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Anti-HIV activity of natural triterpenoids and hemisynthetic derivatives 2004–2009

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Abstract The continued advance of HIV-AIDS makes the development of relatively inexpensive, freely accessible, and mechanistically more diverse antiviral therapies an urgent need. Natural products are, directly or indirectly, an important potential source of compounds meeting these conditions. A review of the recent literature indicates that some hemisynthetic triterpenoid derivatives, particularly belonging to the lupane, oleanane and ursane series, may be nearing a stage where they can be used to complement existing therapeutic approaches. On the other hand, although some natural derivatives of tetracyclic terpenoid families have revealed many novel structures and some promise as anti-HIV substances, their chemical modification to improve their potency and selectivity remains practically untouched. While ongoing work with the more 'classical' pentacyclic triterpenoids will continue to be a fertile field for HIV-AIDS drug discovery, the other structural groups offer unprecedented opportunities for the development of additional substances with useful properties and for the discovery of novel targets for antiviral therapy.

Keywords AIDS · Antiviral activity · Natural products · Triterpenes · Structural modification

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Introduction

As of 2007, about 33 million people worldwide were estimated to be living with HIV-1 infection, of which 22 million inhabited Sub-Saharan Africa and more than 4 million were in South and South-East Asia. Globally, only 27–34% of those in need of antiretroviral therapy were receiving it (WHO 2009). In addition to these tragic figures, the cost of this therapy in most countries and the rapid development of viral resistance to drugs in current use underline the need for less expensive, preferably non-patented, and mechanistically more diverse antivirals is a pressing concern.

Aside from the nucleoside analogue reverse transcriptase inhibitors (Zidovudine, Didanosine, Stavudine, and so on), many classes of natural products and some of their analogues and hemisynthetic derivatives have been tested with varying success for anti-HIV-1 activity. As early as 1989 soybean

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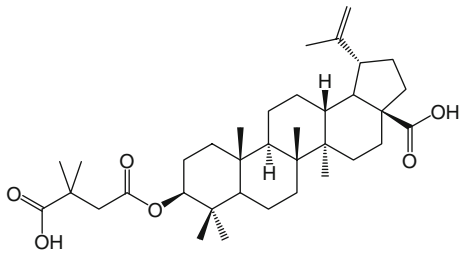
saponins were shown to inhibit the infectivity of HIV-1 in vitro (Nakashima et al. 1989), and a number of other saponins have exhibited similar activities over the years. Although such compounds can be useful to limit transmission of the virus, their pharmacokinetics and the fact that they are generally unacceptable for parenteral use set practically impassable limits to their wider application. From a medicinal chemical viewpoint one would wish for 'druglike' molecules that satisfy Lipinski's 'rule of five' or its extensions, which would be met by most triterpenoid aglycones.

In 1992 a friedo-triterpene, salaspermic acid, was reported to inhibit HIV-1 replication in H9 lymphocytes with $EC_{50} = 5$ μg/ml (10 μM), while its antiproliferative activity in uninfected cells gave an $IC_{50} = 53$ μM (Chen et al. 1992). A major breakthrough occurred 2 years later, when betulinic acid, dihydrobetulinic acid, and platanic acid were shown to be potent and moderately selective HIV inhibitors and that, among various betulinic acid derivatives esterified at C3, the hemisuccinate retained similar potency (Fujioka et al. 1994). This led to the development of the extremely potent 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid, which inhibited HIV replication with $EC_{50} < 0.35$ nM and a selectivity index of 20,000 (Hashimoto et al. 1997) (Bevirimat, PA-457, MPC-4326), and which is now in Phase II clinical studies:

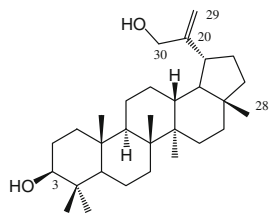


Two important reviews published just before the period we are covering are those of Sun et al. (2003) and Baglin et al. (2003), the latter concentrating on triterpenoids and more specifically betulinic, ursolic and echinocystic acid derivatives. A short paper on the HIV-1 antiviral activity of betulinic acid derivatives is one by Aiken and Chen (2005). An extensive review of the literature up to 2004 on anti-HIV activity of naturally derived compounds is frustratingly brief regarding triterpenoids (Asres et al. 2005). The structure–activity relationships of oleanane- and ursane-derived triterpenoids as anti-HIV agents, covering the literature until 2002, were briefly reviewed (together with several other activities) in 2006 (Sun et al. 2006a). Another recent review presents diverse classes of natural products with anti-HIV activities (Yu et al. 2007a). Most recently, plant anti-HIV agents were considered in the framework of their putative mechanisms of action but, as far as triterpenoids are concerned, the only current development mentioned is one of the papers discussed below on *Schisandra lancifolia* constituents (Cos et al. 2008; Xiao et al. 2006c). Finally, a review has just appeared providing a welcome summary of what is known of the structure–activity relationships of hemisynthetic lupane, ursane and oleanane derivatives, as well as a listing of most of the other anti-HIV natural triterpenoids discussed here (Kuo et al. 2009).

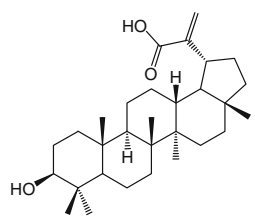
Pentacyclic triterpenoid derivatives

Lupanes

The previously known lup-20(29)-ene-3β,30-diol, 3β-hydroxylup-20(29)-en-30-oic acid and betulinic acid were isolated, together with a series of inactive compounds and a couple of moderately active xanthones, from leaves and twigs of *Cratoxylum arborescens* (Vahl) Blume (Clusiaceae or Guttiferae). The anti-HIV activity of betulinic acid was confirmed, and that of the lupenediol and lupenoic acid was demonstrated by inhibition of the viral reverse transcriptase ($IC_{50} = 10.8$, 14.0, and 8.7 μg/ml, respectively), and of syncytium formation by HIV-infected 1A2 cells (EC_{50} values <3.9, 6.4, and 23.8 μg/ml) with a more favorable therapeutic index versus cytotoxicity (TI = 11.7) in the case of the lupenediol (Reutrakul et al. 2006).



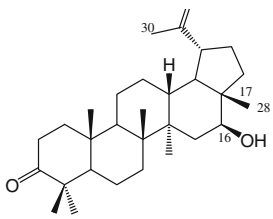
lup-20(29)-ene-3β,30-diol



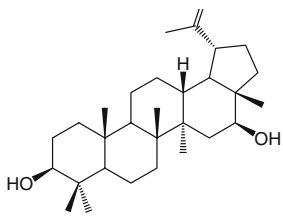
3β-hydroxylup-20(29)-en-30-oic acid

It has been pointed out that hydrogen bond donors on the isopropenyl side chain of betulinic acid result in a severalfold reduction of anti-HIV activity, while diverse ether substituents at this position can be used to improve solubility without affecting antiviral activity (Qian et al. 2009). The results obtained with the *Cratoxylum* metabolites mentioned above suggest that, in lupene derivatives lacking a C-28 carboxylic acid function, hydrogen bond donors at C-30 may be better tolerated, pointing to an alternative to C-28 for functionalization in the quest for novel AIDS drugs.

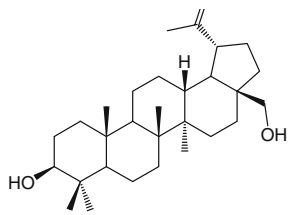
A total of fourteen triterpenoids, three of them previously undescribed, were isolated from the stems of *Stauntonia obovatifoliola* Hayata subsp. *intermedia* (Y. C. Wu) T. Chen (Lardizabalaceae) (Wei et al. 2008). Of the previously known compounds of this plant, two lupanes (lupeol and lupenone) were inactive and resinone, lup-20(29)-ene-3β,16β-diol, and betulin, showed modest activity with IC_{50} = 29.4, 33.0, and 53.0, respectively (Wei et al. 2008).



resinone

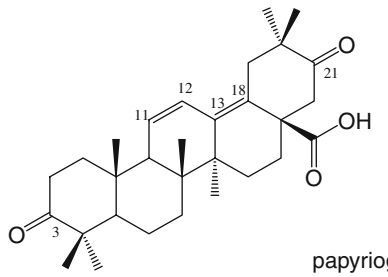


lup-20(29)-ene-3 ,16 -diol



betulin

inhibited p24 antigen production in the H9 T lymphocyte cell line acutely infected with HIV-1_{IIIB}, showing $EC_{50} < 0.8$ μg/ml. Although this compound proved to be fairly toxic to infected cells (IC_{50} = 8.91 μg/ml), its selectivity index (>11.1) was considered promising (Ho et al. 2007).



papyriogenin A

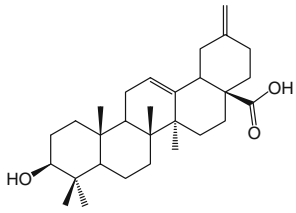
It should be pointed out that papyriogenin A differs from most oleananes (and ursanes) in having a conjugated $\Delta^{11,12,13,18}$ diene moiety. This not only offers uncommon opportunities for modification (both 'chemical' and in vivo enzymatic), but also changes the stereochemistry of the C/D and D/E ring junctions, which may have important consequences for antiviral activity.

It seems worth pointing out that some of the active *Cratoxylum* and *Stauntonia* lupenes have an unoxidized (C-28) methyl group at C-17. Although these compounds are not highly active, they clearly show that, at least in the lupane series of 'classical' pentacyclic triterpenes, a C-28 acid function or some of its extensions is not a prerequisite. If a polar group is necessary near the D/E ring junction, the effect of functionalization at positions not far removed from C-28 such as C-30 or C-16 should be explored further.

