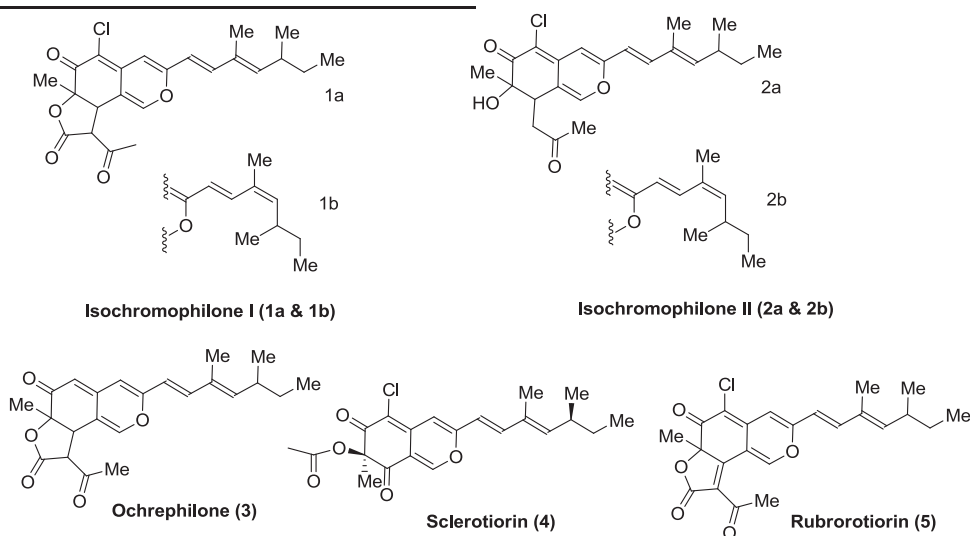
Bioassay-guided isolation of fungus *Emericella aurantiobrunnea*[46] led to the purification of two known sesterterpenes, variecolin[47] [6] and variecolol[48] [7], along with four other new analogs, emericolin[46] A–D. Only variecolin[47,49] [6] and variecolol[48] [7] competed with macrophage inflammatory protein (MIP)-1 R for binding to human CCR5 with IC$_{50}$ values of 9 and 32 μM, respectively, in a scintillation proximity assay (SPA). Variecolin [6] was first reported in 1991 from the fungus *Aspergillus variecolor* by Merck & Co.[47] Yoganathan et al. reported HIV entry inhibitory activity of two new, 10-methoxydihydrofuscin [8] and fuscinarin [9], and a known fungal metabolite fuscin[50,51] [10], isolated from the soil-derived mitosporic fungus *Oidiodendron griseum* (Yoganathan et al. 2003).[50,51] These compounds were effectively competed

concentration > 100 μM) binding assay. Two compounds pericoannosin A [13] and periconiasins F [14] isolated from endophytic fungus *Periconia* sp. showed low anti-HIV activity with IC$_{50}$s of 69.6 and 29.2 μM, respectively against efavirenz standard with an IC$_{50}$ of 1.4 nM.[53] Anibamine [15], a novel pyridine quaternary alkaloid as a TFA salt (plausibly an artifact of the isolation), from *Aniba* sp.[54]; ophiobolin C from fermentation extracts of fungi *Mollisia* sp.; and 19,20-epoxycytochalasin Q from *Xylaria* sp.[55] have shown to exhibit considerable HIV-1 inhibition. The identity of the natural counterion of Anibamine [15] is unknown. Anibamine•TFA competed for the binding of ^{125}I-gp120 to human CCR5 with an IC$_{50}$ of 1 μM. Ophiobolin C [16] and 19,20-epoxycytochalasin Q [17] exhibited binding IC$_{50}$ values of 40 and 60 μM, respectively.

Variecolin (6) **Variecolol (7)** **10-Methoxydihydrofuscin (8)** **Fuscinarin (9)**

Fuscin (10) **Sch 210971 (11)** R$_1$ = CO$_2$H, R$_2$ = OH **Pericoannosin A (13)** **Pericoannosin F (14)**

Sch 210972 (12) R$_1$ = OH, R$_2$ = CO$_2$H

**Anibamine (15)** **Ophiobolin C (16)** **19,20-epoxycytochalasin Q (17)**

with MIP-1 R for binding to human CCR5 with IC$_{50}$ values of 154, 80, and 21 μM, respectively, in the SPA binding assay. The greater activity of fusin [10] with respect to [8] and [9] may be attributed to the presence of multiple reactive sites of the isochromene-5,9-dione unit in [10].

Two tetramic acid type fungal metabolites, Sch 210971 [11] and Sch 210972 [12] isolated from a culture of *Chaetomium globosum*, demonstrated very strong CCR-5 inhibitory activity[52] with IC$_{50}$ values of 1.2 μM and 79 nM, respectively. Compound [11] which is an epimer of [12] showed over 15-fold decrease in it inhibition potency. Their high specificity also confirmed by CCR-2 (not bind even at a very high

RT inhibitors. RT, a critical enzyme in the HIV life cycle, generates complementary DNA from an RNA template. As this process does not occur in normal cell, it has become one of the main targets of anti-HIV chemotherapeutics. There are two functionally distinct classes of RT inhibitors: nucleoside RT inhibitors and nonnucleoside RT inhibitors (NNRTIs).[56] NNRTIs are important components of highly active antiretroviral therapy (HAART). So far there are five NNRTIs nevirapine (NVP), efavirenz (EFV), etravirine (ETR), rilpivirine (RPV), and delavirdine (DLV) approved by FDA for HIV treatment.[57,58] All of these drugs bind to the hydrophobic pocket in the palm domain, adjacent to the thumb domain on the

RT p66 subunit, in blocking allosteric sites of RT.[59–62] Drug resistance is the most important factor of the failure of HAART. NNRTI-resistant mutations exist widely in patients since NVP, EFV, and DLV have been used in clinical settings over the past decade.

indinavir sulfate with an EC_{50} value of 8.18 nM.[66] Inactivity of structurally related metabolite helotialins C [21] from the same extract in assay suggested that the C-13/C-14 olefin is important for their anti-HIV activity.

Stachybotrins D (18)

Helotialins A (19) R = CO$_2$H
Helotialins B (20) R = H

Helotialins C (21)
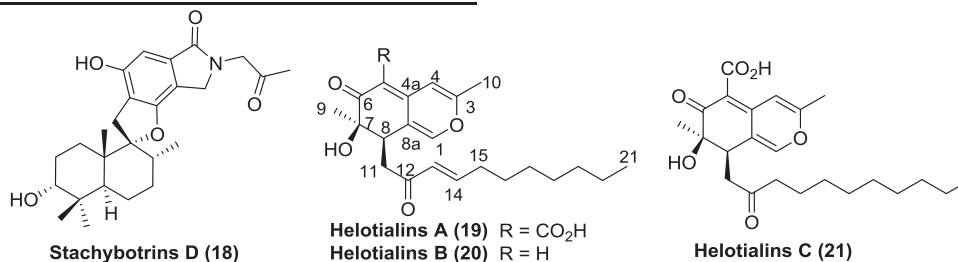
Both clinical and in vitro studies have shown that E138K is a resistant mutation to ETR and RPV, which were approved by the FDA in 2008 and 2012, respectively.[63,64] Therefore, there remains a strong demand for the development of new NNRTIs that can effectively inhibit NNRTI-resistant viruses.

Ma et al. isolated 11 phenylspirodrimanes along with stachybotrins D [18] from the fermented broth of fungus *Stachybotrys chartarum*[65] and studied their anti-HIV against both wild-type HIV-1 and five NNRTI-resistant strains. Only active compound [18] exhibited an inhibitory effect on HIV-1 replication with an EC_{50} value of 8.4 μM with no cytotoxicity at 10 μM. In-depth mechanistic study revealed that compound [18] inhibits the RT RNA-dependent DNA polymerase activity in a dose-dependent manner with an EC_{50} of 50 μM. Compound [18] also exhibited unusually selective activity toward five different NNRTI-resistant HIV-1 pseudovirus strains compared to the wild-type HIV-1. It could block NNRTIs-resistant strains (HIV-1$_{RT-K103N}$, HIV 1$_{RT-L100I,K103N}$, HIV-1$_{RT-K103N,V108I}$, HIV-1$_{RT-K103N,G190A}$, and HIV-1$_{RT-K103N,P225H}$) as well as wild-type HIV-1 (HIV-1 wt) with EC_{50} values of 7.0, 23.8, 13.3, 14.2, 6.2, and

Bioactivity-guided isolation of ethyl acetate extract of a fermentation broth of endophytic fungi *Alternaria tenuissima* QUE1Se and spectroscopic identification resulted in two new metabolites, altertoxins V [25] and VI [26][67] along with three known altertoxins I [22], II [23], and III [24].[68,69] Among these, altertoxins I [22], II [23], III [24] and altertoxins V [25] completely inhibited HIV-1 viral replication in A3.01 cells at concentrations of 0.50, 2.20, 0.30, and 1.50 μM, respectively, compared to standard positive control AZT at 20 μM. Altertoxins VI [26] showed reduced effectiveness probably due to its instability in the cell culture medium. Altertoxin V [25] displayed the lowest IC_{50} value of 0.09 μM whereas altertoxins I [22], II [23], and III [24] exhibited higher IC_{50} values of 1.42, 0.21, and 0.29 μM. All the active compounds are insignificantly cytotoxic at these concentrations. From their IC_{50} and cytotoxicity data therapeutic index of [22], [23], [24], and [25] were determined to be 3, 6.5, 15, and 50 μM, respectively. Their therapeutic index provides a narrow window for further drug development study but surely provide a good platform for the development of synthetically modified nontoxic anti-HIV drug.
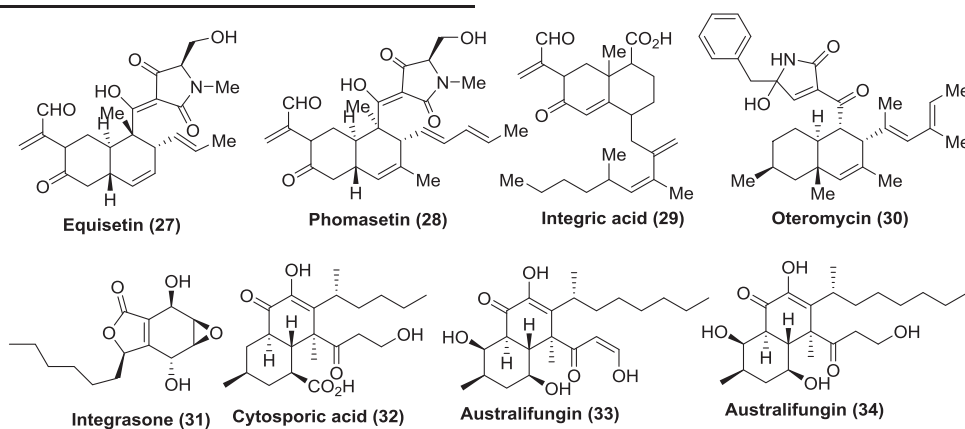

Altertoxins I (22)   **Altertoxins II (23)**   **Altertoxins III (24)**   **Altertoxins V (25)**   **Altertoxins VI (26)**
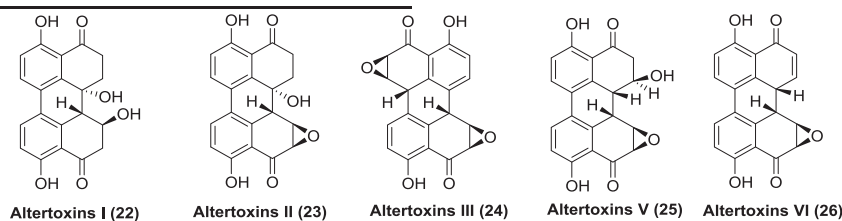
8.4 μM, respectively. Two azaphilones, Helotialins A [19] and Helotialins B [20], isolated from *Helotialean Ascomycete*, showed in vitro HIV-1 replication inhibitory effects in C8166 cells, with EC_{50} values of 8.01 and 27.9 nM, respectively, compared to standard drug

HIV integrase inhibitors. HIV-1 integrase is one of the three enzymes that are critical for viral replication. HIV DNA is inserted into the host genome by a specialized DNA recombination reaction in which the viral integrase is the key player.[70,71] The integration reaction

is composed of three steps, 3'-processing, strand transfer, and gap filling, and integrase catalyzes the first and second steps. The third step is thought to be catalyzed by cellular enzymes. It catalyzes three essential steps that include assembly, endonucleolytic cleavage (3'-end processing) of the viral DNA, and strand transfer of the viral DNA into the host cell DNA.[70,72,73] The absence of HIV integrase enzyme in the host cells and its indispensability for HIV replication cycle makes it an excellent target for the development of a nontoxic antiretroviral therapeutic agent. The inhibitors of the other two key enzymes, HIV RT and HIV protease, have led to many approved drugs that continue to have enormous impact on the control of the spread of HIV-1 infection. But, inhibitors for HIV integrase inhibitors, with only three recently approved drugs,[57] dolutegravir, elvitegravir, and raltegravir (raltegravir potassium) are much underexplored and possess enormous potential to be a vital component in combination therapy (HAART). Also combating future drug resistance for HIV integrase inhibitors[74] requires a sufficiently strong drug discovery pipeline. Thus, research toward development of new HIV integrase inhibitor drugs bears huge importance.[75–77]

strand transfer, and disintegration with IC_{50} values 5–10 μM. Despite being enantiomers equisetin and phomasetin showed almost equal inhibitory activity for both 3' end processing and strand transfer reaction with IC_{50} value 7 and 15 μM and 15–20 μM, respectively.[78] Oteromycin exhibited much reduced inhibition compared to the other three structural homologs.[80]

Integrasone [63],[81] a dihydroxy bicyclic epoxy lactone polyketide fungal metabolite, isolated by same group from an unidentified mycelium, which moderately inhibited the strand transfer reaction of HIV-1 integrase with an IC_{50} value of 41 μM. The aliphatic chain may play a significant role in the activity, as was observed in the case of integric acid.[82] Two polyketides, cytosporic acid [32] isolated from fermentation broth of the filamentous fungus *Cytospora* sp.,[83] and Australifungin [33], isolated from the coprophilus fungus *Sporormiella australis*,[84] almost equally inhibited strand transfer reaction of HIV-1 integrase with an IC_{50} of 20 μM in an in vitro assay. But, Australifungin[84] [34] did not show any inhibition even at 200 μM. The lack of activity of [34] indicates that the α-keto aldehyde of [33] and the carboxyl group of [32] are critical for the activity.

