

REVIEW ARTICLE

Naturally Derived Anti-HIV Agents

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The urgent need for new anti-HIV/AIDS drugs is a global concern. In addition to obvious economical and commercial hurdles, HIV/AIDS patients are faced with multifarious difficulties associated with the currently approved anti-HIV drugs. Adverse effects, 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. This has driven many scientists to look for new anti-retrovirals with better efficacy, safety and affordability. As has always been the case in the search for cures, natural sources offer great promise. Several natural products, mostly of plant origin have been shown to possess promising activities that could assist in the prevention and/or amelioration of the disease. Many of these anti-HIV agents have other medicinal values as well, which afford them further prospective as novel leads for the development of new drugs that can deal with both the virus and the various disorders that characterize HIV/AIDS. The aim of this review is to report new discoveries and updates pertaining to anti-HIV natural products. In the review anti-HIV agents have been classified according to their chemical classes rather than their target in the HIV replicative cycle, which is the most frequently encountered approach. Perusal of the literature revealed that most of these promising naturally derived anti-HIV compounds are flavonoids, coumarins, terpenoids, alkaloids, polyphenols, polysaccharides or proteins. It is our strong conviction that the results and experiences with many of the anti-HIV natural products will inspire and motivate even more researchers to look for new leads from plants and other natural sources. Copyright © 2005 John Wiley & Sons, Ltd.

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INTRODUCTION

An increasing number of patients with HIV infection and/or AIDS cannot use the currently approved anti-HIV drugs, including the reverse transcriptase and protease inhibitors, due to the adverse effects and the emergence of drug resistance. Many antiviral compounds presently in clinical use have a narrow spectrum of activity and limited therapeutic usefulness. Those monitoring the spread of AIDS in many parts of the world know that the cost of treatment is one of the major problems in combatting the disease. In general, the price of antiretroviral drugs is exorbitant for the afflicted population in developing countries, which at present take the lion's share of the world population that is either living with HIV/AIDS or greatly vulnerable to it. For the past two decades, developing nations struggling to break free from poverty have been obliged to address this epidemic as its progress has significantly retarded their socio-economic development, which in turn has resulted in an even more accelerated spread of the disease. Thus, the search for new effective and safe as well as affordable anti-HIV agents is not merely an academic curiosity but rather a necessity. Natural

products are important sources of new drugs and leads besides tailored synthesis (Koprowski, 2002; Liu *et al.*, 2002; Martin and Ernst, 2003). The following is a review of natural products with anti-HIV activity. Attempts were made to cover as many reports as possible on compounds of plant origin that inhibit HIV giving special emphasis to new discoveries and updates. Although many natural products have been reported to exhibit anti-HIV activities to date, none of them are found in the list of conventional antiretroviral drugs. Many natural products are not followed up to see their actual usefulness in combatting the biggest enemy mankind has ever faced. Each new antiviral needs to be addressed not only for its inhibitory profile but its utility to the total HIV/AIDS chemotherapeutic package (Turpin, 2003). However, it is important to note that a number of promising anti-HIV natural products have made it to the clinical level and are anticipated to be available to patients very soon. In the following review, anti-HIV natural products have been classified according to their chemical classes rather than their target in the HIV replicative cycle, which is the most frequently encountered approach. The following natural products can be cited as promising anti-HIV agents of plant origin: baicalin (a flavonoid), calanolides (coumarins), betulinic acid (a triterpene), polycitone A (an alkaloid), lithospermic acid (a polyphenolic), sulphated polysaccharides, and cyanovirin-N, pokeweed antiviral protein and alpha-trichobitacin (proteins).

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FLAVONOIDS

Flavonoids and related polyphenols possess promising anti-HIV activity. A number of flavonoids inhibit reverse transcriptase (RT), induce interferons and inactivate viral protease (Havsteen, 2002), and down-regulate the expression of HIV co-receptors such as CCR2b, CCR3 and CCR5 (Nair *et al.*, 2002). However, the molecular mechanisms underlying the anti-HIV effects of flavonoids and polyphenolic compounds still need to be clearly elucidated (Nair *et al.*, 2002).

Flavonoid constituents of a proprietary grape seed extract which are predominantly flavans and proanthocyanidins significantly downregulated the expression of the HIV-1 entry co-receptors, CCR2b, CCR3 and CCR5 in normal peripheral blood mononuclear cells (PBMC) in a dose dependent manner (93% viability of PBMC

at 5 mg/mL). Analysis of the mechanisms underlying the anti-HIV-1 effects of grape seed extracts may help to identify promising natural products useful in the prevention and/or amelioration of HIV-1 infection. Grape proanthocyanidins have a potential value as an adjunct nutritional supplement, along with the existing conventional therapeutic regimens, in the treatment of HIV infection (Nair *et al.*, 2002).

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 (Ohtake *et al.*, 2004). Baicalin inhibits HIV-1 replication in PBMC in a dose dependent manner with IC_{50} values of 0.2–0.5 $\mu\text{g/mL}$ and a suitable safety profile; well tolerated up to 10 $\mu\text{g/mL}$ (Kitamura *et al.*, 1998; Li *et al.*, 1993). Baicalin also inhibited HIV-1 RT with an IC_{50} value of 2 $\mu\text{g/mL}$ and IC_{100} ~10 $\mu\text{g/mL}$, without

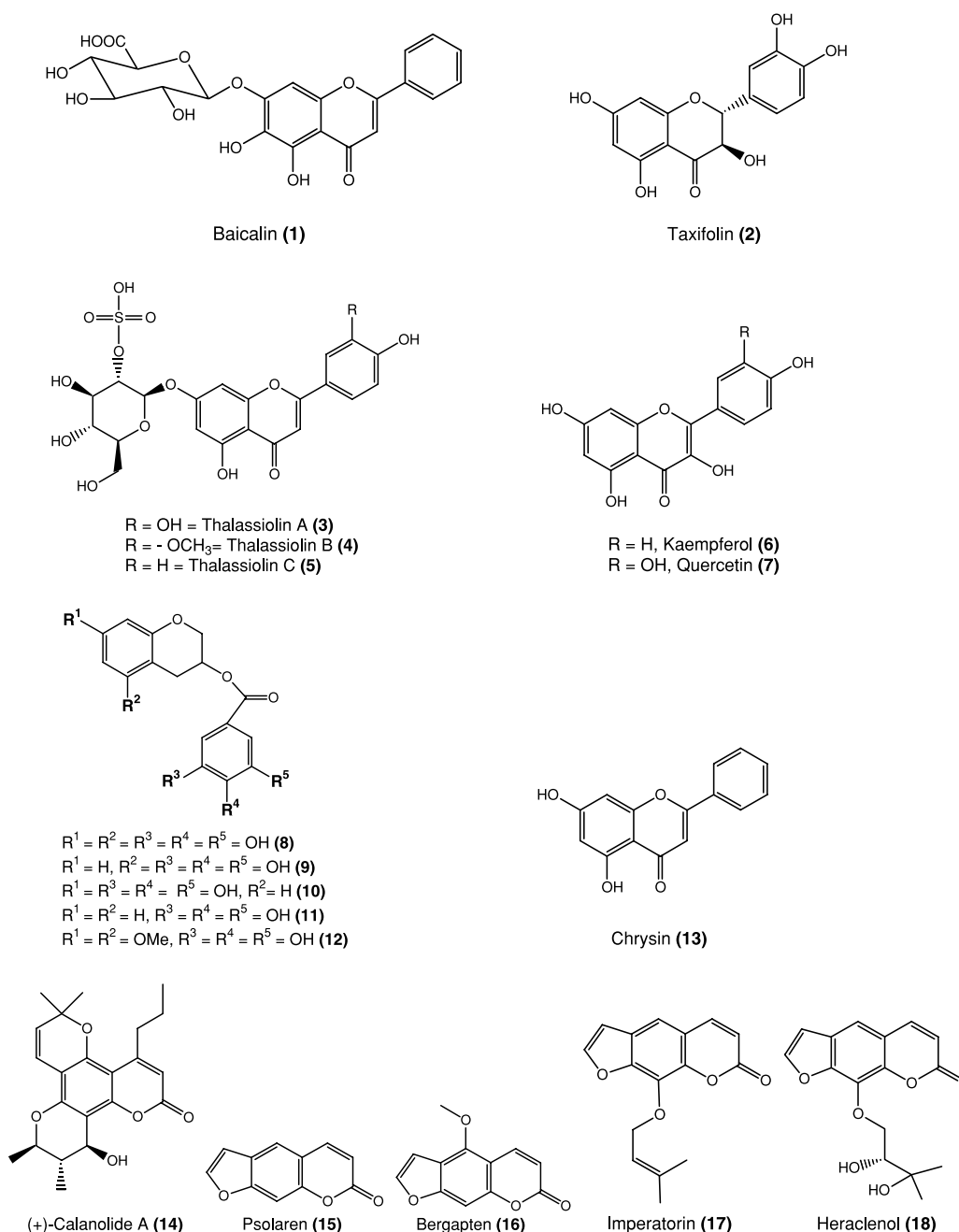


Figure 1. Structural formulae of representative anti-HIV natural products.

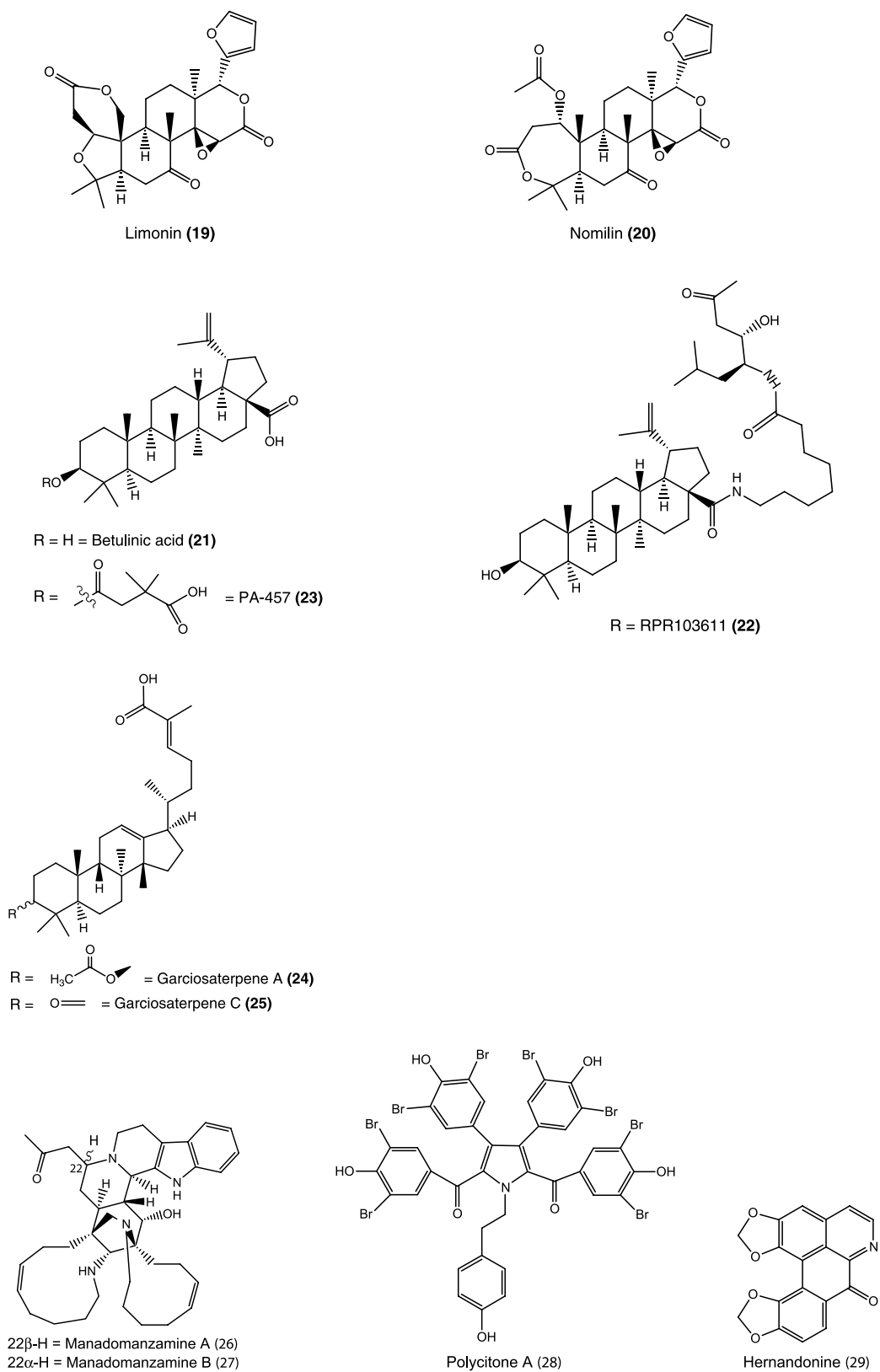


Figure 1. (Continued)

