Inhibitors from Natural Products to HIV-1 Reverse Transcriptase, Protease and Integrase

Y. Jiang[1,3], T.B. Ng[2], C.R. Wang[3], D. Zhang[3], Z.H. Cheng[3], Z.K. Liu[3], W.T. Qiao[1], Y.Q. Geng[1], N. Li[3] and F. Liu*[,1,3]

[1]*Center for AIDS Research, Nankai University, Tianjin, China*

[2]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*

[3]*Department of Microbiology, College of Life Science, Nankai University, Tianjin, China*

Abstract: Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus type 1 (HIV-1) infection, is still one of the most challenging diseases of the early 21st century. Reverse transcriptase (RT), protease (PR) and integrase (IN) are three key enzymes of HIV-1. Despite the shortcomings of chemical drugs such as toxicity, lack of curative and multiple effects, the search for more and better anti-HIV agents has been focused on natural products. Many natural products have been shown to possess promising activities that could assist in the prevention and amelioration of the disease. Most of these natural anti-HIV agents have other medicinal values as well, which afford them further prospective as novel lead compounds for the development of new drugs. These natural products can deal with both the virus and the various disorders that are caused by HIV. In this review, natural inhibitors of RT, PR and IN have been found to be classified and the relationship between structure and inhibitory activity is discussed.

Keywords: HIV-1, RT, PR, IN, natural products.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus type 1 (HIV) infection, is still one of the most challenging diseases of the early 21st century. Recently, the global epidemic is stabilizing but at an unacceptably high level. Globally, there were an estimated 33 million people living with HIV in 2007 (http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/).

The HIV-1 genome encodes three essential enzymes for its replicative cycle, reverse transcriptase (RT), protease (PR) and integrase (IN). RT is the etiological agent of AIDS. It is a heterodimer consisting of a p66 (66 kDa) and a p51 (51 kDa) subunits [1,2] and is a multifunctional enzyme showing RNA-dependent DNA polymerase, DNA-dependent DNApolymerase, and RNase H activities, all of which are required to convert the viral RNA genome into a double-stranded cDNA. Antiretroviral agents that inhibit the HIV-1 RT enzyme can be classified into two main groups, the nucleoside/nucleotide RT inhibitors (N(t)RTIs), which, following two or three phosphorylation steps, act as chain terminators, and the nonnucleoside RT inhibitors (NNRTIs), that inhibit the enzyme in a noncompetitive manner by interacting with the allosteric binding site at the palm region of the RT [3]. PR 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 which are crucial in the life cycle of HIV-1 [4]. IN catalyzes the insertion and integration of viral DNA into the host genome. It is crucial for viral replication and has no counterpart in the host cell. HIV-1 IN is a 32 kDa protein comprised of three independently folded domains. The N-terminal domain (residues 1-50) is characterized by a conserved HHCC zinc binding motif while the dimeric catalytic core domain (residues 50-212) and the C-terminal domain (residues 213-288) are involved in non-specific DNA binding [5].

In the absence of effective vaccines, drugs are the only therapeutic tools that can be used to treat human immunodeficiency virus type 1 (HIV-1) infections. The prevailing strategy for the therapy of AIDS is combinative application of several therapeutic agents, that is, reverse transcriptase inhibitors, such as nucleotides and non-nucleotides used in conjunction with protease inhibitors. But the emergence of drug resistance and the narrow spectrum of activity have limited the therapeutic usefulness of the various reverse transcriptase and protease inhibitors that are currently available on the market. Despite the beneficial effects of chemical drugs in clinical treatment, the increasing virus resistance, toxicity, unavailability and above all the lack of curative and multiple effects are their major shortcomings. As a result, the search for more and better anti-HIV agents has been focused on natural sources.

1. NATURAL INHIBITORS TO HIV-1 RT

AZT is an inhibitor of HIV-1 RT and also the first anti-HIV drug approved by FDA 20 years ago. Recently, the

*Address correspondence to this author at the Department of Microbiology, College of Life Science, Nankai University, Tianjin, China;
Tel: 8622-23509491; E-mail: liufang312@nankai.edu.cn

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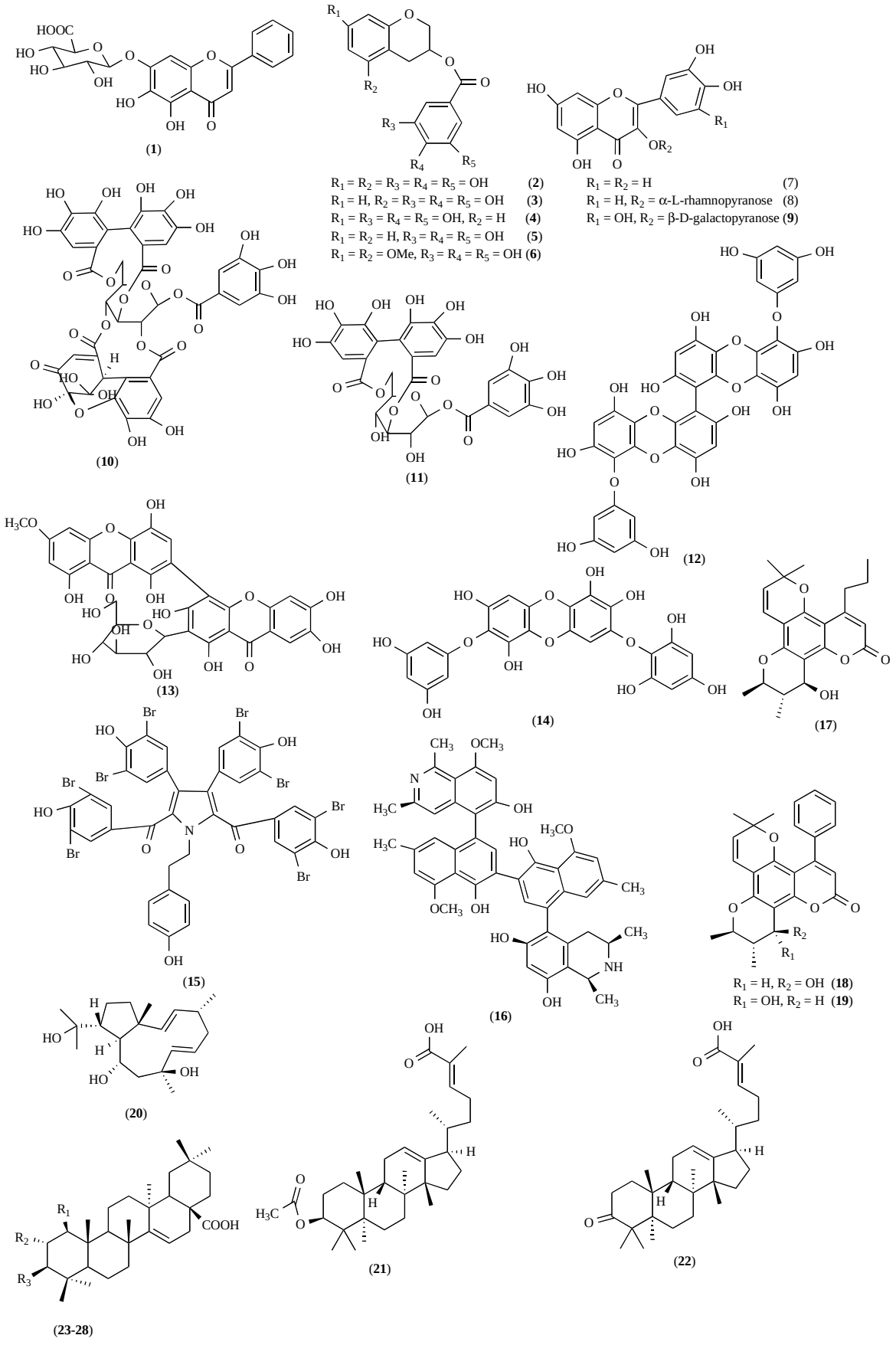


Fig. (1). The structures of natural HIV-1 RT inhibitors.

most popular anti-HIV drugs still target at RT. Similarly, the natural inhibitors of RT have been studied more extensively than inhibitors of PR and IN Fig. (**1**).

1.1. Flavonoids and Polyphenols

Baicalin (**1**) is an anti-HIV flavonoid obtained from *Scutellaria baicalensis* which is one of the seven medicinal plants constituting *Sho-saiko-to*, a traditional Chinese as well as a Japanese medicinal drug [6]. Baicalin inhibited HIV-1 RT with an IC_{50} value of 4.48 μM [7]. Baicalin can discriminate between double-stranded DNA and single-stranded DNA since it binds to DNA through intercalation [8].

Nakane and Ono reported that (–)-epicatechin gallate and EGCG, the two components from the tea plant *Camellia sinensis*, differentially inhibit the activities of RT with IC_{50} values in the range of 2.3×10^{-2}-4.6×10^{-2} μM. Epicatechin gallate and EGCG also inhibited other cellular DNA and RNA polymerases, in cell-free chemical assays. The mode of inhibition of RT and other DNA polymerases was competitive with respect to the template-primer, whereas in the case of RNA polymerase it was with respect to the nucleotide substrate [9]. Then a class of these HIV-1 RT inhibitors (**2-6**) has been recognized. Some of these compounds (**2-4**) inhibited the native as well as the A17 double mutant (K103N Y181C) forms of the enzyme. Substantial separation of polymerase and DNA-strand-transfer inhibition was achieved with compounds **5** and **6** for both wild-type and mutant enzyme. The presence of polar hydroxyl groups in the aromatic ring of the chromanol moiety enhanced polymerase inhibition while complete removal of these hydroxyl groups or their conversion to less polar methyl ether functions resulted in 10- to 80-fold selectivity for DNA-strand-transfer inhibition over polymerase inhibition. Removal of one or more of the hydroxyl groups on the gallic acid moiety led to a loss of both inhibitory activities. Such specific DNA-strand-transfer inhibitors may thus have important therapeutic potential [10].

Fourteen compounds were isolated and identified from the methanol extract of *Alnus firma* leaves. Of these compounds, quercetin (**7**), quercitrin (**8**) and myricetin 3-O-13-D-galactopyranoside (**9**) displayed inhibition against RT, all with IC_{50} values of 60 pM [11].

