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Recent advances in the discovery and development of plant-derived natural products and their analogs as anti-HIV agents*

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Abstract: Chemotherapy for AIDS has progressed steadily in the past decade with the advent of five HIV reverse transcriptase inhibitors, protease inhibitors, and combination of the two. However, new, effective, and less toxic chemotherapeutic agents are still needed. Plants, particularly anti-infective or immunomodulating Chinese herbal medicines, can serve as sources of new active leads to be further developed as anti-AIDS drug candidates. This report describes current new lead discovery and analog development in the authors' laboratory. Several compound classes (for example, DCK and betulinic acid derivatives) are extremely active against HIV replication, with activity rivaling or surpassing that of AZT. Continued progress is anticipated in the discovery of new leads and in the development of these agents as potential anti-AIDS drug candidates.

Acquired immunodeficiency syndrome (AIDS), a degenerative disease of the immune and central nervous systems, is an enormous world-wide health threat. No cure has been found, and research is aimed at developing chemotherapy against the causative agent, human immunodeficiency virus (HIV). The first clinically approved drugs were 2′,3′-dideoxynucleosides, including 3′-azido-3′-deoxythymidine (AZT, Zidovudine), dideoxyinosine (ddI, Didanosine), dideoxycytidine (ddC, Zalcitabine), 2′,3′-dideoxy-3′-thiacytidine (3TC, Lamivudine), and 2′,3′-didehydro-3′-deoxythymidine (d4T, Stavudine). These compounds act at an early stage in viral replication by inhibiting HIV reverse transcriptase (RT). AZT has been the recommended initial therapeutic agent; however, limitations include adverse side-effects, such as bone marrow suppression, anemia, and peripheral neuropathy, and decreased sensitivity due to the rapid emergence of drug resistant mutant virus. Lately, HIV protease inhibitors, including saquinavir (Inverase), ritonavir (Norvir), and indinavir (Crixivan), have also been introduced. However, the most exciting chemotherapeutic development to date is combination therapy. Blood levels of virus dropped below the detectable level (< 200 copies of viral RNA per mL of plasma) in patients treated with a triple-drug cocktail of two nucleoside inhibitors (e.g. ddC and 3TC) and one protease inhibitor. However, the long-term sustainable effects are unknown, increased toxicities can occur due to drug-drug interactions in a person receiving multiple drug therapies, and drug resistance is likely to become an escalating problem due to both use and misuse of drug therapy.

New, effective and less toxic anti-AIDS agents are still needed. Thus, we are continuing our long-term screening of plant extracts, particularly anti-infective or immunomodulating Chinese herbal medicines, and the structural modification of discovered leads. An earlier review was published in 1994 [2]. The latest research developments from our laboratory are summarized below.

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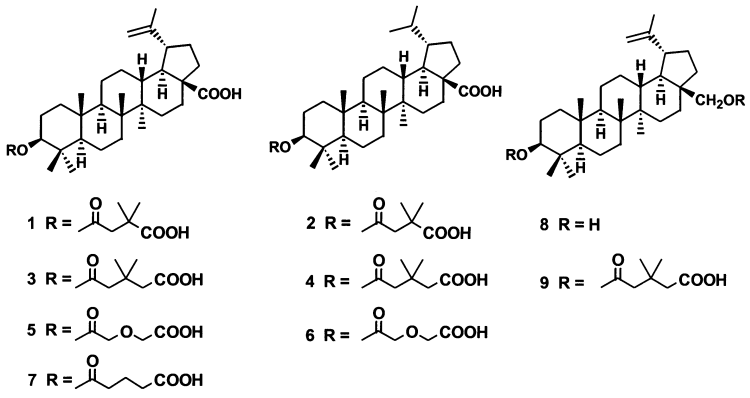
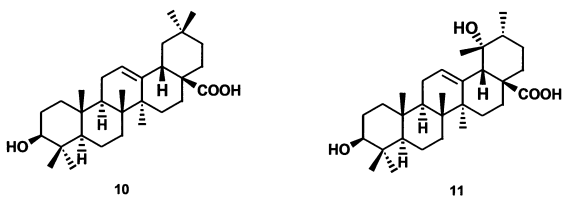
TRITERPENE DERIVATIVES

