Introduction

Almost ten years have passed since our first review “Plant-Derived Leading Compounds for Chemotherapy of Human Immunodefiency Virus (HIV) Infection” [1]. Much research effort has focused on the prevention and therapy of this infection resulting in AIDS, and despite considerable progress being made, still no vaccine that prevents infection or therapy that cures the disease and eliminates all infectious particles is yet available. Nevertheless, research efforts have been tremendous and a keyword search on “HIV” in the ISI Web of KnowledgeSM yielded at this time almost 90,000 published papers in the time frame 1998 – 2007. In addition to biotechnological techniques, molecular modelling, etc., exploitation of natural resources to find new lead compounds against HIV is still a valuable approach. In Planta Medica alone, more than 40 papers on HIV have been published since 1998, including a review paper on HIV reverse transcriptase inhibitors of natural origin [2]. The high number of citations of the previous review on anti-HIV compounds from plants published in 1998 in Planta Medica indicates the importance of natural products research in the battle against HIV. Therefore, we have decided to write an update of our previous review paper, this time covering the time span 1998 – 2007. Part of this material was also included in our review paper published in 2003 on plant substances as antiviral agents, focusing on HIV as well as other viruses, covering the period 1997 – 2001 [3], and in our review published in 2004 focusing on mechanisms of action of plant substances as anti-HIV agents [4].

HIV, the causative agent of AIDS, is a member of the lentivirus subfamily of retroviruses. From the two known HIV-types, HIV-1 is the most pathogenic. Antiretroviral drugs have transformed it from a rapid lethal infection into a chronic condition that can be controlled for many years through combination therapies with different classes of antiviral drugs, also known as highly active antiretroviral therapy (HAART) [5]. Anti-HIV drugs are classified into different groups according to their activity on the replicative cycle of HIV, which can be roughly divided into ten different steps [6]. These are virus-cell adsorption, virus-cell fusion, uncoating, reverse transcription, integration, DNA replication, transcription, translation, budding (assembly/release), and maturation. There are currently 24 compounds approved for the treatment of HIV: a) seven nucleoside reverse transcriptase inhibitors or NRTIs (zidovudine or AZT, didanosine or ddi, zalcitabine or ddC, stavudine or d4T, lamivudine or 3TC, abacavir or ABC, and emtricitabine); b) one nucleotide reverse transcriptase inhibitor (tenofovir disoproxil fumarate); c) four non-nucleoside reverse transcriptase inhibitors or NNRTIs (nevirapine, delavirdine, efavirenz, and...
etravirine); d) ten HIV protease inhibitors or PIs (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir and darunavir); e) a fusion inhibitor (enfuvirtide); f) an entry inhibitor – CCR5 co-receptor antagonist (maraviroc); and g) an HIV integrase strand transfer inhibitor (raltegravir) [7]. NRTIs and NNRTIs inhibit both reverse transcriptases, but they are targeted at the substrate (dNTP) and allosteric non-substrate binding sites, respectively. PIs bind to the active site of the viral protease enzyme, preventing the processing of viral proteins into functional forms. Viral particles are still produced when the protease is inhibited, but these particles are not infectious. Integrase inhibitors block the action of integrase, i.e., an enzyme that catalyzes the integration of viral DNA into host DNA. Besides viral enzymes, the viral entry is an attractive therapeutic target for HIV. A fusion inhibitor is a synthetic peptide that blocks viral/cellular membrane fusion triggered by gp41 and thus suppresses viral proliferation, while the entry inhibitor maraviroc blocks the chemokine receptor CCR5 which HIV uses as a coreceptor to bind and enter a human helper T cell. Due to the rapid emergence of drug-resistant strains, new antiviral therapeutics which act by other mechanisms are highly desirable for the treatment of HIV infections. Taking into account the enormous number and the amazing structural diversity of the currently available plant constituents, the plant kingdom should be further explored as a source of new and diverse antiviral agents. In this review, only those compounds that have been structurally characterised and possess a significant antiviral activity will be discussed. The latter means IC_{50} values lower than 25 μM or μg/mL [8]. An intrinsic component of the antiviral testing is the determination of a selectivity index (SI) towards the supporting host cell. The SI refers to the ratio of the maximum drug concentration causing either 50% or 90% inhibition of growth of normal cells (CC_{50}, CC_{90}) and the minimum drug concentration at which 50% or 90% of the virus is inhibited (IC_{50}, IC_{90}). Reports of antiviral activity of extracts/compounds even at very low concentrations but without SI data are of limited value [8].

**Anti-HIV Plant-Derived Agents**

### Alkaloids

In our first review the naphthylisoquinoline alkaloid dimers, michellamines A (1), B (2) and C (3), from the tropical liana Ancistrocladus korupensis were discussed. They act in a complex manner by at least two antiviral mechanisms, inhibition of reverse transcriptase and inhibition of HIV-induced cellular fusion. In addition, the michellamines were found to inhibit rat brain protein kinase C with IC_{50} values in the 15 – 35 μM range [9]. From the same plant michellamines D – F were obtained, exhibiting in vitro HIV inhibitory activity comparable to michellamine B [10]. Michellamine B has undergone extensive preclinical evaluation as a potential anti-AIDS drug, but has been considered too toxic for advancement to clinical trials. Therefore, synthetic efforts have been made in order to prepare less toxic and more potent derivatives. Jozimine C (4) was the synthetically prepared dimer of dioncophyline C, showing a close structural similarity with the michellamines (Fig. 1). Its anti-HIV activity (HIV-1) was nearly as good as michellamine B, but it also showed a distinct cytotoxicity, limiting its therapeutic range [11]. Octadehydromichellamine (5), a fully dehydrogenated structural analogue, was the first synthetic michellamine without centrochirality. It showed some anti-HIV activity (HIV-1) (IC_{50} = 29 μM) comparable to michellamine B, but with cytotoxicity at CC_{50} = 104 μM [12].

Some nitrogen-containing sugar analogues reported in our first review, such as castanospermine and 1-deoxynojirimycin, were capable of inhibiting N-linked oligosaccharide processing, and inhibited HIV replication [13], [14]. The anti-HIV potency was found to be correlated with the α-glucosidase I inhibitory activity, leading to the hypothesis that the anti-HIV activity was related to the inhibition of α-glucosidase I [15]. However, a series of natural epimers of α-homonojirimycin and N-alkylated derivatives were isolated or synthesised, and it was observed that α-homonojirimycin (6) and N-methyl-α-homonojirimycin (7) were more potent inhibitors of α-glucosidase I than 1-deoxynojirimycin or castanospermine. Nevertheless, only the two latter compounds showed a significant anti-HIV-1 activity [16], suggesting that the HIV-inhibitory activity may be due to other factors than inhibition of α-glucosidase I.