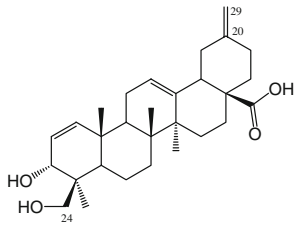
Oleananes

Papyriogenin A, isolated from the aerial parts of *Tetrapanax papyriferus* (Hook) K. Koch (Araliaceae)

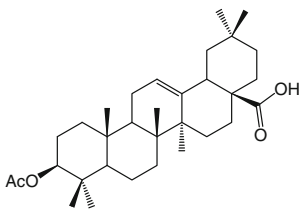
Among the previously known compounds isolated from *Stauntonia obovatifoliola* (vide supra), two noroleananes: 3β-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid and 3α,24-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid, inhibited HIV-1 protease with IC_{50} = 35.0 and 40.8 μg/ml, respectively; and three oleananes: 3-*O*-acetyloleanolic acid, mesenbryanthemoidgenic acid, and 3β,23-dihydroxyolean-12-en-28-oic acid were also active with IC_{50} = 38.0, 28.0, and 36.0 μg/ml, respectively. In this assay, the previously studied



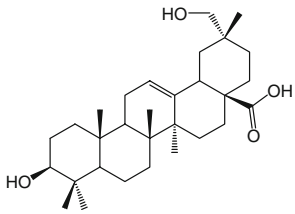
3 -hydroxy-30-noroleana-12,20(29)-dien-28-oic acid



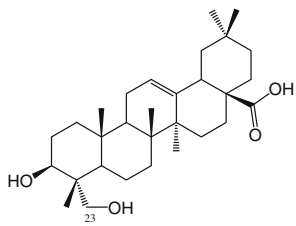
3α,24-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid



3-O-acetyloleanolic acid



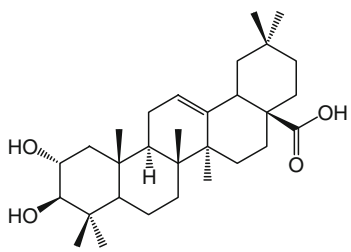
mesenbryanthemoidgenic acid



3β,23-dihydroxyolean-12-en-28-oic acid

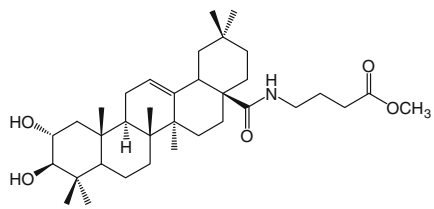
oleanolic acid exhibited $IC_{50} = 24.8$ μg/ml (Wei et al. 2008).

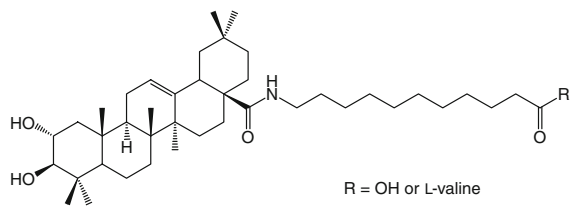
Maslinic acid, which can be isolated on an industrial scale from the wastes of olive oil production, has been shown to inhibit HIV protease (Xu et al. 1996).



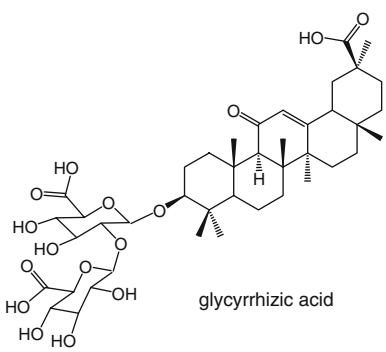
maslinic acid

Recent work demonstrated that coupling with an amino acid or peptide residue at C-28 in most cases reduces the ability of maslinic acid to inhibit HIV replication (though not necessarily via protease inhibition) in MT-2 cells infected with the pNL4-3 HIV-1 clone. Nevertheless, two of these derivatives were at least as potent as maslinic acid itself, inhibiting viral replication by 33–57% at 10 μM (Parra et al. 2009):





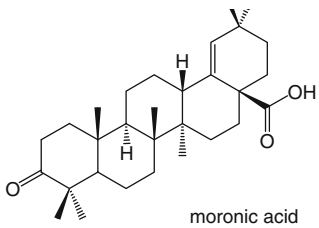
Glycyrrhizic acid, the main saponin of liquorice (*Glycyrrhiza glabra* L.) root extracts, was shown to have anti-HIV activity more than 20 years ago (Ito et al. 1987), although its selectivity (versus cytotoxicity) is poor:



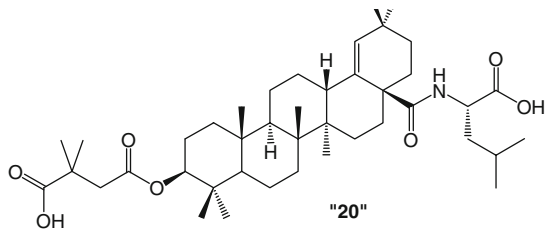
glycyrrhizic acid

Recent publications describe the synthesis of several amino sugar conjugates, heterocyclic and carbocyclic amides of glycyrrhizic acid and their assay in HIV-1-infected MT-4 cell cultures. Some of these new compounds exhibited promising selectivity indices (Kondratenko et al. 2004, 2009).

Moronic acid, isolated from a southern Brazilian propolis (believed to originate primarily from the local *Myrceugenia euosma* (O. Berg) Legrand, Myrtaceae), had been shown to significantly inhibit p24 antigen release from H9 cells infected with HIV-1_{IIIB} ($EC_{50} < 0.1$ μg/ml) with a good therapeutic index (>186) relative to its cytotoxicity (Ito et al. 2001). Structural modification of this natural product led to compounds with more potent anti-HIV activity than the betulinic acid analogue Bevirimat (PA-457, MPC-4326), and HIV maturation inhibitor which is currently in Phase 2 clinical trials. The more promising analogue **"20"** (EC_{50} values of 0.0085, 0.021, and 0.13 μM against different viral strains, the latter resistant to Bevirimat) is depicted below (Yu et al. 2006).



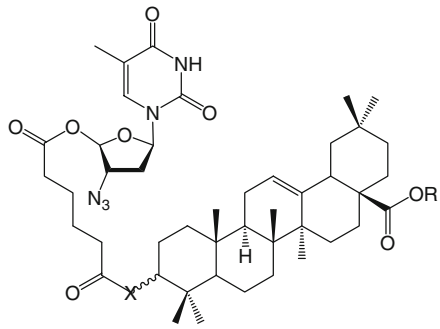
moronic acid



"20"

Moronic acid has an unusual double bond between C-18 and C-19 instead of the common $\Delta^{11,12}$ arrangement. This clearly modifies the geometry of the ring-D/ring-E system as compared to the widespread olean-12-enes (and urs-12-enes), which might be related to the potent antiviral activity of moronic acid derivatives. The similar orientation of the C-28 carboxyl group of 3*β*-hydroxymoronic and betulinic acids has been noted as a possible explanation (Yu et al. 2006). A simple model of oleanolic or ursolic acid also places the C-28 carboxyl group in a similar position (Huang et al. 2007c), but a rigorous conformational comparison of $\Delta^{11,12}$, $\Delta^{18,19}$, and $\Delta^{11,12,13,18}$ oleanane/ursane derivatives with the lupane skeleton is still clearly necessary.

A paper that seems to have been overlooked by the recent reviews is one by Ma et al. (2002), describing the synthesis, viral protease inhibition and anti-HIV assay (in MT-4 cells) of a series of oleanolic acid derivatives conjugated with the nucleoside analogue reverse transcriptase inhibitor zidovudine or azidothymidine (AZT). Although AZT has no effect on the protease, these compounds inhibited the enzyme with IC_{50} values in the 1.2–20 μM range, and showed anti-HIV-1 activity in the 0.370–18.4 μM range, with no obvious correlation between both activities. It should be pointed out that the immediate precursors of the AZT conjugates, i.e. before attaching the nucleoside analogue, also inhibited the viral protease in the 3.0–7.5 μM range. The three most potent anti-HIV-1 AZT conjugates, designated as **"5b"**, **"6b"** and **"7b"** with IC_{50} values of 0.589, 0.370, and 0.469 μM, respectively, and selectivity indices of 200 or more, are depicted below.



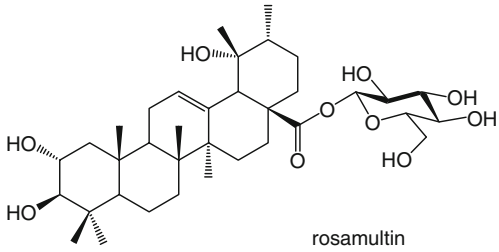
"5b": R = H, X = β-NH

"6b": R = CH_3, X = β-NH

"7b": R = H, X = α-NH

Ursanes

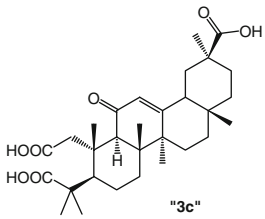
Out of 12 Rosaceae extracts, one prepared from *Rosa rugosa* Thunb. roots inhibited HIV-1 protease at a concentration that warranted further study. Fractionation of this extract led to the isolation of rosamultin, which only inhibited the enzyme by 53% at a concentration of 100 μM (Park et al. 2005).



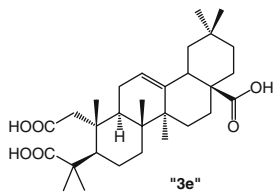
rosamultin

2,3-Seco-lupanes, -oleananes, and -ursanes

Exploiting the common presence of a C-3 hydroxyl group (which allows the preparation of 2,3-seco-2,3-dioic acid derivatives) and a C-28 carboxyl group on the betulinic, oleanolic, and ursolic acid skeletons, Wei et al. (2009c) prepared a series of thirty derivatives with different A ring modifications and with the C-28 carboxyl incorporated into a methyl ester or an L- or D-valine peptide. Aside from two compounds which were practically inactive toward HIV-1 protease, most of the derivatives were moderate to weak inhibitors with IC_{50} values in the 10–90 μM range, and two of them, **"3c"** and **"3e"**, among the least elaborate structures, were more potent, with IC_{50} = 5.7 and 3.9 μM, respectively.



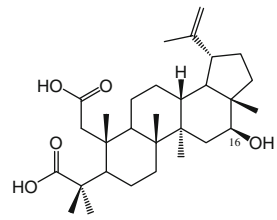
"3c"



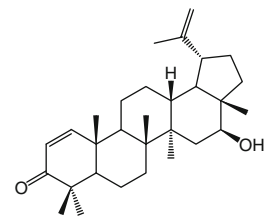
"3e"

The authors note that free carboxyl groups or 'multi-hydrogen bonding capacity' of the A-ring, together with a free carboxyl group at C-28 or C-30, seem to favor HIV-1 protease inhibition, as also pointed out in Kuo et al. (2009). In general, all compounds were very weak inhibitors of hepatitis C virus protease, renin, and trypsin (IC_{50} > 80 μM).