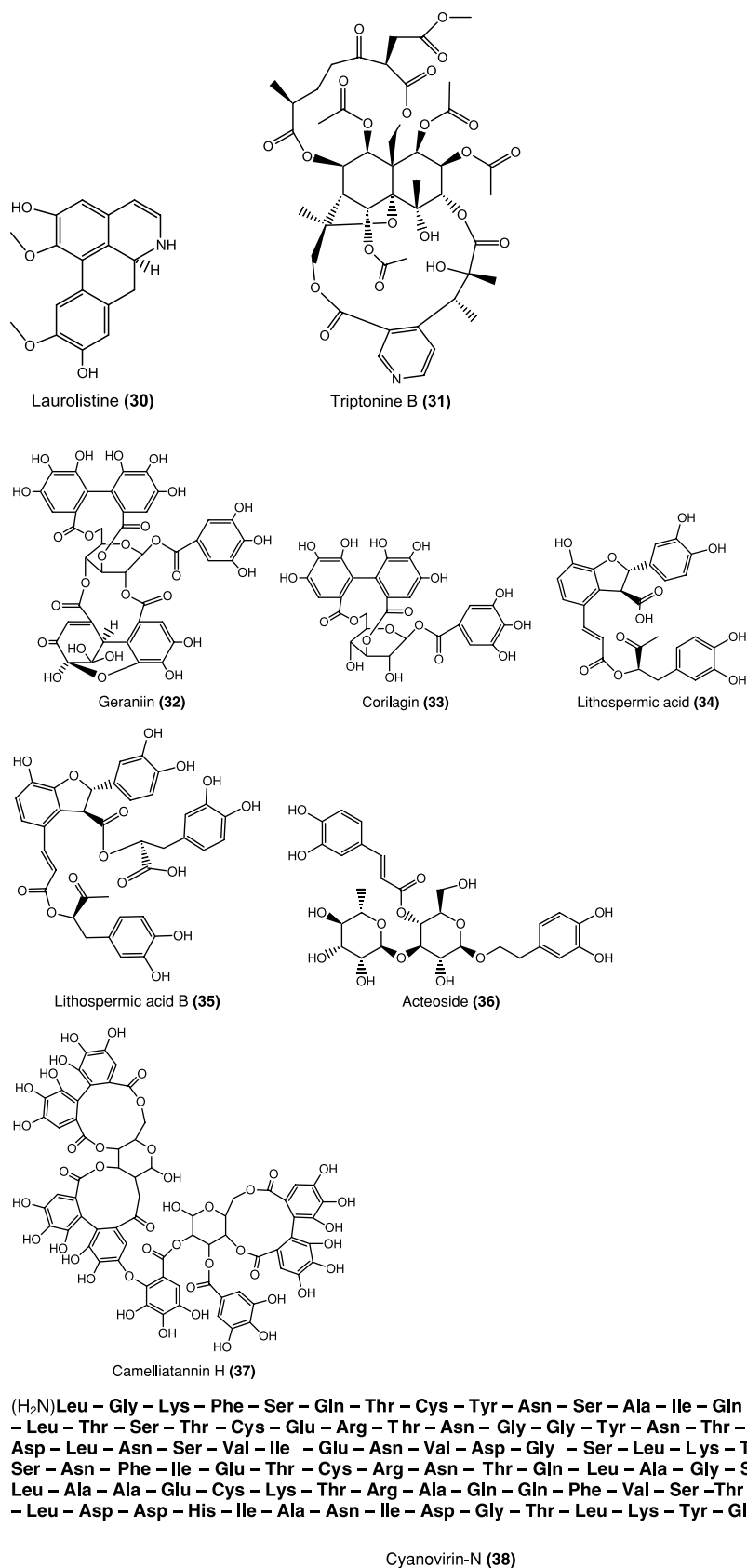


Figure 1. (Continued)

affecting the DNA polymerases α and γ but slightly inhibiting DNA polymerase β (Kitamura *et al.*, 1998). Baicalin can discriminate between double-stranded DNA and single-stranded DNA since it binds to DNA through intercalation (Sun *et al.*, 2004). This monoglycosylated flavonoid inhibited HIV-1 Env protein mediated fusion with both CD4/CXCR4 and CD4/CCR5T cells ($IC_{50} = 4 \mu M$). However, since it did not inhibit binding of HIV-1 gp120 to CD4 it may interact with HIV-1 Env domains repressing their interaction with chemokine co-receptors and block HIV-1 entry of target cells (Li *et al.*, 2000). Reports also indicate that baicalin selectively induces apoptosis in HIV-infected cells (Wu *et al.*, 1995) and human cancer cells (Ikezoe *et al.*, 2001; Ueda *et al.*, 2002). Thus baicalin and its analogues are potentially very useful for developing novel anti-HIV-1 agents.

Two flavonoids, 4,6-dihydroxy-2-methoxy-3-methyl-5(3'-hydroxy)-cinnamoylbenzaldehyde (a chalcone) and lawinal (a flavanone) isolated from *Desmos* spp. inhibited HIV replication with EC_{50} values of 0.022 and 2.30 $\mu g/mL$ and therapeutic indexes (TIs) of 489 and 45.2, respectively. The replacement of the C-2 methoxyl group with a hydroxyl abolished the anti-HIV property indicating the importance of the former for anti-HIV activity. 4,6-Dihydroxy-2-methoxy-3-methyl-5(3'-hydroxy)-cinnamoylbenzaldehyde appears to be an excellent lead for further anti-HIV drug development (Wu *et al.*, 2003a).

The flavonoid taxifolin (**2**) (also called dihydroquercetin) isolated from the stem-bark of *Juglans mandshurica* (Juglandaceae) showed a very potent inhibition of HIV-induced cytopathic activity against MT-4 cells with complete inhibitory concentration (IC_{100}) value of 25 $\mu g/mL$ and a maximum cytotoxic concentration (CC_{100}) value of above 100 $\mu g/mL$ (Min *et al.*, 2002). Mahmood *et al.* (1997) reported a decrease in the infectivity of virus incubated in the presence of the compound for 2 h at 37 °C. Although taxifolin was inactive in aborting syncytium formation, it is still believed to act at an early stage of virus infection since it failed to inhibit virus production from chronically infected H9 cells.

A series of HIV integrase inhibitors, thalassiolins A–C (**3–5**) were isolated from the Caribbean sea grass *Thalassia testudinum*. The thalassiolins contain unique functionality in the form of β -D-glucopyranosyl-2''-sulphate, a substituent that imparts increased potency against HIV-1 integrase, compared with the parent flavones namely, luteolin, chrysoeriol and apigenin, respectively. The thalassiolins are non-cytotoxic ($LD_{50} > 800 \mu M$, on MT-2 cells), water soluble and relatively easy to acquire. The most active of these molecules, thalassiolin A (**3**) inhibited terminal cleavage and Mg^{+2} dependent strand transfer reaction with IC_{50} values of 2.1 and 0.4–2 μM , respectively. It also inhibited *in vitro* HIV infection of MT-2 cells with an IC_{50} of 30 μM . Computational docking studies indicated a favourable binding mode at the catalytic core domain of HIV-1 integrase. Its binding modes overlap with the experimentally determined location of 5CITEP [1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)propenone]. The tetrazole ring of this inhibitor occupies the same binding position as the sulphated glucose of the thalassiolins, and the orientations of the keto-enol and chloroindole moieties of 5CITEP show similarities

with the benzopyranone binding position. The apparent similarities in binding of these structurally different molecules could aid in the future design of inhibitors binding to the integrase active site. However, taking into account the fact that many flavonoids and related compounds such as L-chicoric acid, block viral entry altogether, it is uncertain, at the present, whether the step affected by thalassiolin A during infection *in vivo* is indeed integration. Regardless of the actual target *in vivo*, thalassiolin A may nevertheless serve as a starting point for drug development (Rowley *et al.*, 2002).

A number of kaempferol and quercetin based flavonol glycosides from the leaves of *Thevetia peruviana* have exhibited an appreciable HIV-1 reverse transcriptase-associated RNA-dependent DNA polymerase (RDDP) inhibitory activity with IC_{50} values of 20–43 μM ; quercetin derivatives being more active than those of kaempferol. Moreover, the compounds bearing a feruloyl moiety exhibited higher activity than those bearing a sinapoyl moiety. Quercetin 3-O-[(6-O-feruloyl)- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranoside] and quercetin 3-O-[(6-O-sinapoyl)- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranoside] also inhibited HIV-1 integrase with IC_{50} values of 5 and 7 μM , respectively. With regard to integrase inhibitory activity, compounds possessing a feruloyl or sinapoyl group in the terminal glucose moiety showed more potent inhibitory activity than the unsubstituted ones. The aforementioned flavonols showed higher inhibitory activity than their aglycones, quercetin ($IC_{50} = 43$ and 15 μM , respectively) and kaempferol ($IC_{50} > 100$ and 40 μM , respectively) (Tewtrakul *et al.*, 2002). The tetrahydroxyflavonol kaempferol (**6**) (*Rosa damascena*) effectively reduced the maturation of infectious progeny virus apparently due to selective inhibition of the viral protease ($IC_{50} = 0.8 \mu M$, TI = 62.5 in H9 cells). On the other hand, the pentahydroxyflavonol quercetin (**7**) prevented binding of gp120 to CD4 ($IC_{50} = 10 \mu M$, TI = 10 in H9 cells) (Mahmood *et al.*, 1996). Furthermore, two related flavonol glucosides namely, (-)-4'-methylepigallocatechin-5-O- β -glucopyranoside and (+)-4'-methylepigallocatechin-3'-O- β -glucopyranoside isolated from the Sudanese medicinal plant *Maytenus senegalensis* exhibited 72.9% and 68.2% inhibition of HIV-1 protease, respectively, at a concentration of 100 μM (Hussein *et al.*, 1999).

(-)-Epigallocatechin-3-gallate (EGCG) which is approximately 50% of the total catechin content of green tea represents a potential low-cost inhibitor of HIV infection that could be associated with current anti-HIV therapy. Related compounds like (-)-epicatechin and (-)-epicatechin-3-O-gallate from *Detarium microcarpum* were reported to block HIV infection through an irreversible interaction with the glycoprotein gp120: IC_{50} 2 and 1 $\mu g/mL$, respectively, in C8166 cells and a CC_{50} value of $>100 \mu g/mL$ (Mahmood *et al.*, 1993). Nakane and Ono (1990) 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 0.01–0.02 $\mu g/mL$, whilst Fassina *et al.* (2002) recently reported EGCG to inhibit HIV-1 RT (of both LAI/IIIB and Bal) with doses of 25 and 50 μM giving nearly 100% inhibition. 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 (Nakane and Ono, 1990).

The mechanism by which EGCG interferes with viral infection is not yet clear, however, there are indications that it impinges on various steps of the HIV life cycle, in addition to its inhibitory effect on RT. EGCG destroyed virions by binding to the surface of the viral envelope and deforming of the phospholipids in a manner similar to the effect of polymixin B on bacterial membranes. It does not have an appreciable effect on virus-cell binding (only 20% at 100 μM). EGCG significantly inhibited in a dose-dependent manner, entry of the virus after adsorption in monocytoid cells (THP-1) and monocyte-derived macrophages (MDM) but not in H9 cells. It also inhibited viral production from chronically infected monocytoid cells (IIIB/THP-1) with an IC_{50} value of $\sim 20 \mu\text{M}$ but not in T-lymphoid cells (IIIB/H9, MN/H9). The ability of EGCG to decrease viral mRNA production in lipopolysaccharide (LPS)-activated chronically HIV-1-infected cells (IIIB/THP-1), but not in unstimulated or LPS-stimulated T-lymphoid cells (H9) suggests that its action could be related to the NF- κB pathway, which is activated by LPS-stimulation, and that EGCG may not have a strong and direct downregulatory effect on the HIV-1 promoter, which controls viral gene regulation. Similarly, the anti-protease activity of EGCG was observed only with monocytoid host cells at a concentration higher than 10 μM . Aside from obvious differences in their cell surface receptors, their responses to several stimulators, multi-drug resistance (MDR) pumps, and behaviour during viral infection between monocytes and T-cells, phagocytosis of the EGCG by monocytoid cells might also account for the above observation. Anti-HIV viral activity of EGCG may thus result from an interaction with several steps in the HIV-1 life cycle. Moreover, EGCG was not significantly cytotoxic; $\text{LD}_{50} = 174.8 \mu\text{M}$ for H9; 440.3 μM for THP-1 (Fassina *et al.*, 2002; Yamaguchi *et al.*, 2002).

A new class of HIV-1 RT inhibitors (**8–12**) obtained by the systematic structural simplification of epicatechin and epigallocatechin gallates is now recognized. Some of these compounds (**8–10**) 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 **11** and **12** for both wild-type and mutant enzyme. The last two compounds inhibit DNA-strand-transfer with up to 80-fold selectivity over polymerase activity. The presence of polar hydroxyl groups on 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. DNA-strand-transfer is rate-limiting in the overall process of reverse transcription and critical to recombination-associated mutation of the virus. Such specific DNA-strand-transfer inhibitors may thus have important therapeutic potential (Tillekeratne *et al.*, 2002).