Cepharantine (8) is a bisococlaurine alkaloid, isolated from Stephania cepharantha. It has been shown to possess anti-inflammatory, antiallergic, and immunomodulatory activities in vivo [17], [18]. Its effects on mammalian cells, and the implications for cancer, shock, and inflammatory diseases have recently been reviewed [19]. It is known that several inflammatory cytokines affect the progression and pathogenesis of HIV-1 infection [20]. Therefore, the inhibitory effects of cepharantine on TNF-α and phorbol 12-myristate-13-acetate (PMA)-induced HIV-1 replication in chronically infected monocytic and T lymphocytic cell lines were evaluated. Cepharantine was a highly potent inhibitor of HIV-1 in the monocytic cell line, but not in the T lymphocytic cell line [21]. It also suppressed HIV-1 long terminal repeat (LTR)-driven gene expression through inhibition of NF-κB activation. The related bisbenzylisoquinoline alkaloid cyleanine (9) was evaluated against HIV-1 and HIV-2. It showed activity against HIV-2 with an IC_{50} of 1.83 μg/mL but was at least 10-fold less active against HIV-1. The selectivity index of cyleanine against HIV-2 was 9, with a CC_{50} of 15.68 μg/mL [22].

Two new sesquiterpene pyridine alkaloids, triptonines A (10) and B (11), were isolated from the methanolic extract of the root bark of Tripterygium hypoglaucum [23]. Their IC_{50} values against HIV replication were 2.54 and < 0.10 μg/mL, respectively, with a selectivity index (SI) of > 39.4 and > 1000. The β-carboline skeleton was found to be present in various anti-HIV alkaloids. The well-known β-carboline alkaloid harmine (12) isolated from Symlocos setchuenis, indigenous in southern China, was found to inhibit HIV replication (IC_{50} = 10.7 μM, SI = 10.4) [24]. Substitution of the indole nitrogen with 1-methyl, 7-methoxy, or an alkyl group increased its activity. The most active derivative was N-butylharmane (13) (IC_{50} = 0.037 μM, SI = 210). The new carbazole alkaloid sianemol (14) was isolated from an extract of Murraya siamensis. It inhibited HIV with an IC_{50} value of 2.6 μg/mL, reaching 50 – 60% maximum protection in the XTT-tetrazolium assay, and it was more active than the related β-carboline alkaloid mahanimbibil isolated from the same source [25]. Anti-HIV carbazoles were also obtained from Clausena excavata, used in Thai folk medicine. O-Methylmukonal (15), 3-formyl-2,7-dimethoxycarbazole (16) and clauszoline J (17) displayed anti-HIV-1 activity in a syncytial assay with IC_{50} values of 12, 29.1 and 34.2 μM, respectively, and with a selectivity index of 56.7, 8.0 and 1.6, respectively [26]. The β-carboline derivative 5-methoxycanthinone (18), isolated from Lettneria floridana, a rare tree or shrub restricted to scattered wet sites in the southern Atlantic and Gulf coastal plains of the United States, was a potent...
anti-HIV agent (IC$_{50}$ 0.26 µg/mL; SI > 391) [27]. Also the canthin-4-one drymaritin (19), obtained from Drymaria diandra from Taiwan, showed anti-HIV activity (IC$_{50}$ 0.69 µg/mL; SI 20.6), indicating the potential of canthinones as anti-HIV leads [28]. The Iboga alkaloid congener 18-methoxycoronaridine (20), for which in vitro leishmanicidal and in vivo anti-addiction properties had already been described, also inhibited HIV-1 replication in human peripheral blood mononuclear cells and in monocyte-derived macrophages [29]. According to the test system, IC$_{50}$ values in the range of 9.5 to 23 µM were obtained, with an SI of 14.5 to 34.5. In this concentration range, 18-methoxycoronaridine moderately reduced the polymerase activity of recombinant HIV-1 reverse transcriptase (IC$_{50}$ = 69.4 µM). The antileishmanial activity may be useful for treating HIV-1 infected patients coinfected with Leishmania.

The antiviral properties of Amaryllidaceae alkaloids such as lycoreine (21) had already been reported many years ago [30]. More recently, anti-HIV activity was observed for lycoreine, homocorynie, trisphearine and haemanthamine. IC$_{50}$ values in the range of 0.4 – 7.3 µg/mL were obtained, but the cytotoxicities were similar, leading to a low SI for all alkaloids (1.3 – 1.9) [31]. Remarkably, also a very simple compound such as indole-3-carboxylic acid showed anti-HIV activity, with an IC$_{50}$ of 2.41 µg/mL and an SI of 6.79 [32].

Carbohydrates
The HIV regulatory gene tat is essential for viral replication. The tat protein is released from HIV-1 infected cells, enters new cells in an active form, and stimulates the transcriptional activity of HIV-LTR [33]. Inhibition of tat activity by pentosan polysulphate (22) [34] and heparin (23) [35] leads to anti-HIV-1 activity; selective 2-O-, 6-O-, or N-desulphation of heparin prevented the interaction with tat (Fig. 2). Sulphated dextrin derivatives were also able to inhibit HIV-1 tat, whereas unsulphated dextrin did not [36].