The gallotannins geraniin (**10**) and corilagin (**11**) were isolated from *Phyllanthus amarus*. Geraniin exhibited *in vitro* inhibition of RT at an IC_{50} of 1.9 μM, a potency about 1000 fold higher than that of AZT-TP. At the same time, it effectively blocked viral uptake (EC_{50} < 2.6 μM). Its activities at two distinct sites of the HIV replication are helpful in suppressing the emergence of escape mutants. The activity of geraniin's against RT differs from the approved RT inhibitors in that it is competitive with respect to the primer/template [12]. Corilagin (from *Jatropha curcas* and *Chamaesyce hyssopifolia*) has been reported to inhibit RT *in vitro* at an IC_{50} of 20 μM [13].

Ecklonia cava (EC) (**12**), which is an edible marine brown alga with a broad range of bioactivities, belongs to the family of Laminariaceae. The bioactive 6,6'-bieckol, one of the main naturally occurring phloroglucinol derivatives from this genus, was isolated and characterized by NMR techniques. 6,6'-bieckol selectively inhibited the activity of RT with an IC_{50} lower than 1 μM [14].

The first flavone-xanthone C-glucoside, swertifrancheside (**13**), was isolated from *Swwtia fiancbetiana*, and its structure was elucidated on the basis of spectroscopic analysis as 1,5,8-trihydroxy-3-methoxy-7-(5',7',3",4"-tetrahydroxy-6'-C-β-D-glucopyranosyl-4'-oxy-8'-flavyl)-xanthone. This compound was a moderately potent inhibitor of RT with an ED_{50} of 4.6 μM [15].

The bioassay-directed isolation of a marine brown alga, *Ishige okamurae*, afforded a carmalol derivative, diphlorethohydroxycarmalol (**14**). This compound exhibited inhibitory effects on HIV-1 reverse transcriptase and integrase with an IC_{50} of 9.1 μM and 25.2 μM, respectively [16].

1.2. Alkaloids

Polycitone A (**15**) is one of the most important natural products with interesting activity on RT. It is an aromatic alkaloid isolated from the marine ascidian *Polycitor* sp. The IC_{50} values of polycitone A for inhibition of the RNA- and DNA directed DNA polymerase functions of RT were as low as 245 nM and 470 nM, respectively. Experimental evidence suggests that the inhibition of the DNA polymerase activity is independent of the template-primer used and also does not appreciably affect RNase H function (IC_{50} = 30 μM). Polycitone A, on the other hand, has been shown to interfere with DNA primer extension (IC_{50} = 2.5 μM) as well as with the formation of the RT-DNA complex (IC_{50} = 5-10 μM). It seems that it inhibits RT polymerase by preventing its reassociation with the DNA primer after it disassociates from the template-primer during extension. Furthermore, despite the fact that polycitone A bears no structural relationship to dTTP, it is a competitive inhibitor with respect to dTTP, indicating that the inhibitor binding site on the enzyme may be functionally or spatially related to the substrate binding site. Natural and chemical derivatives, in which some or all of the five phenol groups have been methoxylated, showed substantially decreased inhibition of RT DNA polymerase activity, signifying the importance of the hydroxyl groups of polycitone A [17].

Other alkaloids such as michellamine B (**16**), extracted from tropical liana *Ancistrocladus korupensis*, could also inhibit RT [18]. Michellamine B is a dimeric naphthylisoquinoline alkaloids.

1.3. Coumarins

Coumarins are a class of important anti RT natural products. (+)-Calanolide A (**17**) and related coumarins isolated from various *Calophyllum* spp. represent a novel and distinct subgroup of non-nucleoside reverse transcriptase inhibitors (NNRTI) which have received special attention for the development of new antiretrovirals. NNRTIs are the group of compounds that inhibit the activity of RT by the virtue of their ability to bind irreversibly at the nonsubstrate-binding, allosteric site. Studies have demonstrated that (+)-calanolide A has a favourable safety profile in both animal and human subjects. All adverse effects observed with its use were mild to moderate in intensity and transient. No dose-related pattern in adverse effects or laboratory abnormality incidence was apparent [19].

Calanolides A (**18**) and B (**19**), isolated from *Calophyllum lanigerum*, inhibited RT with an IC_{50} value of 20 and 15 μM, respectively [20]. Inophyllum B and P, isolated from *Calophyllum inophyllum* Linn *clusiaceae*, showed anti RT activities with an IC_{50} value of 38nM and 130 nM, respectively [21]. All of them were NNRTIs.

1.4. Terpenoids

Dolabelladienetriol (**20**), a dollabelane diterpene isolated from the marine alga *Dictyota pfaffii*, could inhibit RT. Studies of the kinetic mode of its action revealed that dolabelladienetriol is a NNRTI, acting as a noncompetitive inhibitor, with a *K*i value of 7.2 μM [22]. It displayed an additive effect with the nucleoside RT inhibitor AZT.

Besides diterpenes, several triterpenoids have also been found to exhibit anti RT activity with different mechanisms of action. Cycloartenol ferulate (IC_{50} = 2.2 μM), 24- methylenecycloartanol ferulate (IC_{50} = 1.9 μM), lupenone (IC_{50} = 2.1 μM), betulin diacetate (IC_{50} = 1.4 μM) and karounidiol 29-benzoate (IC_{50} = 2.2 μM) inhibited purified RT and have been suggested as potential lead compounds [23].

The triterpenes from the bark and stems of *Garcinia speciosa* named garciosaterpene A (**21**) and C (**22**) strongly inhibited HIV-1 RT with the IC_{50} values of 33.3 μM and 28.1 μM, respectively [24].

Pentacyclic triterpenes isolated from *Maprounea Africana* manifested anti RT activities. These compounds comprising maprounic acid (**23**), maprounic acid acetate (**24**), 1β-hydroxymaprounic acid 3-p-hydroxybenzoate (**25**), 2α-hydroxymaprounic acid *2,3-bis-p*-hydroxybenzoate (**26**) and their respective hydrolyzed products (**27 and 28**), demonstrated potent inhibitory activity against RT with the IC_{50} values of 14.9, 136.0, 3.7, 3.7, 4.9 and 3.4 μM, respectively [25].

1.5. Proteins and Peptides

Most of the proteins and peptides that have been found as PR inhibitors were from mushrooms and seeds of vegetables (Table **1**).

2. NATURAL INHIBITORS TO HIV-1 PR

Nearly all the inhibitors to HIV-1 protease used clinically were produced by chemical synthesis. Initial inhibitors created in the laboratory were peptide derivatives which could bond with HIV-1 PR active sites. Later, designers shifted from creation of symmetric inhibitors of this inherently symmetric enzyme to their ultimate conversion to asymmetric compounds. Most of these chemically synthetic drugs were toxic and could not be used at high enough doses which soon engendered the resistant mutant of PR. In recent years, natural low-molecular-weight molecules compounds have drawn the attention of researchers Fig. (**2**).

2.1. Flavonoids and Polyphenols

The tetrahydroxyflavonol kaempferol (**29**) (*Rosa damascena*) effectively reduced the maturation of infectious progeny virus apparently due to selective inhibition of PR (IC_{50} = 0.8 μM, TI = 62.5 in H9 cells) [39].

Two related flavonol glucosides, (–)-4′-methylepigallocatechin-5-*O*-β-glucopyranoside and (+)-4′-methylepigallocatechin-3′-*O*-β-glucopyranoside isolated from the Sudanese medicinal plant *Maytenus senegalensis*, brought about 72.9% and 68.2% inhibition of HIV-1 protease, respectively, at a concentration of 100 μM [40].

Table 1. Proteins and Peptides Inhibitors of HIV-1 RT

Source	Remarks	Size (kD)	IC_{50} (μM)	N-Terminal Sequence	References
Russula paludosa	mushroom	4.5	11	KREHGQHCEF	[26]
Brassica alboglabra	seed	5907	4.9	PEGPFQGPKATKPGDLAXQTWGGWXGQTPKY	[27]
Schizophyllum commune	mushroom	64	1.2	APPNFPLLVPGNDKLLAVISAMTP	[28]
Pleurotus citrinopileatus	mushroom	32.4	0.93	QYSQMAQVME	[29]
Canavalia gladiata	bean	30	35	ADTIVAVELDTYPNTDIGDP	[30]
Glycine max cv.'Dull Black'	bean	40	9.4	DFVIDNEGNPIEDGG	[31]
Agrocybe cylindracea	mushroom	9	60	ANDPQCLYGNVAAKF	[32]
Tricholoma giganteum var. golden blessings	mushroom	27	8.3×10^{-2}	QVHWPMF	[33]
Lentinus edodes	mushroom	9	1.5	CQRAFNNPRDDAIRW	[34]
Brassica campestris	seed	9	4	ALSCGTVSGNLAACAGYV	[35]
Bauhinia variegata	seed	64	1.02	QRNRLTSFTFPNNRS	[36]
Inocybe umbrinella	mushroom	17	4.7	DGVLATNAVA	[37]
Hypsizigus marmoreus	mushroom	9.6	30	AEGTLLGSRATCESGNSMY	[38]