Betulinic acid, a triterpene isolated from *Syzigium claviflorum*, was active against HIV replication in H9 lymphocytes with an EC_{50} of 1.4 μM and a therapeutic index (TI) of 9.3. The related platanic acid has an acetyl rather than an isopropenyl side chain and is less active with a slightly higher EC_{50} value (6.5 μM). Esterification of the C-3 hydroxyl of betulinic acid and its dihydro derivative led to 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (**DSB**) (**1**) and -dihydro-betulinic acid (**DSD**) (**2**), which were more potent ($EC_{50} < 3.5 \times 10^{-4}$ μM) and had better therapeutic indexes (> 20 000 and > 14 000, respectively) than AZT ($EC_{50} = 0.15$ μM, TI = 12 500). Other 3-acylated compounds including 3-*O*-(3′,3′dimethylglutaryl)-betulinic acid (**3**) and -dihydrobetulinic acid (**4**), 3-*O*-diglycolyl-betulinic acid (**5**) and -dihydrobetulinic acid (**6**), and 3-*O*-glutaryl betulinic acid (**7**) were also potent inhibitors of HIV replication with EC_{50} values from 0.04 to 2.3×10^{-3} μM and TI values from 292 to 2344. Activity was also found in a monocyte cell line and in peripheral blood mononuclear cells. In preliminary mechanism of action studies with selected compounds, syncytia formation was completely inhibited at concentrations of 20–40 μM, but HIV RT was not affected. One compound inhibited HIV-induced membrane fusion with an IC_{100} value of 20 μg/mL, although it was less active than other betulinic acid derivatives in the HIV replication assay ($EC_{50} = 2.7$ μM, TI = 6.7) [3,4].

Betulin (**8**) is less potent (≈ 16-fold) than betulinic acid; however, adding 3′,3′-dimethylglutaryl esters at both the C-3 and C-28 hydroxy groups gave an extremely potent compound (**9**) with EC_{50} and TI values of 6.6×10^{-4} μM and 21 515, respectively. These values were better than those of both the unesterified parent compound and betulinic acid. The diesterified betulin was more active than both C-3 esterified betulinic acid and C-28 monoesterified betulin. This order of activity confirms the important of two acyl side chains for maximal potency. Also, compounds that lacked a C-3 acyl group (i.e. 3-keto and 2,3-dehydro derivatives) were less active. 3′,3′-Dimethyl substitution was favored relative to 3′-ethyl-3′-methyl or 3′-tetramethylene substitution. If the isopropylidene group was reduced to an isopropyl group, activity also dropped (Scheme 1) [1,5].

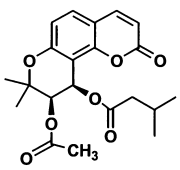


Two other natural triterpenoids from *Rosa woodisii*, *Hyptis capitata*, and other species, oleanolic acid (**10**) and pomolic acid (**11**), had EC_{50} values of 1.7 and 1.4 μg/mL, respectively, in an HIV-1 replication assay in H9 lymphocytes. Related natural and synthetic analogs were also assayed for anti-HIV activity. Improved activity was achieved by the following modifications of oleanolic acid: converting the 3-OH to a 3′,3′-dimethylsuccinate ester, oxidizing the 3-OH to a ketone, and making the potassium salt of the carboxylic acid (Scheme 2) [6].



COUMARIN DERIVATIVES

Suksdorfin [(3′R,4′R)-3′-acetoxy-4′-(isovaleryloxy)-3′,4′-dihydroseselin] (**12**) is a pyranocoumarin derivative isolated from *Lomatium suksdorfii* and *Angelica morii* [7]. Its EC_{50} for inhibiting HIV replication in H9 lymphocytes was 1.3 μM with a TI > 40. In early studies of related coumarins, activity was affected by changing the type and stereochemistry of the 3′ and 4′ acyl groups and by changing the 4′-ester to an azide or amide moiety. The most promising lead compound was [(3′R,4′R)-3′,4′-di-*O*-(−)-camphonyl-(+)-*cis*-khellactone] (**DCK**) (**13**), which showed extremely potent inhibitory activity ($EC_{50} = 0.0004$ μM) and a remarkable TI (136 719). In comparison, these values for AZT in the same assay are 0.15 μM and 12 500; thus, DCK is 366-fold more potent and 11-fold more selective than AZT. DCK was also active in a monocytic cell line and in PHA-stimulated peripheral blood mononuclear cells (PBMCs). This new compound is optically active and was prepared together with the (−)-*cis* diastereoisomer and two *trans* diastereoisomers [8]. Oxidation of seselin with osmium tetroxide gave the *cis*-diols, and reaction with *m*-chloroperbenzoic acid followed by saponification gave the *trans*-diols. Acylation with optically active (−)-camphanoyl chloride allowed separation of all four diastereoisomers. The three diastereoisomers [(−)-*cis*, (+)-*trans*, and (−)-*trans*] were at least ten thousand times less active than DCK [9]. Based on these results, we were prompted to develop a highly selective asymmetric synthesis of DCK. Catalytic asymmetric dihydroxylation of seselin with potassium osmate dihydrate using the enantioselective ligand: hydroquinone 2,5-diphenyl-4,6-pyrimidinediyl diether $(DHQ)_2$-Pyr, resulted in 93% stereoselectivity [10]. Mechanism of action studies on this unique cournarin lead and its dihydroseselin derivatives showed that they do not inactivate virus, block viral entry, alter cellular metabolism, regulate (enhance or suppress) integrated HIV in chronically infected cells, or block viral budding. These compounds do suppress viral replication in HIV-infected T-cells and monocyte/macrophages (Scheme 3).