Sulphated polycarboxylic acids have already been known for a long time as potent inhibitors of HIV-1 and -2 replication in vitro [37], [38]. They are characterised by the following properties: (a) broad activity against enveloped viruses, including HIV and HSV; (2) low induction of viral resistance in cell cultures; (3) inhibition of virus adsorption to the cells; and (4) inhibition of syncytium (giant cell) formation between HIV-infected and normal CD4+ T cells. The latter point may be important for the depletion of CD4+ T cells in AIDS patients. The anti-HIV activity of sulphated polycarboxylic acids is due to shielding off the positively charged amino acid residues in the V3 loop of the viral envelope glycoprotein gp120 [38]. In this way, viral attachment to cell surface heparan sulphate is prevented. This is a primary binding site, followed by a more specific binding to the CD4 receptor. Resistance of HIV-1 to dextransulphate (24) seemed to be located in the env genome of HIV, and specifically in the V3 loop domain [39]. More than 15 years ago, the in vivo activity of dextransulphate against HIV was found to be disappointing, both after oral or intravenous administration [40], [41]. This was due to its poor oral bioavailability, its short plasma half-life, partial inactivation by plasma components, and poor ability to penetrate infected cells.
However, it was reported that dextran sulphate was absorbed rapidly in humans after oral administration and could be found in plasma, lymphocytes, and urine [42]. In an open phase I/II dose-escalation study in which six AIDS patients were treated with intraperitoneally administered dextrin 2-sulphate, a significant decrease in viral load was observed [43]. The question if sulphated polysaccharides can be useful anti-HIV drugs after oral or parenteral administration, or as a gel formulation (e.g., on condoms) in the prevention of sexually transmitted HIV remains a matter of debate [44]. The development of new drug delivery systems such as liposomes may improve the therapeutic efficacy of sulphated polysaccharides.

A sulphated polysaccharide with fucose as the main component was isolated from the water extract of a brown alga *Sargassum horneri*. It showed potent antiviral activity against Herpes simplex virus type 1, human cytomegalovirus and HIV-1 [45]. Time-of-addition experiments suggested that it inhibited not only the initial stages of viral infection, but also intracellular replication stages. The same observations were made for rhamnan sulphate, a natural sulphated polysaccharide isolated from the seaweed *Monostroma latissimum*, composed of large amounts of 1,2- and 1,3-linked α-L-rhamnose residues with small amounts of their branching residues, which was active against the same range of viruses. Rhamnan sulphate and AZT acted synergistically in their anti-HIV effect [46]. The gametic, carposporic and tetrasporic reproductive stages of the Mediterranean red alga *Asparagopsis armata* yielded sulphated galactans with a galactose:3,6-anhydrogalactose:sulphates molar ratio of 1:0.01:1.23, 1:0.04:0.47, and 1:0.01:1.13, respectively. The carposporic polysaccharide with the lowest sulphate ratio was ineffective against HIV-1 replication up to 100 μg/mL, in contrast to the other galactans which inhibited HIV-1 replication at 10 and 8 μg/mL, as measured by HIV-induced syncytium formation and reverse transcriptase activity in cell-free culture supernatant [47]. Calcium spirulan, a sulphated polysaccharide from *Arthrospira platensis* (formerly *Spirulina platensis*), consists mainly of two types of disaccharide repeating units, O-hexuronoxylyranosyl and O-rhamnosyl-3-O-methylrhamnose. A broad spectrum of antiviral activity of calcium spirulan and spirulan-like substances was reported, including herpes viruses, paramyxovirus viruses, influenza viruses, and HIV-1. With regard to herpes viruses, antiviral effects were most pronounced after pre-incubation prior to virus addition, indicating virus entry as the primary target. However, in the case of human cytomegalovirus, it was clearly demonstrated that intracellular steps also contributed to the antiviral effect. In the case of HIV-1, inhibition occurred at a stage later than viral entry [48].

**Coumarins**

A screening program by the U.S. National Cancer Institute (NCI) has led to the isolation of 4-propyldipyranocoumarins or calanolides from *Calophyllum lanigerum*, a tropical rainforest tree [49]. (+)-Calanolide A (25) was the most active against HIV-1 and was classified as an NNRTI, but its HIV sensitivity/resistance profile is different from other NNRTIs (Fig. 3). Other *Calophyllum* coumarins were also evaluated for their antiviral activity.
marins could be classified into three groups, based on the C-4 substituent of the lactone ring: calanolides with an n-propyl group, inophyllums having a phenyl group and cordatolides with a methyl group in this position [50]. The methyl groups at C-10 and C-11, and a free hydroxy group at C-12 are important for the anti-HIV-1 activity [51], [52]. In calanolides, the configuration of C-12 should be S, but can be R or S in inophyllums [53]. Cordatolides A (26) and B (27) were about 50 times less active against HIV-1 reverse transcriptase than (+)-calanolide A, indicating that the nature of C-4 substitution is important [54].

(+)-Calanolide A has been extensively studied because of its unique sensitivity/resistance profile [55]; [56], its synergistic effect in combination with other anti-HIV drugs [57] and its pharmacokinetic profile [58], [59], [60], [61], [62]. The interaction of NNRTIs at the hydrophobic non-nucleoside binding site of HIV-1 reverse transcriptase is highly specific. A single amino acid change in the NNRTI active site drastically changes the anti-HIV-1 activity of these NNRTIs. (+)-Calanolide A and nevirapine exhibited different activities against Y181C- and Y188H-mutated reverse transcriptase. Both compounds bind to reverse transcriptase in the NNRTI common binding site, but in a mechanistically different fashion. (+)-Calanolide A enhanced anti-HIV-1 activity against viruses with Y181C mutation, but the Y188 H substitution in reverse transcriptase resulted in about 30-fold resistance. In contrast, Y181C substitution in reverse transcriptase resulted in about 90-fold resistance to nevirapine, while Y188 H substitution did not decrease the sensitivity to nevirapine at all [55].

(-)-Calanolide B (costatolide) (28) and (-)-dihydrocalanolide B (dihydrocostatolide) (29) were similar to (+)-calanolide A with regard to their sensitivity/resistance profile for viruses with Y188 H or Y181C amino acid changes in their reverse transcriptase [56]. Calanolides act synergistically in combination with lamivudine and nelfinavir in vitro [57]. They represent a distinct subgroup of the NNRTI family and may be useful in combination therapy with other anti-HIV drugs.

The in vivo anti-HIV-1 efficacy of (+)-calanolide A was demonstrated in a hollow fibre mouse model after oral or parenteral administration once or twice daily in the subcutaneous site or the peritoneal cavity [58]. (+)-Calanolide A has been quantified using a validated HPLC method in rat, dog and human plasma. It was demonstrated that epimerisation of the 12-OH group of (+)-calanolide A to its inactive epimer (+)-calanolide B did not occur after oral administration in humans [59]. The pharmacokinetic profile of (+)-calanolide A and (+)-dihydrocalanolide A was comparable, but the oral bioavailability of (+)-dihydrocalanolide A was markedly better than that of (+)-calanolide A [60].