The flavonoid chrysin (**13**) and benzothiofenenes have been shown to prevent HIV expression in latently and chronically infected cells (Critchfield *et al.*, 1996) through the inhibition of casein kinase II, a cellular protein that may regulate HIV-1 transcription by phosphorylating cellular proteins involved in the HIV-1 transcription transactivation process (Critchfield *et al.*, 1997). The mechanism of action of these compounds is independent of the nuclear factor κB -driven transcription pathway and they have demonstrated specificity toward inhibiting HIV-1 transcription (Butera *et al.*, 1995). This unorthodox approach is in contrast with the popular view that dormancy of HIV infected cells is a major obstacle to controlling or curing HIV-1 infection (Blankson *et al.*, 2002; Chun and Fauci, 1999). According to the latter view, breaking latency in HIV-infected cells could reduce the number of latently infected cells by causing them to be directly killed by the cytopathic action of the virus, to be recognized and destroyed by the immune system, or to express proteins that render them susceptible to targeted therapeutics such as immunotoxins (Bocklandt *et al.*, 2003). Chrysin has low oral bioavailability in humans (estimated to be 0.003%–0.02%), mainly due to extensive presystemic intestinal as well as hepatic metabolism and efflux of metabolites (chrysin glucuronide and chrysin sulphate) back into the intestine for hydrolysis and faecal elimination. Other flavonoids as well could possibly have a similar bioavailability profile (Walle *et al.*, 1999; Walle *et al.*, 2001). The usefulness of chrysin and similar HIV transcription inhibitors or latency inducers remains to be assessed.

A flavonoid glucuronide, apigenin 7-*O*- β -D-(4'-caffeyloyl)glucuronide isolated from the flowers of *Chrysanthemum morifolium*, showed strong HIV-1 integrase inhibitory activity ($\text{IC}_{50} = 7.2 \pm 3.4 \mu\text{g/mL}$) and anti-HIV activity in a cell culture assay ($\text{EC}_{50} = 41.86 \pm 1.43 \mu\text{g/mL}$) using HIV-1_{IIIB} infected MT-4 cells (Lee *et al.*, 2003). Similarly flemiphyllin, quercetin, euchretin M and formosanatin C isolated from the methanol extract of *Euchresta formosana* inhibited HIV replication in H9 lymphocyte cells (Lo *et al.*, 2003). Moreover, the flavonoids 6,8-diprenylaromadendrin, 6,8-diprenylkaempferol and lonchocarpol A obtained from *Monotes africanus* were reported to exhibit HIV-inhibitory activity in the XTT-based, whole-cell screen. It is worth noting that all the three flavonoids contain a 5,7-dihydroxy-6,8-diprenyl system in their A ring (Meragelman *et al.*, 2001).

The methanol and ethyl acetate extracts from a new chemotype of *Mentha longifolia* that grows in the Moroccan mountains significantly inhibited ($p < 0.01$) HIV-1BaL infection by approximately 40% and 55%, respectively. In addition, the ethyl acetate extract showed significant ($p < 0.008$) inhibitory activity (50% inhibition) against HIV-1 RT. Chemical analysis of these extracts suggests that flavonoids, mainly flavones may be the major inhibitors of HIV infection (Amzazi *et al.*, 2003).

COUMARINS

(+)-Calanolide A (**14**) 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. De Clercq (2000) has reviewed the anti-HIV activities of these compounds. (+)-Calanolide A has already been the subject of a phase II clinical study in healthy, HIV-negative individuals. 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. The most common adverse effects seen were headache, dizziness, nausea and taste perversion (oily aftertaste). No dose-related pattern in adverse effect or laboratory abnormality incidence was apparent. (+)-Calanolide A seemed to have highly variable plasma levels and absorption profiles, however, no accumulation of the parent compound was seen over a 5-day treatment course. Determination of steady-state trough plasma levels in cohorts taking 600 mg and 800 mg bid for 5 days showed a mean elimination half-life of 15.5 h in men and 35.2 h in women. Such pharmacokinetic properties, together with the benign safety profile, and unique *in vitro* resistance pattern warrant the continued development of this potential antiviral agent (Eiznhamer *et al.*, 2002). Creagh *et al.* (2001) have also reported a related clinical trial on (+)-calanolide A.

In addition to its distinctive HIV-1 resistance profile *in vitro*, (+)-calanolide A has recently been shown to be active against most of the strains of *Mycobacterium tuberculosis* including those resistant to the standard antitubercular drugs. Efficacy evaluations in macrophages revealed that (+)-calanolide A significantly inhibited intracellular replication of *M. tuberculosis* H37Rv at concentrations below the MIC observed *in vitro*; MIC = 3.13 µg/mL and IC₅₀ = 7.60 µg/mL. It inhibits RNA and DNA synthesis followed by an inhibition of protein synthesis (Xu *et al.*, 2004).

Various furanocoumarins obtained from *Prangos tschimganica* have shown anti-HIV activity. The most active of these, psolarene (**15**) and bergapten (**16**), inhibited HIV-1_{IIIB} strain replication in H9 lymphocytes with EC₅₀ values of 0.1 and 0.354 µg/mL (TI = 191 and 69.9), respectively (Shikishima *et al.*, 2001).

A number of coumarin derivatives isolated from the roots of *Ferula sumbul* showed anti-HIV activity. Imperatorin (**17**) and heraclenol (**18**) inhibited HIV replication in H9 lymphocytes with EC₅₀ values of <0.10 µg/mL (TI > 1000) and 0.115 µg/mL (TI = 870), respectively. Another potent coumarin osthol showed similar anti-HIV activity with an EC₅₀ value of 0.155 µg/mL (TI = 75.5) (Zhou *et al.*, 2000). In addition, esculetin isolated from *Fraxinus sieboldiana* var. *angustata* was reported to bind to HIV gp41 at IC₅₀ of 0.5 mg/mL (Kim *et al.*, 2002).

TERPENOIDS

Several triterpenoids have been found to exhibit antiretroviral activity with different mechanisms of action. The limonoids, limonin (**19**) and nomilin (**20**), inhibited HIV-1 replication in PBMC including those with chronic infection and on monocytes/macrophages with EC₅₀ values ranging from 20 to 80 µM. HIV-1 protease seems to be their target

(Battinelli *et al.*, 2003). Another limonoid, clausenolide-1-ethyl ether isolated from the rhizomes and the roots of *Clausena excavata* showed anti-HIV activity in a syncytium assay with an EC₅₀ value of 34.4 µM exhibiting a substantially low cytotoxicity (IC₅₀ = 548 µM). The compound was confirmed to be inactive against HIV-1 RT (Sunthitikawinsakul *et al.*, 2003). On the contrary, cycloartenol ferulate (IC₅₀ = 2.2 µM), 24-methylenecycloartanol ferulate (IC₅₀ = 1.9 µM), lupenone (IC₅₀ = 2.1 µM), betulin diacetate (IC₅₀ = 1.4 µM) and karounidiol 29-benzoate (IC₅₀ = 2.2 µM) inhibited purified HIV-1 RT and have been suggested as potential lead compounds (Akihisa *et al.*, 2001).

3-β-Hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) (**21**) is a pentacyclic lupane-type triterpene that is widely distributed throughout the plant kingdom. Among its many biological activities it is highly regarded for its anti-HIV-1 activity and specific cytotoxicity against a variety of tumour cell lines. Interest in developing even more potent anti-HIV agents based on betulinic acid has led to the discovery of a host of highly active derivatives exhibiting greater potencies and better therapeutic indices than some current clinical anti-HIV agents. While its mechanism of action has not been fully determined, it has been shown that some betulinic acid analogues disrupt viral fusion to the cell in a post-binding step through interaction with the viral glycoprotein gp41, whereas others disrupt assembly and budding of the HIV-1 virus, inhibition of the P24/p2 cleavage site being responsible for the antimaturation activity of the latter group, and a third group capable of inhibiting both steps of the virus replicative cycle has recently been reported. The targets of betulinic acid derivatives are varied, depending primarily on the side chain structures of the compounds (Cichewicz and Kouzi, 2004; Huang *et al.*, 2004).

The betulinic acid derivative RPR103611 (**22**) blocks HIV infection at an IC₅₀ of approximately 10 nM, through inhibition of a post-binding, envelope-dependent step involved in the fusion of the virus with the cell membrane (Mayaux *et al.*, 1994). The target for the anti-HIV action of RPR103611 is the HIV-1 glycoprotein gp41. HIV resistance to RPR103611 is associated with amino acid substitutions at positions 22 (Arg → Ala) and 84 (Ile → Ser) of gp41 (Labrosse *et al.*, 1997). RPR103611 is active against CXCR4-dependent (X4) HIV-1 strains, such as HIV-1_{LAI} (LAI). Other X4 strains, such as HIV-1_{NDK} (NDK), and CCR5-dependent (R5) HIV-1 strains, such as HIV-1_{ADA} (ADA), were totally resistant to RPR103611. A single difference at position 91, leucine in LAI and histidine in NDK, apparently accounted for their sensitivity or resistance to RPR103611 indicating that nonpolar residues in this region are important for the antiviral activity of RPR103611 and are possibly part of its target. However, another mechanism had to be envisaged to explain the drug resistance of ADA, since its gp41 loop region was almost identical to that of LAI. Fusion mediated by chimeric Env consisting of LAI gp120 and ADA gp41, or the reciprocal construct, was fully blocked by RPR103611. The gp120-gp41 complex of R5 strains is stable, relative to that of X4 strains, and this stability could play a role in their drug resistance. Therefore, the antiviral efficacy of RPR103611 depends on the sequence of the gp41 loop and the stability of the gp120-gp41 complex, which could limit the accessibility

of this target (Labrosse *et al.*, 2000). On the other hand, gp120 appears to be the target of the stereoisomer of RPR103611, IC9564 (Holz-Smith *et al.*, 2001).

Hydrogenation of betulinic acid yielded dihydrobetulinic acid, which showed an IC_{50} of 0.9 μM and a selectivity index of 14. 3-*O*-(3',3'-Dimethylsuccinyl)betulinic acid (PA-457) (**23**) and 3-*O*-(3',3'-dimethylsuccinyl)dihydrobetulinic acid have remarkably high anti-HIV activity and selectivity; IC_{50} : <0.35 nM; selectivity index >20 000 and >14 000, respectively (Kashiwada *et al.*, 1996). PA-457 inhibited replication of patient-derived WT viruses; IC_{50} of 10.3 nM, TI >2500. It was also active against a panel of virus isolates resistant to various anti-reverse transcriptases and antiproteases with a mean IC_{50} value of 7.8 nM. PA-457 acts by disrupting a late step in Gag processing involving conversion of the capsid precursor (p25) to mature capsid protein (p24). PA-457 was inactive against the related retroviruses HIV-2_{ROD} and simian immunodeficiency virus (SIV) in cell-based replication assays. PA-457 represents a unique class of anti-HIV compounds referred to as maturation inhibitors. Such compounds act on a previously unexploited viral target, providing additional opportunities for HIV drug discovery (Li *et al.*, 2003). Other betulinic acid derivatives such as LH15 and LH55 exhibit the aforementioned activities combined, i.e. they are antientry like IC9564 and antimaturation like PA-457 (Huang *et al.*, 2004).

Glycyrrhizin (GL) from licorice root (*Glycyrrhiza glabra*) has been known for some time as an antiviral agent, its IC_{50} against HIV-1_{IIIB} in MT-4 cells being 0.15 mM. Its effect was thought to be mediated at least partly through inhibition of protein kinase C (PKC) and interference with virus-cell binding, although the site of interaction of glycyrrhizin at the envelope glycoprotein has not been further characterized (Ito *et al.*, 1988). Hattori *et al.* (1989) have demonstrated its *in vivo* effects in AIDS patients. Glycyrrhizin has the potential to inhibit a non-syncytium-inducing variant of HIV (NSI-HIV) replication in PBMC cultures by inducing the production of β -chemokines (Sasaki *et al.*, 2002–2003). In addition to this, it suppressed *in vitro* UV-induced HIV gene expression in stably transfected HeLa HIV-LTRcat cells, without affecting cell proliferation and viability at doses as high as 2.4 mM. The inhibitory effect correlated with the complete inhibition of binding activities of NF- κ B p65, NF- κ B p50, c-Fos and c-Rel (Cherng *et al.*, 2004). Some of the chemically modified glycyrrhizin derivatives (salts, amides, glycopeptides) were potent HIV-1 and HIV-2 inhibitors *in vitro*. An example of these is niglizin (penta-*O*-nicotinate of GL) (Baltina, 2003). Persons with HIV may have previous or concurrent liver impairment as a result of injection drug use, hepatitis, alcohol abuse and damage from medication. Additional stress is placed on the liver by low-grade opportunistic infections and haemophilia. It is especially important that persons with HIV care for their liver to help this organ remain physiologically normal during chronic and acute management of HIV infection. Readily available liver protectants such as thioctic acid, glycyrrhizin and *Silybum marianum* are very important in this aspect (Hernandez, 1995).