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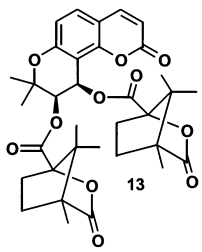

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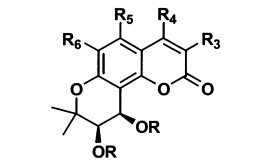
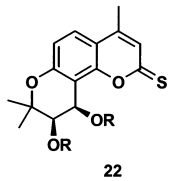
New series of DCK derivatives variously substituted or modified in the coumarin nucleus are under investigation; preliminary results confirm and extend the extremely high anti-HIV activity and selectivity of this compound class. The 3-, 4-, and 5-methyl DCKs (**14–16**) all were more potent than DCK and AZT against HIV-1 replication in H9 lymphocytes with EC_{50} and T1 values of $< 4.23 \times 10^{-7}$ μM and $> 3.72 \times 10^{8}$, respectively. The 6-methyl derivative (**17**) was significantly less active with values ol 0.15 μM and 220, respectively. All four compounds were synthesized asymmetrically from different starting materials [11]. Similar results were found when the substituent was a methoxy group [12]. The order of activity from lowest to highest was 6-methoxy DCK (**18**) ($EC_{50} = 24.5$ μM, TI > 9.68), 3-methoxy DCK (**19**) ($EC_{50} = 0.006$ μM, TI > 25 500), 4-methoxy DCK (**20**) ($EC_{50} = 0.00276$ μM, TI > 51 000), and 5-methoxy DCK (**21**) ($EC_{50} = 0.000138$ μM, TI > 402 632). The latter compound was more potent than the unsubstituted DCK. Changing the ketone carbonyl of DCK to a sulfur also resulted in potent compounds [13], although not as potent as DCK. 4-Methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-khelthiolactone (**22**) was extremely potent with EC_{50} and TI values of 0.00718 μM and > 21 300, respectively. The unsubstituted thiolactone and the 4-propyl and 4-benzyl derivatives were less active in that order (Scheme 4).

OTHER NATURAL PRODUCTS AND THEIR DERIVATIVES

Podophyllotoxin is a potent inhibitor of microtubule assembly, while etoposide and other synthetic analogs are inhibitors of DNA topoisomerase II. To further investigate the range of biological activities



14 $R_3 = CH_3$ 18 $R_6 = OCH_3$
15 $R_4 = CH_3$ 19 $R_3 = OCH_3$
16 $R_5 = CH_3$ 20 $R_4 = OCH_3$
17 $R_6 = CH_3$ 21 $R_5 = OCH_3$



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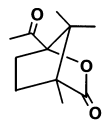
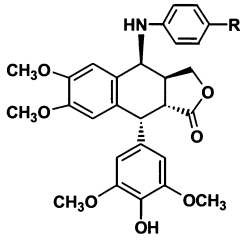
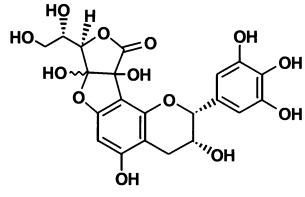
R = 

found in this compound class, we synthesized and evaluated modified podophyllotoxin derivatives as inhibitors of HIV replication. Podophyllotoxin itself had an EC_{50} value of 0.03 μM and a TI of 42.7. The most active compounds (**23**, **24**) ($EC_{50} < 0.001$ μM, TI > 120) had substituted anilino groups at C-4, the methylenedioxy A ring opened and methylated, and a phenolic OH at the 4′-position (Scheme 5) [14].