The safety and pharmacokinetics of (+)-calanolide A was evaluated in both healthy and HIV-infected volunteers [61]. In HIV-negative volunteers, the toxicity of (+)-calanolide A after oral administration was minimal with dizziness, taste perversion, headache and nausea as the most reported adverse effects [62]. In HIV-infected volunteers, the Cmax and AUC increased dose-proportionally, indicating linear pharmacokinetics. No drug accumulation was observed, although (+)-calanolide A has a relatively long elimination half-life (15 – 20 h). The viral load reduction was dose-dependent (mean reduction from baseline of 0.81 log10). However, the pharmacokinetic parameters showed high intrainject variability and the results of a phase IB study were rather disappointing. A significant drug-related toxicity (fever, rash) was observed at the highest dose of 600 mg. A decrease in CD4 cell count was observed in Asian patients, but not in Caucasian and black US patients.

In addition to the calanolides, khellolactone coumarins have also served as lead compounds for new anti-HIV agents. The original lead, suksdorfin (30), was obtained from Lomatium suksdorfii. Its IC50 against HIV-1 was 2.6 μM. The 3’R,4’R-di-O-(-)-camphanolyl-(-)-cis-khellolactone (31) had potent anti-HIV activity (IC50 0.4 nM, SI 136,719), and was selected for preclinical studies [53], [63]. Also this analogues were found to merit attention as anti-HIV agents [64].

Also other types of coumarins showed more or less pronounced anti-HIV activity [26], [32, 63]. Mesuel (32), a naturally occurring 4-phenylcoumarin, was found to inhibit HIV-1 replication by targeting the NF-κB pathway. It inhibited the phosphorylation and the transcriptional activity of the NF-κB p65 subunit in TNFR-stimulated cells [65].

**Flavonoids**

Flavonoids are capable of inhibiting a wide variety of enzymes, including several critical enzymes involved in the HIV life cycle, such as reverse transcriptase [66], viral protease [67], and integrate [68]. Another important enzyme for HIV replication is casin kinase II, a cAMP-, cGMP-, and Ca2+/phospholipid-independent serine/threonine protein kinase that phosphorylates several HIV-1 structural proteins in HIV-1 infected cells. Flavonoids such as quercetin, chrysin, and (–)-epigallocatechin 3-O-gallate (33) were able to inhibit casin kinase II [69], [70], [71] (Fig. 4). However, the biological significance of casin kinase II in HIV-1 replication and its inhibition by flavonoids are not completely understood yet.

The chalcone 2-methoxy-3-methyl-4,6-dihydroxy-5-(3’-hydroxy)cinnamoylbenzaldehyde (34) isolated from the roots of Desmos spp. showed potent anti-HIV activity (IC50 = 0.022 μg/mL, SI = 489) [72]. The compound can now be synthesised in five or six simple steps without any protecting groups [73]. Six chalcones were isolated from the methanolic extract of Boesenbergia pandurata rhizomes and tested for anti-HIV-1 protease activity. Hydroxypanduratin A (35) showed the highest activity with an IC50 value of 5.6 μM. An SAR of these chalcones was summarised as follows: a) a hydroxy moiety at position 4 conferred higher activity than a methoxy group; b) prenylation of dihydrochalcone was essential for activity; c) hydroxylation at position 4” reduced activity; and d) introduction of a double bond at C1’ and C6’ of chalcones gave higher activity [74].

Flavanones bearing an OH group in position C-3’, such as taxifolin (36), inhibited viral protease, reverse transcriptase, CD4/ gp120 interaction by binding to the V3 loop of gp120, and showed binding to non-specific proteins. However, flavanones without this OH group, such as aromadendrin (37), were more specific in their antiviral activity and inhibited the CD4/gp120 interaction, but not viral protease or reverse transcriptase [75]. Another example of non-specific activity is (–)-epigallocatechin 3-O-gallate (33), which exhibited a destructive effect on viral particles and post-adsorption entry and inhibited viral protease and reverse transcriptase [76]. Recently, it was stated that (–)-epigallocatechin 3-O-gallate at concentrations equivalent to those obtained by the consumption of green tea was able to reduce the attachment of gp120 to CD4 by a factor of 10-fold [77]. Thalassiolins A – C are natural flavones isolated from the Caribbean sea grass Thalassia testudinum with HIV integrase inhibition [78]. Thalassiolin A (38) was the most active and inhibited terminal cleavage and Mg2+-dependent strand transfer reaction
at low micromolar concentrations. Long-term passage of cells with thalassiolin A did not lead to resistant viruses.

Some prenylated flavanones and flavones known for their phytoestrogenic activity, also showed moderate anti-HIV activity, but accompanied by a high cytotoxicity and very low selectivity [79], [80]. Xanthohumol (39), purified from hops (Humulus lupulus), is a constituent of beer, a major dietary source of prenylated flavonoids and a natural product with multiple biofunctions. It was shown to inhibit HIV-1 induced cytopathic effects (CPE), and production of viral p24 antigen and reverse transcriptase in C8166 lymphocytes at non-cytotoxic concentrations [81]. The IC50 values were, respectively, 0.82, 1.28 and 0.50 μg/mL and an SI of about 10.8. The target of xanthohumol on HIV-1 may be on the steps post reverse transcription.

Biflavonoids consisting of two apigenin units, such as robustaflavone and hinokiflavone inhibited HIV-1 reverse transcriptase with IC50 values of 65 and 62 μM, while apigenin itself was about seven times less active [82]. Five new flavonoid glycosides with moderate to weak anti-HIV activity were isolated from Ochna integerrima [83] together with two known biflavonoids, ochnaflavone 7″-O-methyl ether (40) and 2″,3″-dihydroochnaflavone 7″-O-methyl ether (41) showing significant anti-HIV-1 activities in the syncytium assay with IC50 values of 2.0 and 0.9 μg/mL, respectively. Their IC50 values for inhibition of HIV-1 reverse transcriptase are comparable to the syncytium assay values, suggesting reverse transcriptase as a potential mechanism of action.

The correlation between anti-HIV activity of flavonoids and their structural properties were studied [84], [85], [86]. Electronegativity, LUMO (the energy of the lowest unoccupied molecular orbital), and the charges on atoms C-3 and C-7, were the molecular parameters allowing one to classify the flavonoids into active and less active anti-HIV agents [84], [86]. Sodium rutin sulphate showed a broad anti-HIV activity against R5 and X4 viruses with an SI ranging from 197 to 575 [87]. Like the sulphated polysaccharides, it has a poor oral bioavailability [88] and a short plasma half-life [89], indicating that it may be a good candidate for topical microbicide development.