Oleanolic acid was identified as an anti-HIV principle from several plants, including *Rosa woodsii* (leaves),

Prosopis glandulosa (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant) and *Ternstroemia gymnanthera* (aerial part). It inhibited HIV-1 replication in acutely infected H9 cells with an EC_{50} value of 1.7 $\mu\text{g}/\text{mL}$ and a TI of 12.8. Pomolic acid, isolated from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV agent (EC_{50} 1.4 $\mu\text{g}/\text{mL}$, TI 16.6). Although ursolic acid did show anti-HIV activity (EC_{50} 2.0 $\mu\text{g}/\text{mL}$), it was shown to be slightly toxic (IC_{50} 6.5 $\mu\text{g}/\text{mL}$). A derivative of oleanolic acid, on the other hand, demonstrated a very potent anti-HIV activity, with an EC_{50} value of 0.0005 $\mu\text{g}/\text{mL}$ and a TI value of 22 400 (Kashiwada *et al.*, 1998).

It has been reported that the use of a sterols/sterolin mixture in HIV infection shifts the balance of type 1 T helper cells to type 2 helper cells (Th1/Th2) towards the more beneficial Th1 and also maintains CD4 cell numbers over an extended period of time in the absence of any anti-retroviral therapy (Breytenbach *et al.*, 2001).

A phase I dose-escalating clinical trial of andrographolide, a diterpenoid lactone (*Andrographis paniculata*) previously reported to inhibit cell to cell transmission, viral replication and syncytia formation in HIV infected cells, revealed a significant rise in the mean CD4⁺ lymphocyte level (from a baseline of 405 cells/mm³ to 501 cells/mm³; $p = 0.002$) of HIV-1 infected subjects after 2 weeks administration of 10 mg/kg andrographolide. No subjects used antiretroviral medications during the trial. Unlike in the sero-positive subjects there was an unexplained decrease in CD4⁺ counts in all sero-negative counterparts, indicating a possible differential effect. The plasma viral load did not significantly decrease throughout the trial. It is proposed that andrographolide may inhibit HIV-induced cell cycle dysregulation, rather than interrupting viral replication directly. The side effects experienced by the cohorts and rated as being mild to moderate include headache, fatigue, rash, bitter/metallic/decreased taste, loose stool/diarrhoea and pruritus. Anaphylactic reactions have also been reported in one HIV-positive subject (Calabrese *et al.*, 2000).

Agents that induce HIV-1 out of latency would be useful adjuvants for currently available anti-retroviral therapy. 12-Deoxyphorbol-13-phenylacetate (DPP), an anti-tumour-promoting phorbol ester originally isolated from the West African plant *Euphorbia poissonii*, induced the expression of HIV-1 in latently infected T cells ($IC_{50} = 4$ nM, TI = 11 500) and rendered them sensitive to killing by an immunotoxin targeted to the viral envelope glycoprotein. DPP also regulates an extensive series of genes under the control of PKC, including several involved in T cell activation and cytoskeleton reorganization, and represses expression of the HIV-1 receptor CD4 ($IC_{50} = 14$ nM) and coreceptor CXCR4 ($IC_{50} = 2.9$ nM). DPP is 20- to 40-fold more potent than the related phorbol ester prostratin, probably due to its more lipophilic aromatic side chain structure at position 13. The combination of high potency and anti-tumour promoting activity make DPP an attractive candidate for the adjunctive therapy of persistent HIV-1 infection (Bocklandt *et al.*, 2003). Another phorbol ester, pedilstatin [13-*O*-acetyl-12-*O*-(2'*Z*,4'*E*-octadienyl)-4 α -deoxyphorbol], an anticancer principle from *Pedilanthus* sp., was shown to afford protection

(to 80%) of human-derived lymphoblastoid CEM-SS cells from infection and cell-killing by HIV-1, at concentrations of 2–5 μM and also inhibited PKC with a K_i of $620 \pm 20 \text{ nM}$ (Pettit *et al.*, 2002). Similarly, 12-*O*-tetradecanoylphorbol-13-acetate from the seeds of *Croton tiglium* was found to be a potent inhibitor of HIV-1-induced cytopathic effect on MT-4 cells with complete inhibitory concentration (IC_{100}) value of 0.48 ng/mL and minimum cytotoxic concentration (CC_0) value of 31.3 $\mu\text{g/mL}$. In addition, it was found to be an effective activator of PKC (96% activation at 10 ng/mL). Also, 12-*O*-acetylphorbol-13-decanoate effectively inhibited the cytopathic effect of HIV-1 on MT-4 cells with IC_{100} value of 7.6 ng/mL and CC_0 value of 62.5 $\mu\text{g/mL}$ (El-Mekkawy *et al.*, 2000). Table 1 summarizes other terpenoid-derived anti-HIV compounds.

ALKALOIDS

Screening of natural products in the search for human CCR5 inhibitors led to the identification of anibamine, a novel pyridine quaternary alkaloid as a trifluoroacetic acid salt, from *Aniba* sp. Anibamine-TFA competed for the binding of 125I-gp120 to human CCR5 with an IC_{50} of 1 μM . Formation of the TFA salt of anibamine is plausibly an artifact of the isolation process (Jayasuriya *et al.*, 2004). Similarly, the pentacyclic guanidine alkaloids crambescidin 826, crambescidin 800 and fromiamycalin isolated from the marine sponge *Monanchora* sp. inhibit HIV-1 envelope-mediated fusion *in vitro* with IC_{50} values of 1–3 μM (Chang *et al.*, 2003). Other marine alkaloids named manadomanzamines A (**26**) and B (**27**) which are structurally related to the manzamine-type alkaloids, have been isolated from an Indonesian sponge *Acanthostrongylophora* sp. (Haplosclerida: Petrosiidae) and characterized to have significant activities against *Mycobacterium tuberculosis* and HIV-1, and moderate activity against several AIDS opportunistic fungal infections. Manadomanzamines A and B are active against HIV-1 with EC_{50} values of 7.0 and 16.5 $\mu\text{g/mL}$, respectively. Manadomanzamine A is also active against human lung carcinoma A-549 and human colon carcinoma H-116, while manadomanzamine B is only active against the latter. In addition, xestomanzamine A another alkaloid from the same sponge species was active against HIV-1 at IC_{50} of 11.2 $\mu\text{g/mL}$. Manadomanzamines A, B and xestomanzamine A did not show cytotoxicity against the normal Vero cell line (African Green Monkey kidney cells) at a concentration of 4.8 $\mu\text{g/mL}$. Manadomanzamine B and xestomanzamine A are also active against the fungus *Cryptococcus neoformans* with IC_{50} values of 3.5 and 6.0 $\mu\text{g/mL}$, respectively. Manadomanzamine A was active against *Candida albicans* with an IC_{50} of 20 $\mu\text{g/mL}$ (Peng *et al.*, 2003).

One of the natural products with interesting activity on RT is polycitone A (**28**), an aromatic alkaloid isolated from the marine ascidian *Polycitor* sp. Polycitone A exhibits potent inhibitory capacity of both RNA- and DNA-directed DNA polymerases, such as reverse transcriptases of various retrovirals, such as HIV-1 (including L74V, Q89G, Y183F and M184L mutants), HIV-2, murine leukaemia virus and mouse mammary tumour virus (MMTV), *Escherichia coli* DNA poly-

merase I and cellular α and β DNA polymerases. The IC_{50} values for inhibition of the RNA- and DNA-directed DNA polymerase functions of HIV-1 RT were as low as 245 nM and 470 nM, respectively. As to its mode and mechanism of inhibition of HIV-1 RT, experimental evidence suggests that the inhibition of the DNA polymerase activity is independent of the template-primer used and also does not appreciably affect the RNase H function ($\text{IC}_{50} = 30 \mu\text{M}$). Polycitone A, on the other hand, has been shown to interfere with DNA primer extension ($\text{IC}_{50} = 2.5 \mu\text{M}$) as well as with the formation of the RT-DNA complex ($\text{IC}_{50} = 5\text{--}10 \mu\text{M}$). To add further detail to this, it seems that polycitone A inhibits the HIV-1 RT polymerase by preventing its reassociation with the DNA primer after it disassociates from the template-primer during extension. Steady-state kinetic studies also demonstrated that polycitone A can be considered as an allosteric inhibitor of HIV-1 RT that decreases the affinity of the enzyme to its substrate. 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 HIV-1 RT DNA polymerase activity, signifying the importance of the hydroxyl groups of polycitone A. In pentamethoxy polycitone A, for instance, the abilities to inhibit DNA primer extension as well as the formation of the RT-DNA complex were absent. Although polycitone A (similar to toxiusol) is a general inhibitor of DNA polymerase, lacking specificity to retroviral reverse transcriptases, its inhibition of the first step in DNA polymerization, i.e. the formation of the RT-DNA complex, and hence, of the overall process, could serve as a model for the rational design of new selective anti-HIV RT derivatives and possibly anti-AIDS drugs (Loya *et al.*, 1999).

The aporphine alkaloids hernandonine (**29**), laurolistine (**30**), 7-oxohernangerine and lindechunine A isolated from the roots of *Lindera chunii* showed significant anti-HIV-1 integrase activity with IC_{50} values of 16.3, 7.7, 18.2 and 21.1 μM , respectively (Zhang *et al.*, 2002).

The alkaloid harman isolated from *Symplocos setchuensis* was found to inhibit HIV replication in H9 lymphocyte cells with an EC_{50} of 10.3 μM and TI of 7.5. It was derivatized to give a compound showing a potent activity with EC_{50} and TI values of 0.037 μM and 210, respectively (Ishida *et al.*, 2001). Similarly, a sesquiterpene pyridine alkaloid triptonine B (**31**) isolated from *Tripterygium hypoglaucum* and a clinically used extract of *T. wilfordii*, demonstrated potent inhibition of HIV-1 replication in H9 cells with an $\text{EC}_{50} < 0.10 \mu\text{g/mL}$ and TI value of >1000 (Duan *et al.*, 2000). In a similar study 1-methoxycanthinone, isolated from *Leitneria floridana* inhibited HIV-1 replication at EC_{50} 0.26 $\mu\text{g/mL}$ and TI >39 (Xu *et al.*, 2000). Aromoline and FK-3000 isolated from the root tuber of *Stephania cepharantha*, completely inhibited the cytopathic effects of HIV-1 on MT-4 cells at 31.3 and 7.8 $\mu\text{g/mL}$, and possessed a CC_0 at 62.5 and 15.6 $\mu\text{g/mL}$, respectively (Ma *et al.*, 2002). Likewise, the carbazole alkaloid, siamenol from *Murraya siamensis*, inhibited HIV-1 induced cytopathic inhibitory activity in an XTT assay

Table 1. Terpenoid derived anti-HIV agents

Compound	Source	Part used	Activity/Target	Potency	Reference
Litsegermacrane, 5-epi-eudesm-4(15)-ene-1 β ,6 β -diol and litseachromolaevanes B (sesquiterpenes)	<i>Litsea verticillata</i>	Leaves and twigs	Inhibited HIV-1 replication	IC ₅₀ values: 27.5, 73.1 and 119.7 μ M, respectively	Zhang et al., 2003a
Dehydroxoperezinone (sesquiterpene quinone)	<i>Aristolochia manshuriensis</i>	Stem	Inhibited HIV-1 replication	EC ₅₀ = 17.5 μ g/mL and TI = 1.43	Wu et al., 2003b
Ovatodioid (a diterpenoid)	<i>Anisomeles indica</i>	Leaves	Inhibited the cytoprothic effects of HIV-1 infection	EC ₅₀ = 0.10 μ g/mL; IC ₅₀ = 1.20 μ g/mL; Maximum cellular protection of 80%–90%	Shahidul Alam et al., 2000
Agastanol and agastaquinone (diterpenoids)	<i>Agastache rugosa</i>	Roots	HIV-1 PR ^a	IC ₅₀ = 360 and 87 μ M, respectively	Min et al., 1999a
Uvaol and ursolic acid (triterpenes)	<i>Crataegus pinnatifida</i>	Leaves	HIV-1 PR	IC ₅₀ = 5.5 and 8.0 μ M, respectively	Min et al., 1999b
Garciosaterpene A (24) and C (25) (triterpenes)	<i>Garcinia speciosa</i>	Bark and stems	HIV-1 RT ^b Inhibition in syncytium assay	IC ₅₀ = 15.5 and 12.2 μ g/mL, respectively EC ₅₀ = 5.8 μ g/mL with TI 3.4, and 37.0 μ g/mL with TI 1.9, respectively	Rukachaisirikul et al., 2003
Vaticinone (triterpenoid)	<i>Vatica cinerea</i>	Leaves and stem	Inhibited HIV-1 replication	IC ₅₀ = 6.5 μ g/mL (15.3 μ M) and TI = 1.4	Zhang et al., 2003b
Moronic acid (triterpenoid)	Propolis	–	Inhibited HIV in H9 cells	EC ₅₀ = <0.1 μ g/mL with TI > 186	Ito et al., 2001
Lancilactones C (a triterpene lactone)	<i>Kadsura lanciliimba</i>	Stems and roots	Inhibited HIV replication	EC ₅₀ = 1.4 μ g/mL and TI > 71.4	Chen et al., 1999
Arganine C (a saponin)	<i>Tieghemella heckelii</i>	Fruits	Inhibited HIV-1 syncytium formation	EC ₅₀ : ~7 μ M; EC ₁₀₀ : 20 μ M; IC ₅₀ > 20 μ M; and TI: > 2.85	Gosse et al., 2002
Actein (a T-type saponin)	<i>Cimicifuga racemosa</i> (blackcohosh)	Rhizome	Inhibited HIV-1 replication	EC ₅₀ = 0.375 mg/mL and TI = 144	Sakurai et al., 2004
Valtrate (an iridoid)	<i>Valeriana fauriei</i>	Roots	Inhibited Rev-mediated nuclear transport Inhibited HIV-p24 production	IC ₅₀ = 3 μ M 44% at 0.5 μ M without showing any cytotoxicity	Murakami et al., 2002
Pheophorbide A (a chlorophyll isolate)	<i>Vatica cinerea</i>	Leaves and stem	Inhibited HIV-1 replication	IC ₅₀ = 1.5 μ g/mL (2.5 μ M) TI > 13	Zhang et al., 2003b

^a HIV-1 protease; ^b HIV-1 reverse transcriptase.

with an EC_{50} of 2.6 $\mu\text{g/mL}$ and TI of 2.4 (Meragelman *et al.*, 2000).