23 $R = NO_2$
24 R = F



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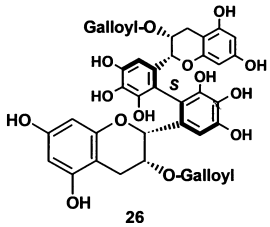
Of 38 polyphenols isolated from various teas, 8-C-ascorbyl-(–)-epigallocatechin (**25**) and theasinensin-D (**26**) were the most potent inhibitors of HIV replication with EC_{50} values of 4 and 8 μg/mL and TI values of 9.5 and 5, respectively [15].

The known shinjulactone C (**27**), isolated from *Ailanthus altissima,* was the most active quassinoid ($EC_{50} = 10.6$ μM, TI > 25) among a series of 18 quassinoid glycosides and nine quassinoids (Scheme 6) [16].



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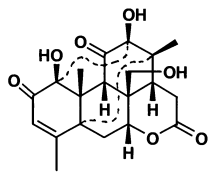
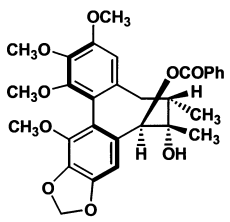


27

Seven of 12 known lignans isolated from *Kadsura interior* inhibited HIV replication; gomisin-G (**28**) was the most potent ($EC_{50} = 0.006$ μg/mL; TI = 600). Schisantherin-D (**29**), kadsuranin (**30**), and schisandrin-C (**31**) were also quite active: the respective EC_{50} and TI values are 0.5, 0.8 and 1.2 μg/mL and 110, 56, and 33.3 (Scheme 7). In the cyclooctane ring, the position and substitution of hydroxy groups were important to enhanced anti-HIV activity. These results prompted a study of related synthetic isomeric biphenyls with bismethylenedioxy, dimethoxy, dimethoxycarbonyl, and bromine substituents. The relative position and types of substituents on the phenolic hydroxy groups of both the natural lignans and the synthetic biphenyls rather than the number of bromines were of primary importance. The 2- (or 2′)-methoxycarbonyl and 4- (or 4′)-methoxy groups composed an essential anti-HIV structural feature. Bromination at the 3- (or 3′)-position could then greatly enhance activity as seen by the EC_{50} and TI values (0.52 μg/mL, > 190 and 0.23 μg/mL, > 480) for 3-bromo- (**32**) and 3,3′-dibromo-4, 4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl (**33**) [17,18].

A new kaurane type diterpene lactone, neotripterifordin (**34**), was isolated from the roots of



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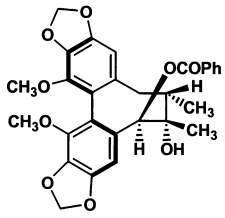

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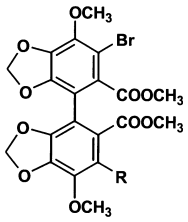
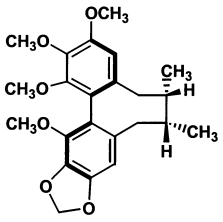


32 R = H
33 R = Br



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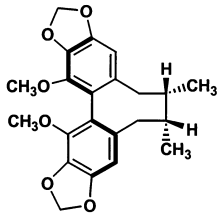

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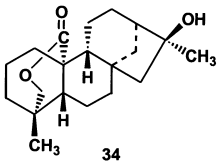
Tripterygium wilfiordii, a poisonous liana found in southern China. It showed potent anti-HIV replication activity in H9 lymphocytes with an EC_{50} of 25 nM and a TI of 125 [19]. Another kaurene diterpene, 16β,17-dihydroxy-*ent*-kauran-19-oic acid (**35**), was identified as an anti-HIV agent ($EC_{50} = 0.8$ μg/mL, TI > 5) from *Annona squamosa* (Scheme 8) [20].