Lignans

The antiviral activity of lignans was reviewed in 1998 by Charlton [90] and was found to be rather moderate for most products [91], [92], [93], [94]. Mechanisms of action included tubulin binding, inhibition of reverse transcriptase, integrase, and topoisomerase. Whereas podophyllotoxin and its derivatives were the most prominent representatives of the tubulin-binding lignans, various classes of lignans, such as dibenzylbutyrolactones, dibenzylbutanes, dibenzylcyclooctadienes, and aryltetralins inhibited reverse transcriptase.

Podophyllotoxin (42) is a lignan isolated from the roots of the North American Podophyllum peltatum Linnaeus, the Tibetan P. emodi Wall, or the Taiwanese species Podophyllum pleinthum (© Fig. 5). Extensive structural modification and anti-tumour studies of podophyllotoxin have resulted in clinically useful anti-cancer drugs, such as etoposide and teniposide. In addition, it is also used as an antiviral agent in treatment of anogenital warts [3]. Recently, several podophyllotoxin derivatives were synthesised that showed potent inhibitory effects on HIV-1 [95], [96].

Bioassay-guided fractionation of an ethanolic extract of the fruits of Schisandra rubriflora led to the isolation of new dibenzocyclooctadiene lignans, called rubrisandrins A and B, together with 11 known lignans [97]. (+)-Gomisin M1 (43) was the most active anti-HIV-1 compound with an IC50 value of less than 0.65 μM and an SI greater than 68. The other lignans possessed a low anti-HIV-1 activity and a low SI. During an HTS campaign to find novel HIV integrase inhibitors from natural products, the MeOH extract from the buds of Eucalyptus globoides was found to be active [98]. Bioassay-guided fractionation led to the purification of a new lignan, globoidan A (44) that inhibited the combined 3’ processing and strand transfer activity of HIV integrase with an IC50 value of 0.64 μM. In contrast to i-chicoric acid, the diketo acid motif containing globoidan A showed no activity in the whole cell anti-HIV assay. Interestingly, despite the presence of the three bis-catechol moieties, no cytotoxicity was observed at the highest test concentration of 50 μM.

Some dibenzylbutyrolactone-type lignans were isolated from Phenax angustifolius and the phenaxolactone 2-hydroxy-2-(3′,4′-dihydroxyphenyl)-methyl-3-(3′,4′-dimethoxyphenyl)-methyl-γ-butyrolactone (45) had an IC50 value of 3 μM and an SI of 37.3, requiring further SAR studies [99]. Moderate anti-HIV activity was observed for their glycosyl derivatives at the
C-4’ position. Preliminary mechanism of action studies suggested inhibition of an early step in the virus replicative cycle.

**Phenolics**

There has been discussion about the real anti-HIV-1 mechanism of dicafeoylquinic acids (DCQAs) and dicafeoyltartaric acids (DCTAs). It was suggested that HIV-1 integrase, an enzyme catalysing the insertion of viral DNA into the genome of the host cell, was their target [100], [101], [102]. In enzymatic assays, DCQAs such as 3,5-dicafeoylquinic acid (46), and DCTAs such as l-chicoric acid (47), showed a ten- to hundred-fold higher preference for inhibition of HIV integrase than of HIV reverse transcriptase (Fig. 6) [100]. In the series of the bis-catechols, l-chicoric acid was the most active inhibitor of HIV integrase, while phenolic acids such as caffeic acid and chlorogenic acid were not active. The inhibition of HIV integrase by DCQAs was irreversible and not dependent on divalent cations [101]. An HIV-1 mutant containing a single glycine-to-serine substitution at position 140 of integrase was resistant to l-chicoric acid, indicating that this compound is likely to interact at residues near the catalytic triad in the integrase active site [102]. However, this mechanism of action has been questioned [103]. HIV strains resistant to l-chicoric acid contained several mutations in the V2, V3, and V4 loop regions of the envelope glycoprotein gp120, but not in the integrase enzyme. Furthermore, l-chicoric acid did not inhibit the replication of viral strains resistant to polyanionic compounds, such as dextran sulphate [103]. Therefore, the primary anti-HIV target of l-chicoric acid and its analogues would be the envelope glycoprotein gp120. However, the most potent classes of integrase inhibitors with anti-HIV activity are still the dicafeoylquinic acids (DCQAs), dicafeoyltartaric acids (DCTAs), and diketo acids (DKAs). Moreover, molecular modelling studies have identified a putative HIV integrase inhibitor-binding pocket for l-chicoric acid and other integrase inhibitors [104], [105].

According to Lipinski’s rule of 5 and Veber’s bioavailability criteria, l-chicoric acid is a rather poor drug candidate for the following reasons: a) limited cell permeability due to the diacid moiety, b) hydrolytic enzymatic instability of the two ester linkages, and c) potential toxicity of the two catechol moieties [106], [107]. On the other hand, the very potent l-chicoric acid is an excellent lead compound for optimisation and indeed, a great number of DCQA and DCTA analogues were synthesised and evaluated as HIV-1 integrase inhibitors [108], [109], [110], [111], [112], [113]. Structure-activity relationship studies on these synthetic compounds demonstrated that l- and d-chicoric acid (48) exhibited similar anti-HIV-1 integrase activity. Removal of one or both carboxylic groups did not result in a significantly lower integrase inhibitory activity [110]. The bis-catechol moieties...
were essential to obtain a high inhibitory activity of integrase [111], but acetylation had only a minor negative effect on the inhibition of HIV integrase [110].

Curcuma longa Linn. (Zingiberaceae) is a medicinal plant widely cultivated in tropical regions of Asia. Curcumin (49) is a yellow pigment isolated from the rhizomes of this plant and possesses a wide variety of biological activities, including anti-inflammatory and antioxidant activities. It is also able to inhibit different enzymatic activities, such as HIV-1 integrase, nuclear factor-kappaB activation and p300-specific HAT/factor acetyltransferase activity [114], [115], [116]. In the latter case, curcumin could inhibit the acetylation of HIV-Tat protein in vitro by p300 as well as proliferation of the virus, as revealed by the repression in syncytia formation upon curcumin treatment in Sup T1 cells [116]. An acetone extract of Helichrysum italicum spp. microphyllum yielded phloroglucinol α-pyrene arzanol (50), a potent NF-kappaB inhibitor [117]. It also inhibited HIV-1 replication in T cells and the release of pro-inflammatory cytokines in LPS-stimulated primary monocytes. This compound is worthwhile investigating for developing a complementary anti-HIV strategy, targeting both viral and cellular factors [4]. From Clerodendron trichotomum, several phenylpropanoid glycosides were isolated and evaluated for their anti-HIV-1 integrase activity [118]. Acteoside (51) and an acteoside isomer (52) showed the highest activity with IC_{50} values of 7.8 and 13.7 μM, respectively. A new dimeric lactone, ardidemin digallate (53) was isolated from the whole plants of Ardisia japonica and inhibited HIV-1 and HIV-2 RNase in vitro with IC_{50} values in the low micromolar concentrations [119].