Equisetin (27) **Phomasetin (28)** **Integric acid (29)** **Oteromycin (30)**

Integrasone (31) **Cytosporic acid (32)** **Australifungin (33)** **Australifungin (34)**
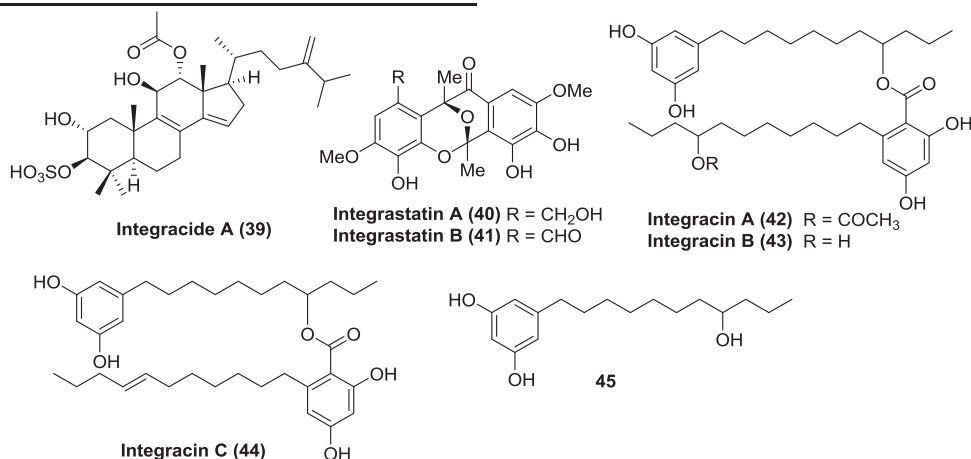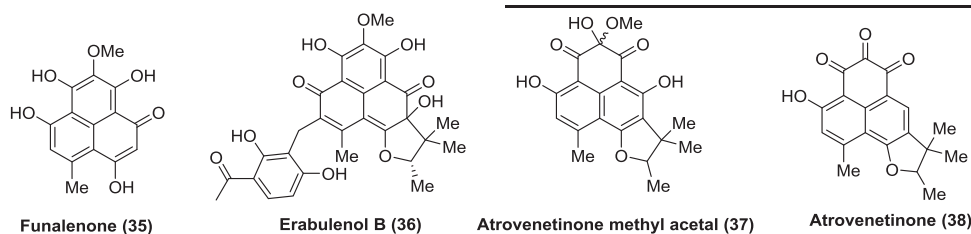
Two enantiomeric homologous fungal metabolites equisetin [27] and phomasetin [28], isolated from the marine fungi *Fusarium heterosporum* and *Phoma* sp. respectively,[78] exhibited strong in vitro HIV-1 integrase inhibitory activity. These fungal metabolites and other two structurally related metabolites integric acid [29], isolated from *Xylaria* sp.[78] and oteromycin [30], isolated from two different strains of unidentified fungus,[79] are structural homologs that comprise a structurally and functionally unique class of integrase inhibitors. A detailed in vitro inhibition assay of these metabolites carried out bySingh et al.[80] revealed that integric acid is the most active in inhibiting three different types of activity of HIV-1 integrase enzyme, e.g. 3' end processing,

Funalenone [35], originally isolated from mycelium of *Aspergillus niger* FO-5904,[85] Erabulenol B [36] isolated from *Penicillium* sp. FO-5637,[86] and atrovenetinone methyl acetal [37] was isolated from a culture broth of *Penicillium* sp. FKI-1463[87] was evaluated for their integrase inhibition and anti-HIV activity.[87] All these metabolites showed considerably strong in vitro anti-HIV activity with IC_{50} values 1.7, 17, and 6.7 μM, respectively. They also inhibited HIV-1 integrase enzyme with IC_{50} values 10, 7.5, and 19 μM, respectively. Compound [35] and [36] are found to be better integrase inhibitors with much less cytotoxicity (IC_{50} 87 and 230 μM, respectively) and high selectivity compared to [37] (cytotoxicity IC_{50} 13 μM). Compound

[37] is a methyl acetal derivative of Atrovenetinone [38], which was initially obtained from oxidation of atrovenetin produced by *Penicillium* sp.[88] and was lately isolated from a culture broth of *Gremmeniella abietina*.[89] All these phenalenone compounds reported to possess many other favorable biological activities.[90]

inhibited coupled reaction of recombinant HIV-1 integrase with an IC_{50} value of 3.2, 6.1, and 3.5 μM, respectively. But these are 10–30-fold less active in strand transfer inhibition with IC_{50} values of 32, 17, and 88 μM, respectively. The monomeric metabolite [77] from same species was not active even at 100 μM.[93]

Funalenone (35) **Erabulenol B (36)** **Atrovenetinone methyl acetal (37)** **Atrovenetinone (38)**

Integracide A (39)

Integrastatin A (40) R = CH$_2$OH
Integrastatin B (41) R = CHO

Integracin A (42) R = COCH$_3$
Integracin B (43) R = H

Integracin C (44)

45

Integracide A [39], four tetracyclic triterpenoids isolated from the fermentation broth of a *Fusarium* sp. together with other three analogs.[91] Integracide A, a sulfated ester, exhibited significant inhibitory activity in both coupled and strand transfer reactions of recombinant HIV-1 integrase with IC_{50} values of 4 and 9 μM, respectively. However, due to their high toxicity (IC_{50} 25 μM), it is not much useful for further therapeutic consideration. The other metabolic analogs, integracide B, C, and D, showed very weak or no inhibitory activity. Integrastatins A [40] and integrastatins B [41] are two novel aromatic [6/6/6/6]-ring system, isolated from fermented extract of unidentified fungus from Mexico, inhibits HIV-1 integrase in coupled and strand transfer assays with IC50 values of 0.6 and 1.1 mM for [40] and 1.04 and 2.5 mM for [41], respectively.[92] Three dimeric alkyl aromatics of polyketide origin, integracins A [42], B [43], and C [44], isolated from *Cytonaema* sp.,[93]

Four unsymmetrical bis-naphtho-γ-pyrone; isochaetochromin B1 [46], isochaetochromin B2 [47], isochaetochromin D1 [48], and oxychaetochromin B [49] isolated from the culture broth of *Fusarium* sp., inhibited both the coupled and strand transfer activity of HIV-1 integrase with IC_{50} values of 1–3 and 4–12 μM, respectively.[94] In in vitro bioassay the dehydro compound, [48] exhibited most inhibition activity with IC_{50} values of 1 and 4 μM in coupled and strand transfer assays, respectively. Compounds [46] and [47] showed similar bioactivity with IC_{50} values of 2 and 12 μM in corresponding assays. Oxychaetochromin B [49] also exhibited nearly equal activity with corresponding IC_{50} values of 3 and 9 μM, respectively. Semisynthetic modification of hydroxyl group of [47] failed to improve the bioactivity, suggesting the necessity of free –OH for bioactivity.
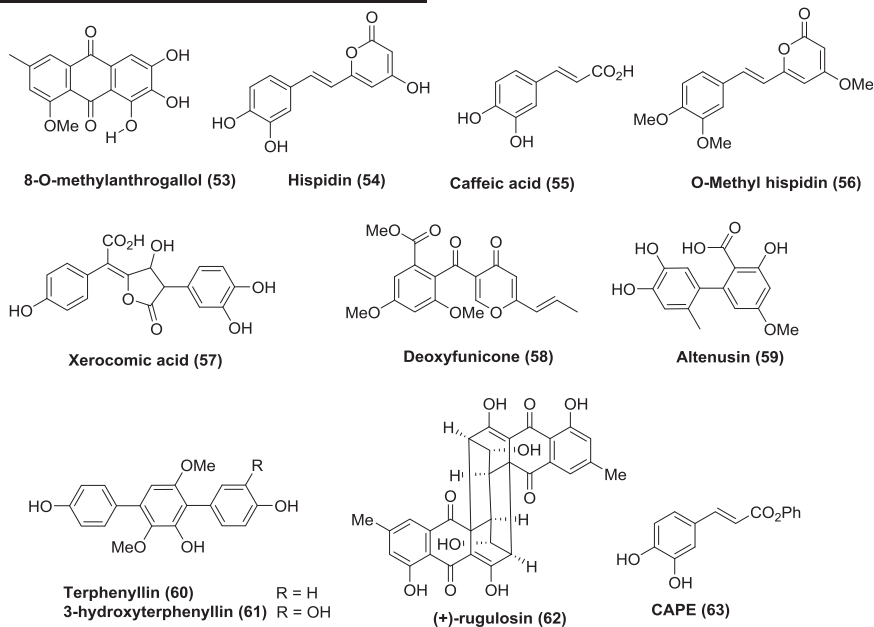
Isochaetochromin B$_1$ (46)   **Isochaetochromin B$_2$ (47)**   **Isochaetochromin D$_1$ (48)**   **Oxychaetochromin B (49)**
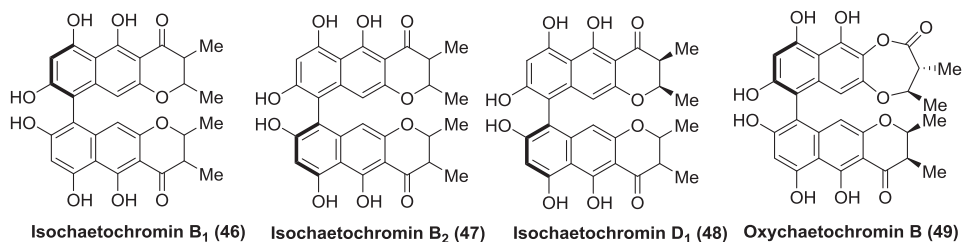
Doxorubicin **[50]**, a fungal metabolite from streptomyces and approved anticancer drug, exhibited HIV inhibitory activity in integrase-mediated DNA cleavage and strand transfer assay with IC$_{50}$ value of 0.9 and 2.4 µM, respectively.[95] Hydroxyrubicine **[51]** showed much reduced activity with corresponding IC$_{50}$ value 14.4 and 11.3 µM, respectively. Aglycone, adriamycinone **[52]**, exhibited no activity in both these assays (IC$_{50}$ > 100).
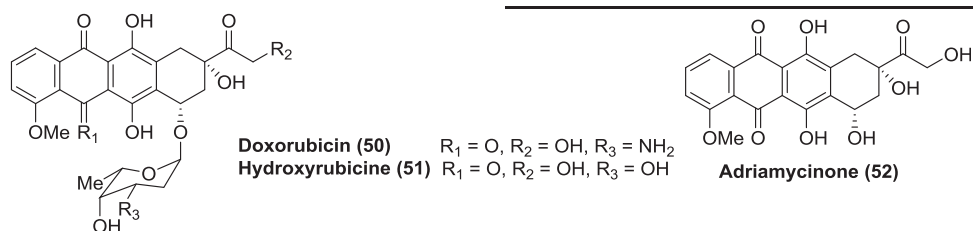
IC$_{50}$ values of 6, 2, 2.8, 1.1, and 2.8 µM, respectively. However, these compounds showed much less activity in strand-transfer assay with IC$_{50}$ values 22, 24, 24, 4.4, 12.1 µM, respectively. Much less activity of these inhibitors in strand-transfer assay suggests that they are cleavage inhibitors in nature. Other compounds in this group deoxyfunicone **[58]**, altenusin **[59]**, terphenyllin **[60]**, and (+)-rugulosin **[62]** were less active in both assays, with IC$_{50}$ values of 11, 19, 17.7, and 19 µM, respectively, in the coupled assay > 140, 25, 47.7, and

Doxorubicin (50)   R$_1$ = O, R$_2$ = OH, R$_3$ = NH$_2$
Hydroxyrubicine (51) R$_1$ = O, R$_2$ = OH, R$_3$ = OH
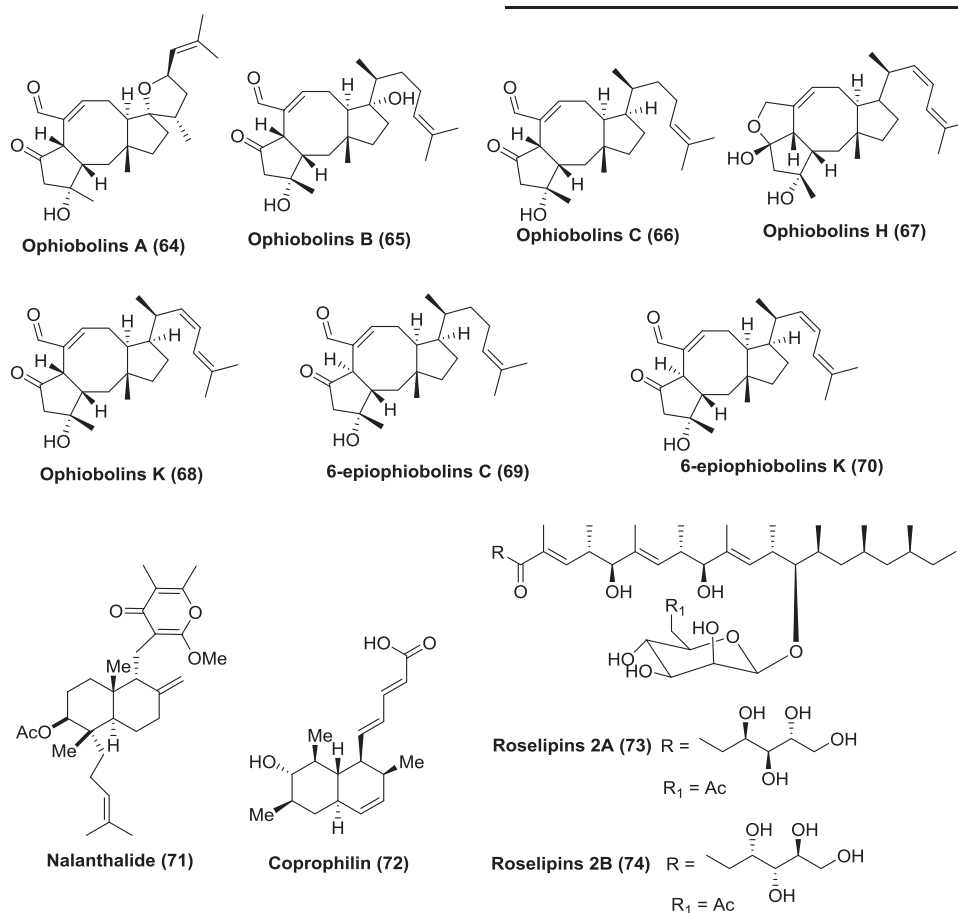
Adriamycinone (52)