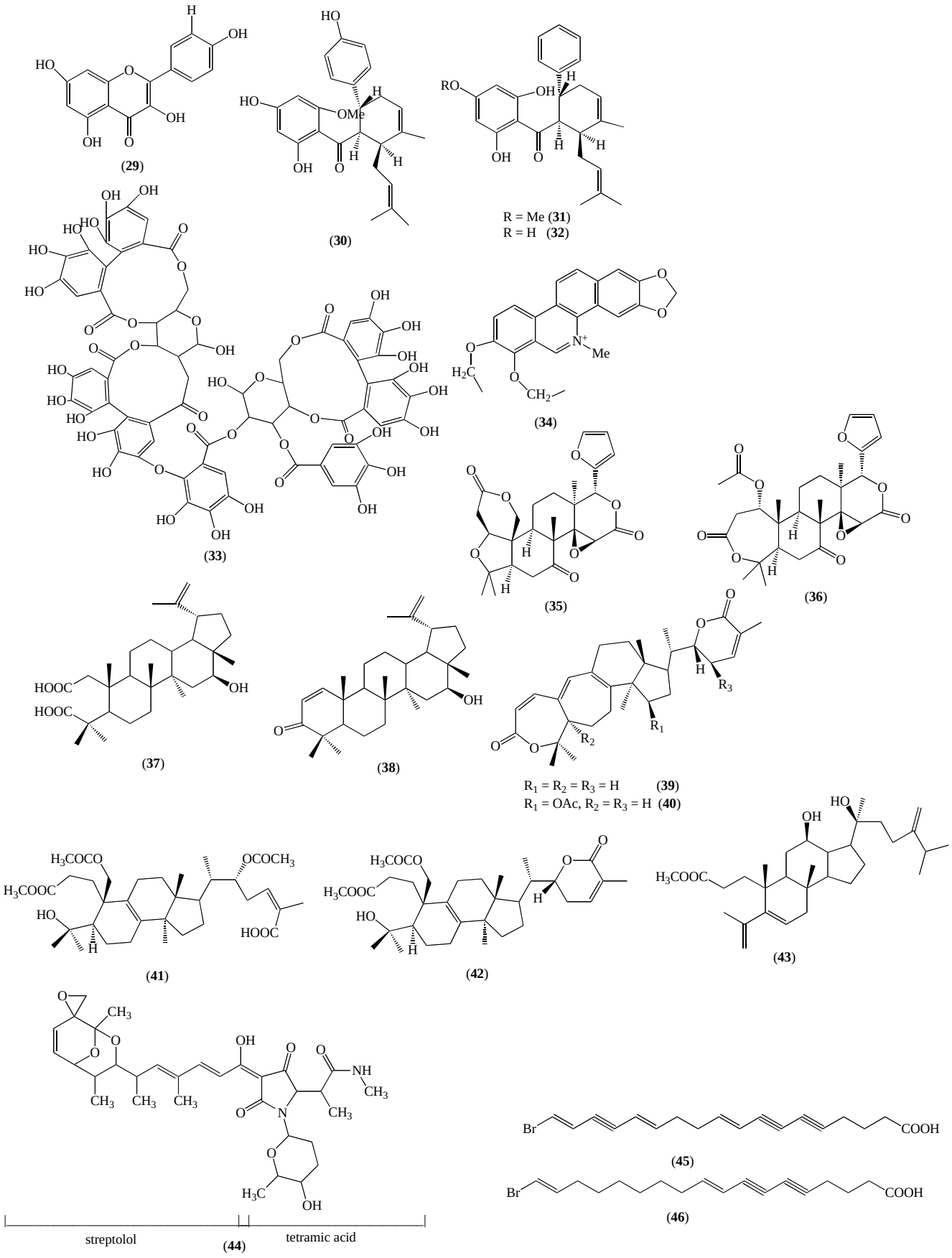


Fig. (2). The structures of natural HIV-1 PR inhibitors.

Searching for anti-HIV-1 protease (PR) inhibitors of Thai medicinal plants *Boesenbergia pandurata* rhizomes led to the isolation of a new cyclohexenyl chalcone named panduratin C (**30**) with an IC_{50} value of 100 μM to PR. The other two chalcone derivatives from the methanol extract were identified to be panduratin A (**31**) and hydroxypanduratin A (**32**) with the IC_{50} values of 18.7 μM and 5.6 μM, respectively. Structure-activity relationships of these compounds on anti-HIV-1 PR activity are summarized as follows: (1) hydroxyl moiety at position 4 conferred higher activity than methoxyl group; (2) prenylation of dihydrochalcone was essential for activity; (3) hydroxylation at position 4000 reduced activity; and (4) introduction of double bond at C10 and C60 of chalcone led to higher activity [41].

Phloroglucinol-1-O-β-D-glucopyranoside from *Maytenus senegalensis*, a compound of polyphenol, showed 68.2% inhibition at 100 μM on PR [40].

Camelliatannin H (**33**) from *Camellia japonica* potently inhibited PR with an IC_{50} value of 0.9 μM [42].

Condensed tannins from *Xanthoceras sorbifolia* exhibited 50% inhibition on PR at about 6.0 μg/ml [43].

2.2. Alkaloids

The methanol extract of *Artemisia caruifolia* yielded N1,N5,N10-tri-*p*-coumaroylspermidine which showed moderate inhibitory activity against PR. Its EC_{50} value was 53 μg/mL. Based on the lead structure, two related amides N1, N5, N10, N14-tetra-*p*coumaroylspermine and N1, N4, N7, N10, N13-penta-*p*coumaroyltetraethylenepentamine, were then synthesized and inhibited PR more potently than N1,N5,N10-tri-*p*-coumaroylspermidine, with EC_{50} values of 27 and 30 μg/mL, respectively [44].

A non-peptidomimetic PR inhibitor named sanguinarine (**34**) from *Chelidonium majus* inhibited PR with an IC_{50} of around 13 μM [45].

2.3. Terpenoids

The diterpenoids named agastanol and agastaquinone extracted from the roots of *Agastache rugosa*, inhibited PR with an IC_{50} value of 360 and 87 μM, respectively [46].

Several triterpenoids have also been found to exhibit anti PR activity with different mechanisms of action. Uvaol and ursolic acid extracted from leaves of *Crataegus pinnatifida* strongly inhibited PR with an IC_{50} value of 5.5 μM and 8.0 μM, respectively [47]. The limonoids, limonin (**35**) and nomilin (**36**) inhibited HIV-1 replication in PBMC. PR seems to be their target [48].

Two triterpenoids, 16b-hydroxy-2,3-seco-lup-20(29)-ene-2,3-dioic acid (**37**) and 16b-hydroxylupane-1,20(29)-dien-3-one (**38**), isolated from stems of *Stauntonia obovatifoliola Hayata subsp*. intermedia (Y.C. Wu) T. Chen [49] inhibited IN with the IC_{50} values of 17.9 and 57.1 μM. Interestingly, the former compound, which possesses a novel 2,3-seco-2,3-dioc acid moiety in ring A showed potent inhibitory activity against PR, indicating that the 2,3-seco-2,3-dioc acid moiety in ring A, might be an important pharmacophore to be considered for designing and synthesizing PR inhibitors. Since some triterpene derivatives with a 3-O-acidic acyl group showed strong anti-HIV activity [50], and 3-O-(30,30-dimethylsuccinyl) betulinic acid (PA-457, bevirimat) was reported to enter phase 2 clinic trial as a new AIDS drug candidate [51], it is of interest to examine the anti-HIV activity of compound 37 and other seco-triterpenes with acidic groups at positions 2 and 3 of A ring.

Schisanlactone A (**39**), colossolactone E (**40**), colossolactone V (**41**), and colossolactone VII (**42**), isolated from the fruiting bodies of the Vietnamese mushroom *Ganoderma colossum*, showed inhibitory activity against HIV-1 protease with an IC_{50} value of 11.8, 16.0, 15.0 and 26.6 μM, respectively. Concerning the compounds containing seven-membered and six-membered lactone rings in rings A and E, respectively, the presence of a hydroxyl group at C-23 or C-5 in the above-mentioned compounds markedly reduced the activity, suggesting that the hydrophobicity of the triterpene core in these compounds may play a significant role in mediating the HIV-1 protease inhibitory activity. The significant influence of the unsaturation pattern on the activity may be due to the double bond(s) that alter the three-dimensional structures of compounds and consequently the spatial arrangement of pharmacophores in the structures [52].

Fourteen compounds were isolated and identified from the methanol extract of *Alnus firma* leaves. Of these compounds, the alnustic acid methyl ester (**43**) exhibited inhibition against PR, with an IC_{50} of 15.8 μM [53].

2.4. Other Natural Inhibitors to PR

Streptolydigin (**44**) from the zymolytic product of *Streptomyces lydigus* potently inhibited PR with an IC_{50} of 66.3 μM [54].

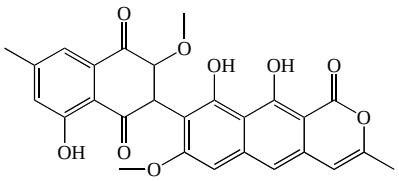
Bromo acetylenic acid (**45**) and (**46**) from sponge *Xertospongia muta* exhibited anti PR activity with the IC_{50} of 6μM and 12μM, respectively [55].

3. NATURAL INHIBITORS TO HIV-1 IN

The integration of viral cDNA into the host genome is an essential step in the HIV-1-life cycle and is mediated by the virally encoded enzyme, IN. There are no cellular homologs to IN and the reactions catalyzed by IN are unique, which allow design of selective inhibitors with little or no side effects. Compared to RT inhibitors, there is a development of IN inhibitors slower. The discovery of β-diketo acid inhibitors played a major role in validating IN as a legitimate antiretroviral drug target. Raltegravir is the only IN inhibitor that has been approved by FDA. Recently, more and more natural products with anti IN potency have been reported Fig. (**3**).