Proteins
Proteins from higher plants active against HIV include ribosome-inactivating proteins (RIPs) and lectins [120]. RIPs are RNA N-glycosidases; they inactivate ribosomes through a site-specific deamination of the large ribosomal RNA [121]. A high number of RIPs have been identified in plants belonging to various families, particularly Caryophyllaceae, Sambucaceae, Cucurbitaceae, Euphorbiaceae, Phytolaccaceae and Poaceae [122]. For some RIPs, sensitisation and IgE induction have been demonstrated, so their allergenic and cross-reactive potential should be considered when applying them in therapy [123].

MAP30 is a plant protein with a molecular weight of 30 kDa isolated from Momordica charantia with anti-tumour and anti-HIV properties [124], [125]. MAP30 was active against tumour-transformed or HIV-infected cells, while it showed no adverse effects on normal cells. In addition to its RNA N-glycosidase activity, MAP30 acted as a DNA glycosylase/apurinic lyase [126]. This may explain its apparent inhibition of HIV-1 integrase by rendering the HIV LTR an unsuitable substrate for HIV integrase as well as DNA gyrase. The DNA glycosylase/apurinic lyase activity of MAP30 [126] and other RIPs [127] suggested that the anti-HIV activity of RIPs was independent of their ribosome inactivation activity. Indeed, endopeptidase digestion of MAP30 and GAP31 resulted in the generation of peptide fragments with full antiviral and antitumour activity [128]. These fragments remained fully active in HIV integrase inhibition and HIV-LTR topological inactivation, but not ribosome inactivation. Therefore, it could be concluded that the antiviral and antitumour activities of MAP30 and GAP31 are independent of their ribosome inactivation activity.

Trichosanthin from the root tuber of Trichosanthes kirilowii was the first RIP found to inhibit HIV in vitro [129]. Clinical trials with trichosanthin showed that it induced anaphylactic reactions in AIDS patients after i.v. administration [130]. In order to reduce its antigenicity, the seven C-terminal amino acid residues were deleted, which resulted in a 2.7-fold decrease in antigenicity, but a 10-fold reduction in in vitro ribosome inactivation [131]. A recent study demonstrated that trichosanthin was more effective in inducing apoptosis in HIV-1 infected cells, which can explain partially the antiviral action [132]. Several plant RIPs, such as agrostin, gelonin, luffin, α-momorcharin, β-momorcharin, saporin, and trichosanthin were evaluated as inhibitors of HIV-1 replication [133]. They exhibited a very weak suppressive effect on HIV-1 reverse transcriptase and HIV-1 protease, but apart from agrostin, all RIPs strongly inhibited HIV-1 integrase. However, it remains to be elucidated whether interference with integrase is the key mechanism for the anti-HIV activity of RIPs.

The glycoproteins gp120 and gp41 are present on the envelope of HIV and mediate the entry of the virus into host cells. Both gp120 and gp41 are heavily glycosylated, surrounding the receptor-binding regions. It is estimated that gp120 consists of N-linked glycans for almost 50% of its molecular weight [134]. The glycans of HIV-1 gp120 consist to 33% of the high-mannose type, 4% of the hybrid type and 63% of the complex type, the latter being predominantly glucosylated and/or sialylated. Carbohydrate-binding agents (CBAs) specifically targeting HIV-1 glycan shields efficiently inhibit HIV infection and prevent virus entry into target cells. In contrast to other existing anti-HIV agents, resistance development of HIV against CBAs may allow efficient immunological suppression of virus replication and virus clearance from the systemic circulation because of the exposure of previously hidden immunogenic epitopes on gp120. They may therefore represent the first available drugs for which chemotherapy may act in concert with an immunological response [135], [136]. Synergistic activity between CBAs and 1-deoxymannojirimycin was recently described [137]. The (α1,2)-mannosidase I inhibitor 1-deoxymannojirimycin was found to potentiate the inhibitory activity of CBAs against wild-type HIV-1. In cell cultures infected with mutant HIV-1, strains containing N-glycan deletions in the gp120 envelope rendered the mutant virus susceptible to the inhibitory activity of 1-deoxymannojirimycin. Moreover, it was able to partially reverse the phenotypic resistance of CBAs to the mutant virus strains. CBAs are almost exclusively of protein nature and can be divided into at least seven distinct groups of molecules depending on their origin: prokaryotes, sea corals, algae, fungi, higher plants, insectivores, and vertebrates [138], [139]. In this review, the (α1–3)- and (α1–6)-mannose- and N-acetylgalactosamine (GlcNAc)-specific plant lectins and the cyanobacterial cyanovirin-N will be discussed in detail.

Plant lectins are the largest group of plant proteins with biological activities, including antimicrobial activity, immunostimulation/repression and anti-HIV activity [120]. Lectins are proteins bearing a non-catalytic domain that binds irreversibly to specific carbohydrates, normally through a monosaccharide-specific mechanism. Most plant lectins with HIV activity are derived from the monocot families Alliaceae, Amaryllidaceae and Orchidaceae or the dicot families Cercropiaceae, Fabaceae, Moraceae and Urticaceae. The vast majority of anti-HIV active plant lectins are directed against mannose oligomers. Two extensively studied mannose-specific plant lectins are Galanthus nivalis agglutinin (GNA) and Hippeastrum hybrid agglutinin (HHA). Recently, a higher inhibitory activity of HHA compared to GNA...
was demonstrated for HIV adsorption to the epithelial cell line HEC-1A. HIV transcytosis through HEC-1A cell line monolayer, HIV adsorption to monocyte-derived dendritic cells (MDDC), and HIV transfer from MDDC to T cells [140].