The methanol extract of *Artemisia caruifolia* yielded N^1, N^5, N^{10} -tri-*p*-coumaroylspermidine which showed a moderate inhibitory activity on HIV-1 protease ($EC_{50} = 53 \mu\text{g/mL}$). Based on the lead structure two related amides, namely, N^1, N^5, N^{10}, N^{14} -tetra-*p*-coumaroylspermine and $N^1, N^4, N^7, N^{10}, N^{13}$ -penta-*p*-coumaroyltetraethylenepentamine, were then synthesized and inhibited HIV-1 protease more potently and N^1, N^5, N^{10} -tri-*p*-coumaroylspermidine, with EC_{50} values of 27 and 30 $\mu\text{g/mL}$, respectively (Ma *et al.*, 2001).

POLYPHENOLS

Chronic administration of polyphenol-rich fruit juices is thought to be favourable to HIV-positive patients due to enhanced phytohaemagglutinin-induced lymphocyte proliferation, which could restore disturbances in T-cell homeostasis. Apoptosis that counterbalances increased lymphocyte proliferation in healthy individuals during juice consumption is absent in the case of HIV patients (Winkler *et al.*, 2004).

The galloannins geraniin (**32**) and corilagin (**33**) isolated from *Phyllanthus amarus* demonstrated a specific inhibition of HIV-1 replication in MT-4 cells with an EC_{50} of 0.24 $\mu\text{g/mL}$ and TI values of 26.8 and 29.3, respectively. Geraniin was shown to be active at two distinct sites of the HIV replication, which is helpful in suppressing the emergence of escape mutants. It effectively blocked viral uptake ($EC_{50} < 2.5 \mu\text{g/mL}$) and also exhibited *in vitro* inhibition of HIV-1 RT at an IC_{50} of 1.9 μM , a potency about 1000 fold higher than AZT-TP. It seems that this has enabled it to inhibit various nucleoside reverse transcriptase inhibitor (NRTI) and NNRTI resistant HIV-1 and HIV-2 strains, making it a good candidate for salvage therapy. Geraniin's activity against RT differs from the approved RT inhibitors by being competitive with respect to the primer/template (Notka *et al.*, 2003). Corilagin (*Jatropha curcas* and *Chamaesyce hysopifolia*) has been reported to inhibit HIV-1 RT *in vitro* at an IC_{50} of 20 μM (Matsuse *et al.*, 1999). In addition, tannins are believed to account for the inhibitory effect on polymerase and ribonuclease activities of HIV-1 RT as demonstrated by an aqueous extract of the leaves of *Terminalia triflora*; IC_{50} 1.6 $\mu\text{g/mL}$ and 1.8 $\mu\text{g/mL}$, respectively. This rare combination of anti-HIV-1 RT occurred with no associated cytotoxicity in HLT4LacZ-1_{III}B (Martino *et al.*, 2002). Min *et al.* (2000) have also reported that the gallic acid derivatives 1,2,6-trigalloylglucopyranose and 1,2,3,6-tertagalloylglucopyranose, isolated from the stem-bark of *Juglans mandshurica*, had strong inhibitory activities against RT; IC_{50} values being 0.067 and 0.040 μM , respectively. The latter also inhibited ribonuclease H activity with an IC_{50} of 39 μM .

Gallic acid and its various derivatives (*Terminalia chebula* and *Euphorbia pekinensis*) inhibited HIV-1 integrase and it has been proposed that the galloyl moiety plays a major role in the inhibition of the 3'-processing of HIV-1 integrase by these compounds (Ahn *et al.*, 2002).

Lithospermic acid (**34**) and lithospermic acid B (**35**) (*Salvia miltiorrhiza*) are two structurally related com-

pounds that demonstrated sound anti-HIV activities without showing cytotoxicity to H9 cells at high concentrations ($CC_{100} > 297 \mu\text{M}$ and $> 223 \mu\text{M}$, respectively). Both inhibitors strongly suppressed the acute HIV-1 infection of H9 cells with IC_{50} values of 2 and 6.9 μM , respectively. These compounds inhibited two HIV-1 integrase activities, 3'-processing and 3'-joining to the 5'-P ends of the target DNA with IC_{50} values in the range 0.37–0.83 μM . Both compounds neither prevent HIV entry into H9 cells nor inhibit RT activity in infected cells. These two selective integrase inhibitors hold promise as a novel class of therapeutic drugs for AIDS based on their high potencies and absence of cytotoxicity (Abd-Elazem *et al.*, 2002). In relation to this, rosmarinic acid methyl ester and rosmarinic acid, isolated from *Coleus parvifolius* (Labiatae), inhibited HIV-1 integrase with IC_{50} values of 3.1 and 5.0 μM , respectively (Kim *et al.*, 1999; Tewtrakul *et al.*, 2003). The HIV-1 integrase inhibitory effects of rosmarinic acid derivatives increase in order of monomers, dimers ($IC_{50} = 5.0 \mu\text{M}$), trimers, e.g. lithospermic acid ($IC_{50} = 1.4 \mu\text{M}$) and tetramers, e.g. lithospermic acid B ($IC_{50} = 1.0 \mu\text{M}$). It was shown that the metal-chelating derivatives were more potent than those that are non-bonding (Tewtrakul *et al.*, 2003).

Polyphenols from *Prunella vulgaris* and *Rhizoma cibotte* potently blocked gp41 six-helix bundle formation (a critical step of HIV-1 fusion with target cells) in a similar way to tannin; 86.2% and 98.3% at 50 $\mu\text{g/mL}$, respectively. Tannin and other polyphenols may be developed as a topical microbicide for the prevention of sexual transmission of HIV (Liu *et al.*, 2002).

St. John's wort potently inhibits UV-induced activation of HIV gene expression in stably transfected HIVcat/HeLa cells, in a dose-dependent manner. Since hypericin is known to exhibit a similar inhibitory property, it is likely to be the active constituent of St. John's wort (Taher *et al.*, 2002).

Polycaphenol, the alkaline extract of cacao husk, effectively inhibited the cytopathic effect of HIV infection in MT-4 cells and also protected mice from lethal infection of *E. coli*. In addition to this, polycaphenol exhibits selective antitumour activities against human oral tumour cells such as human oral squamous cell carcinoma (HSC-2) and human salivary gland tumour (HSG) and also possesses antioxidant properties (Jiang *et al.*, 2001). Thus polycaphenol could be of an immense significance in the management of chronic HIV/AIDS. Table 2 summarizes other anti-HIV polyphenol natural products.

POLYSACCHARIDES

Sulphated polysaccharides of marine origin have been reviewed comprehensively by De Clercq (2000). Sulphated polysaccharides block the binding (adsorption) of retroviruses to cells, which, in the case of HIV, is due to a direct interaction of the sulphated polysaccharides with the v3 loop of the viral envelope gp120. As a consequence, sulphated polysaccharides also block syncytium formation (fusion) between HIV-infected cells expressing the gp120 glycoprotein on their surface and uninfected cells expressing the CD4 receptor for gp120 (Baba *et al.*, 1990). Sulphated

Table 2. Polyphenol derivatives with anti-HIV activities

Protein	Source	Target	Potency (IC ₅₀)	Reference
Acteoside (36) and acteoside isomer	<i>Clerodendron trichotomum</i>	HIV-1 IN ^a	7.8 and 13.7 µM, respectively	Kim et al., 2001a
(-)-3,5-Dicaffeoyl-muco-quinic acid	<i>Aster scaber</i>	HIV-1 IN	7.0 µg/mL	Kwon et al., 2000
Phloroglucinol-1-O-β-D-glucopyranoside	<i>Maytenus senegalensis</i>	HIV-1 PR ^b	68.2% inhibition at 100 µM	(Hussein et al., 1999)
Camelliatannin H (37)	<i>Camellia japonica</i>	HIV-1 PR	0.9 µM	Park et al., 2002
Tellimagrandin I	<i>Eugenia caryophyllata</i>	Syncytia formation	16.12 ± 1.98 µg/mL	Kim et al., 2001b
Calceolarioside B	<i>Fraxinus sieboldiana</i> var. <i>angustata</i>	Bind to HIV gp41	0.1 mg/mL	Kim et al., 2002
Gomisin-G	<i>Kadsura japonica</i>	Inhibited HIV-1 replication in acutely infected H9	EC ₅₀ = 0.006 µg/mL TI = 300	Chen et al., 1997
Schisantherin-D			EC ₅₀ = 0.5 µg/mL TI = 110	
Schisandrin-C			EC ₅₀ = 0.8 µg/mL TI = 56	
Kadsuranin			EC ₅₀ = 1.2 µg/mL TI = 33.3	
Punicalagin	<i>Combretum molle</i>	Inhibited HIV-1 replication in MT-4 cells	EC ₅₀ = 1.2 µg/mL TI = 16	Asres and Bucar, 2005
Unidentified hydrolysable tannin designated as CM-A			EC ₅₀ = 0.96 µg/mL TI = 25	
Condensed tannins	<i>Xanthoceras sorbifolia</i>	HIV-1 PR	~6.0 µg/mL	Ma et al., 2000

^a HIV-1 integrase; ^b HIV-1 protease.

homopolysaccharides are more potent than sulphated heteropolysaccharides. The presence of the sulphate group is necessary for anti-HIV activity, and potency increases with the degree of sulphation (Schaeffer and Krylov, 2000). Their greatest potential may well reside in their topical application, i.e. as a gel formulation in the prevention of sexual HIV transmission (De Clercq, 2000).

Terrestrial non-sulphated polysaccharides also have their share in the list of anti-HIV natural products. A polysaccharide extracted from the leaf of *Rhizophora apiculata* (RAP) inhibited HIV-1, HIV-2 and SIV strains in MT-4, PBMC and MAGI-CCR5 cells in various assay systems including inhibition of viral cytopathogenicity by the MTT assay, antigen expression, p24 production, or the MAGI assay, within an EC₅₀ concentration range of 6.5 to 40.6 µg/mL. For instance, it blocked the expression of HIV-1 antigen in MT-4 cells and abolished the production of HIV-1 p24 antigen in PBMC with EC₅₀ values of 10.7 (TI = 144.5) and 25.9 µg/mL (TI = 44.0), respectively. RAP, at 100 µg/mL, completely blocked the binding of HIV-1 virions to MT-4 cells. RAP also reduced the production of viral mRNA when added before virus adsorption. It inhibited syncytium formation in cocultures of MOLT-4 cells and MOLT-4/HIV-1(IIIB) cells (EC₅₀ = 53.3 µg/mL); gp120 seemed to be its target. These properties may be advantageous should RAP be considered for further development as a vaginal anti-HIV formulation. Moreover, RAP did not prolong activated partial thromboplastin time (APTT) up to 500 µg/mL. The acid polysaccharide RAP had a molecular weight of more than 30 000 and was mainly composed of galactose, galactosamine and uronic acid. Its antiviral activity may be attributed to its carboxylated (polyanionic) character (Premanathan et al., 1999a). A similar anti-HIV polysaccharide extracted from the bark of *Rhizophora mucronata* has also been characterized by Premanathan et al. (1999b).

Various sulphated polysaccharides are now being tested for their clinical efficacy. The experience with topically applied dextrin sulphate in human subjects (both male and female) shows that sulphated polysaccharides do not produce systemic toxicity or genital epithelial disruption (Low-Beer et al., 2002; Van Damme et al., 2002). Another sulphated polymannuroguluronate (SPMG), a marine sulphated polysaccharide extracted from brown algae with specific means of fractionation and chemical modification, has entered Phase II clinical trial in China as the first anti-AIDS drug candidate obtained from marine organisms (Miao et al., 2004). SPMG exhibits a significant inhibitory effect against HIV proliferation in both normal human umbilical vein endothelial cells (HUVEC) and bFGF-treated HUVEC (Wang et al., 2003).

PROTEINS

This is the largest group of natural products with anti-HIV activity. The botanical sources, size and potency of most of these compounds are summarized in Table 3 and those which are considered to be most important are discussed below.