Two of the new compounds from *Stauntonia obovatifoliola* (vide supra) 16*β*-hydroxy-2,3-secolup-20(29)-ene-2,3-dioic acid, and 16*β*-hydroxylupane-1,20(29)-dien-3-one, inhibited HIV-1 protease with IC_{50} = 8.7 and 25.0 μg/ml, respectively (Wei et al. 2008):



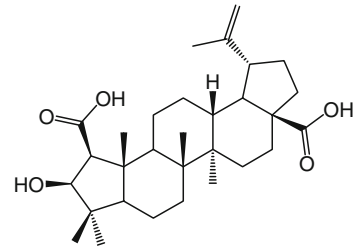
16β -hydroxy-2,3-secolup-20(29)-ene-2,3-dioic acid



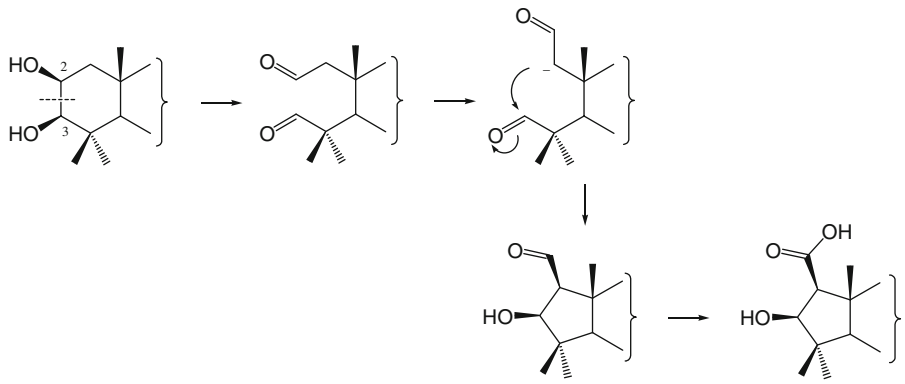
16β-hydroxylupane-1,20(29)-dien-3-one

Here again the more potent compound is a 2,3-seco derivative, presumably formed via a 2,3-dihydroxylupene.

3*β*-HydroxynorlupA(1)-20(29)-en-2*β*,28-dioic acid was isolated from a methanol extract of the thorns of *Gleditsia sinensis* Lam. (Fabaceae, Caesalpinioideae):



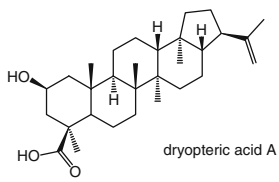
This unusual compound, which inhibited syncytia formation by HIV-1-infected C8166 cells with the unusually potent EC_{50} < 0.064 μg/ml (Li et al. 2007), can be viewed as derived from a 2,3-dihydroxylupene by oxidation to a seco derivative followed by reclosure of the A-ring, e.g.



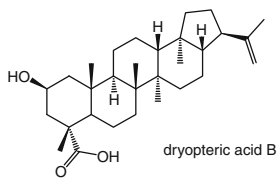
The presence of a neighboring hydroxyl and carboxyl group on ring A suggests that the 'multi-hydrogen bonding capacity' in this region of the molecule might be a general feature of triterpenoids with enhanced anti-HIV activity. In fact, this might also be extended to less highly modified triterpenoids such as the 2,3-dihydroxylated maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid) which is a well documented, fairly potent inhibitor of HIV protease (Xu et al. 1996).

Hopanes

An 80% methanol extract of the rhizome of *Dryopteris crassirhizoma* Nakai (Dryopteridaceae, formerly Aspidiaceae) gave a total of eighteen secondary metabolites, of which two were previously unrecorded diastereomeric triterpenes, dryopteric acids A and B. Of the isolated compounds, ursolic acid and dryopteric acids A and B inhibited HIV-1 protease with IC_{50} values of 8.9, 26.5, and 44.5 μM. It is noteworthy that acetylation of the dryopteric acids increased their inhibitory activities to $IC_{50} = 1.7$ and 10.8 μM, respectively (Lee et al. 2008).



dryopteric acid A

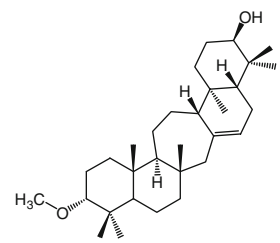


dryopteric acid B

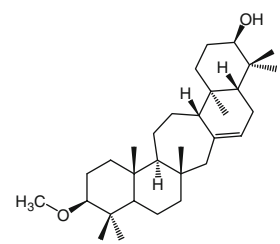
It may be significant that in these compounds two hydrogen-bonding functions are placed in close proximity (particularly in the more potent dryopteric acid A and its acetate) on ring A, a feature vaguely reminiscent of the situation pointed out for moronic acid, the hemisynthetic and natural 2,3-seco-compounds mentioned above, and the unusual 3β-hydroxynorlupA(1)-20(29)-en-2β,28-dioic acid of *Gleditsia sinensis*.

Serratanes

3α-Methoxyserrat-14-en-21β-ol and 3β-methoxyserrat-14-en-21β-ol are abundant triterpenoids in *Picea* species (Pinaceae), accounting for more than one-third of the chloroform extract of the bark of *P. jezoensis* (Sieb. et Zucc.) Carr. var. *jezoensis*, *P. jezoensis* (Sieb. et Zucc.) Carr. var. *hondoensis* (Mayr) Rehder, and *P. glehni* (Fr. Schm.) Masters (Tanaka et al. 2000).



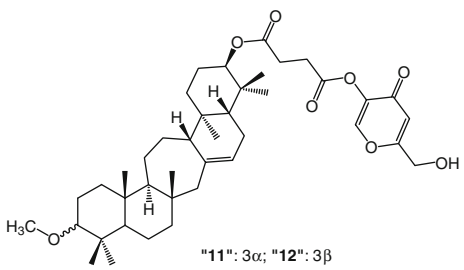
3α-methoxyserrat-14-en-21 β-ol



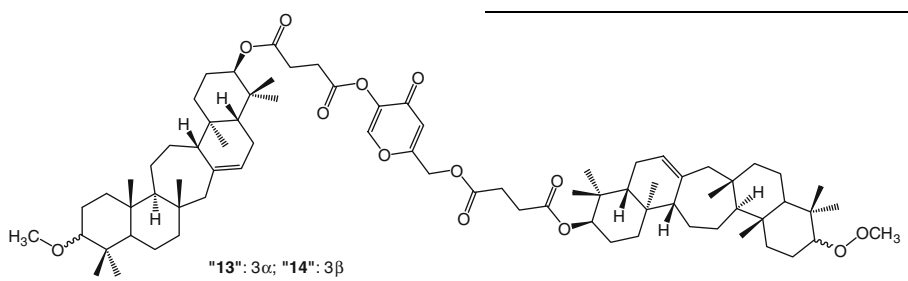
3β-methoxyserrat-14-en-21 β-ol

The 3α-methoxy isomer has been recently shown to decrease the size of adenomas and other tumors in a rat carcinogenesis model (Yamaguchi et al. 2008), and the abundance of these two compounds seems to have prompted the synthesis of a collection of eighteen of their conjugates with the antioxidant natural products curcumin, kojic acid, quercetin, and baicalein, using malonic and succinic acids as linkers, and their assay as HIV-1 reverse transcriptase inhibitors. The natural

products were inactive (and non-cytotoxic) in the C8166-CCR5 cell line, as was the case for all the curcumin derivatives synthesized, but some of their kojic acid derivatives and, to a smaller measure, quercetin and baicalein derivatives, were of interest. Kojic acid linked through its 5-hemisuccinate to the α- and β-methoxyserrat-14-en-21β-ols gave products **"11"** and **"12"**, with EC_{50} = 4.13 and 6.75 μg/ml, respectively, and low selectivities.



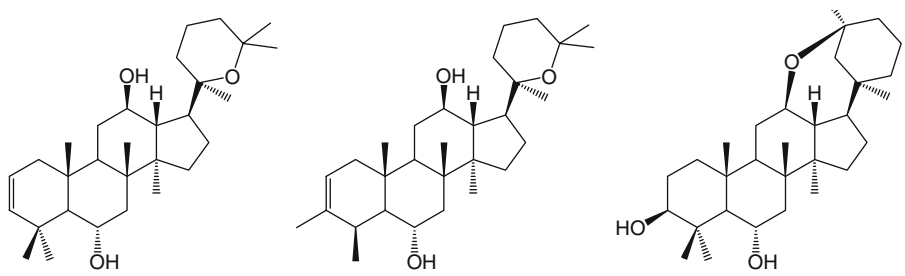
Kojic acid linked through its bis-hemisuccinate to the 3β-methoxy compound (**"13"**) was of particular interest, being quite highly potent (EC_{50} = 0.12 μg/ml) although with modest selectivity (SI = 35). The 3α-methoxy analogue of **"13"** (**"14"**) was less potent (EC_{50} = 5.94 μg/ml SI > 17 (Tanaka et al. 2009).



Tetracyclic triterpenoid derivatives

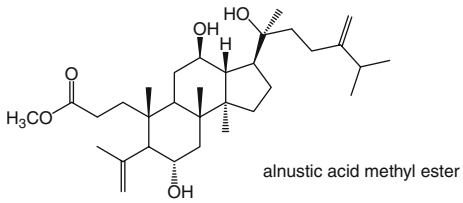
Dammaranes

An acid hydrolysate of a methanol extract of *Panax ginseng* C. A. Meyer (Araliaceae) roots was fractionated to afford the presumed artefacts (20*R*)-20,25-epoxydammar-2-en-6α,12β-diol (**1**), (20*R*)-20,25-epoxy-3-methyl-28-nordammar-2-en-6α,12β-diol (**2**), and isodehydroprotopanaxatriol (**3**), along with (20*R*)-panaxadiol, (20*R*)-panaxatriol, and oleanolic acid. Panaxadiol and -triol were inactive as inhibitors of HIV-1 protease, but the new compounds exhibited IC_{50} values in the narrow 10.3–12.3 μg/ml range, being somewhat less potent than oleanolic acid (6.3 μg/ml) in the current assay (Wei et al. 2009a).



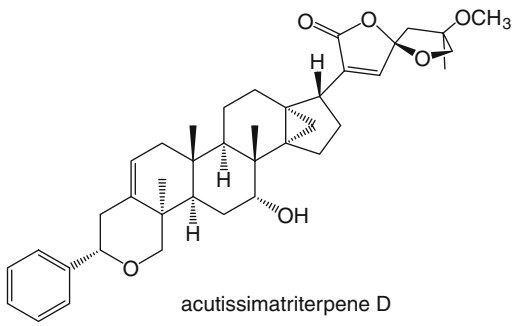
1 **2** **3**

A methanol extract of *Alnus firma* Sieb. et Zucc. (Betulaceae) leaves yielded, among a series of inactive compounds and reverse transcriptase-inhibiting flavonoids, the secodammarane alnustic acid methyl ester, which inhibited HIV-1 protease with IC_{50} = 15.8 μM. This triterpenoid did not inhibit the viral reverse protease or α-glucosidase (Yu et al. 2007b).