Systemic use of plant lectins to inhibit HIV infection may be questionable due to their antigenic (immunogenic) properties and short plasma half-life. However, local (intravaginal) application as a gel or cream formulation may avoid these disadvantages and may open novel perspectives to develop plant lectins as microbicides. Therefore, Balzarini et al. investigated GNA and HHA for their potential as microbicides [141], [142]. Both proteins inhibited a wide variety of HIV-1 and -2 strains and clinical isolates in different cell types. Short exposure of the lectins to cell-free virus particles or persistently HIV-infected HUT-78 cells markedly decreased HIV infectivity and increased the microbical activity of the plant lectins. Selection of HIV-1 strains with different levels of resistance to the two mannose-specific lectins showed that there was no cross-resistance to any other HIV entry inhibitor, including T-20 and cyanovirin [142]. They also exhibited desirable properties for formulation studies, such as stability at high temperatures and low pH for prolonged time periods, odorless and tasteless, and they can be easily formulated in gel preparations [141].

The only GlcNAc-specific plant lectin (Urtica dioica agglutinin or UDA) with a pronounced anti–HIV activity was isolated from Urtica dioica. It is an 8.5-kDa monomeric protein having two carbohydrate-binding sites with different affinities and ranks among the smallest plant lectins. It inhibited HIV-1 and -2-induced cytopathogenicity and syncytium formation of HIV-infected HUT-78 cells and CD4+ MOLT-4 cells [143]. In contrast to the mannose-binding proteins, which had a 50- to 100-fold decreased antiviral activity as the UDA-exposed mutant viruses, UDA only showed a slightly lower antiviral activity, even against those mutant virus strains lacking about 40% of the glycosylation sites in their gp120 envelope [135]. UDA was free of toxicity when given intravenously to mice at doses up to 25 mg/kg body weight. It has also been reported that UDA at high concentrations did not agglutinate human red blood cells in cell culture and was poorly mitogenic.

Another interesting lectin was isolated from the rhizomes of Polygonatum cyrtonema Hua and was 10- to 100-fold more potent than other tested CBAs including GNA, while it was about 10-fold less toxic than GNA in MT-4 and CEM cells [144]. In contrast to other lectins from the Liliaceae family, it exhibited a higher affinity for both mannose and sialic acid. Lectins from Phaseolus vulgaris, Momordica charantia, and Ricinus communis were able to inhibit HIV-1 reverse transcriptase [145], while lectins from Myrianthus holstii [146] showed an inhibitory activity against the HIV envelope protein gp120. Two plant proteins, MRK29 [147] and ginkobilobin [148], have been isolated from the fruits of Momordica charantia and the seeds of Ginkgo biloba, respectively. Both compounds inhibited HIV-1 reverse transcriptase, but MRK29 was about hundred times more active.

Cyanovirin-N, originally isolated from an aqueous extract of the cyanobacterium Nostoc ellipsosporum, is the first prokaryotic mannos-specific lectin with a potent anti-HIV activity [149], [150]. This protein consists of a single chain containing 101 residues and its amino acid sequence shows obvious duplication. The protein is highly resistant to degradation and shows no loss of structural integrity or antiviral activity after treatment with detergents, denaturants, organic solvents, freezing and heating up to 100 °C [149]. Until now, cyanovirin-N is the only CBA for which efficacy and safety was demonstrated in a chimeric simian immunodeficiency virus (SIV)/HIV-1 virus infection in monkey studies when applied intravaginally or rectally as a topical microbicide [151], [152]. By exposing HIV-1 infected CEM cell cultures to increasing concentrations of cyanovirin-N, a total of eight different amino acid mutations exclusively located at N-glycosylation sites in the envelope surface gp120 were observed. The extent of the decrease of antiviral activity against the mutated virus strains was markedly less pronounced than observed for the (α1,3)- and (α1,6)-mannose-specific plant lectins, Hippeastrum hybrid agglutinin and Galanthus nivalis agglutinin, pointing to the existence of a higher genetic barrier for cyanovirin-N [138]. Whereas the antiviral and in vitro antiproliferative activity of cyanovirin-N can be efficiently reversed by mannane, its pronounced mitogenic activity on peripheral blood mononuclear cells remained unaffected. Therefore, careful monitoring of potential side effects should be required if applied as a microbicial drug. Nowadays, studies are ongoing to express and release cyanovirin-N in commensal lactobacilli or Streptococcus gordonii to create a microbicidal environment in the vaginal ecology [153], [154], [155]. Other algal lectins with a significant anti-HIV activity are stevorin, Microcystis viridis lectin, and griffithsin [156], [157], [158].

Quinones/Xanthones

From several plants, new and known xanthones were isolated and evaluated for their anti-HIV activity [79], [159], [160], [161]. In general, most xanthones showed a weak or moderate activity against HIV, mostly due to their toxicity.

Tannins

The vegetable tannins can be divided into two classes: hydrolysable and non-hydrolysable or condensed tannins [162]. The first group consists of polymers of gallic and hexahydroxydiphenic acids (gallotannins and ellagittannins, respectively). The condensed tannins are oligomers and polymers composed of flavan-3-ol moieties, commonly referred to as proanthocyanidins [163].

A proanthocyanidin polymer fraction (MW 1500–2000 Da) from Cupressus sempervirens showed antiviral activity against the retroviruses HIV and HTLV IIIB (IC50 values from 1.5 to 15 μg/mL and 5 to 25 μg/mL, respectively) [164]. Epigallocatechin (4β-8,2′→6,3′)-epicatechin (54) inhibited HIV-1 protease at 70 μg/mL, but proanthocyanidin A5 (55) was not active up to a concentration of 100 μg/mL (Fig. 7) [165]. However, a structure-anti-HIV-1 activity relationship study of a series of proanthocyanidol oligomers showed that proanthocyanidin A5 was the most interesting compound with an SI of 24 [166]. Proanthocyanidin A5, (56), which only differs from proanthocyanidin A5 in its terminal (+)-catechin unit, only had an SI of 10, which is still larger than procyandin with a single linkage (the B-series). The catechins and theflavins are two groups of natural polyphenols found in green and black tea. In a comparative study, the theflavin derivatives showed the highest anti-HIV-1 activity [167]. Tea polyphenols with a galloyl moiety were more active than those without a galloyl moiety and the number of galloyl groups was correlated with the anti-HIV-1 activity. Theflavin 3,3′-digallate (57) inhibited HIV-1 replication, HIV-1-mediated virus-cell fusion and cell-cell fusion, and gp41 six-helix bundle formation at low micromolar concentrations. Computer-aided molecular docking studies indicated that theflavin 3,3′-
Digallate may fit in the hydrophobic pocket with its phenyl groups interacting with the hydrophobic residues in the pocket. Out of six hydrolysable tannins, two ellagitannins, corilagin (58) and repandusinic acid (59), inhibited HIV-1 protease (IC₅₀ values 20.7 μM and 12.5 μM, respectively), which indicates the importance of the hexahydroxydiphenoyl unit [67]. From a gallotannin-containing fraction of *Phyllanthus amarus*, the isolated ellagitannins geraniin and corilagin were shown to be the most active antiretroviral compounds [168]. These tannins blocked the interaction of HIV-1 gp120 with its primary cellular receptor CD4 and inhibited the enzymes integrase, reverse transcriptase and protease at low μg/mL concentrations.