3.1. Flavonoids and Polyphenols

Quercetin 3 - *O*- [(6-*O*-feruloyl) - β – D - glucopyranosyl- (1→2) - β -Dgalactopyranoside] and quercetin 3 – *O* - [(6-*O*-sinapoyl) - β – D – glucopyranosyl - (1→2) - β – D - galactopyranoside] extracted from *Thevetia peruviana* inhibited IN with IC_{50} values of 5 and 7 μM, respectively. With regard to IN inhibitory activity, compounds possessing a feruloyl or sinapoyl group in the terminal glucose moiety showed a more potent inhibitory activity than the unsubstituted ones. The aforementioned flavonols showed higher



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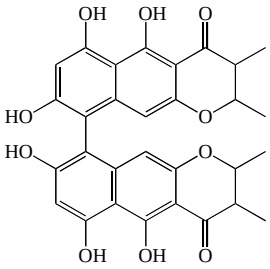

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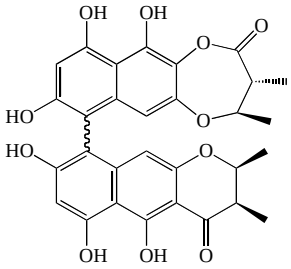

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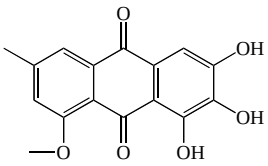

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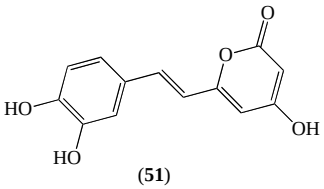

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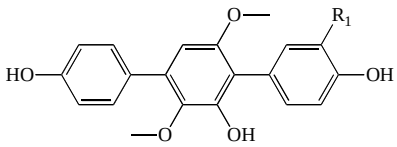
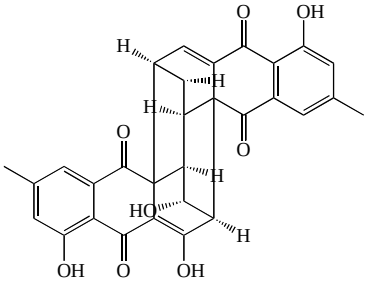


R_1 = H (**52**)
R_1 = OH (**53**)



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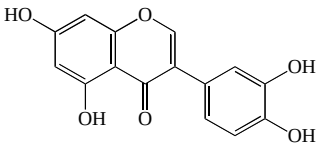

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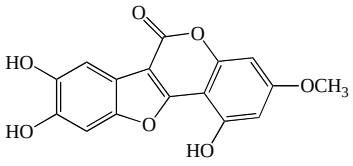

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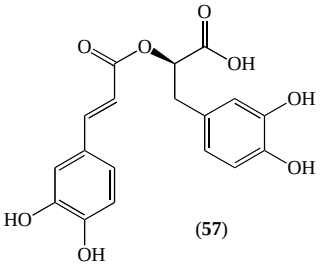

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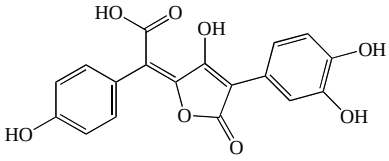

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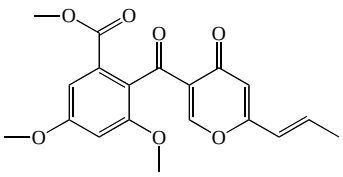

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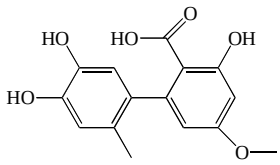

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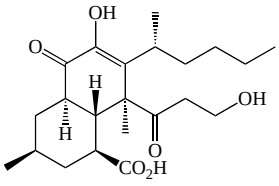

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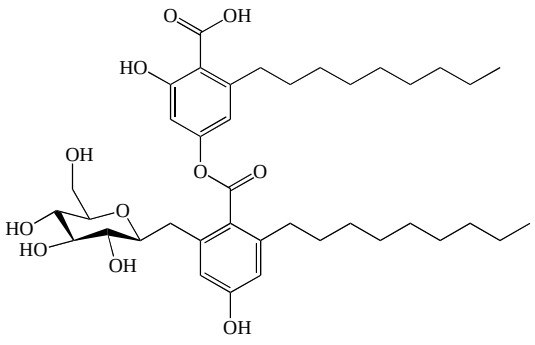
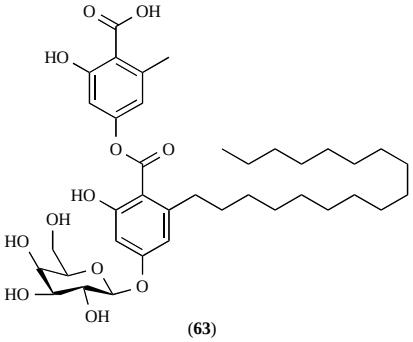


(**62**)

Fig. (3). Contd…..



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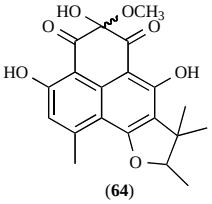

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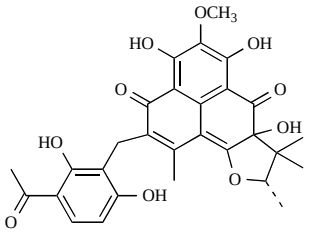

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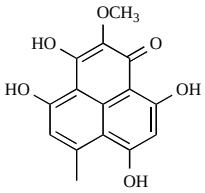

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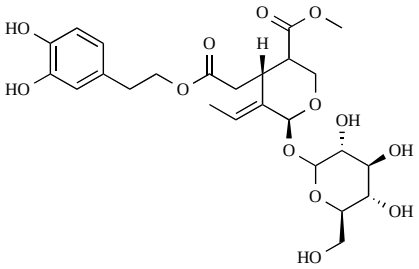

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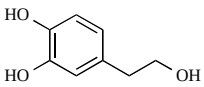

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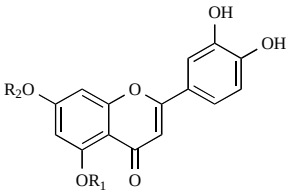
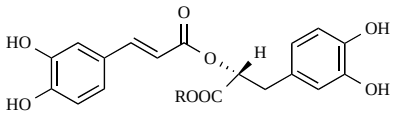
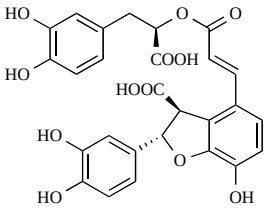


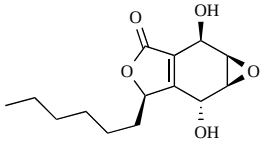
R_1 = Glucose, R_2 = H (**69**)
R_1 = R_2 = H (**70**)
R_1 = H, R_2 = CH_3 (**71**)
R_1 = H, R_2 = Glucuronic acid (**72**)
R_1 = Glucose, R_2 = CH_3 (**73**)



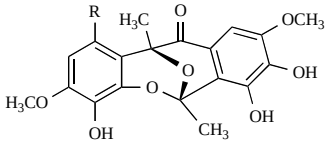
R = H (**74**)
R = CH_3 (**75**)



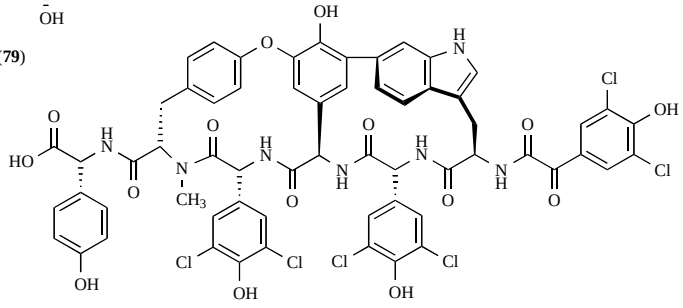
(**76**)



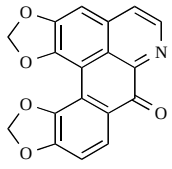
(**79**)



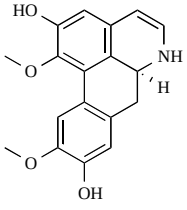
R = CH_2OH (**77**)
R = CHO (**78**)



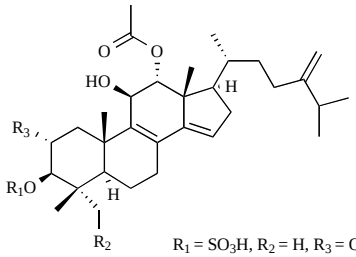
(**80**)



(**81**)



(**82**)



R_1 = SO_3H, R_2 = H, R_3 = OH (**83**)
R_1 = R_2 = H, R_3 = OH (**84**)

Fig. (3). Contd…..

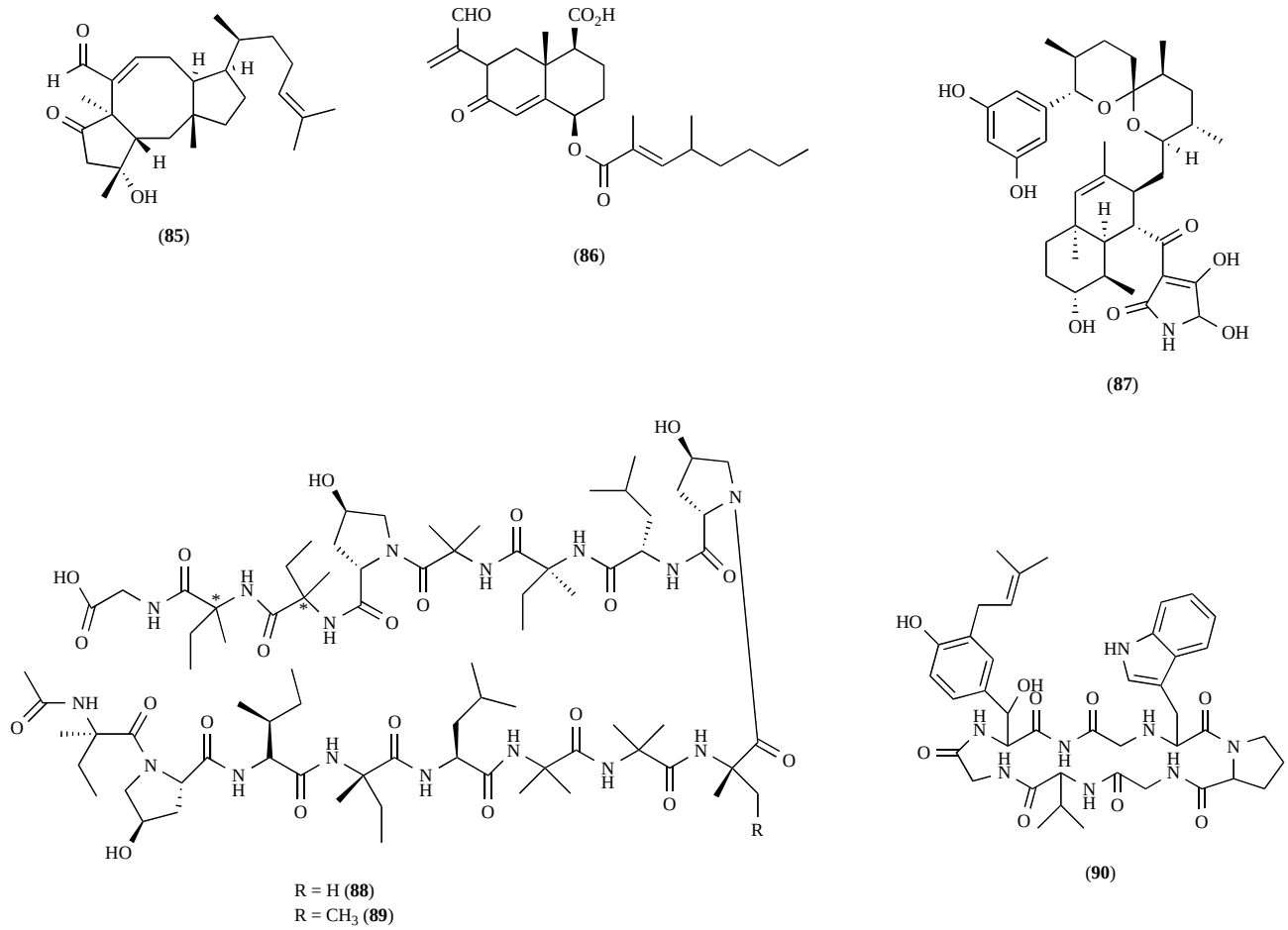


Fig. (3). The structures of natural HIV-1 IN inhibitors.

inhibitory activity than their aglycones, quercetin (IC_{50} = 43 and 15 μM, respectively) and kaempferol (IC_{50} > 100 and 40 μM, respectively) [56].