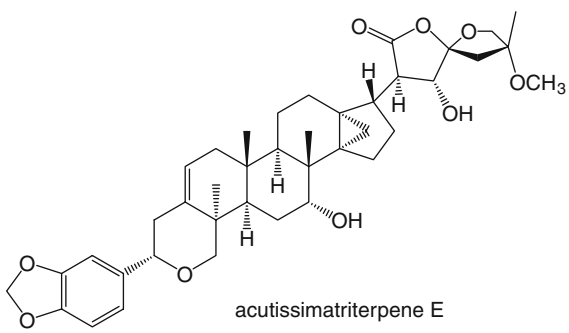


alnustic acid methyl ester

Acutissimatriterpenes D and E, isolated from the aerial parts of *Phyllanthus acutissimus* Miq. (Phyllanthaceae, formerly Euphorbiaceae), inhibited syncytia formation in 1A2 cells with $EC_{50} = 5.1$ and <3.9 μg/ml, respectively, the former with negligible selectivity versus cytotoxicity, but the latter with a promising selectivity index (SI) >8.1. Acutissimatriterpene D inhibited HIV-1 reverse transcriptase by only 37.8% at 200 μg/ml, and acutissimatriterpene E was inactive in this assay, suggesting that these compounds act by a mechanism other than RT inhibition (Tuchinda et al. 2008).

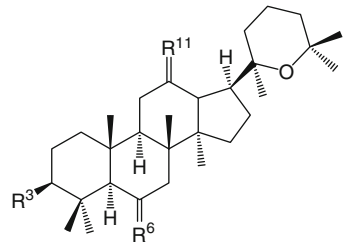


acutissimatriterpene D



acutissimatriterpene E

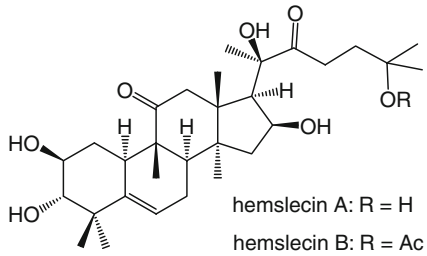
In what appears to be a first effort to discover hemisynthetic tetracyclic triterpene derivatives with anti-HIV-1 activity, Wei et al. (2009b) used (20*R*)-panaxadiol and (20*R*)-panaxatriol from a *Panax ginseng* extract hydrolysate to prepare various 2, 3-seco, A-nor, and 3′,3′-dimethylsuccinyl derivatives. Although the latter were reasonably potent inhibitors of HIV-1 protease, with IC_{50} values in the 2-7-10.9 μM range, several other compounds prepared by these authors inhibited the enzyme with IC_{50}'s in the 10.0–42.3 μM range and very few were practically inactive. Some of these products were also potent-to-moderate inhibitors of hepatitis C virus protease. The more highly anti-HIV-1 active esters are depicted below (DMS = 3′,3′-dimethylsuccinyloxy) with their IC_{50} values (μM).



R^3 = DMS; R^6 = H, H; R^{11} = H, β-OH	2.7
R^3 = DMS; R^6 = H, H; R^{11} = O	6.5
R^3 = DMS; R^6 = H, α–OH; R^{11} = H, β-OH	3.9
R^3 = OH; R^6 = H, α-DMS; R^{11} = H, β-OH	2.7
R^3 = DMS; R^6 = H, α–DMS; R^{11} = H, β-OH	5.4
R^3 = DMS; R^6 = H, α–DMS; R^{11} = O	10.9

Cucurbitanes

Hemsleya is a genus of Cucurbitaceae that is widely distributed in China, used in traditional Chinese medicine, and an extensively studied source of cucurbitane derivatives. The antibacterial activities of 23,24-dihydrocucurbitacin F (hemslecin A) and its 25-acetate (hemslecin B) were demonstrated more than two decades ago, and the effectiveness of the former was demonstrated in clinical trials (Nie and Chen 1986). Hemslecins A and B, now isolated from the tubers of *Hemsleya pengxianensis* L. T. Shen et W. J. Chang var. *jinfushanensis* have been shown to inhibit syncytia formation in C8166 cells induced by HIV-1_{IIIB} with $EC_{50} = 3.09$ and 2.53 μg/ml, respectively, inhibit p24 antigen production in acutely infected C8166 and MT2 cells, and also inhibit cell-to-cell fusion of C8166 cells with chronically infected H9/HIV-1_{IIIB} cells. Hemslecin A was severalfold more potent in all these assays. The hemlecins had no effect on viral protease and reverse transcriptase (Tian et al. 2008).

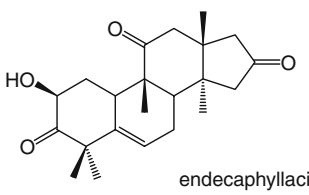


hemslecin A: R = H

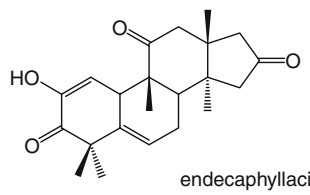
hemslecin B: R = Ac

Two new octanorcucurbitacins and six previously described cucurbitacins, isolated from the tubers of *Hemsleya endecaphylla* C. Y. Wu, were tested for the inhibition of syncytia formation in HIV-1-infected C8166 cells. All compounds were at least moderately active, but cucurbitacin B showed very potent anti-HIV-1 activity (EC_{50} = 0.09 μg/ml) with a selectivity index of 16.7 and the somewhat less potent 23, 24-dihydrocucurbitacin D and cucurbitacin I (EC_{50} = 0.13 and 0.70 μg/ml, respectively) were more selective (SI = 20.6 and 50.0, respectively) (Chen et al. 2008a).

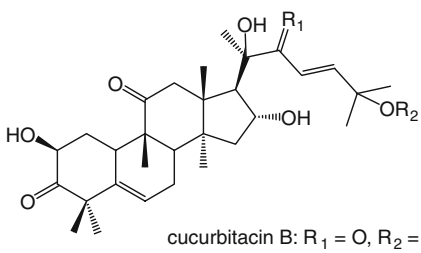
Momordica charantia L. (Cucurbitaceae) is widely distributed in tropical and subtropical Africa and Asia, its fruit (ku gua, karela, bitter melon, bitter gourd, African cucumber or balsam pear) is a popular vegetable in China, India and Pakistan, and is well documented as a source of drugs that improve glucose tolerance. This plant is known to contain more than 50 cucurbitacins and cucurbitane glycosides. Two recent studies on the roots and aerial parts of this plant led to the isolation and structure elucidation of 19 new members of this structural class, kuguacins A–S. Of these, only kuguacins C, E, Q and S showed moderate to strong activities in preventing cell death in HIV-1-infected C8166 cells (EC_{50} values of 8.45, 25.62, 7.2, and 3.7 μg/ml, respectively). Kuguacins C and E exerted minimal cytotoxicity against uninfected cells (IC_{50} > 200 μg/ml), pointing to selectivity indices >23.68 and 7.81, respectively, while the corresponding indices of kuguacins Q and S were 7.6 and 13.3, respectively. Six previously known cucurbitacins were also tested,



endecaphyllacin A

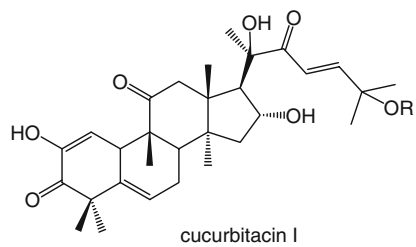


endecaphyllacin B

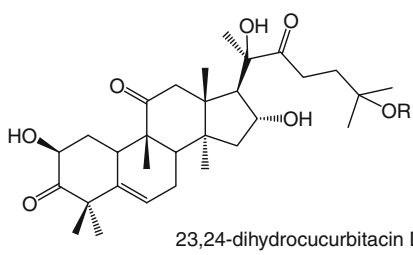


cucurbitacin B: R_1 = O, R_2 = Ac

22-deoxocucurbitacin D: R_1 = H_2, R_2 = H

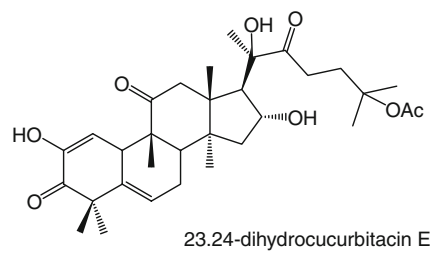


cucurbitacin I



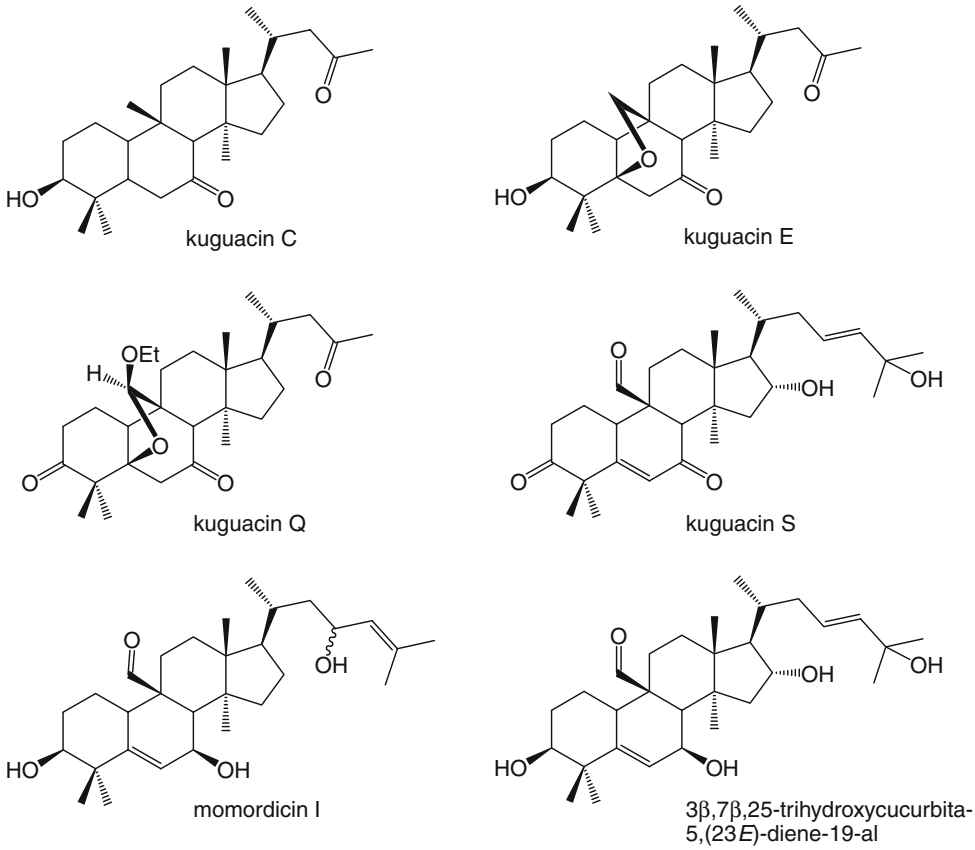
23,24-dihydrocucurbitacin D: R = H

23,24-dihydrocucurbitacin B: R = Ac



23.24-dihydrocucurbitacin E

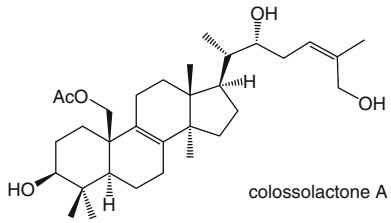
of which 3β,7β,25-trihydroxycucurbita-5,(23*E*)-diene-19-al and momordicine I showed promising activities, with EC_{50} values of 5.67 and 5.37 μg/ml, respectively, but also with low selectivity (Chen et al. 2008b, 2009).