In their recent search for HIV-1 integrase inhibitor Singh et al. isolated bunch of new and known metabolites of different classes, from fungal species of various genera[96] and studied their HIV-1 integrase inhibition capability. Among those, metabolites containing more than one phenolic group, 8-O-methylanthrogallol **[53]**, hispidin **[54]**, caffeic acid **[55]**, xerocomic acid **[57]**, and 3-hydroxyterphenyllin **[61]**, having catechol residue were the most active in the coupled assay and had
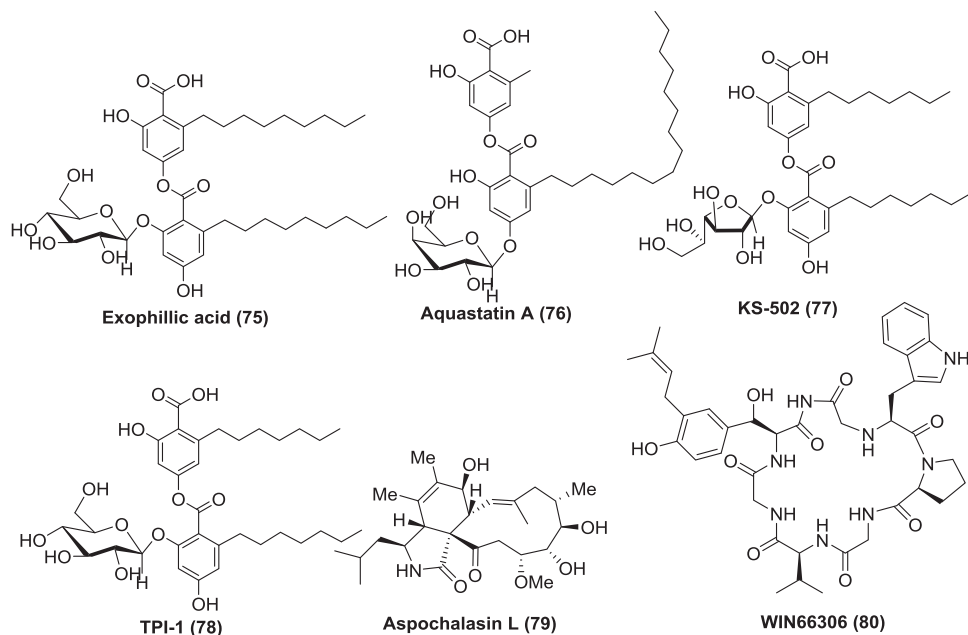
25 µM, respectively, in the strand-transfer assay. The completely abolished activity of **[56]**, where all phenolic –OH of hispidine **[54]** are capped by methyl groups, indicates the critical role of phenolic groups in the integrase inhibitory activity. A derivative of caffeic acid, caffeic acid phenethyl ester **[63]**, was reported to inhibit the strand transfer reaction with IC$_{50}$ = 18.9 µM and was a surprisingly poor inhibitor of the cleavage reaction (IC$_{50}$ = 220 µM).[95,97]

8-O-methylanthrogallol (53)   **Hispidin (54)**   **Caffeic acid (55)**   **O-Methyl hispidin (56)**

Xerocomic acid (57)   **Deoxyfunicone (58)**   **Altenusin (59)**

Terphenyllin (60)   R = H
3-hydroxyterphenyllin (61)   R = OH

(+)-rugulosin (62)   **CAPE (63)**

The other metabolites, **[64–74]**, are terpenoid/ polyketide and without any phenolic group. Among these, ophiobolins A **[64]**, B **[65]**, C **[66]**, H **[67]**, and K **[68]** exhibited IC_{50} values of 48, 21.5, 6.7, 30, and 23 μM, respectively in coupled assay and > 125, 125, 33, > 120, and > 120 μM, respectively in strand-transfer assay.[96] Ophiobolin C **[66]** with cis-fused A/B ring exhibited the highest inhibitory activity in both these assay. Epiophiobolins C **[69]** and K **[70]** exhibited HIV-1-integrase inhibitory activities with IC_{50} values of 29 and 33 μM, respectively, in the coupled assay but both were inactive in the strand-transfer assay ($IC_{50} > 120$ μM). The equisetin-type decalin-containing nalanthalide **[71]** and coprophilin **[72]** were equipotent and were 2.5-fold better inhibitors of the coupled reaction than of the strand transfer reaction. The 1:1 mixture of gycosylated triterpens, **[73]** and **[74]** exhibited an IC_{50} value of 8.7 μM in a coupled assay.

Exophillic acid **[75]**, a novel dimeric 2,4-dihydroxy alkyl benzoic acid, derived from fungus *Exophiala pisciphila*,[98] and structurally related to another fungal metabolite aquastatin A **[76]**, isolated from *Fusarium aquaeductuum*,[99] moderately inhibit the strand transfer reaction of HIV-1 integrase with IC_{50} values of 68 and 50 μM, respectively. No HIV-1 integrase inhibitory data are available for two other structurally similar fungal metabolites, KS-502 **[77]**, isolated from *Sporothrix* sp.[100] and TPI-1 **[78]** from *Hypomyces* sp. and *Nodulisporium* sp. Bioassay-guided isolation from the fermentation broth of a soil-derived fungal culture of *Aspergillus flavipes* led to the isolation of aspochalasin L **[79]**[101] and the known cyclohexapeptide WIN66306 **[80]**.[102,103] Compounds **[79]** and **[80]** inhibited HIV integrase with IC_{50} values of 71.7 and 32.1 μM, respectively, and exhibited CC_{50} values of 70 and 75 μM, respectively.[101] In HIV-1 replication inhibition assays in HuT78 cells, none of these has shown any effect at concentrations less than their CC_{50}s.

Ophiobolins A (64)   **Ophiobolins B (65)**   **Ophiobolins C (66)**   **Ophiobolins H (67)**

Ophiobolins K (68)   **6-epiophiobolins C (69)**   **6-epiophiobolins K (70)**

Nalanthalide (71)   **Coprophilin (72)**

Roselipins 2A (73) R = R_1 = Ac

Roselipins 2B (74) R = R_1 = Ac

Exophillic acid (75)   **Aquastatin A (76)**   **KS-502 (77)**

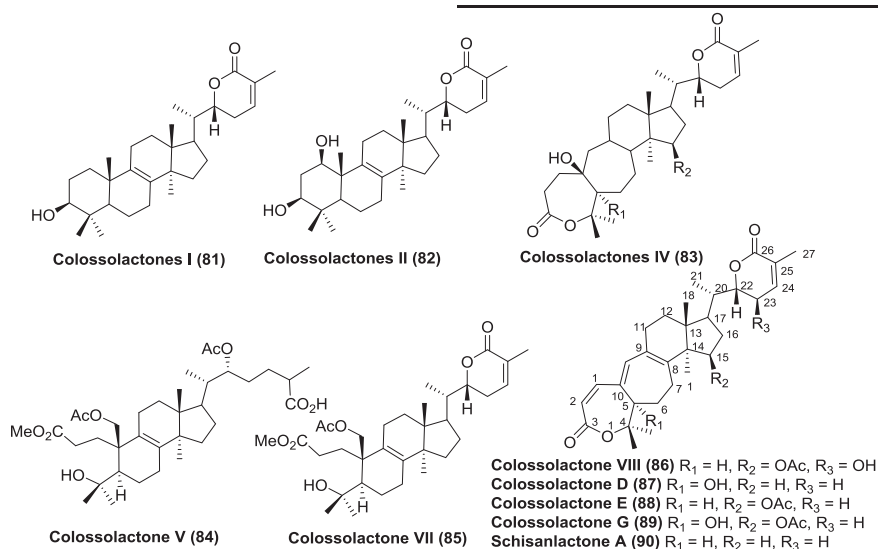**TPI-1 (78)**   **Aspochalasin L (79)**   **WIN66306 (80)**

HIV-1 protease (HIV-1 PR) inhibitors. HIV-1 PR is one of the important enzymes necessary for the replication of HIV. This enzyme belongs to the aspartyl protease class and functions as a dimer of 99 amino acids each. This enzyme cleaves the HIV gag and gag-pol polyprotein backbone at nine specific cleavage sites to produce shorter, functional proteins.[104] Protease inhibitors and RT inhibitors form the basis of HAART, which has been successful in improving survival rates and quality of life for HIV-infected individuals.[105] Intense research from academia and industry for around two decades resulted in eight commercially available HIV-1 PR inhibitors which include saquinavir, nelfinavir, ritonavir, indinavir, amprenavir, lopinavir, atazanavir, and fosamprenavir.[57,106] The loss of sensitivity to protease inhibitors usually occurs because the resistant viral strains encode for protease molecules containing specific amino acid mutations that lower the affinity for the inhibitors yet maintains sufficient affinity for the substrate and sufficient catalytic efficiency.[107] The development of more powerful inhibitors with a lower susceptibility to mutations and reduced side effects remains a major challenge. Searching for HIV-1 PR inhibitors from natural sources has thus become a promising approach in this respect. Secondary metabolites of fungal origin with considerable HIV protease activity are described here.
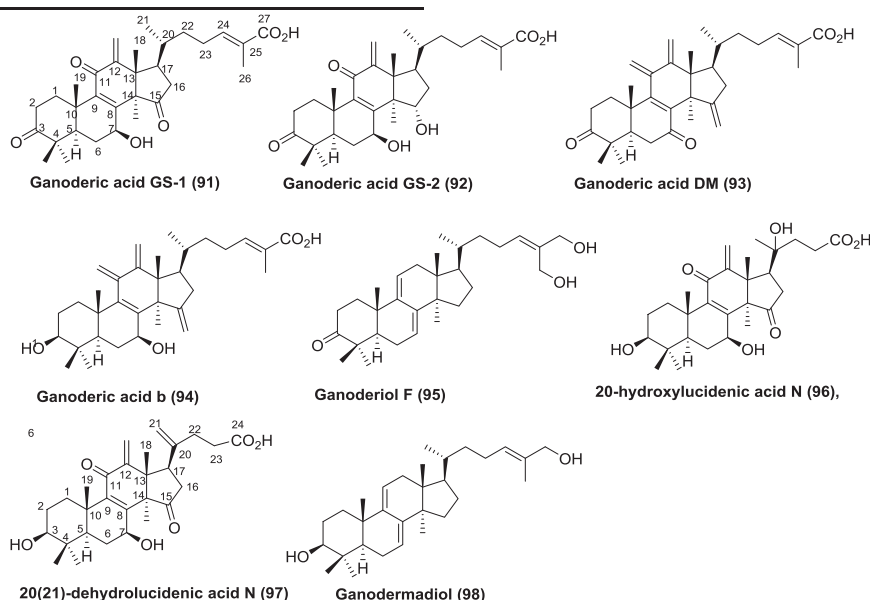
Triterpenes are most prevalent and therapeutically important class of compounds present in various ganoderma species. Many of these have exhibited remarkable inhibitory activity against HIV-1 PR. A group of triterpenoids isolated from *Ganoderma colossum* by Dine et al.[108–110] *called* colossolactones, characterized by the presence of a six-membered α, β-unsaturated δ-lactone group in their side chain with or without a seven-membered lactone ring as ring A. Most of the compounds from this mushrooms inhibited HIV-1 PR in a dose-dependent manner. Compounds colossolactone I [81] and colossolactone II [82] *found* to be the most potent against HIV-1 PR with IC_{50} values of 4.1 and 4.4 μM, respectively. Among other metabolites, schisanlactone A [90], colossolactones IV [83], colossolactones V [84], colossolactones E [88], colossolactones VII [85], and colossolactones D [87] inhibited HIV-1 PR with IC_{50} values of 10.8, 12.0, 14.5, 15.3, 24.7, and 29.1 μM, respectively. Compounds [86–90] have same basic skeleton containing seven-membered and six-membered lactone rings, respectively, with different substituents attached to it. The presence of a hydroxyl group at C-5 or C-23 of compounds [89] and [86] strikingly reduced their bioactivity (i.e. [86] versus [88] and [89] versus [90]). The reduction in bioactivity suggests that the hydrophobicity of the triterpene skeleton might have a significant role in their HIV-1 PR inhibitory activity. Kinetic and virtual docking study revealed that compound schisanlactone A [90] is a dimerization inhibitor of HIV-1 PR.
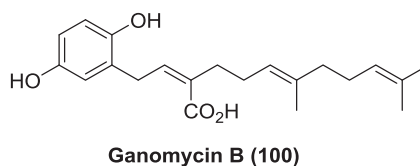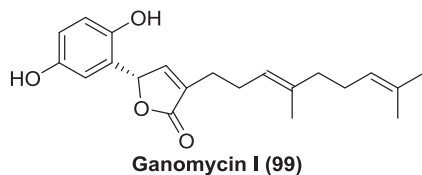
Colossolactones I (81) **Colossolactones II (82)** **Colossolactones IV (83)**