Cyanovirin-N (CV-N) (38) is a 101 residue (11 kDa) protein originally isolated from the blue-green alga

Table 3. Protein derived anti-HIV agents

Protein	Source	Part of plant	Size (kDa)	Target	Potency (IC ₅₀)	Remarks	Reference
Angularin	<i>Vigna angularis</i> (adzuki bean)	Seeds	8	HIV-1 RT ^a	27.5% at 70 μM	• Antifungal • Inhibit translation in RRLS ^b	Ye and Ng, 2002c
Ascalin	<i>Allium ascalonicum</i>	Bulbs	9.5	HIV-1 RT	10 μM	• Antifungal	Wang and Ng, 2002b
α-Basrubrin	<i>Basella rubra</i> (Ceylon spinach)	Seeds	4.3	HIV-1 RT	79.4% and 10.56% at 400 and 40 μM respectively	• Antifungal • Inhibit translation in RRLS	Wang and Ng, 2001a
β-Basrubrin			5		54.6% and 2.12% at 400 and 40 μM, respectively		
<i>Castanopsis</i> thaumatin-like protein	<i>Castanopsis chinensis</i>	Seeds	30	HIV-1 RT	1.6 μM	• Antifungal	Chu and Ng, 2003b
Chickpea cyclophilin-like antifungal protein	<i>Cicer arietinum</i> (chickpea)	Seeds	18	HIV-1 RT	69.8% at 27.7 μM and 97.8% at 277.7 μM	• Antifungal • Mitogenic in MSC ^c • Weakly inhibited cell-free translation	Ye and Ng, 2002e
Chrysancorin	<i>Chrysanthemum coronarium</i> var. <i>spatiosum</i>	Seeds	13.4	HIV-1 RT	84.56% and 0.0% at 22% 2.2 μM	• Antifungal • Mitogenic in MSC	Wang et al., 2001
Contraivirin	<i>Dorstenia contrajerva</i>	Leaves	5	Bind to gp41 Bind gp120 Inhibited HIV-1 induced cytopathogenicity	0.59 μM 0.20 μM EC ₅₀ = 1.0 μM and IC ₅₀ > 4.9 μM		Bokesch et al., 2004
Cowpea α-antifungal protein	<i>Vigna unguiculata</i> (Cowpea)	Seeds	28	HIV-1 RT α-Glucosidase	54.3% at 5 mg/mL 55.2% at 5 mg/mL	• Antifungal • Inhibit translation in RRLS	Ye et al., 2000
Cowpea β-antifungal protein	<i>V. unguiculata</i>	Seeds	12	HIV-1 RT α-Glucosidase β-Glucosidase	66.4% at 5 mg/mL 76.6% at 5 mg/mL 35.6% at 5 mg/mL		
Delandin	<i>Delandia unbellata</i> (rice bean)	Seeds	28	HIV-1 RT	44.5%, 32%, 13.4% at 180, 18, 1.8 μM, respectively	• Antifungal • Inhibit translation in RRLS • Mitogenic in MSC	Ye and Ng, 2002d
Fabin	<i>Vicia faba</i> (broad bean)	Seeds	34	HIV-1 RT	34 μM	• Antifungal • Inhibit translation in RRLS	Ng and Ye, 2003
Ginkbilobin	<i>Ginkgo biloba</i>	Seeds	13	HIV-1 RT	75.1% at 2 mg/mL	• Antifungal • Antibacterial • Anti-mitogenic in MSC	Wang and Ng, 2000b
Ground bean lectin	<i>Vigna sesquipedalis</i> (ground bean)	Seeds	~60	HIV-1 RT	73 μM	• Hemagglutinating activity inhibited by polygalacturonic acid but not galacturonic acid and simple monosaccharides • Decreased viability of hepatoma (HepG2), leukaemia (L1210), and leukaemia (M1) cells • Mitogenic in MSC	Wong and Ng, 2003
A homodimeric lectin	<i>Phaseolus vulgaris</i> (red kidney beans)	Seeds	67	HIV-1 RT and α-glucosidase	80.2% at 5 mg/mL 71.8% at 5 mg/mL	• Antifungal	Ye et al., 2001b

Table 3. (Continued)

Protein	Source	Part of plant	Size (kDa)	Target	Potency (IC ₅₀)	Remarks	Reference
Hypogin	<i>Arachis hypogaea</i> (peanut)	Seeds	7.2	HIV-1 RT α - and β -glucosidases	58.9% at 5 mg/mL 61.9% at 5 mg/mL 25.7% at 5 mg/mL	<ul style="list-style-type: none"> • Antifungal • Anti-mitogenic in MSC 	Ye and Ng, 2001b
Kiwi fruit thaumatin-like protein	<i>Actinidia chinensis</i>	Fruits	21	HIV-1 RT	30.6% at 27 μ M	<ul style="list-style-type: none"> • Antifungal 	Wang and Ng, 2002a
A laccase	<i>Tricholoma giganteum</i>	Fresh fruiting bodies of the mushroom	43	HIV-1 RT	2.2 μ M		Wang and Ng, 2004
Lentin	<i>Lentinus edodes</i> (shitake mushroom)	Fruiting bodies	27.5	HIV-1 RT	1.5 μ M	<ul style="list-style-type: none"> • Antifungal • Inhibited proliferation of leukaemia cells 	Ngai and Ng, 2003
Lilin	<i>Lilium brownii</i>	Bulbs	14.4	HIV-1 RT	97.93% at 120 μ M 26.7% at 12 μ M	<ul style="list-style-type: none"> • Antifungal • Mitogenic activities 	Wang and Ng, 2002c
Lyophyllin	<i>Lyophyllum shimeji</i>	Fruiting bodies of the mushroom	20	HIV-1 RT	7.9 nM	<ul style="list-style-type: none"> • An RIP^d • Antifungal • Inhibit translation in RRLS • Anti-mitogenic in MSC 	Lam and Ng, 2001a
Lyophyllum antifungal protein	<i>Lyophyllum shimeji</i>	Fruiting bodies of the mushroom	14	HIV-1 RT	5.2 nM	<ul style="list-style-type: none"> • Antifungal • Inhibit translation in RRLS 	
A mannose-binding lectin	<i>Allium tuberosum</i>	Inner shoots	13	HIV-1 RT	67.7% at 0.1 mg/mL 41.8% at 0.01 mg/mL	<ul style="list-style-type: none"> • Mitogenic in MSC 	Lam and Ng, 2001b
Mollisin	<i>Castanea mollissima</i>	Seeds	28	HIV-1 RT	14 μ M	Antifungal	Chu and Ng, 2003a
Pananotin	<i>Panax notoginseng</i> (sanchi ginseng)	Root	35	HIV-1 RT	35.8% at 12.6 μ M and 24.7% at 1.26 μ M	<ul style="list-style-type: none"> • Antifungal • Inhibit translation in RRLS 	Lam and Ng, 2002a
Phasein A	<i>Phaseolus vulgaris</i> (pinto beans)	Seeds	28	HIV-1 RT	29 μ M	<ul style="list-style-type: none"> • Antifungal • Inhibit translation in RRLS 	Ye and Ng, 2002f
Phasein B			32	HIV-1 RT	8 μ M	<ul style="list-style-type: none"> • Increased nitrite production by murine peritoneal macrophages 	
Quinqueginsin	<i>Panax quinquefolium</i> (American ginseng)	Roots	53	HIV-1 RT	Not determined	<ul style="list-style-type: none"> • Anti HIV-1 RT activity potentiated after chemical modification with succinic anhydride • Antifungal • Inhibit translation in RRLS 	Wang and Ng, 2000a
Rice bean antifungal peptide	<i>Delandia unbellata</i> (rice bean)	Seeds	5	HIV-1 RT	39.0% at 1.02 mM and 24.1% at 0.10 mM	<ul style="list-style-type: none"> • Antifungal • Inhibit translation in RRLS • Mitogenic in MSC 	Ye and Ng, 2002b
Treculavirin	<i>Treculia obovoidea</i>	Bark	10	Inhibited HIV-1 induced cytopathogenicity Bind to gp41	EC ₅₀ < 0.02 μ M and IC ₅₀ > 2.5 μ M 0.20 μ M		Bokesch et al., 2004
A trypsin-chymotrypsin inhibitor peptide	<i>Vicia faba</i> (broad bean)	Seeds	13	HIV-1 RT	32 μ M	<ul style="list-style-type: none"> • Antifungal • Mitogenic in MSC 	Ye and Ng, 2002a

Table 3. (Continued)

Protein	Source	Part of plant	Size (kDa)	Target	Potency (IC ₅₀)	Remarks	Reference
A trypsin-chymotrypsin inhibitor peptide	<i>Vicia faba</i> (broad bean)	Seeds	7.5	HIV-1 RT	100, 87.8, 58.2, 26.6% at 196.1, 98, 49, 24.5 μM respectively	<ul style="list-style-type: none"> • Antifungal • Mitogenic in MSC 	Ye <i>et al.</i> , 2001a
Unguilin	<i>Vigna unguiculata</i>	Seeds	18	HIV-1 RT, α- and β-glucosidases	84.8% at 5 mg/mL 76.9% at 5 mg/mL 40.8% at 5 mg/mL	<ul style="list-style-type: none"> • Antifungal • Inhibited methyl-3H-thymidine uptake by MSC • Weakly inhibited translation in RRLS 	Ye and Ng, 2001a
Velutin	<i>Flammulina velutipes</i>	Fruiting bodies of the mushroom	13.8	HIV-1 RT β-glucosidase β-glucuronidase	100% at 5 μM 62.3% at 5 μM 64.7% at 5 μM	<ul style="list-style-type: none"> • An RIP • Inhibit translation in RRLS • Chemical modification with succinic anhydride potentiated Anti-RT activity 	Wang and Ng, 2001b
Vulgin	<i>Phaseolus vulgaris</i> (pinto beans)	Seeds	28	HIV-1 RT	58 μM	<ul style="list-style-type: none"> • Antifungal • Inhibited translation in RRLS • Mitogenic in MSC 	Ye and Ng, 2003
A xylanase	<i>Panax notoginseng</i> (sanchi ginseng)	Root	15	HIV-1 RT	10 μM	<ul style="list-style-type: none"> • Did not inhibit translation in RRLS 	Lam and Ng, 2002b

^a HIV-1 reverse transcriptase; ^b rabbit reticulocyte lysate system; ^c mouse splenocytes; ^d ribosome inactivating protein.

Nostoc ellipsosporum. At low nanomolar concentrations it acts as a virucide and irreversibly inactivates both laboratory strains and primary isolates of HIV-1 and HIV-2. It has been shown to inhibit virus-to-cell fusion and cell-to-cell fusion between CD4⁺ cells and HIV-1 envelope-expressing cells. Its antiviral activity has been attributed at least in part, to unique and firm interactions of the protein with the viral surface envelope glycoprotein gp120, which must result in a reduced infectivity of the virus as well as reduced capacity of virus-infected cells to fuse with uninfected cells (Boyd *et al.*, 1997). The exact mechanism by which cyanovirin-N exerts its antiviral action is still being worked out. Although, post-CD4 inhibition of fusion was proposed initially (Boyd *et al.*, 1997; Mariner *et al.*, 1998), subsequent findings have repeatedly unfolded new dimensions in this regard. The following is a summary of *in vivo* and *in vitro* observations (Boyd *et al.*, 1997; Dey *et al.*, 2000; Esser *et al.*, 1999; Mariner *et al.*, 1998; Mori and Boyd, 2001; O'Keefe *et al.*, 2000).

1. CV-N impaired the binding of virion-associated gp120 to cell-associated CD4.
2. CV-N preferentially inhibited binding of the glycosylation-dependent neutralizing monoclonal antibody 2G12 (MAb 2G12) to gp120. However, MAb 2G12 pretreatment did not prevent subsequent CV-N binding to soluble gp120 (sgp120).
3. CV-N did not interfere with the interactions of soluble CD4 (sCD4) with either sgp120 or virion-associated gp120.
4. Prebinding of sgp120 to sCD4 did not block the subsequent binding of CV-N with the sgp120.
5. CV-N impairs both CD4-dependent and CD4-independent binding of sgp120 to the target cells. CV-N also impairs interaction of sCD4-activated Env with the coreceptor CCR5.
6. CV-N blocks the sCD4-induced binding of sgp120 with cell-associated coreceptor CXCR4.
7. CV-N dissociates bound sgp120 from target cells.
8. Pretreatment of CV-N with either sgp120 or sgp41 abrogated the neutralizing activity of CV-N against intact infectious HIV-1 virions.

CV-N-gp120 interactions are in part mediated by N-linked complex carbohydrates present on gp120, i.e. carbohydrate-dependent interaction. CV-N can exist in solution either as a monomer or a dimer. In the monomer form it contains a novel carbohydrate binding site that selectively binds to Man₉GlcNAc₂ and the D1D3 isomer of Man₈GlcNAc₂ of gp120 with nanomolar affinity. Besides this high-affinity binding site, it has also a non-essential lower-affinity binding site that facilitates (at μM or higher concentrations) cross-linking of the CV-N-oligomannose complex (CV-N-gp120 complex). The two binding sites of differing affinities mapped to opposite ends of the molecule; C-terminal and N-terminal, respectively (Bewley, 2001; Bewley and Otero-Quintero, 2001; Bewley *et al.*, 2002; Bolmstedt *et al.*, 2001; Botos *et al.*, 2002; Chang and Bewley 2002; Shenoy *et al.*, 2001; Shenoy *et al.*, 2002). In addition to the interactions of CV-N with specific gp120 oligosaccharides, discrete protein-protein interactions might also play an important ancillary role in the CV-N/gp120 binding event (Han *et al.*, 2004).