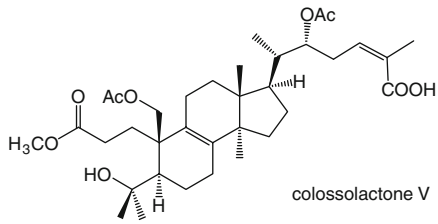
Some of these results have been briefly reviewed by Lee et al. (2009).

Lanostanes

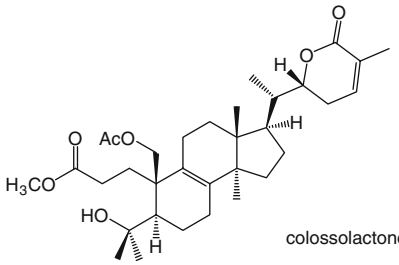
Several lanostane triterpenes named colossolactones have been isolated from the fruiting bodies of the white rot (wood-decaying) fungus *Ganoderma colossum* (Fr.) C. F. Baker (Ganodermataceae) (Kleinwächter et al. 2001; El Dine et al. 2008). A number of these were tested for inhibition of HIV-1 protease, and several showed IC_{50} values in the 5–39 μg/ml range, with colossolactone G, schisanlactone A, and colossolactone V exhibiting values below 10 μg/ml (El Dine et al. 2008). An $IC_{50} = 20$ mg/ml had been reported earlier for schisanlactone A (Sun et al. 2006b).



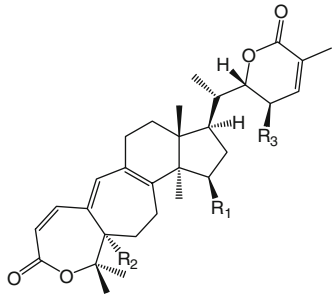
colossolactone A



colossolactone V



colossolactone VII

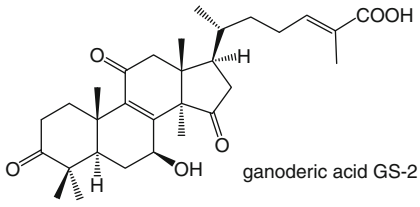


colossolactone VIII: R_1 = OAc, R_2 = H, R_3 = OH

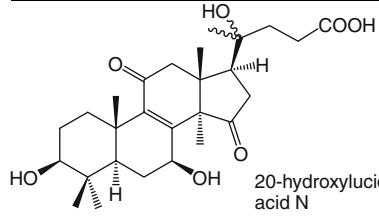
schisanlactone A: $R_1 = R_2 = R_3$ = H

colossolactone G: R_1 = OAc, R_2 = OH, R_3 = H

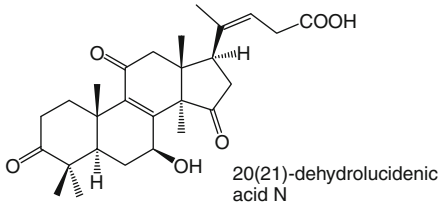
Five new and six previously known lanostane-type triterpenoids were isolated from the fruiting body of *Ganoderma sinense* and tested for inhibition of HIV-1 protease (Sato et al. 2009). Of these, the new ganoderic acid GS-2, and the previously described 20-hydroxylucidenic acid N, 20(21)-dehydrolucidenic acid N, and ganoderiol F, were active in the 20–40 μM range:



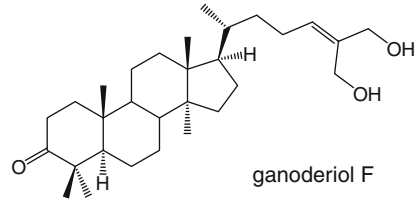
ganoderic acid GS-2



20-hydroxylucidenic acid N



20(21)-dehydrolucidenic acid N



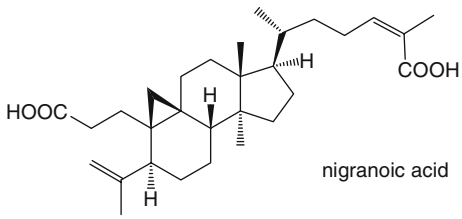
ganoderiol F

Nortriterpenoids of the Schisandraceae

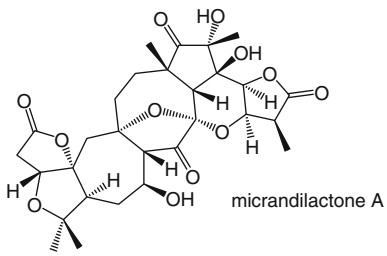
The Schisandraceae constitute a small "primitive" angiosperm family with only two or three genera: *Schisandra* (25 species), *Kadsura* (22 species), and *Illicium* (42 species), the latter placed by some in the separate family Illiciaceae. Most of the *Schisandra* species are used in traditional Chinese medicine, for a

wide variety of ailments and conditions, and many of these plants have been studied from a chemical viewpoint, including some bioassays, mainly by Chinese investigators. Since the isolation of schisanlactone A from an unnamed *Schisandra* species almost three decades ago (Liu et al. 1983), these medicinal herbs have yielded a very considerable number of unusual nortriterpenoids, several of which have documented anti-HIV-1 activity.

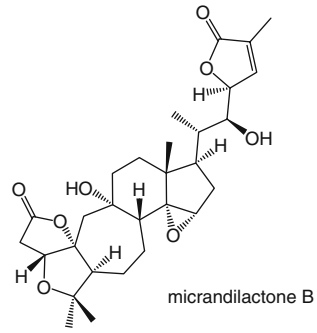
The earliest example of these HIV-active compounds, nigranoic acid, from the stems of *Schisandra sphaerandra* Stapf. was shown to inhibit the viral reverse transcriptase with $IC_{50} = 74.1$ μg/ml (Sun et al. 1996).



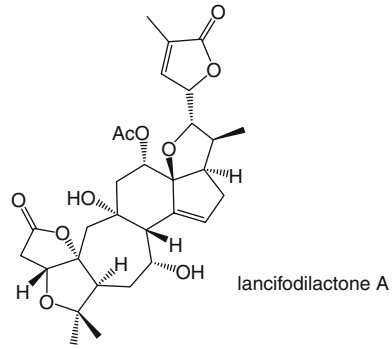
In 2003 the structure of the highly oxygenated nortriterpenoid micrandilactone A with a norcycloartane-derived skeleton and an unusual eight-membered ring, isolated from the stems and leaves of *Schisandra micrantha* A. C. Smith, was described (Li et al. 2003b).



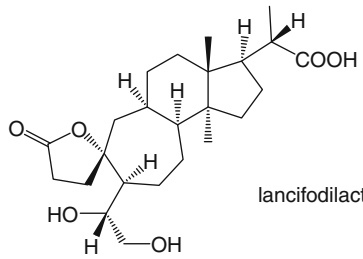
Shortly thereafter its analogues micrandilactones B and C were isolated from this plant. Micrandilactone C exhibited an EC_{50} value of 7.71 μg/ml (SI > 25.94) against HIV-1 with minimal cytotoxicity, and this relatively potent anti-HIV-1 activity and its unique structural features and potency suggested it as a promising lead for therapeutic development (Li et al. 2005). On the other hand, micrandilactone B proved to be a weak inhibitor of HIV-1-induced syncytium formation, with $EC_{50} > 50$ μg/ml (Huang et al. 2007a). The absolute configuration of micrandilactone B has been determined (Huang et al. 2007a), and in this review all the compounds with analogous structures are assumed to have the same stereochemistry in the conserved moieties.



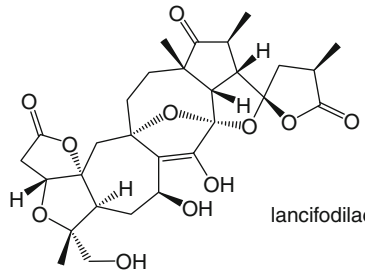
Lancifodilactone A was isolated from a 70% acetone extract of the stems and leaves of the Chinese medicinal plant *Schisandra lancifolia* (Rehd. et Wils) A. C. Smith in 2003, but it does not seem to have been assayed against HIV-1 (Li et al. 2003a).



This work was followed by the isolation of lancifodilactones B–E, structurally related to the micrandilactones (Li et al. 2004a). Subsequently, lancifodilactone F was isolated and shown to exert minimal cytotoxicity against C8166 cells ($CC_{50} > 200$ μg/ml) while showing moderate anti-HIV activity with $EC_{50} = 20.69 \pm 3.31$ μg/ml and a possibly low selectivity index (>6.62). (Xiao et al. 2005a). Lancifodilactone G, with a spirocyclic moiety, was also isolated from this plant. It exerted minimal cytotoxicity against C8166 cells ($CC_{50} > 200$ μg/ml) and showed weak anti-HIV activity with $EC_{50} = 95.47 \pm 14.19$ μg/ml (Xiao et al. 2005b).