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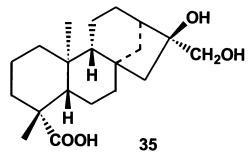

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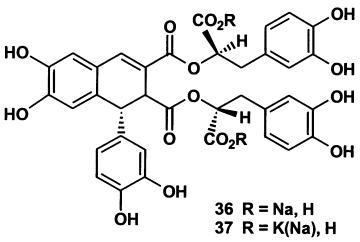
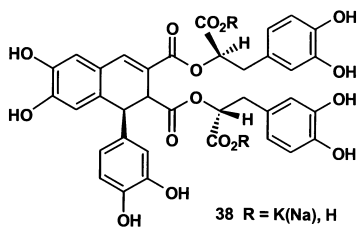
Three monosodium and monopotassium salts of isomeric caffeic acid tetramers (**36–38**) were isolated and characterized from *Arnebia euchroma,* which is used in Chinese prescriptions for anti-inflammatory, anti-pyretic, anti-bacterial and anti-hepatitis purposes. The anti-HIV EC_{50} values were 2.8, 4.0 and 1.5 μg/mL, respectively, and the TI values were 19.6, 12.5, and 33.3. Acidic hydrolysis gave the free caffeic acid tetramers, which were less active than, but had similar toxicity to, the salt forms (Scheme 9) [21].



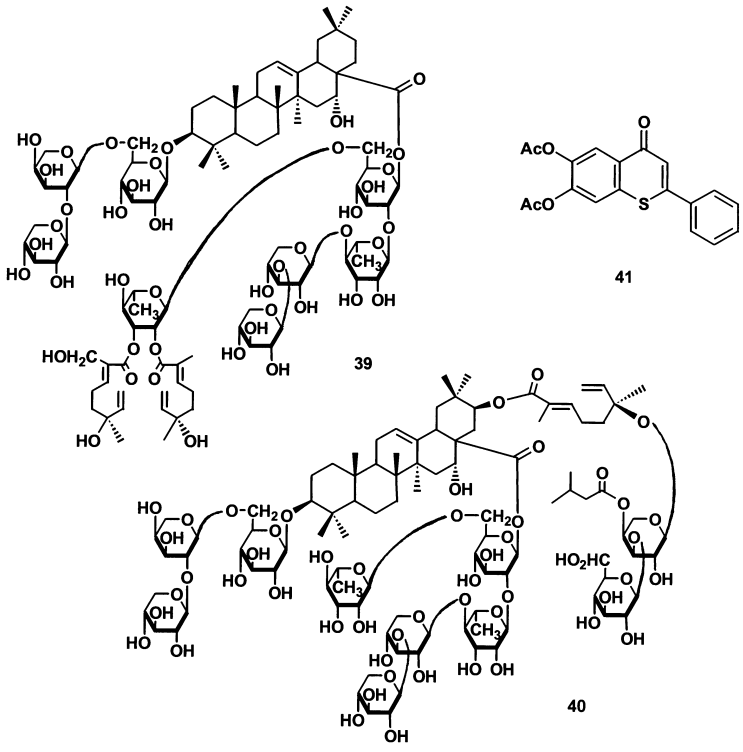
36 R = Na, H
37 R = K(Na), H



38 R = K(Na), H

Two known saponins, gleditsia saponin C (**39**) and gymnocladus saponin G (**40**), were isolated from fruits of the oriental plants *Gleditsia japonica* and *Gymnocladus chinensis, respectively.* These compounds inhibited HIV replication with EC_{50} values of 1.1 and 2.7 μM, respectively; however, their TI values were low (8.9 and 5.2). A unique monoterpenyl group was crucial for anti-HIV activity as the prosapogenins and derivatives without this group were inactive [22].

6,7-Diacetyl-2-phenylthiochromen-4-one (**41**, $EC_{50} = 0.65$ μg/mL, TI = 5) and related compounds were synthesized and evaluated as anti-HIV agents. Compound **41** can be regarded as an analog of apigenin-7-*O*-β-D-glucopyranoside and chrysin, which were isolated as anti-HIV flavonoids from *Kummerowia striata* and *Chrysanthemum morifolium,* respectively, by our laboratory [23].



CONCLUSION

In summary, both the coumarin derivative DCK and the betulinic acid derivatives DSB and DSD, as well as their related compounds, have exciting potential as anti-HIV chemotherapeutic agents. Patents for these compound classes have been awarded or are being reviewed. As noted, several compounds are extremely active against HIV replication, rivalling or surpassing the activity of AZT, a primary anti-HIV drug. Continued progress is anticipated in the development of these agents and the discovery of new leads.

ACKNOWLEDGEMENT

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