A flavonoid glucuronide, apigenin 7 – *O* – β – D - (4′-caffeoyl) glucuronide isolated from the flowers of *Chrysanthemum morifolium*, showed strong IN inhibitory activity (IC_{50} = 7.2 ± 3.4 μg/mL) [57].

A novel polyketide fungal metabolite from the xanthoviridicatin family was isolated from *Penicillium chrysogenum* and evaluated for its ability to inhibit IN catalytic activity. Xanthoviridicatins (**47**) inhibited the 3'-processing reaction with an IC_{50} value of 6 μM. However, this compound did not show any inhibition against the strand transfer reaction (IC_{50} >100 μM), indicating that these compounds are mainly cleavage inhibitors [58].

A set of two kinds of novel fungal bis-naphtho-γ-pyrones, belonging to the chaetochromin and ustilaginoidin family, were isolated from the *Fusarium* species. Isochaetochromin (**48**) inhibited both 3'-processing and strand transfer reactions with an IC_{50} value of 2 and 12 μM, respectively, while Oxychaetochromin (**49**) did so at 3 and 9 μM. Several acetylated or methylated semisynthetic derivatives of these compounds were less active or inactive which indicated a requirement of the phenolic groups for activity [59].

8-O-methylanthrogallol (**50**) isolated from a strain of *Cylindrocarpon ianthothele* inhibited the 3'-processing and strand transfer reactions of IN with an IC_{50} value of 6 and 22 μM, respectively. Apart from the catechol group, it is possible that the keto-enol arrangement in 8-O-methylanthrogallol accounts for its inhibitory activity [60].

Hispidin has earlier been isolated from *Polyporus hispidus*. It is also found in various related fungal species, exemplified by *Inonotus hispidus* and *Phellinus pomaceus*. Hispidin (**51**) inhibited both the 3'-processing and strand transfer reactions with an IC_{50} value of 2 and 24 μM, respectively. The trimethyl ether of hispidin was inactive in both the 3'-processing and strand transfer assays, indicating the importance of a catechol group for IN inhibitory activity [61,62].

Two compounds, terphenyllin (**52**) and hydroxyterphenyllin (**53**), were isolated from a strain of *Aspergillus candidus*. Hydroxyterphenyllin (**53**), which contains a catechol group, inhibited the 3'-processing and strand transfer reactions of IN with an IC_{50} value of 2.8 and 12.1 μM, respectively. Rugulosin (**54**), isolated from a strain of *Penicillium islandicum*, showed moderate activity against IN by inhibiting the 3'-processing and strand transfer reactions with an IC_{50} value of 19 and 25 μM, respectively [63].

Two compounds, orobol (**55**) and wedelolactone (**56**), extracted from the plant *Eclipta prostrate*, showed high activity against IN by inhibiting the 3'-processing and strand transfer reactions with an IC_{50} value of 8.1 μM and 4.0 μM, respectively [64].

Gallic acid and its various derivatives, from *Terminalia chebula* and *Euphorbia pekinensis*, inhibited IN and it has been proposed that the galloyl moiety plays a major role in the inhibition of the 3′- processing of IN by these compounds [65].

Rosmarinic acid methyl ester and rosmarinic acid (**57**), isolated from *Coleus parvifolius* (Labiatae), inhibited the 3'-processing and strand transfer reactions of IN with an IC_{50} value of 3.1 and 5.0 μM, respectively [66]. The HIV-1 integrase inhibitory effects of rosmarinic acid derivatives increase in the order monomers, the dimers (IC_{50} = 5.0 μM), the trimers, e.g. lithospermic acid (IC_{50} = 1.4 μM) and tetramers, e.g. lithospermic acid B (IC_{50} = 1.0 μM). It was shown that the metal-chelating derivatives were more potent than those that are non-bonding [67].

Xerocomic acid (**58**), isolated from a strain of *Xeromphalina junipericola*, showed strong inhibitory potency against IN catalytic activities. Its IC_{50} values for 3'-processing and strand transfer were 1.1 and 4.4 μM, respectively. Deoxyfunicone (**59**), isolated from *Penicillium* species, selectively inhibited the 3'-processing activity of IN with an IC_{50} value of 11 μM and was inactive in the strand transfer assay (>140 μM) [68]. Altenusin (**60**) isolated from *Talaromyces flavus* contains a catechol group and a salicylic moiety. It moderately inhibited the 3'-processing and strand transfer reactions with IC_{50} values of 19 and 25 μM, respectively [69].

Cytosporic acid (**61**), a polyketide-derived novel natural product isolated from a fermentation broth of the filamentous fungus *Cytospora* sp, inhibited strand transfer reaction of HIV-1 integrase with an IC_{50} of 20 μM [70].

Exophillic acid (**62**), a novel dimeric 2, 4-dihydroxy alkyl benzoic acid isolated from *Exophiala pisciphila*, showed modest inhibitory activity against the strand transfer reaction of IN with an IC_{50} value of 68 μM [71]. Aquastatin A (**63**), an unsymmetric galactopyranoside isolated from *Fusarium aquaeductuum* as an inhibitor of adenosine triphosphatase [72], is structurally related to exophillic acid (**62**) and also inhibited the strand transfer reaction of IN with an IC_{50} value of 50 μM.

Three natural phenalenones were isolated from fungal extracts. Atrovenetinone methyl acetal (**64**) was isolated from a cultured broth of the *Pencillium* sp. The natural phenalenone 6 inhibited the strand transfer activity of IN with an IC_{50} value of 19 μM. Erabulenol B (**65**) and funalenone (**66**) inhibited the strand transfer activity of IN with an IC_{50} value of 7.9 and 10 μM, respectively [73].

Oleuropein (**67**) and hydroxytyrosol (**68**) from olive leaf inhibited IN 3'-processing activity with the IC_{50} value of 46 and 54 nM, and strand-transfer activity as with an IC_{50} value of 56 and 43 mM [74].

Seven compounds from *Coleus parvifolius* including luteolin 5-*O*-β -D-glucopyranoside (**69**), luteolin (**70**), luteolin 7-methyl ether (**71**), luteolin 5-*O*-β-D-glucuronide (**72**), 5-*O*-β -D-glucopyranosyl-luteolin 7-methyl ether (**73**), rosmarinic acid (**74**), rosmarinic acid methyl ester (**75**) inhibited activities on IN with an IC_{50} values of 58.0, 11.0, 11.0, 20.0, 70.0, 5.0 and 3.1 μM, respectively [68].

Lithospermic acid (**76**) extracted from the rhizome of *Salvia miltiorrhiza* Bunge inhibited 3'-processing and strand transfer activities with an IC_{50} value of 0.83 and 0.48 μM, respectively [75].

Integrastatin A (**77**) and integrastatin B (**78**) are two novel aromatic natural products derived from an unidentified fungus named ATCC74478, which possess a novel [6/6/6/6]-ring system and are racemic despite having two asymmetric centers. These compounds inhibited the strand transfer reaction of HIV-1 integrase with IC_{50} values of 1.1–2.5 μM [76].

Scientists at Merck reported the isolation and absolute structure determination of a polyketide-derived and epoxyquinone-based natural product integrasone (**79**) from an unidentified sterile mycelium. It has been shown to inhibit the strand transfer reaction of IN with an IC_{50} of 41 μM [77].

Isocomplestatin (**80**) from *Streptomyces* sp. MA7234, inhibited *in vitro* IN coupled and strand transfer activities with IC_{50} values of 0.2 and 4.0 μM. The hydrolytic fragments with intact macrocyclic rings retained almost all of the inhibitory activities. However, opening of one of the macrocyclic rings caused a significant decrease in the integrase inhibitory activity. It should be noted that in contrast to the diketoacid inhibitors, which are much weaker inhibitors of 3' processing than strand transfer, isocomplestatin and its derivatives inhibited 3' processing with a characteristically 10-fold lower IC_{50} than the IC_{50} measured in strand transfer. These data suggest that isocomplestatin may preferentially bind to the uncomplexed enzyme, unlike the diketoacid, which requires binding to the viral DNA end. Also, unlike the diketoacid, isocomplestatin inhibits the HIV-1 disintegration activity of both the intact protein and the catalytic core domain of integrase (amino acids 50-212) with comparable potency (IC_{50}=0.5 μM). It indicates that isocomplestatin binds to the core domain of integrase [78].

3.2. Alkaloids

The aporphine alkaloids hernandonine (**81**), laurolistine (**82**), 7-oxohernangerine and lindechunine A isolated from the roots of *Lindera chunii* showed significant anti-IN activity with an IC_{50} value of 16.3, 7.7, 18.2 and 21.1 μM, respectively [79].

3.3. Terpenoids

Integracides are 4,4-dimethylergostane derivatives that are moderately potent and selective inhibitors of IN. Integracide A (**83**), a 3-sulfate ester of integracide B (**84**), is the most active member of the five natural products reported.