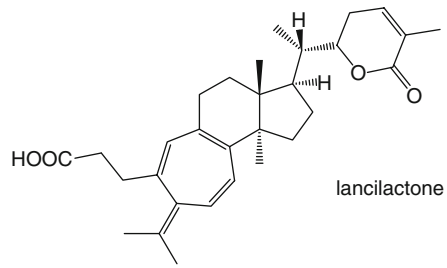
lancifodilactone F



lancifodilactone G

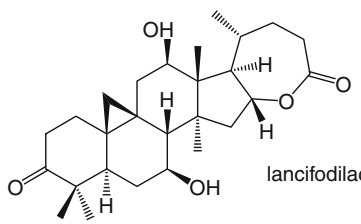
The isolation of six additional new nortriterpenoids, lancifodilactones I–N, closely related to lancifodilactone G, as well as nine known ones, was described a year later. All the new compounds were tested for anti-HIV-1 activity exhibiting EC_{50} values against C8166 cells in the 77–100 μg/ml range and low cytotoxicity (Xiao et al. 2006d). Structurally similar compounds (henridilactones A–D) were isolated from the leaves and stems of *Schisandra henryi* var. *yunnanensis* A. C. Smith, but apparently were not assayed against HIV-1 or for cytotoxicity (Li et al. 2004b).

The less profoundly modified trinorcycloartane triterpenoid lancifodilactone H, and the A ring-secocycloartane triterpenoid lancifoic acid A, as well as the previously known nigranoic acid, were isolated later from the same *S. lancifolia* extract. These three exhibited moderate anti-HIV-1 activity (cytopathic effect in HIV-1-infected C8166 cells: EC_{50} = 16.6, 16.2, 10.3 μg/ml; CC_{50}—same cell line—greater than 200, 104.9, 88.0 μg/ml) (Xiao et al. 2006c).

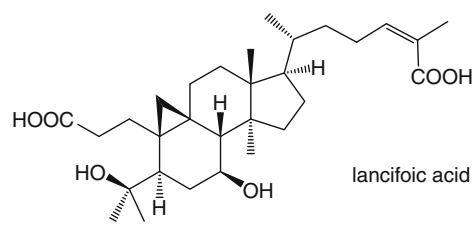


lancilactone C

Kadsura heteroclita (Roxb.) Craib (=*Schisandra crassifolia* Pierre ex Finet et Gagnep.) was studied quite recently, leading to the isolation of sixteen compounds from an acetone extract of the stems, mainly dibenzocyclooctadiene lignans, but including the triterpenoid named longipedlactone J (Pu et al. 2008). This compound possesses a novel skeleton found originally in leaves and stems of *K. longipedunculata* Finet et Gagnep., but the previously described longipedlactones A–I were only examined



lancifodilactone H

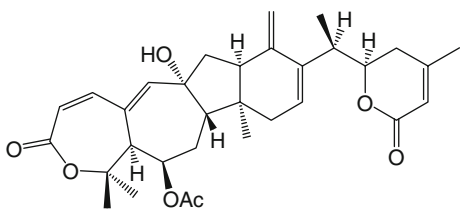


lancifoic acid

These results are generally disappointing when compared with the activity of lancilactone C, from an ether extract of the related plant *Kadsura lancilimba* How. (cytopathic effect in H9 cells: EC_{50} = 1.4 μg/ml; CC_{50} > 100 μg/ml) (Chen et al. 1999).

for cytotoxicity (Pu et al. 2006). Longipedlactone J exhibited moderate cytotoxicity (against C8166 cells) and inhibition of the cytopathic effects of HIV-1 with CC_{50} = 7.3 μg/ml and EC_{50} = 3.8 μg/ml, respectively, while the most active and selective compounds

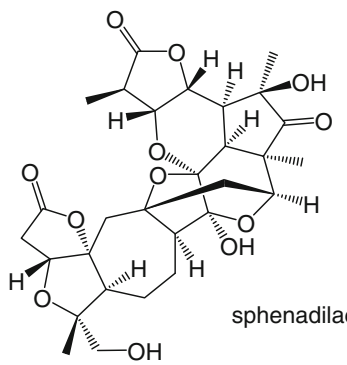
proved to be the flavonoids taxifolin and quercetin (Pu et al. 2008).



longipedlactone J

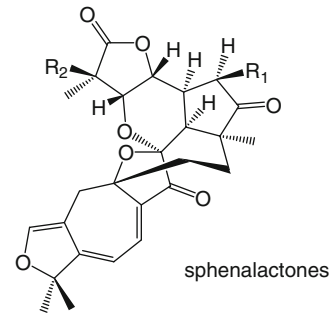
Longipedlactones A, B, C, F, and H, with the same α, β, χ, δ unsaturated moiety conjugated with the seven-membered lactone as in longipedlactone J, exhibited fairly potent cytotoxicity against the A549, HT-29, and K562 cell lines, but apparently were not tested for anti-HIV-1 activity (Pu et al. 2006). The cytotoxicity of these compounds could well be associated with low therapeutic indices, making them relatively uninteresting as HIV drugs.

Two highly oxidized nortriterpenoids with a novel skeleton, sphenadilactones A and B, were isolated from leaves and stems of *Schisandra sphenanthera* Rehd. et Wils. Both compounds were tested for their cytotoxicities against K562, A549, and HT-29, and found to be inactive at 100 μg/ml. Sphenadilactone A was also shown to have very weak anti-HIV-1 activity, with EC_{50} = 137.0 μg/ml (Xiao et al. 2006b).



sphenadilactone A

Sphenalactones A–D are additional representatives of this class of nortriterpenoids, showing anti-HIV-1 activity in the EC_{50} range 35.5–89.2 μg/ml (Xiao et al. 2007b).



sphenalactones

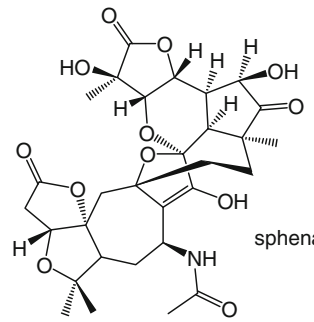
A: R_1 = OH, R_2 = H

B: R_1 = H, R_2 = OH

C: R_1 = OH, R_2 = OH

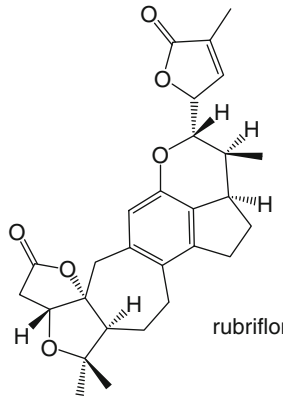
D: R_1 = H, R_2 = H

Sphenadilactone C, with a skeleton related to the sphenalactones, and sphenasin A, structurally similar to the trinorcycloartane lancifodilactone H, together with four known dibenzocyclooctadiene lignans, were subsequently isolated from *S. sphenanthera*. Sphenadilactone C, which features a partial enol moiety and an acetamide group in its structure, exhibited weak anti-HIV-1 activity with EC_{50} = 29.5 μg/ml and a therapeutic index of 6.68. The four lignans were more potent than sphenadilactone C and marginally more selective (Xiao et al. 2008).

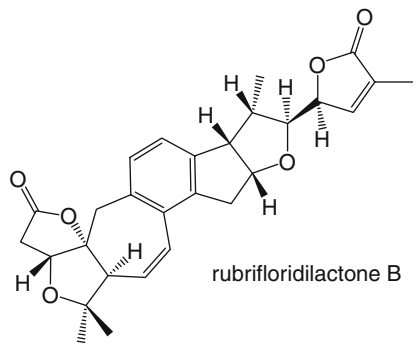


sphenadilactone C

Rubriflordilactones A and B, two novel highly unsaturated rearranged bisnortriterpenoids, were isolated from leaves and stems of *S. rubriflora*. Neither compound was cytotoxic toward K562 cells at 200 μg/ml. Rubriflordilactone B exhibited an EC_{50} value of 9.75 μg/ml in an assay determining the inhibition of HIV-1-induced syncytium formation in C8166 cells, while rubriflordilactone A was somewhat less potent. Both showed 60–80% protective activity against HIV-1-induced lysis of MT-4 cells at 40 μg/ml (Xiao et al. 2006a).



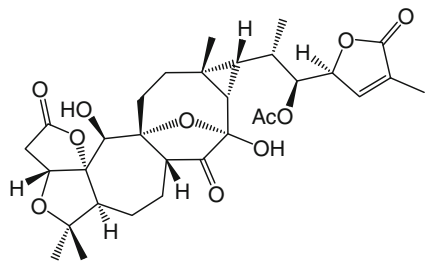
rubrifloridilactone A



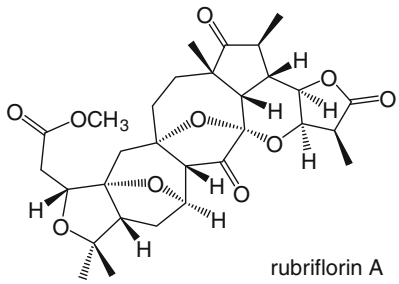
rubrifloridilactone B

The same plant yielded rubriflorins A–C, together with the related micranthidilactone A, lancifolidilactones C and D, henridilactones A and B, and micranthidilactone G. The rubriflorins differ from the previously recorded compounds of this class by having an opened A ring. The cytopathic activities of the new compounds in HIV-1-infected C8166 cells were determined as (EC_{50} values) 10.0, 16.2, and 81.3 μg/ml, respectively, but the two more potent analogues were also found to be more cytotoxic, with CC_{50} values of 89.1 and 100.3 μg/ml (Xiao et al. 2007a).