Colossolactone V (84) **Colossolactone VII (85)**

Colossolactone VIII (86) R$_1$ = H, R$_2$ = OAc, R$_3$ = OH
Colossolactone D (87) R$_1$ = OH, R$_2$ = H, R$_3$ = H
Colossolactone E (88) R$_1$ = H, R$_2$ = OAc, R$_3$ = H
Colossolactone G (89) R$_1$ = OH, R$_2$ = OAc, R$_3$ = H
Schisanlactone A (90) R$_1$ = H, R$_2$ = H, R$_3$ = H

Sato et al.[111] isolated three triterpene-farnesyl hydroquinone conjugates[112] along with many lanostane-type triterpenoids from the fruiting bodies of *Ganoderma sinense*. Several other lanostane-type triterpenoids isolated from spores and fruiting bodies of *Ganoderma lucidum* by El-Mekkawy et al. and Min et al. are also have been studied for their HIV-1 PR inhibitory activity. Structural comparison of these compounds with their protease inhibition capability revealed that, among, 24(25) unsaturated ganoderic acids (major constituents of *G. sinense*), compound Ganoderic acid GS-1 **[91]**, Ganoderic acid GS-2 **[92]**, Ganoderic acid DM **[93]**, with 3-keto moiety exhibited better bioactivities (IC$_{50}$ values 58, 30, and 38 μM, respectively), than Ganoderic acid b **[94]** (IC$_{50}$ 116) and other analogs with 3-β-hydroxy groups (IC$_{50}$ > 200 μM). Among the ganoderma alcohols,

compounds having unsaturation at 24(25) were more inhibitory than 24-hydroxy compounds. The 23-oxo ganoderic acids (the major compounds of *G. lucidum*) were failed to show any activity against HIV PR. Intermolecular hydrogen bonding between a 23-oxo group and a neighboring 26-carboxylic acid in the side chain, which could prevent interaction with amino acid residues of enzyme molecules, might be responsible for this inactivity. Thus, substituents (free hydroxy and oxo groups) at C-3 in the A ring and a terminal functional group in the side chain seem to have critical roles in their anti-HIV-1 PR activity. Ganoderiol F **[95]**, 20-hydroxylucidenic acid N **[96]**, ganodermadiol **[98]**, and 20(21)-dehydrolucidenic acid N **[97]** were comparatively better inhibitors against HIV-1 PR, with IC$_{50}$ values of 22, 25, 29, and 48 μM, respectively.

Ganoderic acid GS-1 (91) **Ganoderic acid GS-2 (92)** **Ganoderic acid DM (93)**

Ganoderic acid b (94) **Ganoderiol F (95)** **20-hydroxylucidenic acid N (96),**

20(21)-dehydrolucidenic acid N (97) **Ganodermadiol (98)**
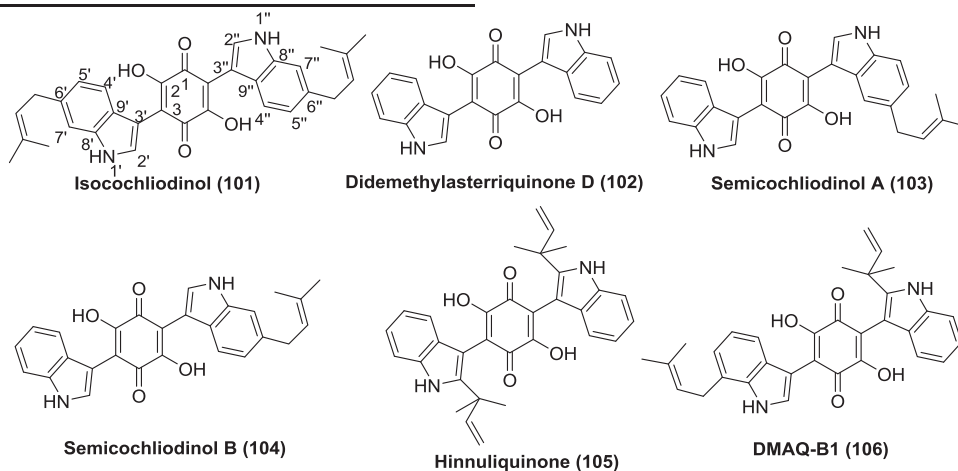
Two farnesyl hydroquinones, ganomycin I [99] and ganomycin B [100], isolated from the chloroform extract of the same mushroom by the same group[110] showed HIV-1 PR inhibition with IC_{50} values of 7.5 and 1.0 µg/ml, respectively. Through kinetics and virtual docking study, it has been found that compound [100] competitively inhibits the active site of the enzyme whereas the tetracyclic triterpene schisanlactone A [90], was a dimerization inhibitor,[108] with an IC_{50} value of 5.0 µg/ml.

showed hydrogen bonding between the dihydroxybenzoquinone moiety of [101] and [102] to both active site aspartic acids (Asp25/Asp25′) of the protease and the indole parts of the inhibitors filling the P2 and P2′ pockets of the protease. Hinnuliquinone, a C2-symmetric dimeric bisindolylquinone, initially isolated as pigment from fungus *Nodulisporium hinnuleum*,[116] again recently isolated from an unidentified fungus and found to show significant HIV-1 PR activity with an IC_{50} of 2.5 µM. It also showed remarkable inhib-

Ganomycin I (99)

Ganomycin B (100)

Bisalkylated 2,5-dihydroxybenzoquinones or asteriquinones are a class of fungal metabolites very prevalent among various fungal species.[113,114] Many of these asteriquinones found to exhibit remarkable inhibitory activities when tested against HIV-1-protease. Isocochliodinol[115] [101] and didemethylasterriquinone D [102] initially isolated from *Chaetomium* sp. and later isolated from *Chrysosporium merdarium*[60] together with semicochliodinol A [103] and B [104] and tested for their HIV-1 PR inhibitory activity. All these asteriquinones showed very strong inhibitory activity against HIV-1 PR with mean IC_{50} values of 0.24, 0.18, 0.37, and 0.5 µM, respectively. Molecular modeling of the HIV-1 PR inhibitor complexes
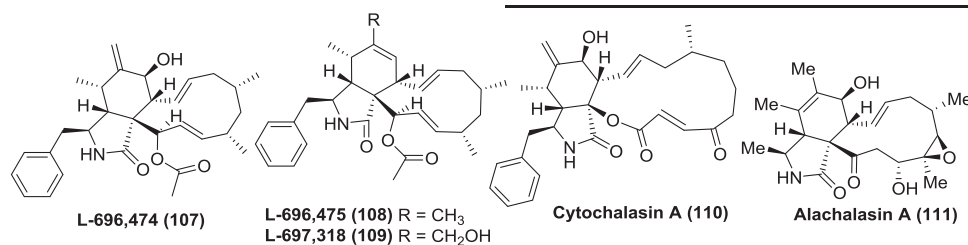
ition against highly resistant mutant enzyme A44[117] with an IC_{50} of 1.8 µM. It exerted high competitive binding affinity (Ki values of 0.97 and 1.25 µM) toward isolated protease enzymes of both wild-type and mutant HIV virus (A-44). X-ray crystallographic study of hinnuliquinone [105] and enzyme cocrystal and in comparison with structurally similar fungal metabolites demethylasterriquinone B-1 (DMAQ-B1) [106][118,119] ($IC_{50} = 57$ µM), and other structurally related metabolites (IC_{50} values of 180- > 500 nM)[120] it has been found that symmetric dimeric structure of hinnuliquinone and no substitution at 5, 6, and 7 positions are two major structural features behind its high HIV-1 PR inhibitory activity.

Isocochliodinol (101)

Didemethylasterriquinone D (102)

Semicochliodinol A (103)

Semicochliodinol B (104)

Hinnuliquinone (105)

DMAQ-B1 (106)

Cytochalasins are another important class of fungal secondary metabolites containing highly substituted isoindolone ring with a benzyl group at the C-3 position and fused to an 11- to 14-membered macrocyclic ring. So far, more than 80 cytochalasins, isolated from various fungal species, including *Aspergillus*, *Phomopsis*, *Penicillium*, *Zygosporium*, *Chaetomium*, *Phoma*, *Xylaria*, *Hypoxylon*, and *Rhinocladiella* sp.[121,122] These compounds exhibit a broad spectrum of bioactivities.[123]
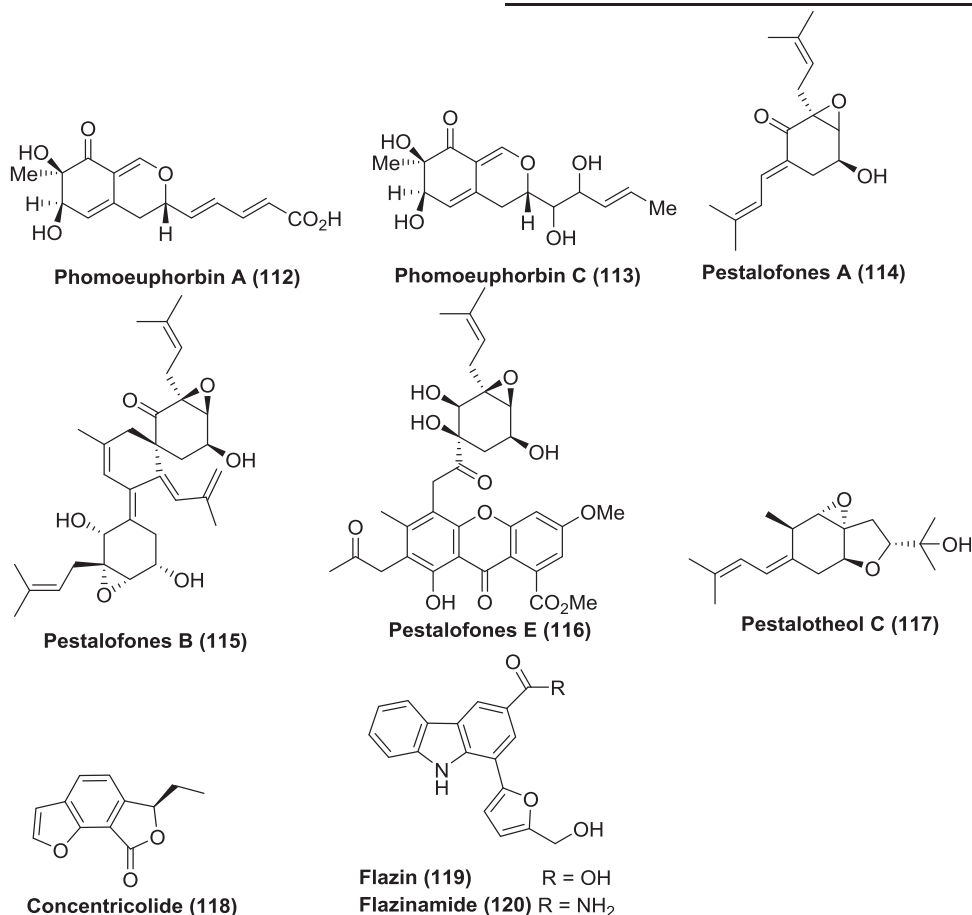
Ondeyka et al.[124,125] studied the HIV PR activity of three newly isolated cytochalasins L-696,474 [107], L-697,318 [108], and L-696,475 [109] from fermented broth of *Hypoxylon fragiforme* along with several other commercially available cytochalasins. Compounds L-696,474 [107] showed fast, specific, and competitive HIV-1 PR inhibitory activity with an IC_{50} of 3 µM and the mode of inhibition was competitive with respect to substrate (apparent $Ki = 1$ µM).[126] The inhibition of [107] was also independent of the HIV-1 PR concentration. Two other structurally related cytochalasins isolated from same fungus, [108] and [109] failed to show any such activity. The striking difference in protease inhibitory activity of [107] with other structurally similar compound [108] and [109] suggests that hydrogen bonding capability C-7 hydroxyl group of [107] might have a critical role to play in such activity. Protease activity assay with other commercially available cytochalasin showed that only cytochalasin A, containing 14-membered lactone ring showed almost similar activity with compound [107] containing an 11-membered carbocyclic ring. Seven new cytochalasin type of metabolites have been isolated from cultures of an isolate of *Stachybotrys charatum*.[127] Alachalasin A [111] displayed inhibitory effect on HIV-1$_{LAI}$ replication in C8166 cells with EC_{50} and CC_{50} values of 8.01 and 101.55 µM, respectively (selectivity index: 12.68; positive control indinavir showed EC_{50} value of 8.18 nM).

with $EC_{50} = 79$ and 71 µg/ml.[128] They exerted minimal cytotoxicity against C8166 cells ($CC_{50} > 200$ µg/ml) and are considered unfavorable for further drug development because of their low therapeutic index. Liu et al.[129] reported moderate inhibitory activity of pestalofones A [114], B [115], and E [116] on HIV-1 replication in C8166 cells. These new cyclohexanone derivatives, isolated from cultures of the plant endophytic fungus *Pestalotiopsis fici* along with five other structurally related new and known metabolites, showed inhibitory effects with EC_{50} values of 90.4, 64.0, and 93.7 µM, respectively, without any toxicity even at higher concentration (>200 µM). Pestalotheol C [117] along with other three new metabolites isolated from cultures of a plant endophytic fungus *Pestalotiopsis theae*. Modified Mosher method is used for determination of its absolute stereochemistry. Pestalotheol C [117] showed an inhibitory effect on HIV-1$_{LAI}$ replication in C8166 cells, with EC_{50} and CC_{50} values of 16.1 and 163 µM, respectively (selectivity index, SI = 10.1) compared to the positive control indinavir sulfate with an EC_{50} value of 8.18 nM. A novel benzofuran lactone, concentricolide [118], isolated from fruiting bodies of the xylariaceous ascomycete *Daldinia concentrica*,[130] showed HIV-1 inhibitory effect with an EC_{50} value of 0.31 µg/ml in cytopathic inhibition assay with a high (TI = 247) therapeutic index. Further investigation revealed that concentricolide [118] blocks syncytium formation between HIV-1 infected cells and normal cells with an EC_{50} value of 0.83 µg/ml. A β-carboline compound, flazin [119], isolated from *Suillus granulatus*[131] has been shown weak anti-HIV-1 activity but its synthetic amido analog flazinamide[132] [120] exhibited 6.2-fold more HIV-1 inhibitory activity and 4.1-fold lower toxicity compared to [120] (therapeutic index value increased from 12.1 to 312.2). Flazinamide potently reduced syncytium formation induced by HIV-1IIIB with an EC_{50} value of 0.38 µM and also inhibited HIV-2 ROD and HIV-2 CBL-20 infection with EC_{50}

L-696,474 (107)

L-696,475 (108) R = CH₃
L-697,318 (109) R = CH₂OH

Cytochalasin A (110)

Alachalasin A (111)

Other fungal metabolites with HIV inhibitory activity. Phomoeuphorbins A [112] and C [113], two new azaphilones isolated from cultures of an endophytic fungus *Phomopsis euphorbiae* along with two other new azaphilones, exhibited weak in vitro inhibitory activities against HIV replication in C8166 cells

values of 0.57 and 0.89 µM, respectively. Compound [120] though efficiently block the fusion between normal cells and HIV-1/HIV-2 chronically infected cells, it exhibits very weak activities on recombinant HIV-1 RT, protease or integrase and only at higher concentrations.