A set of four oxygenated tetracyclic triterpenoids of the 4,4-dimethylergostane family were isolated from *Fusarium* species. Integracide A (**83**), a sulfated ester, showed strong IN inhibitory activity by inhibiting the 3'-processing and

strand transfer reactions with an IC_{50} value of 5 and 9 μM, respectively. The presence of the sulfate group in integracide A is critical for its IN inhibitory activity [80].

Like integracide A (**83**), all natural products with a sulfate ester at the C-3 position were essentially equipotent and exhibited IC_{50} values of 5-6 μM in the coupled assay. A bulky ester group at C-2 did not have any impact on the coupled reaction inhibitory activity. While these compounds were essentially equipotent in their ability to inhibit the coupled reaction, they showed significant differences in their ability to inhibit the strand transfer reaction. Oxygenation (e.g., C-25 hydroxy and 23,24- epoxide) of the C-17 side chain didn't affect the activity of these compounds. Like the sulfated esters, compounds with either a free carboxyl group or a free amino group showed variable levels of activities in the coupled and the strand transfer assays. The activity observed by the charged compounds would indicate that these compounds may potentially interact with bivalent metal ions (e.g., Mg2+) at the active site [81].

Ophiobolins C (**85**), a sesterterpenoid isolated from a *Bipolaris* species, showed activity against IN by inhibiting the 3'-processing and strand transfer reaction with an IC_{50} value of 6.7 and 33 μM, respectively. Among all the ophiobolins tested, the cis-fused A/B ring ophiobolin C (29) was the most potent IN inhibitor [63].

Integric acid (**86**), a novel eremophilane sesquiterpenoid from a *Xylaria* sp., inhibited 3'-end processing, strand transfer and disintegration reactions catalyzed by HIV-1 integrase with IC50 values of 3-10 μM [82].

3.4. Other Natural Inhibitors of IN

Integramycin (**87**) extracted from *Actinoplanes* sp. (ATCC202188) was a novel hexacyclic natural product that inhibited integrase strand transfer reaction with an IC_{50} value of 4 μM [83].

Integramides A (**88**) and B (**89**) are two novel 16-mer linear peptides rich in *C*r-methyl amino acids that were isolated from fungal extracts of *Dendrodochium* sp. Integramides A and B inhibited the coupled reaction of HIV-1 integrase with an IC_{50} value of 17 and 10 μM, respectively [84].

A cyclohexapeptide (**90**) isolated from the fermentation broth of a soil-derived fungal culture of *Aspergillus flavipes* demonstrated IN 3'-processing inhibitory activity (IC_{50}=32.1 μM) [85].

CONCLUSION

AIDS is one of the leading infectious causes of death in the world. So far, untreated disease caused by HIV still has a case fatality rate that approaches 100%. Numerous compounds have been tested for anti-HIV activities, but few are efficient. Even though some chemical antiretroviral drugs can bring about the suppression of serum load of the virus to undetectable levels, economical, commercial and political barriers have limited their accessibility to a good part of the population suffering from the disease, especially in the developing countries. The emergence of resistance and adverse reactions limited the utility of nearly all the conventional drugs. RT, PR and IN are the three key enzymes in HIV life cycle. They are traditional and effective targets for drug screening.

Natural compounds exhibit inhibitory activities on RT, PR and IN, containing flavonoids, polyphenols, alkaloids, coumarins, terpenoids, peptides, and so on. Most of these natural products are Flavonoids and polyhenols, following by terpenoids. Many flavonoids and polyphenols compounds show efficient inhibition on RT, PR and IN at the same time. Comparing to flavonoids and polyphenols, alkaloids inhibitors are fewer. Terpenoids are also the important source of anti HIV inhibitors, in which diterpene and triterpene are the main two kinds of inhibitors. Fungi and plants are the main reservoir of these compounds.

Nearly all the natural inhibitors of RT are NNRTIs which can inhibit the activity of RT directly and with lower toxicity. These micromolecular inhibitors can be absorbed by organism efficiently. Many mechanism of their inhibit activities have been certificated. On the contrary, the precise structures of most peptide inhibitors are not clear, to say nothing of their inhibit mechanisms.

While numerous peptide-derived inhibitors of PR have been described, the identification of nonpeptide natural inhibitors remains an important goal. Most of the natural products with anti PR activities are micromolecular compounds with variety of structures and potency as drug leads.

According with the development of IN structure researches, more and more researchers have devoted their efforts on IN inhibitors. Recently, numerous IN inhibitors especially natural products have been reported. The multivariate structures made them the best leads of new drug designing.

The results and experiences with many of the anti-HIV three key enzymes natural products will inspire and motivate even more researchers to look for new leads from natural sources. It is difficult in improving proteins or peptides inhibitors owing to their unknown structures and complex mechanisms. What's more, how to maintain the activities of these exogenous biomacromolecule remains a crucial problem. The micromolecular inhibitors are prospective. Many of them have other medicinal values and can be extracted or synthetized easily. These types of compounds may also be of interest as they can deal with both the virus and the various disorders that characterize HIV/AIDS.

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REFERENCES

[1] Arts, E.J.; Mak, J.; Kleiman, L.; Wainberg, M.A. Mature reverse transcriptase (p66/p51) is responsible for low levels of viral DNA found in human immunodeficiency virus type 1 (HIV-1). *Leukemia*., **1994**, *8*, 175-178.

[2] Hottiger, M.; H¨ubscher, U. Human immunodeficiency virus type 1 reverse transcriptase. *Biol. Chem. Hoppe.-Seyler*., **1996**, *377*, 97-120.

[3] Pomerantz, R.J.; Horn, D.L. Twenty years of therapy for HIV-1 infection. *Nat. Med*., **2003**, *9*, 867-873.

[4] Cheenpracha, S.; Karalai, C.; Ponglimanont, C.; Subhadhirasakul, S.; Tewtrakul, S. Anti-HIV-1 protease activity of compounds from

Boesenbergia pandurata. *Bioorg. Med. Chem.*, **2006**, *14*, 1710-1714.

[5] Pommier, Y.; Cherfils, J. Intefacial inhibition of macromolecular interactions: nature's paradigm for drug discovery. *Trends Pharmacol. Sci*., **2005**, *26*, 138-145.

[6] Ohtake, N.; Nakai, Y.; Yamamoto, M.; Sakakibara, I.; Takeda, S.; Amagaya, S.; Aburada, M. Separation and isolation methods for analysis of the active principles of Sho-saiko-to (SST) oriental medicine. *J. Chromatogr. B.*, **2004**, *812*, 135-148.

[7] Kitamura, K.; Honda, M.; Yoshizaki, H.; Yamamoto, S.; Nakane, H.; Fukushima, M.; Ono, K.; Tokunaga, T. Baicalin, an inhibitor of HIV-1 production in vitro. *Antiviral Res*., **1998**, *37*, 131-140.

[8] Sun, Z.; Ma, Z.; Zhang, W.; Wang, X.; Fan, C.; Li, G.. Electrochemical investigations of baicalin and DNA-baicalin interactions. *Anal. Bioanal. Chem*., **2004**, *379*, 283-286.

[9] Nakane, H.; Ono, K. Differential inhibitory effects of some catechin derivatives on the activities of human immunodeficiency virus reverse transcriptase and cellular deoxyribonucleic and ribonucleic acid polymerases. *Biochemistry*, **1990**, *29*, 2841-2845.

[10] Tillekeratne, L.M.; Sherette, A.; Fulmer, J.A.; Hupe, L.; Hupe, D.; Gabbara, S.; Peliska, J.A.; Hudson, R.A. Differential inhibition of polymerase and strand-transfer activities of HIV-1 reverse transcriptase. *Bioorg. Med. Chem. Lett*., **2002**, *12*, 525-528.

[11] Yu, Y.B.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Park, J.C. Effects of triterpenoids and flavonoids isolated from *Alnus Firma* on HIV-1 viral enzymes. *Arch. Pharm. Res.*, **2007**, *30*, 820-826.

[12] Notka, F.; Meier, G.R.; Wagner, R. Inhibition of wild-type human immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by *Phyllanthus amarus*. *Antiviral Res*., **2003**, *58*, 175-186.

[13] Matsuse, I.T.; Lim, Y.A.; Hattori, M.; Correa, M.; Gupta, M.P. A search for anti-viral properties in Panamanian medicinal plants: The effects on HIV and its essential enzymes. *J. Ethnopharmacol*., **1999**, *64*, 15-22.

[14] Artan, M..; Li, Y.; Karadeniz, F.; Lee, S.H.; Kim, M.M.; Kim, S.K. Anti-HIV-1 activity of phloroglucinol derivative, 6,60-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem*., **2008**, *16*, 7921-7926.

[15] Wang, J.N.; Hou, C.Y.; Liu, Y.L. Swertifrancheside, an HIV-reverse transcriptase inhibitor and the first flavone-xanthone dimer, from *Swertia franchetiana*. *J. Nat. Prod.*, **1994**, *57*, 211-217.

[16] Ahn, M.J.; Yoon, K.D.; Kim, C.Y.; Kim, J.H.; Shin, C.G.; Kim, J. Inhibitory activity on HIV-1 reverse transcriptase and integrase of a carmalol derivative from a Brown Alga, *Ishige. okamurae*. *Phytother. Res.*, **2006**, *20*, 711-713.

[17] Loya, S.; Rudi, A.; Kashman, Y.; Hizi, A.; Polycitone, A. A novel and potent general inhibitor of retroviral reverse transcriptases and cellular DNA polymerases. *J. Biochem*., **1999**, *344*, 85-92.

[18] Hallock, Y.F.; Manfredi, K.P.; Dai, J.R.; Cardellina, J.H.; Gulakowski, R.J.; Mcmahon, J.B.; Schaffer, M.; Stahl, M.; Gulden, K.P.; Bringmann, G.; Francois, G.; Boyd, R. Michellamines D-F, New HIV-inhibitory dimeric naphthylisoquinoline alkaloids, and korupensamine E, a New antimalarial monomer, from *Ancistrocladus korupensis*. *J. Nat. Prod*., **1997**, *60*, 677-683.

[19] Clercq, D.E. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. *Med. Res. Rev*., **2000**, *20*, 323-349.