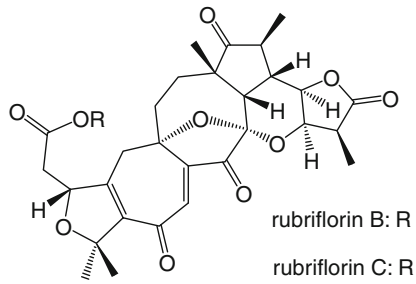
showed anti-HIV-1 activity with EC_{50} = 13.81 μg/ml, while the corresponding value for schindilactone A was >50 μg/ml (Huang et al. 2007a).



pre-schisanartanin



rubriflorin A

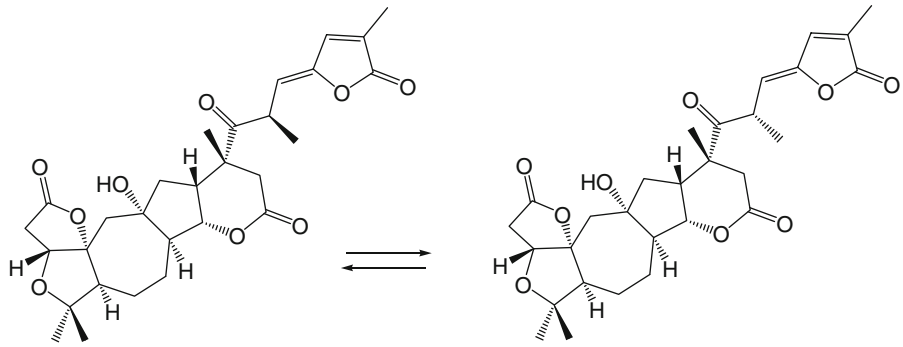


rubriflorin B: R = CH_3

rubriflorin C: R = C_2H_5

Extraction of *Schisandra chinensis* (Turcz.) Baill. (known in traditional Chinese medicine and cooking as "wu wei zi") allowed the isolation and structure elucidation of two additional compounds belonging to this group of nortriterpenoids, named pre-schisanartanin and schindilactone A, the former with an unprecedented carbon skeleton. Pre-schisanartanin

This plant also afforded two nortriterpenoids that constitute a spontaneously interconverting diastereomeric pair, designated as schintrilactones A and B. These compounds inhibited HIV-1-induced syncytium formation with EC_{50} = 17.9 and 36.2 μg/ml, respectively (Huang et al. 2007b).



Concluding remarks

An outstanding feature of recent research is the abundance of papers on the structure elucidation and anti-HIV-1 activity of highly oxygenated, rearranged, nortriterpenoids. Although this activity has been demonstrated in many cases, it is rather disappointingly low compared with that of the more classical lupanes, oleananes and ursanes and, in particular, some of their hemisynthetic derivatives. The complex structures of these triterpenoids, generally isolated from plants belonging to the traditionally appreciated Schisandraceae, pose very attractive synthetic challenges, but at the same time might be an obstacle to the rapid development of compounds that could become clinically useful.

In this sense, less profoundly modified derivatives of the tetracyclic terpenoid families, i.e. lanostanes, cucurbitanes, cycloartanes, dammaranes, ergostanes, etc., appear to be more promising. The anti-HIV-1-active natural products belonging to these types should be examined more closely in an attempt to discover structural patterns associated with this activity and, hopefully, its specific mechanisms. Unfortunately, the different screens used make it very risky to compare results from different laboratories and develop anything resembling structure–activity relationships. Nevertheless, there seems to be a trend toward higher anti-HIV activity in compounds with two or more oxygenated functions on ring A and the biogenetically related A-seco derivatives. Thus, the possibility of oxidizing, opening or even doing away with ring A of these skeletons should be considered, and also perhaps exploring the synthesis of analogues derived from steroids, making use of the extensively studied chemistry of these substances. In the next few years the numerous opportunities offered by these almost unexploited chemical types should open up an abundance of avenues of research, hopefully leading to new families of compounds exhibiting useful antiviral activities and possibly revealing novel targets for the therapy of HIV infection.

Acknowledgments This work was supported by FONDECYT grant 1080174 and ICM grant P05-001-F.

References

Aiken C, Chen C-H (2005) Betulinic acid derivatives as HIV-1 antivirals. Trends Mol Med 11:31–36

Asres K, Seyoum A, Veeresham C, Bucar F, Gibbons S (2005) Naturally derived anti-HIV agents. Phytother Res 19:557–581

Baglin I, Mitaine-Offer A-C, Nour M, Tan K, Cavé C, Lacaille-Dubois M-A (2003) A review of natural and modified betulinic, ursolic and echinocystic acid derivatives as potential antitumor and anti-HIV agents. Mini Rev Med Chem 3:525–539

Chen K, Shi Q, Kashiwada Y, Zhang D-C, Hu C-Q, Jin J-Q, Nozaki H, Kilkuskie RE, Tramontano E, Cheng Y-C, McPhail DR, McPhail AT, Lee K-H (1992) Anti-AIDS agents. 6. Salaspermic acid, an anti-HIV principle from *Tripterygium wilfordii*, and the structure-activity correlation with its related compounds. J Nat Prod 55:340–346

Chen D-F, Zhang S-X, Wang H-K, Zhang S-Y, Sun Q-Z, Cosentino LM, Lee K-H (1999) Novel anti-HIV lancilactone C and related triterpenes from *Kadsura lancilimba*. J Nat Prod 62:94–97

Chen J-C, Zhang G-H, Zhang Z-Q, Qiu M-H, Zheng Y-T, Yang L-M, Yu K-B (2008a) Octanorcucurbitane and cucurbitane triterpenoids from the tubers of *Hemsleya endecaphylla* with HIV-1 inhibitory activity. J Nat Prod 71:153–155

Chen J-C, Tian R, Qiu M-H, Lu L, Zheng Y-T, Zhang Z-Q (2008b) Trinorcucurbitane and cucurbitane triterpenoids

from the roots of *Momordica charantia*. Phytochemistry 69:1043–1048

Chen J-C, Liu W-Q, Lu L, Qiu M-H, Zheng Y-T, Yang L-M, Zhang X-M, Zhou L, Li Z-R (2009) Kuguacins F-S, cucurbitane triterpenoids from *Momordica charantia*. Phytochemistry 70:133–140

Cos P, Maes L, Vlietinck A, Pieters L (2008) Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection—an update (1998–2007). Planta Med 74:1323–1337

El Dine RS, El Halawany AM, Ma C-M, Hattori M (2008) Anti-HIV-1 protease activity of lanostane triterpenes from the vietnamese mushroom *Ganoderma colossum*. J Nat Prod 71:1022–1026

Fujioka T, Kashiwada Y, Kilkuskie RE, Cosentino LM, Ballas LM, Jiang JB, Janzen WP, Chen IS, Lee K-H (1994) Anti-AIDS agents. 11. Betulinic acid and platanic acid as anti-HIV principles from *Syzygium claviflorum* and the anti-HIV activity of structurally related triterpenoids. J Nat Prod 57:243–247

Hashimoto F, Kashiwada Y, Cosentino LM, Chen C-H, Garrett PE, Lee K-H (1997) Anti-AIDS agents–XXVII. Synthesis and anti-HIV activity of betulinic acid and dihydrobetulinic acid derivatives. Bioorg Med Chem 5:2133–2143

Ho J-C, Chen C-M, Row L-C (2007) Oleanane-type triterpenes from the flowers, pith, leaves, and fruit of *Tetrapanax papyriferus*. Phytochemistry 68:631–635

Huang L, Yu D, Ho P, Lee K-H, Chen C-H (2007a) Synthesis and anti-HIV activity of bi-functional triterpene derivatives. Lett Drug Des Discov 4:471–478

Huang S-X, Li R-T, Liu J-P, Lu Y, Chang Y, Lei C, Xiao W-L, Yang L-B, Zheng Q-T, Sun H-D (2007b) Isolation and characterization of biogenetically related highly oxygenated nortriterpenoids from *Schisandra chinensis*. Org Lett 9:2079–2082

Huang S-X, Yang J, Huang H, Li L-M, Xiao W-L, Li R-T, Sun H-D (2007c) Structural characterization of schintrilactone, a new class of nortriterpenoids from *Schisandra chinensis*. Org Lett 9:4175–4178

Ito M, Nakashima H, Baba M, Pauwels R, De Clercq E, Shigeta S, Yamamoto N (1987) Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. Antiviral Res 7:127–137

Ito J, Chang FR, Wang HK, Park YK, Ikegaki M, Kilgore N, Lee KH (2001) Anti-AIDS agents. 48. (1) Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from Brazilian propolis. J Nat Prod 64:1279–1281

Kleinwächter P, Anh N, Kiet TT, Schlegel B, Dahse HM, Härtl A, Gräfe U (2001) Colossolactones, new triterpene metabolites from a Vietnamese mushroom *Ganoderma colossum*. J Nat Prod 64:236–239

Kondratenko RM, Baltina LA, Mustafina SR, Visil'eva EV, Pompei R, Deidda D, Plyasunova OA, Pokrovskii AG, Tolstikov GA (2004) The synthesis and antiviral activity of glycyrrhizic acid conjugates with alpha-D-glucosamine and some glycosylamines. Bioorg Khim 30:308–315

Kondratenko RM, Baltina LA, Baltina LA Jr, Plyasunova OA, Pokrovskii AG, Tolstikov GA (2009) Synthesis of new hetero- and carbocyclic aromatic amides of glycyrrhizic acid as potential anti-HIV agents. Pharmac Chem J 43:383–388

Kuo R-Y, Qian K, Morris-Natschke SL, Lee K-H (2009) Plant-derived triterpenoids and analogues as antitumor and anti-HIV agents. Nat Prod Rep 26:1321–1344

Lee J-S, Miyashiro H, Nakamura N, Hattori M (2008) Two new triterpenes from the rhizome of *Dryopteris crassirhizoma*, and inhibitory activities of its constituents on human immunodeficiency virus-1 protease. Chem Pharm Bull (Tokyo) 56:711–714

Lee SY, Eom SH, Kim YK, Park NI, Park SU (2009) Cucurbitane-type triterpenoids in *Momordica charantia* Linn. J Med Plants Res 3:1264–1269

Li R-T, Li S-H, Zhao Q-S, Lin Z-W, Sun H-D, Lu Y, Wang C, Zheng Q-T (2003a) Lancifodilactone A, a novel bisnortriterpenoid from *Schisandra lancifolia*. Tetrahedron Lett 44:3531–3534

Li R-T, Zhao Q-S, Li S-H, Han Q-B, Sun H-D, Lu Y, Zhang L-L, Zheng Q-T (2003b) Micrandilactone A: a novel triterpene from *Schisandra micrantha*. Org Lett 5:1023–1026

Li R-T, Xiang W, Li S-H, Lin Z-W, Sun H-D (2004a) Lancifodilactones B-E, new nortriterpenes from *Schisandra lancifolia*. J Nat Prod 67:94–97

Li R-T, Shen Y, Xiang W, Sun H-D (2004b) Four novel nortriterpenoids isolated from *Schisandra henryi* var. yunnanensis. Eur J Org Chem 2004:807–811

Li R-T, Han Q-B, Zheng Y-T, Wang R-R, Yang L-M, Lu Y, Sang S-Q, Zheng Q-T, Zhao Q-S, Sun H-D (2005) Structure and anti-HIV activity of micrandilactones B and C, new nortriterpenoids possessing a unique skeleton from *Schisandra micrantha*. Chem Commun (Camb) 2005: 2936–2938