CV-N is well tolerated by human cells (CEM-SS and PBL) at high concentrations (e.g. 9000 nM). The

biological activity of CV-N is extremely resistant to physicochemical degradation and can withstand treatment with denaturants, detergents, organic solvents, multiple freeze-thaw cycles and heat (up to 100 °C) with no apparent loss of antiviral activity (Boyd *et al.*, 1997). CV-N is further pursued as a topical (vaginal or rectal) microbicide to prevent sexual transmission of HIV, and preclinical development is under investigation. Efforts are also underway to produce its functional homologues in prokaryotic and eukaryotic hosts, which would enable their large-scale production (Boyd *et al.*, 1997; Mori *et al.*, 1998; Mori *et al.*, 2002). CV-N could also be expressed as surface protein as well as secreted as soluble protein by the human commensal bacterium *Streptococcus gordonii* providing an alternative means to deliver and maintain an effective concentration of the microbicide in the vaginal mucosa (Giomarelli *et al.*, 2002). Moreover, by coupling it with *Pseudomonas* exotoxin, a conjugate molecule capable of selectively killing HIV-infected gp120-expressing cells has been produced (Mori *et al.*, 1997).

Pf-gp6, a 6 kDa anti-degranulation glycoprotein purified from the extract of *Perilla frutescens* inhibited HIV-1-induced cytopathic effect and proviral DNA synthesis. Its IC₅₀ for various HIV-1 strains, including clinical isolates and CCR5-using (R5) HIV-1, ranged between 1.3 and 71.0 µg/mL, depending on the combination of viral strain and host cell. It also inhibited HIV-2_{ROD} in MT-4 with an IC₅₀ of 7.8 µg/mL. Pf-gp6 did not directly inactivate infectious viral particles. A time-of-addition experiment revealed that Pf-gp6 lost its activity before zidovudine but after the CXCR-4 antagonist AMD3100 during the early stage of viral infection. Although the pinpoint target of Pf-gp6 remains to be elucidated, it may interfere with a step between viral entry and reverse transcription (Kawahata *et al.*, 2002).

A comparative study of a variety of antifungal proteins from the seeds of leguminous plants including French bean, cowpea, field bean, mung bean, peanut and red kidney bean, show that nearly all proteins examined were able to inhibit HIV-1 RT, protease and integrase enzymes, to different extents (Ng *et al.*, 2002).

Pokeweed antiviral proteins (PAPs) are related broad-spectrum antiviral proteins isolated from the leaves of the pokeweed plant, *Phytolacca americana*. They are single-chain ribosome-inactivating proteins that catalytically depurinate ribosomal as well as viral RNA including that of HIV-1. There are three well-known different pokeweed antiviral protein (PAP) isoforms, namely, PAP-I from the spring leaves, PAP-II from the early summer leaves and PAP-III from the late summer leaves. PAP-I, PAP-II and PAP-III effectively inhibit the replication of HIV-1_{HTLV-III_B} in human peripheral blood mononuclear cells at concentrations which do not inhibit the protein synthesis of host cells; IC₅₀ values; 17 nM, 25 nM and 16 nM, respectively (Irvin and Uckun, 1992; Rajamohan *et al.*, 1999a; Rajamohan *et al.*, 1999b).

Depurination alone did not account for the high potency of PAP against HIV-1 and its mechanism is not yet fully understood. Molecular modelling studies predicted a more potent anti-HIV activity for PAP-III due to its unique surface topology and more favourable charge distribution in its 20 Å-long RNA binding active centre cleft. In accordance with this prediction,

PAP-III was more potent than PAP-I in depurinating HIV-1 RNA. Residues Tyr(69), Tyr(117), Glu(172) and Arg(175) are expected to define the active site of PAP-III (Rajamohan *et al.*, 1999b; Kurinov and Uckun, 2003).

A molecular model of PAP-RNA interactions was used to rationally engineer FLP-102(¹⁵¹AA¹⁵²) and FLP-105(¹⁹¹AG¹⁹²) as nontoxic PAPs with potent anti-HIV activities. These proteins depurinate HIV-1 RNA much better than rRNA. They are more potent anti-HIV agents (up to 14.5- and 4.1-fold, respectively) and substantially less toxic to BALB/c mice than native PAP. They were non-toxic to BALB/c mice at dose levels as high as 8.2 mg/kg. They also exhibited potent *in vivo* inhibition against genotypically and phenotypically NRTI-resistant HIV-1 in a surrogate human peripheral blood lymphocyte (Hu-PBL) SCID mouse model of human AIDS. FLP-102 and FLP-105, because of their potent anti-HIV activity and lack of systemic toxicity, may provide the basis for effective salvage therapies for patients harbouring highly drug-resistant strains of HIV-1 (Uckun *et al.*, 2003). Similarly an anti-CD7 immunoconjugate of PAP designated as TXU-PAP has also shown a potent anti-HIV activity both *in vitro* and *in vivo*, with an appreciably low toxicity. Moreover, TXU-PAP showed favourable pharmacokinetics such as a long plasma elimination half-life (up to 12.4 ± 1.4 h) in chimpanzees (HIV-1 infected and healthy) and HIV-1-infected adult patients (Uckun *et al.*, 1998; Uckun *et al.*, 1999). TXU-PAP is a promising anti-HIV agent should it be given further attention.

In a rabbit model study, PAP treatment of semen had no adverse effect on gestation length, pregnancy rate, perinatal outcome, and growth and development of the offspring. It was nontoxic to human sperm and female genital tract epithelial cells even at a concentration 2000 times higher than its IC₅₀ value against HIV-1. PAP is further pursued as a nonspERMICIDAL intravaginal microbicide and as a prophylactic antiviral agent that can inactivate infective viruses and virus-infected cells in semen. It is anticipated to be clinically useful in assisted reproduction in HIV-1 discordant couples (D'Cruz and Uckun, 2001a; D'Cruz and Uckun, 2001b; D'Cruz *et al.*, 2004a). Findings of a recent study, however, indicate that careful monitoring of vaginal irritation might be required in the clinical development of PAP as a nonspERMICIDAL microbicide (D'Cruz *et al.*, 2004b).

The clinical use of native PAP is limited due to inherent difficulties in obtaining sufficient quantities of a homogeneously pure and active PAP preparation with minimal batch to batch variability from its natural source. However, it has been shown that PAP can be produced by DNA recombination in *E. coli* (Rajamohan *et al.*, 1999c) and *Pichia pastoris* (Rajamohan *et al.*, 2000).

A number of anti-HIV proteins have been isolated from Thai bitter gourd, i.e. *Momordica charantia*. Among these is the 20 amino acid protein MRK29 (28.6 kD) isolated from the ripe fruit and seeds. MRK29 inhibited the HIV-1 RT with an IC₅₀ of 18 µg/mL. It also exerted 82% reduction of viral replication at 0.175 µg/mL in HIV-infected cells. It is postulated that MRK29 might also have a modulatory role on immune cells, because it increased 3-fold TNF activity (Jiratchariyakul *et al.*, 2001). In addition to this, a ribosome inactivating protein (RIP) designated MAP30

(*Momordica* anti-HIV protein, 30 kDa) acts as a DNA glycosylase/apurinic lyase, and was therefore able to inhibit HIV-1 integrase and irreversibly relax supercoiled DNA. The toxicity of MAP30 is specific to tumour-transformed or viral-infected cells. It shows no adverse effects on normal cells since it can not penetrate them (Lee-Huang *et al.*, 1990; Lee-Huang *et al.*, 1995; Wang *et al.*, 1999). Furthermore, MAP30 inhibits the proliferation of BC-2, an AIDS-related primary effusion lymphoma (PEL) cell, latently infected with Kaposi's sarcoma-associated herpes virus (KSHV), also known as human herpes virus 8 (HHV8). MAP30 modulates the expression of both viral and cellular genes involved in Kaposi's sarcoma pathogenesis. It downregulates the expression of viral cyclin D (vCD), viral interleukin-6 (vIL-6) and viral FLIP (vFLIP), genes involved in cell cycle regulation, viral pathogenesis and apoptosis. MAP30 also downregulates the expression of various cellular genes involved in mitogenesis, tumorigenesis and inhibition of apoptosis in NF κ B and p53 signalling pathways, while it upregulates the proapoptotic-related genes Bax, CRADD and caspase-3 (Sun *et al.*, 2001). RIPs in general, and MAP30 in particular, hold potential as both anti-tumour and anti-HIV agents. MAP30 may also be useful as a nonspermicidal prophylactic against sexual transmission of HIV since it did not alter the viability and motility of human spermatozoa even at a dose 1000 times the maximum effective concentration that inhibit HIV-1 and herpes simplex virus (Schreiber *et al.*, 1999). Another anti-HIV protein, α -momorcharin from the same plant was reported to possess remarkable anti-HIV-1 properties (Zheng *et al.*, 1999).

α -Trichobitacin, a novel RIP from the root tubers of *Trichosanthes kirilowii*, exhibited anti-HIV activities virtually similar to those of α -momorcharin. α -Trichobitacin greatly suppressed HIV-1 induced syncytial cell formation (IC₅₀ = 5 μ g/L) and markedly reduced both the replication of HIV-1 and the number of HIV antigen positive cells (IC₅₀ of 0.09 mg/L) in acutely but not chronically HIV-1 infected culture (Zheng *et al.*, 2000). Au *et al.* (2000) reported that inhibition of HIV-1 integrase appears to account partly for the anti-HIV properties of common RIPs.

Palicouren, a 37 amino acid cyclotide isolated from the tropical tree *Palicourea condensata*, inhibited the *in vitro* cytopathic effects of HIV-1_{RF} infection of CEM-SS cells with EC₅₀ and IC₅₀ values of 0.1 μ M and 1.5 μ M, respectively (Bokesch *et al.*, 2001). Hallock *et al.* (2000) have reported four novel macrocyclic peptides containing 28–31 amino acid residues, named cycloviolins A–D, isolated from the hitherto tropical plant *Leonia cymosa* (Violaceae) that suppress HIV-1 replication in CEM-SS cells with an EC₅₀ ~0.13 μ M. Similarly, four new macrocyclic polypeptides designated cirulins C–F, from another tropical tree *Chassalia parvifolia*, inhibited the cytopathic effects of *in vitro* HIV-1 infection with EC₅₀ values of 50–275 nM. Cirulins C–F are 29–30 amino acid cyclotides (Gustafson *et al.*, 2000).

Mannose-specific lectins from the bulbs of wild *Narcissus* spp. growing in Spain could suppress HIV-1 infection of MT-4 without significant cytotoxicity. Their haemagglutination and anti-HIV-1 activities showed no significant correlation. Multiple isolectin composition has been suggested to account for this dissociation between the two activities (Lopez *et al.*, 2003).

A lectin from *Myrianthus holstii* designated *M. holstii* lectin repressed CEM-SS infection by HIV-1_{RF} with an EC₅₀ value of 150 nM. Delaying the addition of the lectin for up to 8 h after initial exposure of CEM-SS cells to virus did not result in a loss of the antiviral activity; however, a 16 h or more delay resulted in a marked decrease in the antiviral activity. The lectin bound to a virus-free, soluble form of the viral envelope protein gp120 but did not inhibit the subsequent binding to a cell-free, soluble form of the cellular receptor CD4 (Charan *et al.*, 2000). Lectins from *Phaseolus vulgaris*, *Momordica charantia*, *Ricinus communis* and *Agaricus bisporus* were reported to inhibit HIV-1 RT (Wang and Ng, 2001c).

MISCELLANEOUS

In an *in vitro* XTT-based anti-HIV assay, 2–5 μ g/mL of the polyacetylenic acid, minquartynoic acid [(–)-17-hydroxy-9,11,13,15-octadecatetraenoic acid] from *Ochanostachys amentacea*, effectively inhibited human lymphoblastoid cell killing by HIV-1 (Rashid *et al.*, 2001).

Olive leaf extract (OLE) inhibits acute infection of MT-2 cells with HIV and cell-to-cell transmission of HIV-1. It also inhibits HIV-1 replication in infected H9 cells. These anti-HIV effects of OLE are dose dependent, with EC₅₀ values of around 0.2 μ g/mL. In the effective dose range, no cytotoxicity on uninfected target cells was detected (TI > 5000). HIV-1 infection modulates the expression patterns of cellular genes involved in apoptosis, stress, cytokine, PKC and hedgehog signalling. HIV-1 infection upregulates the expression of the heat-shock PKC proteins hsp27 and hsp90, the DNA damage inducible transcript 1 gadd45, the p53-binding protein mdm2, and the hedgehog signal protein patched 1, while it downregulates the expression of the anti-apoptotic BCL2-associated X protein Bax. Treatment with OLE reverses many of these HIV-1 infection-associated changes. It also upregulates the expression of the apoptosis inhibitor proteins IAP1 and 2, as well as the calcium and PKC pathway signalling molecules IL-2, IL-2R α , and ornithine decarboxylase ODC1 (Lee-Huang *et al.*, 2003).