Terpenes
Some sesquiterpenes isolated from *Litsea verticillata*, collected in Vietnam, inhibited HIV-1 replication with IC₅₀ values ranging from 38.1 to 91.0 μM, but the most active compound was a butenolide, 3-epi-litsenolide D₂ (60) with an IC₅₀ of 9.9 μM (Fig. 8) [169]. Several diterpenes from plants showed anti-HIV activity [170], [171], [172]. The diterpene 8,10,18-trihydroxy-2,6-dolabelladiene (61), a constituent of the Brazilian brown alga *Dictyota paffii*, was found to inhibit HIV-1 reverse transcriptase, with an IC₅₀ value of 16.5 μM. It inhibited HIV-1 replication (IC₅₀ 8.4 μM), and blocked HIV-1 infection in macrophages with IC₅₀ values below 2 μM. It was suggested that this compound also blocked HIV-1 replication at a post-transcriptional step [173], [174]. HIV-1 reverse transcriptase-inhibiting diterpenes were also obtained from the alga *Dictyota menstrualis* [175].

Various triterpenes have been shown to exhibit anti-HIV activity. Two prostanoids, garciosaterpenes A and C, inhibited HIV-1 reverse transcriptase, and were active in the syncytium assay with an IC₅₀ of 5.8 and 37.0 μg/mL, respectively, but with a low SI (3.4 and 1.9, respectively) [176]. The limonoids limonin and nomilin were found to inhibit HIV-1 replication in several cell systems used, e.g., PBMC isolated from healthy donors and infected with HIV-1 after incubation with limonin and nomilin; relatively large IC₅₀ values of 60.0 and 52.2 μM were obtained [177]. An unusual triterpene lactone, lancilactone C (62) from the stems and roots of *Kadsura lancilimba* inhibited HIV-1 replication (IC₅₀ 1.4 μg/mL) with a SI of more than 71.4 [178]. A trinorcoycloartane triterpenoid, lancifodilactone H, and the A ring secocycloartane triterpenoids, lancifolic acid A and nigranoic acid, from *Schisandra lancifolia*, showed weak anti-HIV-1 activity, with IC₅₀ values of 16.6, 16.2 and 10.3 μg/mL, and CC₅₀ values of > 200, 104.9 and 88 μg/mL, respectively [179].

Anti-HIV triterpenes can be classified into five different categories according to their target and mechanism of action: entry inhibitors (by blocking virus adsorption of membrane fusion), reverse transcriptase inhibitors, protease inhibitors, virus maturation inhibitors, and products with an unknown mechanism of action [180]. Research on triterpenes has mainly focused on derivatives of oleanolic acid, betulinic acid and ursolic acid. The parent compound oleanolic acid inhibited HIV-1 replication in hu-
man peripheral mononuclear cells (IC_{50} values: 22.7 – 24.6 μM) and monocyte/macrophages (IC_{50} value: 57.4 μM) [181]. Olea-nolic acid inhibited HIV-1 protease activity in vitro. Because of the structural resemblance of oleanolic and betulinic acid, and because derivatisation of the OH group in position 3 with an ester functionality strongly increased the anti-HIV activity of betulinic acid [182], semi-synthetic oleanolic acid derivatives with different ester groups at C-3 were prepared [183]. Oleanolic acid 3-O-3′,3′-dimethyl succinate (63) was the most potent inhibitor of HIV, with an IC_{50} value of 0.5 ng/mL, and a very high SI of 22 400. In contrast, 3-O-(3′,3′-dimethyl succinyl)-ursolic acid displayed only a weak anti-HIV activity (IC_{50} 2.1 μM, SI 23.6) [184]. 3′-O-[3′,3′-dimethyl succinyl]-betulinic acid was found to be a maturation inhibitor. It is responsible for disruption of the late-stage viral maturation processes of the Gag protein, resulting in a defective core structure around the viral RNA, and a non-infectious virus. This compound was the first member of a new class of anti-HIV drug candidates [185], [186]. Esterification of both the C-3 hydroxy and the C-28 carboxylic acid functionalities of betulinic acid resulted in even more potent compounds; e.g., the di-O-dimethyl succinyl derivative showed an IC_{50} Value of 0.87 nM and an SI of 42 400 [182], [187], [188].

In a structure-activity relationship study on betulinic acid derivatives, RPR103611 (64), a statin derivative, was found to be inactive against HIV-1 protease, reverse transcriptase, and integrase, but it acted as a fusion inhibitor [189], [190]. More recently, it was suggested that its antiviral activity was dependent on the stability of the gp120/gp41 complex [199]. Gp120 was proposed as the primary target for the anti-HIV activity of a steriosomer of RPR103611, IC95 64 (65) [192]. Both compounds appeared to be equally potent in their anti-HIV-1 and anti-fusion activities. However, the drug development process of RPR103611 was stopped due to its poor pharmacodynamic properties [193]. The combination of a 3,3-dimethyl succinyl side chain at C-3 and an aminoalkanoid side chain at C-28 resulted in very active bi-functional anti-HIV compounds with EC_{50} values in the nanomolar range. They showed antifusion activity as well as maturation inhibition [194], [195].

Conclusion

This review clearly shows that the field of plant-derived compounds for chemotherapy of HIV is still booming. Many constit- uents have been isolated, identified and evaluated in vitro for anti-HIV activity, but in vivo studies are still scarce. It is only through carefully designed and conducted clinical trials with the purified active substance that the efficacy and safety of the compound can be unequivocally established. Therefore, it will be interesting to see if any of these putative anti-HIV compounds will ever reach the patient.

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