Li W-H, Zhang X-M, Tian R-R, Zheng Y-T, Zhao W-M, Qiu M-H (2007) A new anti-HIV lupane acid from *Gleditsia sinensis* Lam. J Asian Nat Prod Res 9:551–555

Liu J-S, Huang M-F, Arnold GF, Arnold E, Clardy J, Ayer WA (1983) Schisanlactone A, a new type of triterpenoid from a *Schisandra sp*. Tetrahedron Lett 24:2351–2354

Ma C-M, Nakamura N, Hattori M, Kawahata T, Otake T (2002) Inhibitory effects of triterpene-azidothymidine conjugates on proliferation of human immunodeficiency virus type 1 protease. Chem Pharm Bull 50:877–880

Nakashima H, Okubo K, Honda Y, Tamura T, Matsuda S, Yamamoto N (1989) Inhibitory effect of glycosides like saponin from soybean on the infectivity of HIV in vitro. AIDS 3:655–658

Nie R-L, Chen Z-L (1986) The research history and present status on the chemical components of the genus *Hemsleya* (Cucurbitaceae). Acta Bot Yunnan 8:115–124

Park J-C, Kim S-C, Choi M-R, Song S-H, Yoo E-J, Kim S-H, Miyashiro H, Hattori M (2005) Anti-HIV protease activity from rosa family plant extracts and rosamultin from *Rosa rugosa*. J Med Food 8:107–109

Parra A, Rivas F, López PE, García-Granados A, Martínez A, Albericio F, Márquez N, Muñoz E (2009) Solution and solid-phase synthesis and anti-HIV activity of maslinic acid derivatives containing amino acids and peptides. Bioorg Med Chem 17:1139–1145

Pu J-X, Li R-T, Xiao W-L, Gong N-B, Huang S-X, Lu Y, Zheng Q-T, Lou L-G, Sun H-D (2006) Longipedlactones

A-I, nine novel triterpene dilactones possessing a unique skeleton from *Kadsura longipedunculata*. Tetrahedron 62:6073–6081

Pu J-X, Yang L-M, Xiao W-L, Li R-T, Lei C, Gao X-M, Huang S-X, Li S-H, Zheng Y-T, Huang H, Sun H-D (2008) Compounds from *Kadsura heteroclita* and related anti-HIV activity. Phytochemistry 69:1266–1272

Qian K, Yu D, Chen CH, Huang L, Morris-Natschke SL, Nitz TJ, Salzwedel K, Reddick M, Allaway GP, Lee KH (2009) Anti-AIDS agents. 78. Design, synthesis, metabolic stability assessment, and antiviral evaluation of novel betulinic acid derivatives as potent anti-human immunodeficiency virus (HIV) agents. J Med Chem 52: 3248–3258

Reutrakul V, Chanakul W, Pohmakotr M, Jaipetch T, Yoosook C, Kasisit J, Napaswat C, Santisuk T, Prabpai S, Konsaeree P, Tuchinda P (2006) Anti-HIV-1 constituents from leaves and twigs of *Cratoxylum arborescens*. Planta Med 72:1433–1435

Sato N, Zhang Q, Ma C-M, Hattori M (2009) Anti-human immunodeficiency virus-1 protease activity of new lanostane-type triterpenoids from *Ganoderma sinense*. Chem Pharm Bull 57:1076–1080

Sun H-D, Qiu S-X, Lin L-Z, Wang Z-Y, Lin Z-W, Pengsuparp T, Pezzuto JM, Fong HHS, Cordell GA, Farnsworth NR (1996) Nigranoic acid, a triterpenoid from *Schisandra sphaerandra* that inhibits HIV-1 reverse transcriptase. J Nat Prod 59:525–527

Sun I-C, Kashiwada Y, Morris-Natschke SL, Lee K-H (2003) Plant-derived terpenoids and analogues as anti-HIV agents. Curr Top Med Chem 3:155–169

Sun H, Fang W-S, Wang W-Z, Hu C (2006a) Structure-activity relationships of oleanane- and ursane-type triterpenoids. Bot Stud 47:339–368

Sun Q-Z, Chen D-F, Ding P-L, Ma C-M, Kakuda H, Nakamura N, Hattori M (2006b) Three new lignans, longipedunins A-C, from *Kadsura longipedunculata* and their inhibitory activity against HIV-1 protease. Chem Pharm Bull 54:129–132

Tanaka R, Kinouchi Y, Tokuda H, Nishino H, Matsunaga S (2000) Bioactive terpenoids from the stem bark of *Picea glehni*. Planta Med 66:630–634

Tanaka R, Tsujii H, Yamada T, Kajimoto T, Amano F, Hasegawa J, Hamashima Y, Node M, Katoh K, Takebe Y (2009) Novel 3α-methoxyserrat-14-en-21β-ol (PJ-1) and 3β-methoxyserrat-14-en-21β-ol (PJ-2)-curcumin, kojic acid, quercetin, and baicalein conjugates as HIV agents. Bioorg Med Chem 17:5238–5246

Tian R-R, Chen J-C, Zhang G-H, Qiu M-H, Wang Y-H, Du L, Shen X, Liu N-F, Zheng Y-T (2008) Anti-HIV-1 activities of hemslecins A and B. Chin J Nat Med 6:214–218

Tuchinda P, Kornsakulkarn J, Pohmakotr M, Kongsaeree P, Prabpai S, Yoosook C, Kasisit J, Napaswad C, Sophasan S, Reutrakul V (2008) Dichapetalin-type triterpenoids and lignans from the aerial parts of *Phyllanthus acutissima*. J Nat Prod 71:655–663

Wei Y, Ma C-M, Chen D-Y, Hattori M (2008) Anti-HIV-1 protease triterpenoids from *Stauntonia obovatifoliola* Hayata subsp. intermedia. Phytochemistry 69:1875–1879

Wei Y, Ma C-M, Hattori M (2009a) Anti-HIV protease triterpenoids from the acid hydrolysate of *Panax ginseng*. Phytochem Lett 2:63–66

Wei Y, Ma C-M, Hattori M (2009b) Synthesis of dammarane-type triterpene derivatives and their ability to inhibit HIV and HCV proteases. Bioorg Med Chem 17:3003–3010

Wei Y, Ma C-M, Hattori M (2009c) Synthesis and evaluation of A-seco type triterpenoids for anti-HIV protease activity. Eur J Med Chem 44:4112–4120

World Health Organization (2009) http://www.who.int/hiv/data/en/

Xiao W-L, Li R-T, Li S-H, Li X-L, Sun H-D, Zheng Y-T, Wang R-R, Lu Y, Wang C, Zheng Q-T (2005a) Lancifodilactone F: a novel nortriterpenoid possessing a unique skeleton from *Schisandra lancifolia* and its anti-HIV activity. Org Lett 7:1263–1266 (Erratum in: Org Lett 8: 801)

Xiao W-L, Zhu H-J, Shen Y-H, Li R-T, Li S-H, Sun H-D, Zheng Y-T, Wang R-R, Lu Y, Wang C, Zheng Q-T (2005b) Lancifodilactone G: a unique nortriterpenoid isolated from *Schisandra lancifolia* and its anti-HIV activity. Org Lett 7:2145–2148 (Erratum in: Org Lett 8:801)

Xiao W-L, Yang L-M, Gong N-B, Wu L, Wang R-R, Pu J-X, Li X-L, Huang S-X, Zheng Y-T, Li R-T, Lu Y, Zheng Q-T, Sun H-D (2006a) Rubriflordilactones A and B, two novel bisnortriterpenoids from *Schisandra rubriflora* and their biological activities. Org Lett 8:991–994

Xiao W-L, Pu J-X, Chang Y, Li X-L, Huang S-X, Yang L-M, Li L-M, Lu Y, Zheng Y-T, Li R-T, Zheng Q-T, Sun H-D (2006b) Sphenadilactones A and B, two novel nortriterpenoids from *Schisandra sphenanthera*. Org Lett 8:1475–1478 (Erratum in: Org Lett 8:4669)

Xiao W-L, Tian R-R, Pu J-X, Li X, Wu L, Lu Y, Li S-H, Li R-T, Zheng Y-T, Zheng Q-T, Sun H-D (2006c) Triterpenoids from *Schisandra lancifolia* with anti-HIV-1 activity. J Nat Prod 69:277–279

Xiao W-L, Huang S-X, Zhang L, Tian R-R, Wu L, Li X-L, Pu J-X, Zheng Y-T, Lu Y, Li R-T, Zheng Q-T, Sun H-D (2006d) Nortriterpenoids from *Schisandra lancifolia*. J Nat Prod 69:650–653

Xiao W-L, Li X-L, Wang R-R, Yang L-M, Li L-M, Huang S-X, Pu J-X, Zheng Y-T, Li R-T, Sun H-D (2007a) Triterpenoids from *Schisandra rubriflora*. J Nat Prod 70:1056–1059

Xiao W-L, Yang L-M, Li L-M, Pu J-X, Huang S-X, Weng Z-Y, Lei C, Liu J-P, Wang R-R, Zheng Y-T, Li R-T, Sun H-D (2007b) Sphenalactones A-D, a new class of highly oxygenated trinortriterpenoids from *Schisandra sphenanthera*. Tetrahedron Lett 48:5543–5546

Xiao W-L, Huang S-X, Wang R-R, Zhong J-L, Gao X-M, He F, Pu J-X, Lu Y, Zheng Y-T, Zheng Q-T, Sun H-D (2008) Nortriterpenoids and lignans from *Schisandra sphenanthera*. Phytochemistry 69:2862–2866

Xu H-X, Zeng F-Q, Wan M, Sim K-Y (1996) Anti-HIV triterpene acids from *Geum japonicum*. J Nat Prod 59:643–645

Yamaguchi C, Wanibuchi H, Kakehashi A, Tanaka R, Fukushima S (2008) Chemopreventive effects of a serratane-type triterpenoid, 3α-methoxyserrat-14-en-21β-ol (PJ-1), against rat lung carcinogenesis. Food Chem Toxicol 46:1882–1888

Yu D, Sakurai Y, Chen C-H, Chang F-R, Huang L, Kashiwada Y, Lee K-H (2006) Anti-AIDS agents. 69. Moronic acid

and other triterpene derivatives as novel potent anti-HIV agents. J Med Chem 49:5462–5469

Yu D, Morris-Natschke SL, Lee K-H (2007a) New developments in natural products-based anti-AIDS research. Med Res Rev 27:108–132

Yu Y-B, Miyashiro H, Nakamura N, Hattori M, Park J-C (2007b) Effects of triterpenoids and flavonoids isolated from *Alnus firma* on HIV-1 viral enzymes. Arch Pharm Res 30:820–826