Phomoeuphorbin A (112)　　**Phomoeuphorbin C (113)**　　**Pestalofones A (114)**

Pestalofones B (115)　　**Pestalofones E (116)**　　**Pestalotheol C (117)**

Concentricolide (118)

Flazin (119)　　R = OH
**Flazinamide (120)** R = NH$_2$

Inhibitors of influenza virus

Influenza is a viral respiratory disease that affects mainly the nose, throat, bronchi and, sometimes lungs. The causative agent influenza A virus (IAV), being a genus of the Orthomyxoviridae family, reproduces rapidly, mutates frequently, and occasionally crosses species barriers.[133] Emergence of a new mutated influenza virus with fatal pathogenicity also are able to unleash a major pandemic with a greater morbidity and mortality. The worldwide spread of the swine-originated influenza virus A (H1N1) in human in 2009 had attracted great attention all over the world. At present antiviral drugs that block the activities of M2 protein and neuraminidase (NA), such as adamantanes (M2 protein), zanamivir, and oseltamivir (NA) can stop the infection effectively.[134,135] Despite these successes, the emergence of drug resistance viral strain and toxicity of present drugs remain as major concerns.[136] Therefore, development of novel antiviral agent with high efficacy and low toxicity bears a great importance.

Two novel compounds with pentacyclic moiety containing *cis*-fused decalin, stachyflin **[121]** and acetylstachyflin **[122]**, are isolated through solid-state fermentation of *Stachybotrys* sp.[137] Their in vitro antiviral activities against IAV and cytotoxicities were tested using MDBK cells and compared with two known drug, amantadine and zanamivir. Stachyflin **[121]** exhibited about 77 times more antiviral activity against influenza A/W SN/33 (H1N1) virus with an IC$_{50}$ value of 0.003 μM than acetylstachyflin **[122]** with an IC$_{50}$ value of 0.23 μM. Structural correlation with bioactivity suggested that the hydroxyl group at the C-3 position might be important for the antiviral activity. Inhibitory activity stachyflin **[121]** was around 1760 and 250 times more than that of two known anti-influenza drug amantadine (IC$_{50}$ of 5.3 μM) and zanamivir (IC$_{50}$ of 0.75 μM), respectively. Stachyflin being a fusion inhibitor[138,139] between the viral envelope and the endosome constituting the cell membrane is mechanistically different from amantadine (NA inhibitor) and zanamivir (ion channel inhibitor). The cytotoxicities of **[121]** and **[122]** against MDBK cells were 65 and 44 μM, respectively.

Neosartoryadins A [123] and B [124], together with three biogenetically related compounds were isolated from the endophytic fungus *Neosartorya udagawae*.[140] Both these compounds displayed activity against influenza virus A (H1N1) with IC_{50} values of 66 and 58 µM, respectively, versus positive control ribavirin with $IC_{50} = 94$ µM. Among 14 different compounds isolated from ethyl acetate extract of the culture of mangrove-derived fungus *Cladosporium* sp.[141] oxoglyantrypine [125], norquinadoline A [126], deoxynortryptoquivaline [127], deoxytryptoquivaline [128], tryptoquivaline [129], and quinadoline B [130] exhibited significant activities against influenza virus A (H1N1), with IC_{50} values of 85, 82, 87, 85, 89, and 82 µM, respectively.

(H1N1) with IC_{50} values of 42.07 and 62.05 µg/ml, respectively, in cytopathic effect (CPE) inhibition using MDCK cells (ribavirin used as a positive control, IC_{50} 24.60 µg/ml). Cladosins C [133] along with four new other hybrid polyketides were isolated from the deep-sea-derived fungus *Cladosporium sphaerospermum*.[143] The structures and stereochemistry of all these compounds were determined by spectroscopic data, chemical conversion using both Mosher's and Marfey's methods. Cladosins C exhibited activity against influenza A H1N1 virus with an $IC_{50} = 276$ µM in comparison to ribavirin as a positive control, IC_{50} 131 µM. A cyclic tetrapeptide, asperterrestide A [134] isolated along with two new and 10 other known compounds, from fermentation broth of the
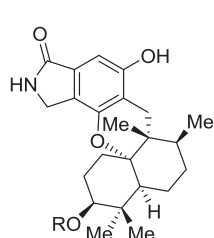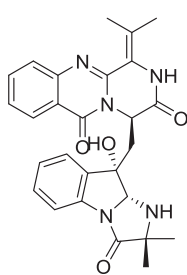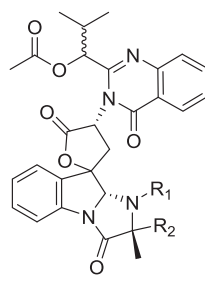

Stachyflin (121) R = H
Acetylstachyflin (122) R = Ac
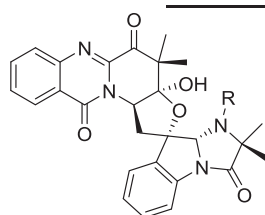
Neosartoryadins A (123) R = H
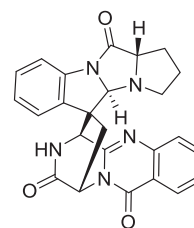**Neosartoryadins B (124)** R = OH
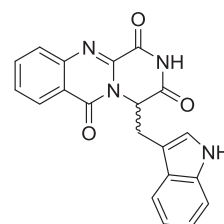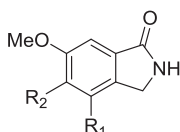
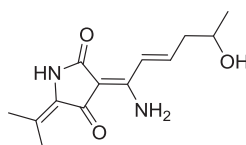**Oxoglyantrypine (125)** (14S)-2

Norquinadoline A (126) R=H

Deoxynortryptoquivaline (127)
R1=H, R2= H, (2R,3S,12R,15S,27S)
Deoxytryptoquivaline (128)
R1=H, R2= Me, (2R,3S,12R,27S)
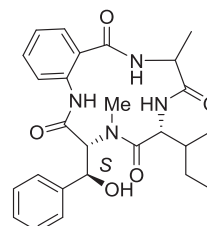**Tryptoquivaline (129)**
R1=OH, R2= Me, (2R,3S,12R,27S)

Quinadoline B (130)

Emerimidine A (131) R₁=OH, R₂=OMe
Emerimidine B (132) R₁=OMe, R₂=OH

Cladosins C (133)

Asperterrestide A (134)

Isolation of six new isoindolones derivatives along with six known compound is reported from endophytic fungus *Emericella* sp. (HK-ZJ) of mangrove plant *Aegiceras corniculatum*.[142] Among these only compounds emerimidine A [131] and emerimidine B [132] showed moderate inhibitory activity against IAV
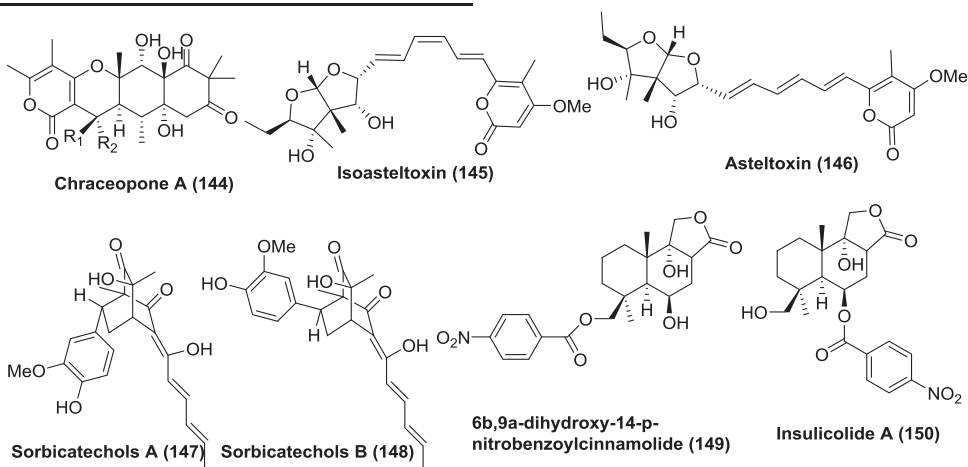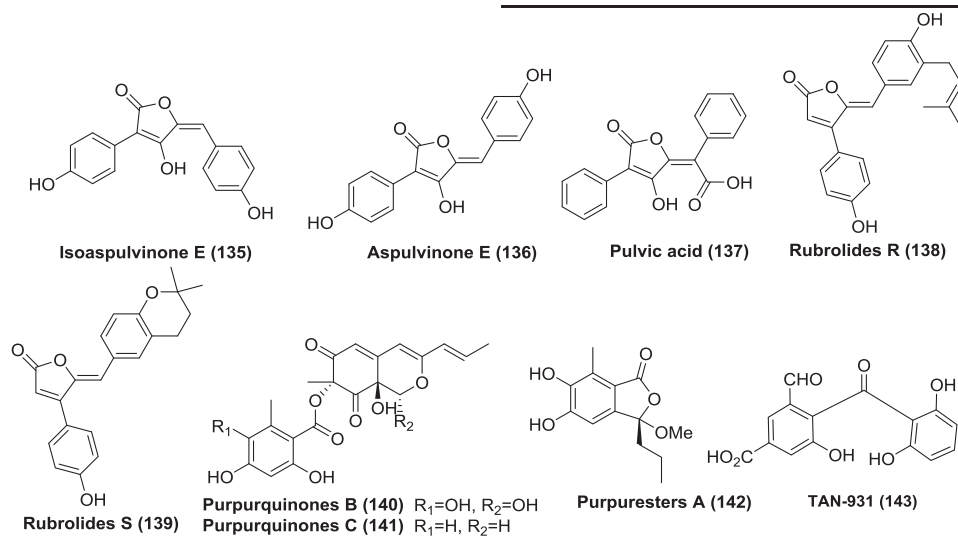
marine-derived fungus *Aspergillus terreus*.[144] Their structures were elucidated by spectroscopic analysis, and the absolute configuration of [134] was determined by the Mosher ester technique and analysis of the acid hydrolysates using a chiral phase HPLC column. Compound [134] showed inhibitory effects on the

influenza virus strains A/WSN/33 (H1N1) and A/Hong Kong/8/68 (H3N2) with IC_{50} values of 15 and 8.1 μM.

One new butenolide isoaspulvinone E [135] and two known butenolides aspulvinone E [136] and pulvic acid [137] have recently been isolated by Gao et al.[145] from the marine-derived fungus, *A. terreus* Gwq-48. All these compounds were moderately active against influenza A H1N1 virus with IC_{50} values of 32.3, 56.9, and 29.1 μg/ml, respectively, versus two positive controls ribavirin and zanamivir with $IC_{50} = 24.6$ μg/ml and 28.4 ng/ml, respectively. Among these only compound [135] showed significant H1N1 viral NA inhibition activity. Docking experiment with active site of enzyme with these compounds showed that *trans* orientation of aryl group is necessary for this activity.

Two new rubrolides, rubrolides R [138] and S [139] were isolated from the fermentation broth of the marine-derived fungus *A. terreus* OUCMDZ-1925 showed anti-influenza A (H1N1) virus activity with IC_{50} values 221.6, 87.1 μM, respectively, compared to positive control ribavirin with IC_{50} value of 118.8 μM in CPE inhibition assay.[146]

Wang et al.[147] isolated total of nine compounds from ethyl acetate extract of an acid-tolerant fungus, *Penicillium purpurogenum*, and tested their activity against influenza virus. Among these compounds purpurquinones B [140], purpurquinones C [141], purpuresters B [142], and TAN-931 [143] exhibited significant antiviral activity against H1N1, with IC_{50} values of 61.3, 64.0, 85.3, and 58.6 μM, respectively.