[20] Kashman, Y.; Gustafson, K.R.; Fuller, R.W.; Cardelina, J.H.; McMohan, J.B.; Currense, M.J.; Buckheit, J.R.W.; Hughes, S.H.; Cragg, G.M.; Boyd, M.R. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from tropical rainforest tree, *Calophyllum lanigerum*. *J. Med. Chem*., **1992**, *35*, 2735-2743.

[21] Patil, A.D.; Freyer, A.J.; Eggleston, D.S.; Haltiwanger, R.C.; Bean, M.F.; Taylor, P.B.; Caranfa, M.J.; Breen, A.L.; Bartus, H.R.; Johnson, R.K.; Hertzberg, R.P.; Westley, J.W. The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from Malaysian tree, *Calophyllum inophyllum* Linn. *J. Med. Chem*., **1993**, *36*, 4132-4138.

[22] Santos, C.C.C.; Souoza, T.M.L.; Teixeira, V.L.; Fontes, C.F.L.; Rebello, M.A.; Castello, B.L.R.R.; Abreu, C.M.; Tanuri, A.; Frugulhetti, I.C.P.P.; Bou, H.D.C. The dolabellane diterpene dolabelladienetriol is a typical noncompetitive inhibitor of HIV-1 reverse transcriptase enzyme. *Antiviral Res*., **2008**, *77*, 64-71.

[23] Akihisa, T.; Ogihara, J.; Kato, J.; Yasukawa, K.; Ukiya, M.; Yamanouchi, S.; Oishi, K. Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase. *Lipids*, **2001**, *36*, 507-512.

[24] Rukachaisirikul, V.; Pailee, P.; Hiranrat, A.; Tuchinda, P.; Yoosook, C.; Kasisit, J.; Taylor, W.C.; Reutrakul, V. Anti-HIV-1 protostane triterpenes and digeranylbenzophenone from trunk bark and stems of *Garcinia speciosa*. *Planta Med*., **2003**, *69*, 1141-1146.

[25] Pengsuparp, T.; Cai, L.; Fong, H.H.S.; Kinghorn, A.D.; Pezzuto, J.M. Pentacyclic triterpenes derived from Maprounea Africana are potent inhibitors of HIV-1 reverse transcriptase. *J. Nat. Prod*., **1994**, *57*, 415-418.

[26] Wang, J.B.; Wang, H.X.; Ng, T.B. A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*. *Peptides*, **2007**, *28*, 560-565.

[27] Lin, P.; Ng, T.B. A novel and exploitable antifungal peptide from kale (Brassica alboglabra) seeds. *Peptides*, **2008**, *29*, 1664-1671.

[28] Han, C.H.; Liu, Q.H.; Ng, T.B.; Wang, H.X. A novel homodimeric lactose-binding lectin from the edible split gill medicinal mushroom *Schizophyllum commune*. *Biochem. Biophys. Res. Commun*., **2005**, *336*, 252-257.

[29] Li, Y.R.; Liu, Q.H.; Wang, H.X.; Ng, T.B. A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*. *Biochim. Biophys. Acta*, **2008**, *1780*, 51-57.

[30] Wong, J.H.; Ng, T.B. Isolation and characterization of a glucose/mannose/rhamnose- pecific lectin from the knife bean *Canavalia gladiata*. *Arch. Biochem. Biophys*., **2005**, *439*,91-98.

[31] Lin, P.; Ng, T.B. A stable trypsin inhibitor from Chinese dull black soybeans with potentially exploitable activities. *Process Biochem*., **2008**, *43*, 992-998.

[32] Ngai, P.H.K.; Zhao, Z.; Ng, T.B. Agrocybin, an antifungal peptide from the edible mushroom *Agrocybe cylindracea*. *Peptides*, **2005**, *26*, 191-196.

[33] Guo, Y.X.; Wang, H.X.; Ng, T.B. Isolation of trichogin, an antifungal protein from fresh fruiting bodies of the edible mushroom *Tricholoma giganteum*. *Peptides*, **2005**, *26*, 575-580.

[34] Ngai, P.H.K.; Ng, T.B. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. *Life Sci*., **2003**, *73*, 3363-3374.

[35] Lin, P.; Xia, L.X.; Wong, J.H.; Ng, T.B.; Ye, X.Y.; Wang, S.Y.; Shi, X.Z. Lipid transfer proteins from *Brassica campestris* and mung bean surpass mung bean chitinase in exploitability. *J. Pept. Sci*., **2007**, *13*, 642-648.

[36] Lin, P.; Ng, T.B. Preparation and biological properties of a melibiose binding lectin from *Bauhinia variegata* seeds. *J. Agric. Food Chem*., **2008**, *56*, 10481-10486.

[37] Zhao, J.K.; Wang, H.X.; Ng, T.B. Purification and characterization of a novel lectin from the toxic wild mushroom *Inocybe umbrinella*. *Toxicon*, **2009**, *53*, 360-366.

[38] Wong, J.H.; Wang, H.X.; Ng, T. B. Marmorin, a new ribosome inactivating protein with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the mushroom *Hypsizigus marmoreus*. *Appl. Microbiol. Biotechnol*., **2008**, *81*, 669-674.

[39] Mahmood, N.; Piacente, S.; Pizza, C.; Burke, A.; Khan, A.I.; Hay, A.J. The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascena*. *Biochem. Biophys. Res. Commun*., **1996**, *229*, 73-79.

[40] Hussein, G.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Kawhata, T.; Otake, T.; Kakiuchi, N.; Shimothno, K. Inhibitory effects of Sudanese plant extracts on HIV-1 replication and HIV-1 protease. *Phytother. Res*., **1999**, *13*, 31-36.

[41] Cheenpracha, S.; Karalai, C.; Ponglimanont, C.; Subhadhirasakulb, S.; Tewtrakul, S. Anti-HIV-1 protease activity of compounds from *Boesenbergia pandurata*. *Bioorg. Med. Chem*., **2006**, *14*, 1710-1714.

[42] Park, J.C.; Hur, J.M.; Park, J.G.; Hatano, T.; Yoshida, T.; Miyashiro, H.; Min, B.S.; Hattori, M. Inhibitory effects of Korean medicinal plants and camelliatannin H from *Camellia japonica* on human immunodeficiency virus type 1 protease. *Phytother. Res*., **2002**, *16*, 422-426.

[43] Ma, C.; Nakamura, N.; Hattori, M.; Kakuda, H.; Qiao, J.; Yu, H. Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. *J. Nat. Prod*., **2000**, *63*, 238-242.

[44] Ma, C.; Nakamura, N.; Hattori, M. Inhibitory effects on HIV-1 protease of tri-p-coumaroylspermidine from *Artemisia caruifolia* and related amides. *Chem. Pharm. Bull*., **2001**, *49*, 915-917.

[45] Cheng, T.J.; Goodsell, D.S.; Kan, C.C. Identification of sanguinarine as a novel HIV protease inhibitor from high- throughput screening of 2,000 drugs and natural products with a cell-based assay. *Lett. Drug Des. Discov*., **2005**, *2*, 364-371.

[46] Min, B.S.; Hattori, M.; Lee, H.K.; Kim, Y.H. Inhibitory constituents against HIV-1 protease from *Agastache rugosa*. *Arch. Pharm. Res*., **1999**, *22*, 75-77.

[47] Min, B.S.; Jung, H.J.; Lee, J.S.; Kim, Y.H.; Bok, S.H.; Ma, C.M.; Nakamura, N.; Hattori, M.; Bae, K. Inhibitory effect of triterpenes from *Crataegus pinatifida* on HIV-I protease. *Planta Med*., **1999**, *65*, 374-375.

[48] Baltina, L.A. Chemical modification of glycyrrhizic acid as a route to new bioactive compounds for medicine. *Curr. Med. Chem*., **2003**, *10*, 155-171.

[49] Wei, Y.; Ma, C.M.; Chen, D.Y.; Hattori, M. Anti-HIV-1 protease triterpenoids from *Stauntonia obovatifoliola Hayata subsp*. intermedia. *Phytochemistry*, **2008**, *69*,1875-1879.

[50] Kashiwada, Y.; Hashimoto, F.; Cosentino, L.M.; Chen, C.H.; Garret, P.E.; Lee, K.H. Betulinic acid and dihydrobetulinic acid derivatives as potent anti-HIV agents. *J. Med. Chem*., **1996**, *39*, 1016-1017.

[51] Beatty, G.L.J.; Eron, J.; Pollard, R.; Saag, M.; Doto, J.; Salzwedel, K.; Wild, C.; Allaway, G..; Jacobson, J.; Martin, D. *Safety and antiviral of PA-457, the first in class maturation inhibitor, in a 10-day monotherapy study in HIV-1 infected patients*. Presented at 45th Conference on Antimicrobial Agents and Chemotherapy, Panacos: Washington D C, December. **2005**.

[52] Dine, R.S.E.; Halawany, A.M.E.; Ma, C.M.; Hatori, M. Anti-HIV-1 Protease Activity of lanostane triterpenes from the Vietnamese Mushroom *Ganoderma colossum*. *J. Nat. Prod*., **2008**, *71*, 1022-1026.

[53] Yu, Y.B.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Park, J.C. Effects of triterpenoids and flavonoids isolated from *Alnus firma* on HIV-1 Viral Enzymes. *Arch. Pharm. Res.*, **2007**, *30*, 820-826.

[54] Li, J.T.; Li, Y.N.; Yuan, Y.J. Study of Molecular docking of an HIV-1 protease inhibitor—streptolydigin. *Acta Chim. Sin*., **2006**, *64*, 2491-2495.

[55] Patil, A.D.; Kokke, W.C.; Cochran, S.; Francis, T.A.; Tomszek, T.; Westley, J.W. Brominated polyacetylenic acids from the marine sponge *Xestospongia muta*: inhibitors of HIV protease. *J. Nat. Prod*., **1992**, *55*, 1170-1177.

[56] Tewtrakul, S; Nakamura, N; Hattori, M; Fujiwara, T; Supavita, T. Flavanone and flavonol glycosides from the leaves of *Thevetia peruviana* and their HIV-1 reverse transcriptase and HIV-1 integrase inhibitory activities. *Chem. Pharm. Bull*., **2002**, *50*, 630-635.