The aqueous extracts of *Ocimum gratissimum* (leaves), *Ficus polita* (leaves), *Clausena anisata* (leaves), *Alchornea cordifolia* (fruits and seeds) and *Elaeophorbium drupifera* (leaves) are effective inhibitors of HIV-1 and HIV-2 replication. Most of the plant extracts inhibited HIV-1_{HTLV-III(B)} cytopathicity with EC₅₀ values in the range of 0.01 to 0.03 mg/mL, and TI values, 18 to 110. The leaves of *O. gratissimum* and the seeds of *A. cordifolia* had the highest TIs; 110 and 90, respectively. Except those of *A. cordifolia*, the other plant extracts inhibited HIV-2 strain GH1 replication in Molt-4 clone 8 cells with EC₅₀ values in the range <0.005–0.110 mg/mL, and TI values, 12.7–>260. Pertaining to anti-HIV-2 activity *F. polita* showed the highest activity accompanied with the highest selectivity; EC₅₀ < 0.005 mg/mL and TI > 260. The plant extracts, unlike AZT, were able to achieve significant inhibition of viral cytopathicity even at high MOI when treatment was delayed for 2 h. Early fusion of chronically HIV-infected cells with uninfected cells has been shown

to be inhibited by all the plant extracts. In addition, the extract of *E. drupifera* has been identified to be selectively toxic to chronically infected cells at concentrations that are not toxic to uninfected cells. *O. gratissimum*, *F. polita* and *A. cordifolia* (fruits) caused 90% reduction in HIV-1 RT activity at concentrations between 0.013 and 0.020 mg/mL. HIV-1 proviral DNA copying as determined in a polymerase chain reaction, was completely inhibited by *O. gratissimum* and *F. polita* at 0.011 and 0.015 mg/mL, respectively. The aforementioned plants thus appear to be promising sources for new antiretroviral compounds (Ayisi and Nyadedzor, 2003).

A polyherbal preparation, designated Immu-25, was evaluated for clinical efficacy and safety in HIV-infected patients, with confirmed HIV infection and a CD4 count <500 cells/ μ L. The polyherbal test preparation produced good symptomatic improvement within 6 months. The incidence and severity of symptoms such as diarrhoea, fatigue, anorexia, cough and fever decreased with drug treatment. There was a significant decrease in the mean viral load. The decrease in viral load was associated with an increase in mean CD4 count. With the exception of mild gastrointestinal adverse effects, the drug was well tolerated. Both patients and investigators rated the treatment as good or very good. It has been suggested that this herbal drug may have a good immunomodulatory effect and has potential as a co-therapeutic agent in the management of HIV infection (Usha *et al.*, 2003).

Momordica charantia (Thai bitter gourd) is a popular medicinal plant that is used for the treatment of various diseases. Among others it has antiviral, antitumour and immune system boosting properties. Chronic administration of a combination of juices and decoction of the leaves and fruits of Thai bitter gourd is reported to have increased the CD4 count and later normalized the CD4/CD8 ratios in an HIV-infected man in California (Rebutan, 1995).

CD4⁺ T cell counts in HIV-1-infected patients treated with only Korean red ginseng (KRG) were maintained or even increased for a prolonged period and also the development of resistance mutations in RT to zidovudine (ZDV) was delayed by combined therapy with KRG and ZDV. It is suggested that the maintenance of CD4⁺ T cell counts by ZDV and KRG intake for a prolonged period might be indirectly associated with delayed development of resistance to ZDV by KRG intake (Cho *et al.*, 2001).

It has been reported that a component of garlic called ajoene protects CD⁺ cells from attack by HIV early in the viral life cycle. At low concentrations, the drug appears to have little toxicity, and its anti-HIV activity is 45 times more powerful than dextran sulphate. Ajoene is found only in fresh garlic and is not readily available. It has been found that garlic impairs the activity of the liver enzymes that process protease inhibitors and raises the protease inhibitor levels (Anonymous, 1998).

Bioavailability of the protease inhibitor, saquinavir, was said to increase with consumption of grapefruit juice, while its clearance was not affected. Inhibition of cytochrome P450 3A4, an intestinal and liver enzyme, which breaks down saquinavir, was suggested to be responsible for this observation (Kupferschmidt *et al.*, 1998). However, in a similar type of study, it was found that concomitant administration of grapefruit juice

increases gastric pH and delayed indinavir absorption but did not uniformly affect the systemic bioavailability of indinavir in HIV-infected subjects (Shelton *et al.*, 2001).

European mistletoe (*Viscum album*) has been used parenterally for more than 80 years as an anticancer medication with significant immunomodulating action. Since 1984, clinical experience with a *Viscum album* extract (*Viscum album* Quercus Frischsaft) among HIV-positive patients has suggested that it inhibits HIV disease progression (Gorter *et al.*, 1999). Table 4 summarizes anti-HIV medicinal plant extracts with unidentified constituents.

CONCLUSION

Next to malaria, acquired immunodeficiency syndrome (AIDS) is the leading infectious cause of death in the world. Untreated disease caused by the human immunodeficiency virus (HIV) has a case fatality rate that approaches 100%. Up to now, the complete suppression of HIV replication in patients has not been more than a wildly ambitious idea. Even though antiretroviral drugs have been discovered that can bring about the suppression of the 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, which indisputably is found in the developing countries. The emergence of resistance and adverse reactions have also limited the utility of many of the conventional antiretroviral drugs. Patient compliance is also one of the challenges in combatting the disease. The impact that the progress of HIV/AIDS has in developing countries is multidimensional and most of all is a vicious circle. Needless to stress that there is an urgent need for a rapid and sustainable solution that must be functional in the developing countries. One of the sources for this kind of solution is the rich wealth of medicinal plants that many developing countries are endowed with. As long as their use is backed up with scientific proof, it could be of an immense economic importance for the people of the developing countries to resort to plant remedies. The aforementioned reports show that many plants, most of which are traditionally used for the treatment of different ailments in different parts of the world, are active against HIV replication at least *in vitro*. These reports are invaluable since they are the milestones for the discovery of new lead compounds and decision making at different levels. However, where many questions remain, it is premature to expect too much from the plethora of available leads at present. First of all it is important to note that a significant number of these studies are proof-of-concept experiments whose significance is not yet clear. Many of these studies have not also been pursued to the point that would enable pharmaceutical companies interested in this area to designate them as leads. Another fact in relation to this is that many findings are exclusively based on *ex vivo* biochemical or *in silico* assays. Such findings, if not confirmed further in cells and *in vivo* systems could potentially terminate in wasted effort. The cytotoxicity or selectivity profile of an antiviral compound or preparation should also be thoroughly

Table 4. Anti-HIV medicinal plant extracts with unidentified constituents

Source	Part of plant extract	Activity/Target	Potency	Reference
<i>Ailanthus altissima</i>	Stem bark	HIV-1 fusion inhibition	74.9% at 100 µg/mL of extract	Chang and Woo, 2003
<i>Atractylodes japonica</i>	Root	HIV-1 fusion inhibition HIV-1 PR ^a	72.8% at 100 µg/mL of extract 40.3% at 100 µg/mL of extract	Min <i>et al.</i> , 2001
<i>Agrimonia pilosa</i>	Whole plant	HIV-1 RT ^b RNase H HIV-1 PR	IC ₅₀ = 8.9 µg/mL IC ₅₀ = 98.4 µg/mL 35% inhibition at 100 µg/mL of extract	Min <i>et al.</i> , 2001
<i>Cornus kousa</i>	Stem and leaf	HIV-1 RT	IC ₅₀ = 6.3 µg/mL	
<i>Limonium tetragonum</i>	Root	HIV-1 RT	IC ₅₀ = 7.5 µg/mL	
<i>Mallotus japonicus</i>	Stem	HIV-1 RT	IC ₅₀ = 11.9 µg/mL	
<i>Clematis heracleifolia</i>	Whole plant	HIV-1 PR	45.3% inhibition at 100 µg/mL of extract	
<i>Syneilesis palmata</i>	Whole plant	HIV-1 PR	38.8% inhibition at 100 µg/mL of extract	
<i>Crinum asiaticum</i> var. <i>japonicum</i>	Root	Inhibited HIV-1 induced cythopathogenicity	ED ₅₀ = 12.5 µg/mL; SI = 16	
<i>Hyssopus officinalis</i>	Leaves	Inhibited HIV-1 induced cythopathogenicity	Active at 50–100 µg/mL without appreciable cytotoxicity	Bedoya <i>et al.</i> , 2002
<i>Ditrichia viscosa</i>	Aerial parts	Inhibited HIV-1 replication (estimated targets: early steps of virus replication, including virus-cell attachment, virus-cell fusion and cell-to-cell fusion)	Active at 25–400 µg/mL without appreciable cytotoxicity	Sanchez <i>et al.</i> , 2002
<i>Baccharis trinervis</i>	Aqueous extract; part not specified		Active at 10–400 µg/mL without cytotoxicity	
<i>Combretum paniculatum</i>	Leaves	Inhibited HIV-1 induced cythopathogenicity	EC ₅₀ = 5.2 µg/mL; SI = 6.4	Asres <i>et al.</i> , 2001
<i>Dodonaea angustifolia</i>	Leaves	Inhibited HIV-2 induced cythopathogenicity	EC ₅₀ = 3.0 µg/mL; SI = 32	
<i>Ximenea americana</i>	Stem bark	Inhibited HIV-1 induced cythopathogenicity	EC ₅₀ = 21.3 µg/mL SI = 4.9	
<i>Bersama abyssinica</i>	Root bark	Inhibited HIV-1 induced cythopathogenicity	EC ₅₀ = 8.3 µg/mL SI = 4.5	
<i>Tuberaria lignosa</i>	Aqueous extract (part not specified)	Inhibit <i>in vitro</i> HIV-1 infection	EC ₅₀ = 3.1 µg/mL SI = 3.8	Bedoya <i>et al.</i> , 2001
<i>Sanguisorba minor magnolii</i>	Root	HIV-1 IN ^c	Active at 50 µg/mL without appreciable cytotoxicity	
<i>Paeonia suffruticosa</i>	Flowers		EC ₅₀ = 15 µg/mL (After removal of polyphenolic compounds)	Au <i>et al.</i> , 2001
<i>Prunella vulgaris</i>	Whole plant	HIV-1 PR	EC ₅₀ = 45 µg/mL (After removal of polyphenolic compounds)	
<i>Scutellaria baicalensis</i>	Root	HIV-1 PR	93.5% inhibition at 200 µg/mL of extract	Lam <i>et al.</i> , 2000
<i>Hyptis lantanifolia</i>	Aerial parts	HIV-1 RT	91.1% inhibition at 200 µg/mL of extract	Lam <i>et al.</i> , 2000
<i>Tetrapteris macrocarpa</i>	Aerial part		IC ₅₀ = 7 µg/mL	Matsuse <i>et al.</i> , 1999
<i>Combretum hartmannianum</i>	Leaves	HIV-1 RT	IC ₅₀ = 8 µg/mL	
<i>Rubus rigidus</i>	Root	Inhibited HIV-1 induced cythopathogenicity	IC ₁₀₀ = 66 µg/mL (non selective, equally towards p56 ^{lck} tyrosine kinase)	Ali <i>et al.</i> , 2002
<i>Acacia nilotica</i>	Pods	HIV-1 PR	IC ₁₀₀ ≥ 31.5 µg/mL; SI ~ 2	Tshibangu <i>et al.</i> , 2002
<i>Batantes aegyptiaca</i>	Bark	HIV-1 PR	Complete inhibition at 100 µg/mL	
<i>Euphorbia granulata</i>	Bark	Inhibited HIV-1 induced cythopathogenicity	63.1% inhibition at 100 µg/mL	
	Leaves	Inhibited HIV-1 induced cythopathogenicity	IC ₁₀₀ ≥ 31.5 µg/mL; SI ~ 2	
	HIV-1 PR		IC ₁₀₀ ≥ 62.50 µg/mL; SI ~ 2	
<i>Maytenus senegalensis</i>	Stem-bark	HIV-1 PR	48.5% inhibition at 100 µg/mL	
			56.8% inhibition at 100 µg/mL	

^a HIV-1 protease; ^b HIV-1 reverse transcriptase; ^c HIV-1 integrase.

assessed before any further attention is given to it. Moreover, its compatibility and drug interaction of any sort with conventional antiretrovirals and other drugs commonly administered to AIDS patients should be studied as well.

Nevertheless it should be stressed that a number of natural products mainly derived from plants have proven effective in suppressing HIV replication and progress. Calanolide derivatives, pokeweed antiviral proteins and sulphated polysaccharides are only but a few of the compounds with excellent and promising

antiviral activities. It is very much anticipated that anti-HIV cures and prophylactic preparations containing these natural products would soon be available. The results and experiences with many of the anti-HIV natural products will inspire and motivate even more researchers to look for new leads from plants and natural sources. Many of the anti-HIV natural products have other medicinal values. 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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