Isoaspulvinone E (135) Aspulvinone E (136) Pulvic acid (137) Rubrolides R (138)

Rubrolides S (139) Purpurquinones B (140) R_1=OH, R_2=OH Purpuresters A (142) TAN-931 (143)
Purpurquinones C (141) R_1=H, R_2=H

Chraceopone A (144) Isoasteltoxin (145) Asteltoxin (146)

Sorbicatechols A (147) Sorbicatechols B (148) 6b,9a-dihydroxy-14-p-nitrobenzoylcinnamolide (149) Insulicolide A (150)

Recent bioassay-guided isolation of an Antarctic soil-derived fungus, *Aspergillus ochraceopetaliformis* by Wang et al.[148] resulted in isolation of six new and two known metabolites. Among these compounds, chraceopone A [144], isoasteltoxin [145], and asteltoxin [146] exhibited antiviral activities against the H1N1 and H3N2 influenza viruses with IC$_{50}$ values of > 20.0/12.2, 0.23/0.66, and 0.54/0.84 μM, respectively. Sorbicatechols A [147] and B [148], two new sorbicillinoids, together with other two known compounds, protocatechuic acid methyl ester and caffeic acid methyl ester were isolated from the culture of the marine sediment-derived fungus *Penicillium chrysogenum* PJX-17.[149] Their structures were assigned by spectroscopic data and TDDFT ECD calculations. Compounds [147] and [148] exhibited activities against influenza virus A (H1N1), with IC$_{50}$ values of 85 and 113 μM, respectively. Two nitrobenzoyl sesquiterpenoids, 6β,9α-dihydroxy-14-*p*-nitrobenzoylcinnamolide [149], and a known analog, insulicolide A [150] were isolated from extracts of the culture of marine-derived fungus *Aspergillus ochraceus* Jcma1F17.[150] The new compound [150] also exhibited moderate inhibitory activity against H3N2 viruses, with IC$_{50}$ values of 17.0 μM, but structurally related compound [150] failed to show any activity. Three triterpenes, ganodermadiol [98], lucidadiol [151], and applanoxidic acid G [152] isolated by Mothana et al.[151] from the European Basidiomycete *Ganoderma pfeifferi* showed antiviral activity against influenza virus type A in dye uptake assay.[152,153] These compounds showed activity against influenza type A virus infection on MDCK cells with ED$_{50}$ values > 0.22, 0.22, and 0.19 μM, respectively.
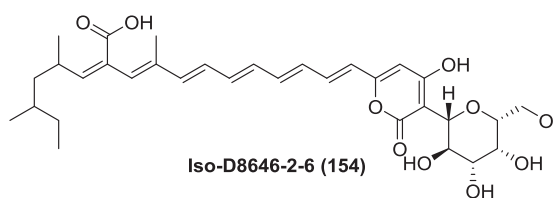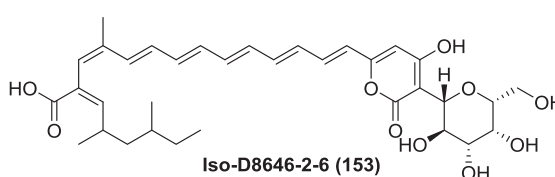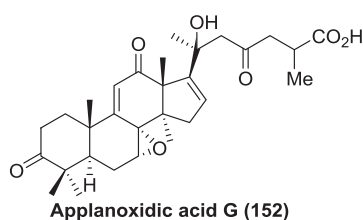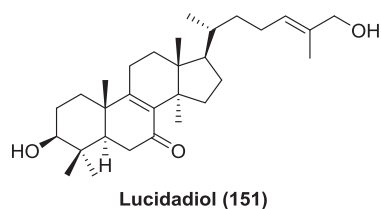
significant anti-IAV (H1N1) inhibitory activity with IC$_{50}$ values of 91.5 and 101.3 μM (ribavirin as a positive control, IC$_{50}$ 114.8 μM) in CPE inhibition assay.
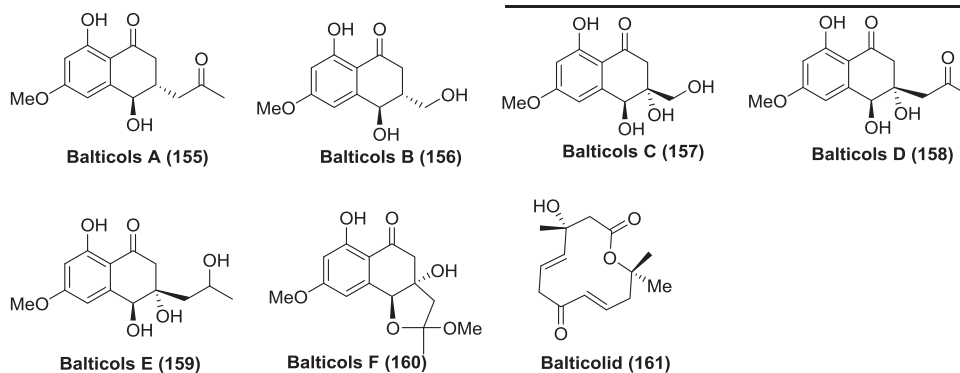
Inhibitors for herpes viruses

Herpes simplex viruses (HSVs) inhibitors. HSVs are very prevalent human pathogens that belong to the Herpesviridae family. There are two serotypes of human HSVs, namely HSV-1 and HSV-2. These are characterized by four main constituents, namely an icosapentahedral capsid, an amorphous layer of proteins around the capsid, an envelope, and an electron-dense core with large double-stranded DNA. HSV-1 is the main causative agent for facial infections with visible cold sores or fever blisters and HSV-2, in general, is associated with genital infection and causes genital herpes,[155,156] but both strains can cause infection in either area.[157] HSV can have both lytic and latent infection cycles. After initial infection, HSV-1 and HSV-2 are transported along sensory nerves to the sensory nerve cell bodies, where they establish lifelong latency of the human host. HSV exits latency periodically and is transported to the body surface where recurrent infection occurs.[158] Though several viral genes critical for HSV replication have been identified,[159,160] HSV treatment is generally limited to few synthetic antivirals as acyclovir, penciclovir, and famciclovir.[161,162] Thus, the search for new antivirals with novel modes of action remains unavoidable.

Six new naphthalenone derivatives, balticols A–F [155–160] and a known metabolite altechromone A[163] were isolated from the ethyl acetate extract of the

Lucidadiol (151)

Iso-D8646-2-6 (153)

Applanoxidic acid G (152)

Iso-D8646-2-6 (154)

A pyronepolyene C-glucoside, named iso-D8646-2-6 [153] together with known compound D8646-2-6 [154], was isolated[154] from the sponge-associated fungus *Epicoccum* sp. JJY40. Both these compounds showed

culture broth of Ascomycota fungal strain 222 obtained from driftwood collect from the coast of the Greifswalder Bodden, Baltic Sea, Germany.[164] All isolated balticols A–F [155-160] exhibited strong anti-HSV-1 activity with IC$_{50}$ values 0.01–1 μM/ml.
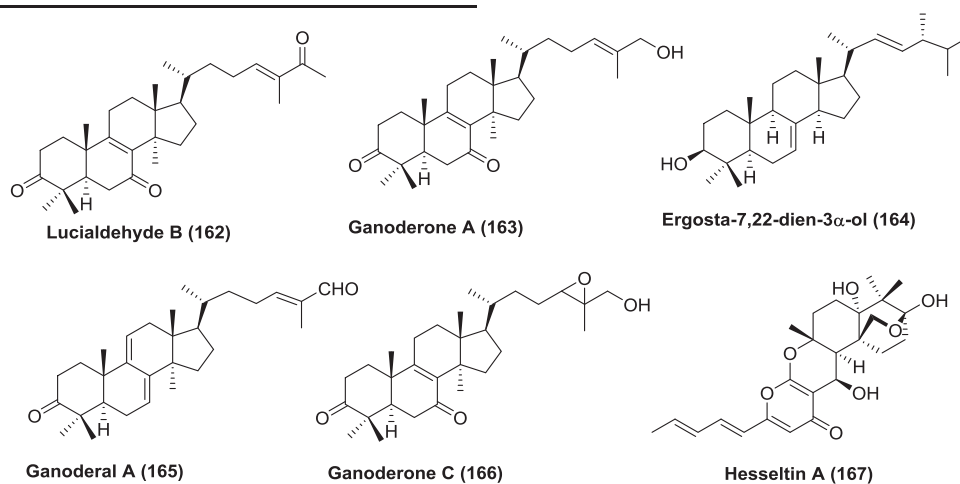
Balticol E [159] exhibited 10 times more activity against Herpes type I viruses with an IC_{50} value of 0.01 µg/ml in comparison to the standard aciclovir (IC_{50} 0.1 µM). However, balticol D [158] and balticol F [160] exhibited similar activity with the standard. Balticol C [157], balticol D [158], and balticol F [160] also showed considerable activity against influenza viruses with IC_{50} values 1, 0.1, and 1 µg/ml, respectively, compared to the positive standard amantadine sulfate with IC_{50} 15 µg/ml. But the activity of balticol E [159] is only very specific to HSV-1 and showed no activity against influenza virus. Another 12-membered macrolide, balticolid [161], from same strain displayed anti-HSV-1 activity with an IC_{50} value of 0.45 µM compared to positive control aciclovir with IC_{50} value of 0.44 µM.[165]

New compound ganoderone A [163] and known ergosta-7,22-dien-3α-ol [164] and ganoderal A [165], also showed very strong activity against HSV with IC_{50} values 0.3, 0.03, 0.03 µg/ml, respectively. Ganoderone C [166] and [162] and [164] exhibited considerably strong activity against IAV with IC_{50} values 2.6, 3.0, and 0.78 µg/ml, respectively, compared to the positive control amantadine sulfate with IC_{50} 15 µg/ml. Another triterpene ganodermadiol [98] isolated from same species by Mothana et al.[151] exhibited activity against HSV-1 infection on Vero cells with an ED_{50} value 0.068 µM. Other two triterpenes lucidadiol [151] and applanoxidic acid G [152] simultaneously isolated together from same species failed to show any such activity. Hesseltin A [167], a novel compound of

Balticols A (155)   **Balticols B (156)**   **Balticols C (157)**   **Balticols D (158)**

Balticols E (159)   **Balticols F (160)**   **Balticolid (161)**

Among four sterols and 10 triterpenes were isolated from the fruiting bodies of *Ganoderma pfeifferi* by Niedermeyer et al.,[166] known metabolite lucialdehyde B [162][167] exhibited strongest inhibitory activity against HSV with an IC_{50} of 0.075 µg/ml compared to the positive control aciclovir ($IC_{50} = 0.1$ µg/ml).[166]

mixed polyketide–terpenoid origins was isolated from the filamentous fungus *Penicillium hesseltinei*.[168] It exhibited 25–50% inhibition of HSV-1 viral growth at 300 µg/ml, but more significantly [167] did not show any cytotoxicity at that concentration.[168]

Lucialdehyde B (162)   **Ganoderone A (163)**   **Ergosta-7,22-dien-3α-ol (164)**

Ganoderal A (165)   **Ganoderone C (166)**   **Hesseltin A (167)**

Monorden [168], tetrahydromonorden [169], several resorcylic acid lactones pochonins A–E [170–174] were isolated from cultures of the clavicipitaceous hyphomycete *Pochonia chlamydosporia* var. *catenulata* strain P 0297.[169] Fermentation of P0297 in bromide containing culture media yielded monocillins II [175] and III [176] as major metabolites and monorden [168] as well as monocillins F [156] as minor metabolites. In the HSV1 replication assay, monorden [168] showed inhibitory activity in the nM (IC$_{50}$ 0.2–0.8 μM) range accompanied by cytostatic effects. The other metabolites with

Seventeen new and known lactones including eight territrem and nine butyrolactone derivatives were isolated from a marine-derived fungus *A. terreus* SCSGAF0162 under solid-state fermentation of rice.[170] Among these, four butyrolactone derivatives, 11 a-dehydroxyiso-terreulactone A [178], isobutyrolactone II [179], aspernolide A [180], and arisugacin A [181] exhibited moderate HSV-1 antiviral activity with IC$_{50}$ values of 16.4, 21.8, 28.9, and 6.34 μg/ml, respectively, under their noncytotoxic concentrations (TC$_0$) on Vero cell line.

Monorden (168)

Tetrahydromonorden (169)

Pochonin A (170) R1 = Cl, R2 = H;
Pochonin B (171) R1 = Cl, R2 = OH;
Monocillin III (176) R1 = H, R2 = H;

Pochonin C (172)

Pochonin D (173) R = H;
Pochonin E (174) R = OH

Monocillin II (175) R1 = H, R2 = H
Monocillin F (177) R1 = H, R2 = OH

R$_1$=H, R$_2$=OH **11a-dehydroxyiso-terreulactone A (178)**
R$_1$=CO$_2$Me, R$_2$=OH **Isobutyrolactone II (179)**
R$_1$=CO$_2$Me, R$_2$=H **Aspernolide A (180)**

Arisugacin A (181)
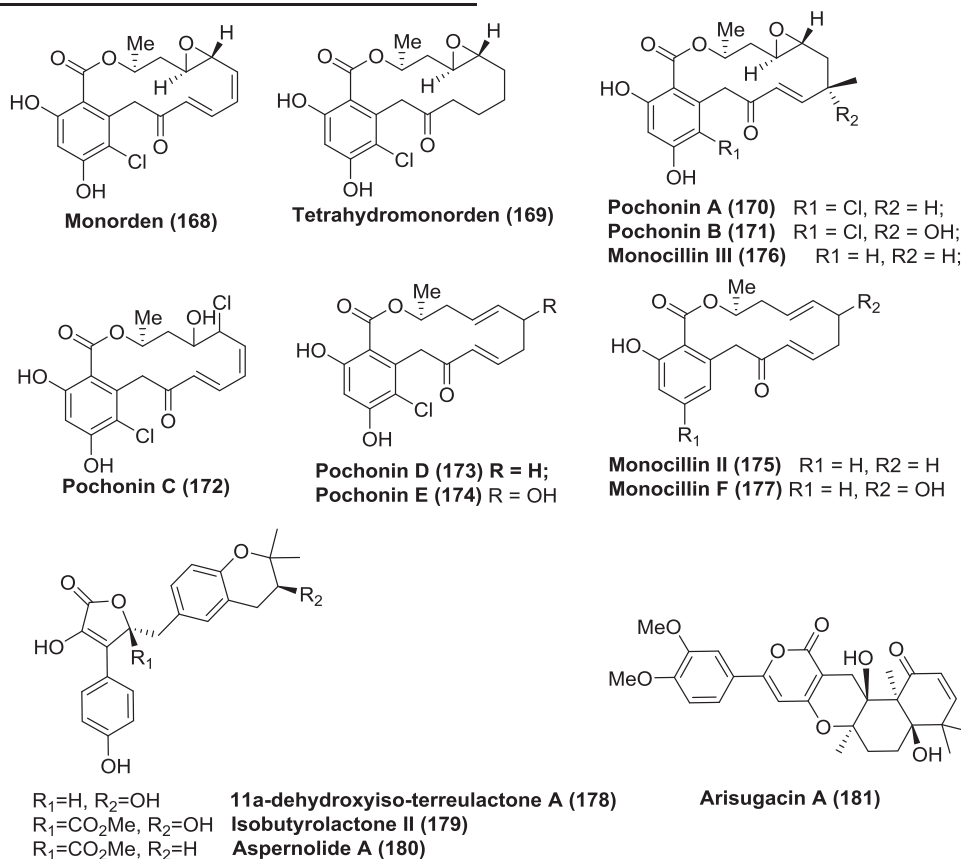
epoxide moieties, [169], [170], [171], and [176] exhibited bioactivities with IC$_{50}$ values 1.5, 2, 10, and 0.4 μM, respectively. Monocillin III [176], without any chlorine substituent, showed inhibitory activity in the nanomolar range, which suggest that the chlorine substituent is not essential for the antiviral activity. Pochonins E and monocillin F [177] with an allyl alcohol moiety showed moderate inhibitory activity. Inhibition of HSV was generally accompanied by weak cytostatic effects on the host cell, as observed microscopically. This results in rather low tolerability in vitro described by the selectivity indices (SIs).