[57] Meragelman, K.M.; McKee, T.C.; Boyd, M.R. Anti-HIV prenylated flavonoids from *Monotes africanus*. *J. Nat. Prod*., **2001**, *64*, 546-548.

[58] Singh, S.B.; Zink, L.D.; Guan, Z.; Clooado, J.; Pelaez, F.; Felock, P.; Hazuda, D.J. Isolation, structure, and HIV-1 integrase inhibitory activity of Xanthoviridicatin E and F, two novel fungal metabolites produced by *Penicillium chrysogenum*. *Helv. Chem. Acta*, **2003**, *86*, 3360-3385.

[59] Singh, S.B.; Zink D.L.; Bills G.F.; Teran A.; Silverman, K.C.; Lingham, R.B.; Felock, P.; Hazuda, D.J. Four novel bis-(naphthogamma-pyrones) isolated from *Fusarium* species as inhibitors of HIV-1 integrase. *Bioorg. Med. Chem. Lett* ., **2003**, *13*, 713-717.

[60] Singh, S.B.; Jayasuriya, H.; Dewey, R.; Polishook, J.D.; Dombrowski, A.W.; Zink, D.L.; Guan, Z.; Collado, J.; Platas, G.; Pelaez, F.; Felock, P.J.; Hazuda, D.J. Isolation, structure, and HIV-1-integrase inhibitory activity of structurally diverse fungal metabolites. *J. Ind. Microbiol. Biotechnol*., **2003**, *30*, 721-731.

[61] Ali, N.A.A.; Jansen, R.; Pilgrim, H.; Liberra, K.; Lindequist, U. Hispolon, a yellow pigment from *Inonotus hispidus*. *Phytochemistry*., **1996**, *41*, 927-929.

[62] Klaar, M.; Steglich, W. Fungus Pigments, 0.27. Isolation of Hispidin and 3,14'-Bihispidinyl from *Phellinus-Pomaceus* (Poriales). *Chem. Ber.-Rec.*, **1977**, *110*, 1058-1062.

[63] Dayam, R.; Deng, J.X.; Neamati, N. HIV-1 integrase inhibitors 2003-2004 update. *Med. Res. Rev.*, **2006**, *26*, 271-309.

[64] Tewtrakul, S.; Subhadhirasakul, S.; Cheenpracha, S.; Karalai, C. HIV-1 protease and HIV-1 integrase inhibitory substances from *Eclipta prostrate*. *Phytother. Res*., **2007**, *21*, 1092-1095.

[65] Ahn, M.J.; Kim, C.Y.; Lee, J.S.; Kim, T.G.; Kim, S.H.; Lee, C.K.; Lee, B.B.; Shin, C.G.; Huh, H.; Kim, J. Inhibition of HIV-1 integrase by galloyl glucoses from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis*. *Planta Med*., **2002**, *68*, 457-459.

[66] Kim, H.K.; Lee, H.K.; Shin, C.G.; Huh, H. HIV integrase inhibitory activity of *Agastache rugosa*. *Arch. Pharm. Res*., **1999**, *22*, 520-523.

[67] Tewtrakul, S.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Kawahata, T.; Otake, T.; Yoshinaga, T.; Fujiwara, T.; Supavita, T.; Yuenyongsawad, S.; Rattanasuwon, P.; Dej, A.S. HIV-1 Integrase inhibitory substances from *Coleus parvifolius*. *Phytother. Res.*, **2003**, *17*, 232-239.

[68] Nakanishi, S.; Toki, S.; Saitoh, Y.; Tsukuda, E.; Kawahara, K.; Ando, K.; Matsuda, Y. Isolation of myosin lightchain kinase inhibitors from microorganisms-Dehydroaltenusin, Altenusin, Atrovenetinone, and Cyclooctasulfur. *Biosci. Biotechnol. Biochem*., **1995**, *59*,1333-1335.

[69] Uchida, R.; Tomoda, H.; Dong, Y.; Omura, S. Alutenusin, a specific neutral sphingomyelinase inhibitor, produced by *Penicillium* sp. FO-7436. *J. Antibiot.* (Tokyo)., **1999**, *52*, 572-574.

[70] Jayasuriya, H.; Guan, Z.; Polishook, J.D.; Dombrowski, A.W.; Felovk, P.J.; Hazuda, D.J.; Singh, S.B. Isolation, Structure, and HIV-1 integrase inhibitory activity of cytosporic acid, a fungal metabolite produced by a *Cytospora* sp. *J. Nat. Prod.*, **2003**, *66*, 551-553.

[71] Ondeyka, J.G.; Zink, D.L.; Dombrowski, A.W.; Polishook, J.D.; Felock, P.J.; Hazuda, D.J.; Singh, S.B. Isolation, structure and HIV-1 integrase inhibitory activity of exophillic acid, a novel fungal metabolite from *Exophiala pisciphila*. *J. Antibiot* .(Tokyo)., **2003**, *56*, 1018-1023.

[72] Hamano, K.; Kinoshita, O.M.; Minagawa, K.; Haruyama, H.; Kinoshita, T.; Hosoya, T.; Furuya, K.; Kinoshita, K.; Tabata, K.; Hemmi, A. Aquastatin A, an inhibitor of mammalian adenosine triphosphatases from *Fusarium aquaeductuum*. Taxonomy, fermentation, isolation, structure determination and biological properties. *J. Antibiot.* (Tokyo)., **1993**, *46*, 1648-1657.

[73] Shiomi, K.; Matsui, R.; Isozaki, M.; Chiba, H.; Sugai, T.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Chiba, T.; Yan, H.; Kitamura, Y.; Sugiura, W.; Omura, S.; Tanaka, H. Fungal phenalenones inhibit HIV-1 integrase. *J. Antibiot.* (Tokyo)., **2005**, *58*, 65-68.

[74] Huang, S.L.; Huang, P.L.; Zhang, D.; Lee, J.W.; Bao, J.; Sun, Y.T.; Chang, Y.T.; Zhang, D.; Huang, P.L. Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part II. Integrase inhibition. *Biochem. Biophys. Res. Commun*., **2007**, *354*, 879-884.

[75] Wang, X.B.; Natscbke, S.L.M.; Lee, K.H. New developments in the chemistry and biology of the bioactive constituents of Tanshen. *Med. Res. Rev.*, **2007**, *27*, 133-148.

[76] Singh, S.B.; Zink, D.L.; Quamina, D.S.; Pelaez, F.; Teran, A.; Felock, P.; Hazuda, D.J. Integrastatins: structure and HIV-1 integrase inhibitory activities of two novel racemic tetracyclic aromatic heterocycles produced by two fungal species. *Tetrahedron. Lett.*, **2002**, *43*, 2351-2354.

[77] Mehta, G..; Roy, S. Enantioselective total synthesis of a novel polyketide natural product (+)-integrasone, an HIV-1 integrase inhibitor. *Chem. Commun*., **2005**, *7*, 3210-3212.

[78] Singh, S.B.; Jayasuriya, H.; Salituro, G.M.; Zink, D.L.; Shafiee, A.; Heimbuch, B. The complestatins as HIV-1 integrase inhibitors efficient isolation, structure elucidation, and inhibitory activities of isocomplestatin, chloropeptin I, New complestatins, A and B, and acid-hydrolysis products of chloropeptin I. *J. Nat. Prod.* **2001**, *64*, 874-882.

[79] Zhang, C.F.; Nakamura, N.; Tewtrakul, S.; Hattori, M.; Sun, Q.S.; Wang, Z.T.; Fujiwara, T. Sesquiterpenes and alkaloids from *Lindera chunii* and their inhibitory activities against HIV-1 integrase. *Chem. Pharm. Bull*., **2002**, *50*, 1195-1200.

[80] Singh, S.B.; Zink, D.L.; Dombrowski, A.W.; Polishook, J.D.; Ondeyka, J.G.; Hirshfield, J.; Felock, P.; Hazuda, D.J. Integracides: Tetracyclic triterpenoid inhibitors of HIV-1 integrase produced by *Fusarium* sp. *Bioorg. Med. Chem.*, **2003**, *11*:1577-1582.

[81] Singh, S.B.; Ondeyka, J.G.; Schleif, W.A.; Felock, P.; Hazuda, D.J. Chemistry and structure-activity relationship of HIV-1 integrase inhibitor integracide B and related natural products. *J. Nat. Prod*., **2003**, *66*, 1338-1344.

[82] Singh, S.B.; Zink, D.; Polishook, J.; Valentino, D.; Shafiee, A.; Silverman, K.; Felock, P.; Teran, A.; Vilella, D.; Hazuda, D.J.; Lingham, R.B. Structure and absolute stereochemistry of HIV- 1 integrase inhibitor integric acid. A novel eremophilane sesquiterpenoid produced by a *Xylaria* sp. *Tetrahedron Lett*., **1999**, *40*, 8775-8779.

[83] Singh, S.B.; Zink, D.L.; Heimbach, B.; Genilloud, O.; Teran, A.; Silverman, K.C.; Lingham, R.B.; Felock, P.; Hazuda, D.J. Structure, stereochemistry, and biological activity of integramycin, a novel hexacyclic natural product produced by *Actinoplanes sp*. that inhibits HIV-1 integrase. *Org. Lett*., **2002**, *4*, 1123-1126.

[84] Singh, S.B.; Herath, K.; Guan, Z.; Zink, D.L.; Dombrowski, A.W.; Polishook, J.D.; Silverman, K.C.; Lingham, R.B.; Felock, P.J.; Hazuda, D.J. Integramides A and B, two novel non-ribosomal linear peptides containing nine C-methyl amino acids produced by fungal fermentations that are inhibitors of HIV-1 integrase. *Org. Lett*., **2002**, *4*, 1431-1434.

[85] Rochfort, S.; Ford, J.; Ovenden, S.; Wan, S.S.; George, S.; Wildman, H.; Tait, R.M.; Meurer, G..B.; Cox, S.; Coates, J.; Rhodes, D. A novel aspochalasin with HIV-1 integrase inhibitory activity from *Aspergillus flavipes*. *J. Antibiot*. (Tokyo), **2005**, *58*, 279-283.

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