A series of lypophilic linear peptides, halovirs A–E [182-186], isolated from saline fermentation of a marine-derived fungus *Scytidium* sp.[171] have displayed potent antiviral activity against HSV-1 with ED$_{50}$ values 1.1, 3.5, 2.2, 2.0, and 3.1 μM, respectively, in virus-induced CPE assay on Vero cell.[171] Halovir A [182] exhibited equal inhibition toward replication of both HSV-1 and HSV-2 with an ED$_{50}$ value of 280 nm in standard plaque reduction assay but did not show any cytotoxicity toward the host cell even at 0.85 μM. Further mechanistic studies revealed that

halovirs kill the virus in direct contact and in time-dependent manner before it can affect the host cell. Though the mechanism of virucidal activity of halovirs was not clear but it is presumed to happen through membrane destabilization of virus.
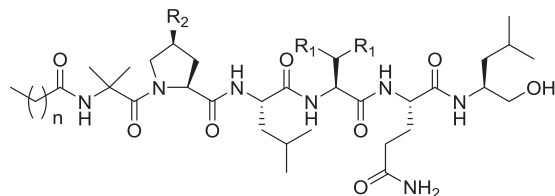

Halovir A (182) R_1 = Me, R_2 = OH, n = 12
Halovir B (183) R_1 = H, R_2 = OH, n = 12
Halovir C (184) R_1 = Me, R_2 = H, n = 12
Halovir D (185) R_1 = Me, R_2 = OH, n = 10
Halovir E (186) R_1 = Me, R_2 = H, n = 10

Cytomegalovirus (CMV) inhibitors. CMV is a double-stranded DNA virus and is another member of the Herpesviridae family. It has the largest genome of the herpes viruses. Human CMV grows only in human cells and replicates best in human fibroblasts. CMV usually causes an asymptomatic infection or produces mild flu-like symptoms; afterward, it remains latent throughout life and may reactivate. In immunocompromised individuals (by HIV infection, solid-organ transplantation, or bone marrow transplantation) symptomatic disease usually manifests as a mononucleosis syndrome.[172] Symptomatic CMV disease can affect almost every organ of the body, resulting in fever of unknown origin, pneumonia, hepatitis, encephalitis, myelitis, colitis, uveitis, retinitis, and neuropathy. Retinitis is the most common manifestation of CMV disease in patients who are HIV positive. In patients coinfected with HIV, CMV infection leads to progression to AIDS and eventually death, even in those receiving highly active antiretroviral therapy (HAART).[173] At least 60% of the US population has been exposed to CMV,[174] with a prevalence of more than 90% in high-risk groups (e.g. babies from infected mothers, people with HIV, male homosexuals etc.).[175,176] The prevailing age of infection varies worldwide. In developing countries, most infections are acquired during childhood, whereas, in developed countries, up to 50% of young adults are CMV seronegative. The incidence of CMV seropositivity rises with age and in a US-based study was reported to increase from 36% in children aged 6–11 years to 91% in individuals older than 80 years.[177] Other factors associated with CMV seropositivity include ethnicity (77% in Mexican Americans and 71% in blacks),[178] female sex, foreign-born status, and low socioeconomic status.[178] CMV is transmitted from person to person via close contact with an individual who is excreting the virus. It can be spread through the placenta, blood transfusions, organ transplantation, and breast milk. It can also be spread through sexual transmission. In the United States, congenital CMV transmission from a mother with acute infection during pregnancy is a significant cause of neurological abnormalities and deafness in approximately 8000 newborns annually.[179] The drug of choice for prevention of CMV disease in solid-organ transplant patients is valganciclovir.[180] Other than CMV retinitis, however, ganciclovir remains the mainstay of treatment, at least initially. Second-line treatments include foscarnet, cidofovir, or maribavir. Currently, there is no vaccine to prevent CMV infection.

A spiro compound having fused benzodihydrofuran and decalin moiety Sch 65676 **[205]** was isolated from the fermentation broth of an unidentified fungal culture. It exhibited in vitro inhibitory activity against the CMV protease with an IC_{50} value of 9.8 μg/ml.[181]
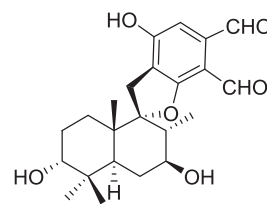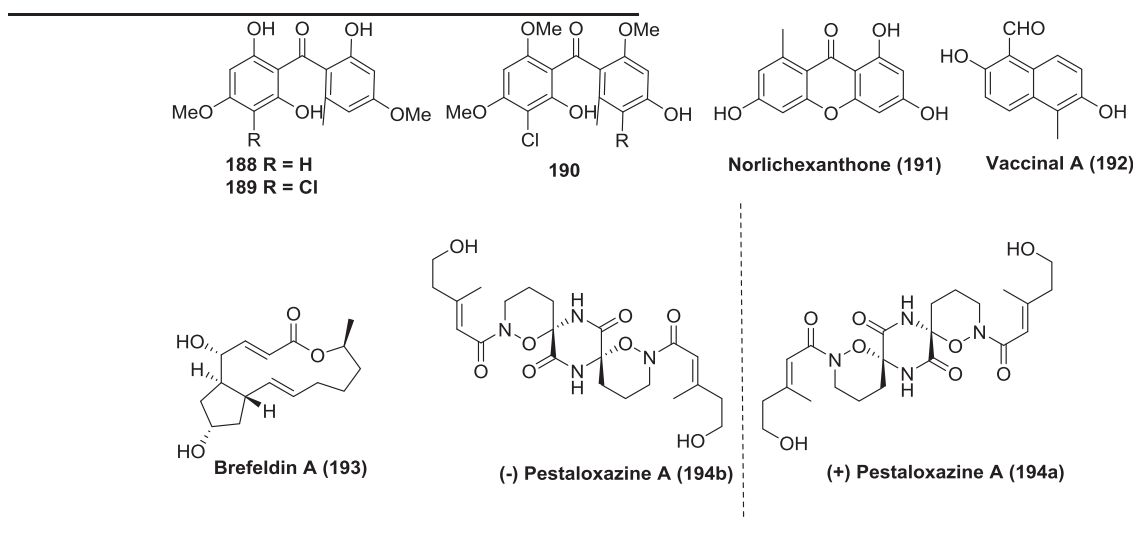

Sch 65676 (187)

Inhibitors of Enterovirus 71 (EV71). EV71 is a small, nonenveloped, single-positive stranded, RNA virus from the Enterovirus genus of the Picornaviridae family.[182] It is a common causative agent in hand, foot, and mouth disease (HFMD) and sometimes provokes severe and fatal neurological complications in young children and infants,[183] which may result in acute flaccid paralysis and even death.[184,185] In 2014, the World Health Organization showed that over 2.8 million cases were diagnosed with HFMD in the Western Pacific Region,[186] with widespread occurrence in China.[187] Biochemical pathway of viral replication is explored in considerable detail[188] and other strategies have been employed to develop antiviral drugs on the basis of the molecular characteristics of the virus.[189] In spite of huge scientific effort so far, no direct targeting vaccines or antivirals are available to treat severe EV71 infections. Many natural products and their synthetic analogs and derivatives have exhibited interesting inhibitory activity.[190] Bioactivity of some secondary metabolites from fungal sources against EV71 has been described here.

Four new xanthone derivatives **[188]**, **[189]**, **[190]**, and **[191]** along with 11 other new and known

xanthones and terpenoid derivatives were isolated from the cultures of sponge-derived fungus *Stachybotry* sp.[191] Compounds [189], [190], and [191] displayed activities against intestinal virus EV71 with IC_{50} values of 30.1, 50.0, and 40.3 µM, respectively. Compounds [188], [189], and [191] also exhibited significant inhibitory activity against cyclooxygenase (COX-2) with IC_{50} values of 10.6, 8.9, and 34.3 µM, respectively. A new naphthalene derivative, vaccinal A [192], together with 12 other new and known phenol derivatives were isolated from a mangrove endophytic fungus *Pestalotiopsis vaccinii*.[192] Vaccinal A [192] exhibited activity against EV71 and COX-2 virus with an IC_{50} value of 19.2 and 1.8 µM, respectively. Brefeldin A (BFA) [193] is a lactone antibiotic

[194b], with symmetric spiro-[oxazinane-piperazine-dione] skeleton, were isolated from a soft coral-derived fungus *Pestalotiopsis* sp.[197] Pure enantiomers (+) Pestaloxazine A [194a], (−) Pestaloxazine A [194b], and their mixture showed different antiviral activity against EV71 with IC_{50} values of 14.2, 69.1, and 16.0 µM, respectively, in in vitro CPE inhibition assay on the Vero cell line. Most promising (+) Pestaloxazine A [194a] exhibited 18-fold stronger activity than the positive control ribavirin ($IC_{50} = 256.1$ µM). Their SIs of anti-EV71 activity of [194a], [194b], and mixture of [194a] and [194b] were 9.2, 2.1, and 7.9 µM, respectively. The difference in antiviral activity and selectivity in two enantiomers indicates the importance of the stereo orientation of the spiro-center.

188 R = H
189 R = Cl

190

Norlichexanthone (191)

Vaccinal A (192)

Brefeldin A (193)

(-) Pestaloxazine A (194b)

(+) Pestaloxazine A (194a)

produced by fungal organisms *Eupenicillium brefeldianum* and later isolated from several other fungal species.[193,194] BFA, an inhibitor of COPI activity, has been shown to strongly inhibit viral RNA replication of poliovirus and EV11.[195,196] BFA inhibited EV71 replication in a dose-dependent fashion, without noticeable cytotoxicity. However, 20 ng/ml BFA inhibited EV71 replication effectively. Replication of EV71 fell substantially to 11% compared to the negative control, when 100 ng/ml BFA was used.[188] BFA inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus indirectly by preventing the formation of COPI-mediated transport vesicles.
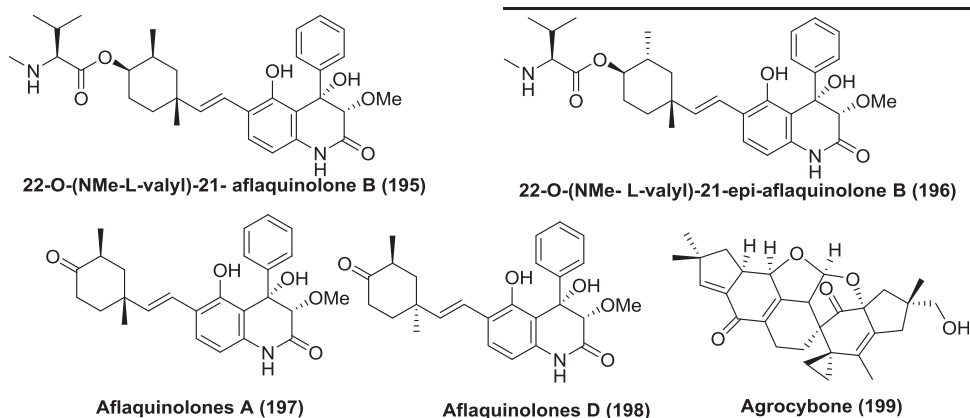
Nitrobenzoyl sesquiterpenoids, 6β,9α-dihydroxy-14-*p*-nitrobenzoylcinnamolide [149] exhibited moderate inhibitory activity against EV71, with an IC_{50} value of 9.4 µM but structurally analogous insulicolide A [150] from same fungal species did not show such activity.[150] A pair of enantiomeric alkaloid dimers, (+) Pestaloxazine A [194a] and (−) Pestaloxazine A

Inhibitors of respiratory syncytial virus (RSV)

Human RSV, a member of the genus *Pneumovirus* within the family Paramyxoviridae, is the leading cause of lower respiratory tract infection in infants and young children worldwide.[198] This negative (−) strand RNA virus with approximately 15 kilobases has 10 genes, which are responsible for the production of 11 viral proteins. Infection is mediated, in part, by an initial interaction between attachment protein (G) and a highly sulfated heparin-like glycosaminoglycan (Gag) located on the cell surface. Palivizumab and ribavirin are only therapeutic options available for treatment of RSV infections. Use of these drugs is also limited due to their toxicity in prolonged use and ineffectiveness against resistant strain. Therefore, there is a pressing need for development of new effective anti-RSV drugs[199] with less toxicity and novel mechanism of action.

Bioassay-guided separation of an extract of a gorgonian-derived fungus *Aspergillus* sp. led to isolation 22-O-(*N*Me-L-valyl)-21-epi-aflaquinolone B [195] along with one new epimer, 22-O-(*N*Me-L-valyl)-21-aflaquinolone B [196] and two other known analogs, aflaquinolones A [197] and D [198].[200] Structure and stereochemistry of the new compounds were determined by spectroscopic methods, ECD spectra, chemical conversion, and Marfey's method. Compound [196] and [197] exhibited around 500- and three-fold stronger anti-RSV activity with IC$_{50}$ value of 42 nM and 6.6 μM, respectively, compared to the positive control ribavirin (IC$_{50}$ = 20 μM) in CPE assay. Outstanding anti-RSV activity with high therapeutic ratio (TC$_{50}$/IC$_{50}$ = 520) of compound [196] makes it a promising lead compound for anti-RSV drug discovery. Large increase of anti-RSV activity of [196] compared to [197] may be attributed to the presence of additional *N*-Me-L-Val residue. No antiviral activity of [195] and [197] suggest that the configuration of the cyclohexane unit play a critical role in anti-RSV activity. Agrocybone [199], a novel illudane–illudane bis-sesquiterpene, isolated from the basidiomycete *Agrocybe salicacola*, was found to exhibit weak antiviral activity against RSV with IC$_{50}$ value of 100 μM in CPE and plaque reduction assays.[201]

small, enveloped, positive (+) strand RNA virus infects pigs and causes respiratory illness and a major failure in the reproduction of sows. Experimental infections have mirrored the clinical signs observed in natural outbreaks, including abortions, premature furrowing, mummified pigs, stillborn pigs, and elevated preweaning mortality.[203–207] By destroying macrophages it weakens a major part of the body's defense mechanism and allows bacteria and other viruses to proliferate and do damage. The high transmissibility of PRRSV[208] through a number of routes, including oral, intranasal, intramuscular, intraperitoneal, and vaginal, etc. increases the risk of its infection. Due to the complex epidemiologic profile, it has been especially difficult to control under the usual conditions of commercial swine production. An RNA genome of about 15 kilobases in size is responsible for the coding of two principal polyproteins, namely 1a and 1ab.[209] PRRSV classifies into two subgroups, namely A and B, representing the North American and European strains, which have different level of virulence. PRRSV transmission can happen in many ways such as equipment, hands, needles, and footwear associated with mechanical transmitters, including flies and mosquitoes. Despite the prevalence of vaccines, control of the virus infection

22-O-(NMe-L-valyl)-21- aflaquinolone B (195)

22-O-(NMe- L-valyl)-21-epi-aflaquinolone B (196)

Aflaquinolones A (197)

Aflaquinolones D (198)
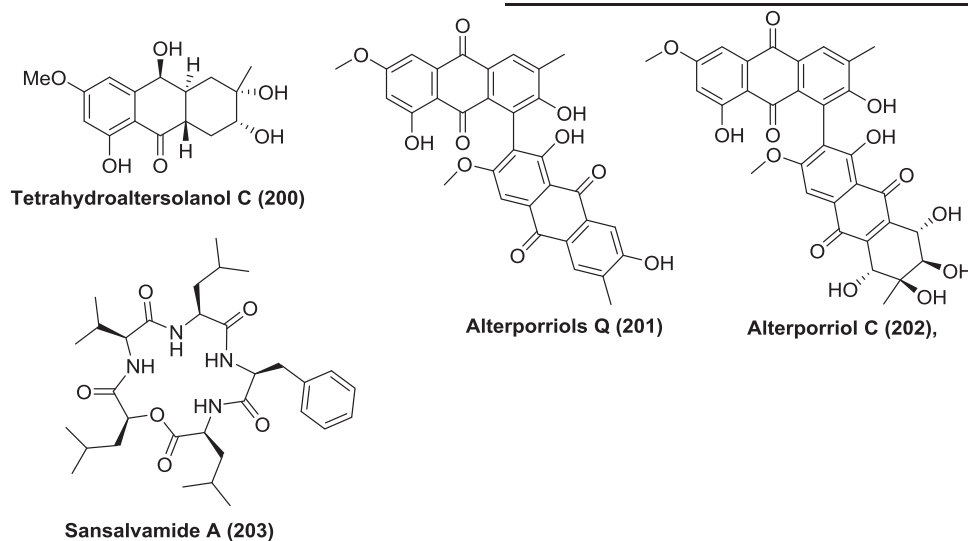
Agrocybone (199)

Inhibitors of porcine reproductive and respiratory syndrome (PRRS)

PRRS is one of the most economically devastating diseases affecting swine industry worldwide.The causative virus PRRS virus (PRRSV), like other members of the Arteriviridae family, has the ability to infect macrophages[202] and to persist in tissues for at least several months after the acute stage of infection subsides. This

in usual swine conditions has always been a major hindrance to food industries.

Eleven new and seven known compounds were isolated from the culture broth and the mycelia of *Alternaria* sp. ZJ-2008003, a fungus obtained from a *Sarcophyton* sp. soft coral collected from the South China Sea.[210] Compounds [200], [201], and [202] exhibited antiviral activity against the PRRSV, with IC$_{50}$ values of 65, 22, and 39 μM, respectively.

Tetrahydroaltersolanol C (200)

Alterporriols Q (201)

Alterporriol C (202),

Sansalvamide A (203)

Inhibitors of Molluscum contagiosum virus (MCV)

MCV is an unclassified pathogenic member of the Poxviridae, which replicate in human epidermis to create a common skin infection through augmentation of epidermal cell mitosis and disruption of cell differentiation. This linear, double-stranded DNA virus is characterized by lateral bodies, a core, an envelope, and a surface membrane.To escape from immune surveillance of it does not pass the basement membrane of the epidermis. Four different types (MCV I–VI) of this virus is identified. MCV I caused 96.6% of infections, and MCV II caused 3.4%, whereas MCV III and VI are very rare.[211,212] MCV infection in healthy people causes only small papules that are easily treated. In AIDS patients, however, MCV causes severe lesions that are essentially untreatable.[213,214] Cells near the surface of lesions become many times larger than normal, forming papules that become filled with a granular mass called "molluscum bodies." Untreated papules in healthy people usually disappear spontaneously within several months, but in AIDS patients dense crops can persist, disfiguring infected patients.[215]
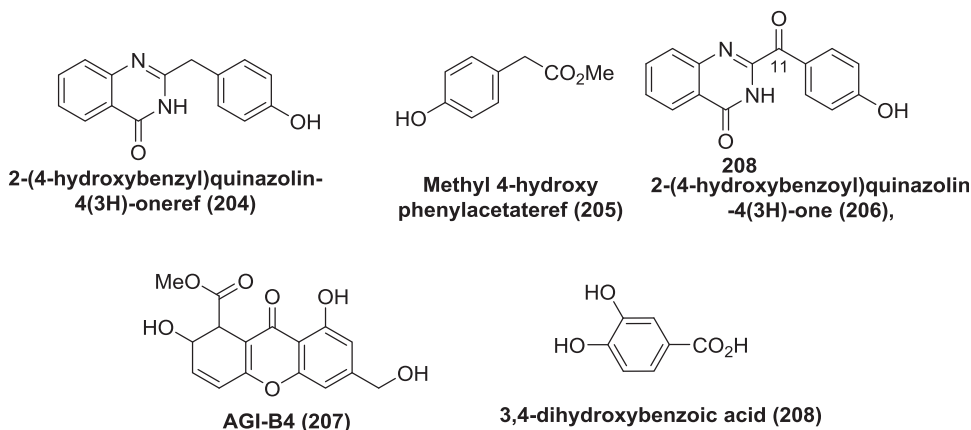
A cyclic depsipeptide, sansalvamide A [203], isolated from a marine fungus *Fusarium* sp., exhibited in vitro MCV topoisomerase inhibitory activity in concentration-dependent fashion with an IC$_{50}$ of 124 μM. It is found to inhibit topoisomerase-catalyzed DNA relaxation and thus viral replication.[216]

Inhibitors of tobacco mosaic virus (TMV)

TMV *is* a rod-like plant virus, with a complex capsid and positive (+) strand RNA of approximately 6.4 kilobases. This virus does not usually kill the plant that is infected; it causes damage to flowers, leaves, and fruit through discoloration and mosaic mottling and stunts a plant's growth.[217,218] TMV is named for the first plant in which it was discovered but can infect over 400 different types of plants including vegetables, weeds, and flowers. The virus does not produce spores but spreads mechanically, entering plants via wounds. Till date prevention is the best control as there is no chemical treatment that effectively protects plants from TMV.[219–221] Therefore, a discovery of a naturally occurring chemical agent can be an important option for treatment of this virus.

Among five different compounds that have been isolated from a marine fungus *Penicillium oxalicum* 0312F1 by Shen et al.,[222] two known compounds 2-(4-hydroxybenzyl)quinazolin-4(3H)-one[223] [204] and methyl 4-hydroxyphenylacetate [205] showed potent inhibitory activity against TMV with EC$_{50}$ values 100.80 and 137.78 μg/ml, respectively, whereas new 2-(4-hydroxybenzoyl)quinazolin-4(3H)-one [206] has exhibited moderate inhibitory activity (41.77%) against TMV. Structural comparison of [204] and [206] with their anti-TMV activity clearly indicates the effect of C-11 ketone for reduced bioactivity of [206]. Two known compounds, AGI-B4 [207] and 3,4-dihydroxybenzoic acid [208] were isolated together with eight other new and known compounds from the culture of a marine-derived fungus *Neosartorya fischeri* 1008F1.[220] Among these AGI-B4 [207] and 3,4-dihydroxybenzoic acid [208] showed potent inhibitory activity on replication of TMV with IC$_{50}$ values 0.26 and 0.63 μg/ml, respectively.

2-(4-hydroxybenzyl)quinazolin-4(3H)-oneref (204)

Methyl 4-hydroxy phenylacetateref (205)

208 2-(4-hydroxybenzoyl)quinazolin-4(3H)-one (206),

AGI-B4 (207)

3,4-dihydroxybenzoic acid (208)

Conclusion and perspective

Natural products with their great complexity and enormous diversity remain as inexhaustible source and inspiration of many drugs for various diseases.[17] Such products from fungal sources also played an important role to substantially enrich the present-day drug repertoire.[27] Current antiviral chemotherapy, driven by mainly synthetic drugs is gradually becoming ineffective due to rapid emergence of drug resistance viral strain[9,225] and their adverse side effect in prolonged use.[226–228] Thus, there is an urgent need for new compounds with novel mode action of which can target different therapeutic sites.[40,229] Due to gradual attrition rate in finding novel synthetic drug lead,[230,231] present-day drug industry once again increasingly being forced to rely on structurally diverse secondary metabolites from nature.[232] Fungal metabolites with great diversity and preapproved biocompatibility can be a potential source for new antiviral drug lead. Considering, very small fraction of fungal species has been discovered and only few percent of these extracts are tested for various viral diseases[233,234] there remains an enormous potential for finding new fungal metabolites as drug leads with novel mechanism of action. Thus, an energetic endeavor toward identification and collection of unknown fungal species and developing better culture methods is required to expedite the discovery process considerably.

In many cases, crude extracts of new fungal species have been studied but less effort has been expended for further isolation of their active metabolites. With better instrumentation and advanced database, characterization of new metabolites became comparatively easier and robotics also brought amazing efficiency in bioactivity screening. But, purification of metabolites from extracts still remained one of the most time-consuming steps in natural product drug discovery. Therefore, development of easy, inexpensive but effective and automated purification techniques for fungal metabolites is very crucial to accelerate bioactivity screening.[235] Due to lack of sophisticated and comprehensive viral screening facilities and lack of funding and stringent biodiversity rule in many countries, laboratories having access to various new fungal species hardly find opportunity to screen their metabolites against various viral diseases. Establishment of more and more sophisticated international antiviral screening facilities will be very helpful to deal such situation and will be a big boost to future antiviral research.

Most of the study of fungal metabolites reported so far mainly is done on in vitro level. Serious in vivo study of metabolites with high therapeutic index is essential to properly assess their strength and weaknesses in biological system. Bioactivity-guided gradual synthetic modification of new bioactive metabolites and subsequent structure–activity optimization study of generated analogs/derivatives is another area of crucial importance in drug discovery and has delivered many semisynthetic drugs in past.[17,236] Metabolites or their semisynthetic derivatives with appealing in vivo activity will be required in large amount for their clinical studies and further drug development process. In such case, exploration and engineering of biosynthetic pathway for efficient production of important metabolite will be another important area of research for mass production of metabolite with established bioactivity.[237]

Though there are many fungal metabolite drugs being used for many other diseases, so far no antiviral drug has been discovered from fungal metabolites. One of the main reasons might be only a small fraction of the total isolated metabolites have been tested for antiviral screening due to lack of proper infrastructure for antiviral assay in many developing countries with high fungal biodiversity and fear associated with handling various viral strains. Therefore, besides identification and exploration of new fungal species and metabolites, effort should be devoted to comprehensive antiviral screening of many known but not-tested metabolites.

Reported metabolites with high in vitro therapeutic index should be taken up for in vivo screening and subsequent bioassay-guided structural optimization study. Promising lead metabolites can be taken up for further serious drug discovery research. Considering high antiviral activity of large number of reported fungal metabolites discussed in this review, enormity of estimated fungal biodiversity, number and structural diversity of untested fungal metabolites and gradual development in various facet of drug discovery process, eventual discovery of novel better antiviral drug from fungal metabolites should be a reality in near future. It is hoped that this review would be an inspiration and helpful resource for future research in the field of fungal metabolite-based antiviral drug discovery.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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