Plant Substances as Anti-HIV Agents Selected According to Their Putative Mechanism of Action[⊥]

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Despite the continuous advances made in antiretroviral combination therapy, AIDS has become the leading cause of death in Africa and the fourth worldwide. Today, many research groups are exploring the biodiversity of the plant kingdom to find new and better anti-HIV drugs with novel mechanisms of action. In this review, plant substances showing a promising anti-HIV activity are discussed according to the viral targets with which they interact. Most of these compounds, however, interfere with early steps in the HIV replication, such as the virus entry steps and the viral enzymes reverse transcriptase and integrase, whereas until now almost no plant compounds have been found to interact with the many other viral targets. Since some plant substances are known to modulate several cellular factors, such as NF-kappa B and TNF-α, which are also involved in the replication of HIV, their role as potential anti-HIV products is also discussed. In conclusion, several plant-derived antiviral agents are good candidates to be further studied for their potential in the systemic therapy and/or prophylaxis of HIV infections, most probably in combination with other anti-HIV drugs.

Introduction

In 1928, Alexander Fleming discovered by serendipity the first antibiotic penicillin, and many other antibiotics have been developed since. In contrast, with the exception of amantadine for the treatment of influenza A virus infection, the search for antiviral chemotherapeutics only started in earnest in the 1970s. During the past decade, we have witnessed a remarkable growth in the development of antiviral therapies for human immunodeficiency virus (HIV), herpes simplex virus (HSV), cytomegalovirus (CMV), hepatitis B virus (HBV), and influenza virus infections.[1,2] However, there is a constant medical need for the development of new antiviral drugs, since these drugs are not always efficacious or well-tolerated and drug-resistance is rapidly emerging. Most of the current antivirals have been discovered through in vitro screening of small molecular weight chemical molecules. In that respect, additional chemical diversity can be included with products from natural sources, such as fungi, marine fauna and flora, bacteria, and plants. Research on antiviral natural products is mainly focused on plants, since, among other reasons, they can be selected on the basis of their ethnomedicinal use.[3,4] The importance of this strategy is clearly demonstrated by the increasing number of reviews on plant products as antiviral agents.[5−11]

Antiviral research has been focused on compounds that interfere with various parts of the viral life cycle. For example, most of the current anti-HIV drugs are targeted against proteins encoded by the virus itself, i.e., reverse transcriptase (RT) and protease. However, since several cellular factors are assumed to be involved in the replication of HIV or in HIV pathogenesis, it would seem logical to develop a complementary anti-HIV strategy, targeting both viral and cellular factors. Due to their amazing structural diversity and their broad range of biological activities, plant substances should be explored further as a source for a complementary anti-HIV strategy. In any case, the currently available antiretroviral combination therapies have certainly improved the quality of life for HIV-infected people in developed countries, but their high cost and limited availability exclude patients in developing countries to benefit from these combination therapies. Considering that about 80% of people living in developing countries rely almost completely on traditional medicinal practices for their primary health care,[12] it would seem logical that substances from higher plants should be considered and become part of a potential complementary strategy against HIV disease.

AIDS and Antiretroviral Therapy

Acquired immunodeficiency syndrome (AIDS), which is caused by HIV, is an immunosuppressive disease that results in life-threatening opportunistic infections and malignancies. First reported in 1981 in the United States, AIDS has become a major worldwide epidemic. The United Nations Program on AIDS (UNAIDS) estimates that at the end of 2002 nearly 42 million will have died of AIDS. During 2002, about 3 million people became infected. AIDS is presently the leading cause of death in Africa and the fourth leading cause of death worldwide.

HIV is a member of the lentivirus family of animal retroviruses. Retroviruses carry their genome as RNA, packaged in a protein capsid and surrounded by a lipid envelope. One of the proteins encoded by the retroviral genome is the enzyme RT, which is responsible for the synthesis of a complementary DNA molecule, using viral RNA as a template. Of the two known HIV types, HIV-1 is most pathogenic and causes over 99% of HIV infections, while HIV-2 is much less pathogenic and is endemic in West Africa.

Anti-HIV compounds can be classified into different groups according to their activity on the replicative cycle of HIV. The HIV life cycle can be roughly divided into 10

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Table 1. List of Antiviral Drugs Approved for the Treatment of HIV Infections[a]

antiviral drug	trade name	mechanism of action
zidovudine or AZT	Retrovir	NRTI
didanosine or ddI	Videx	NRTI
zalcitabine or ddC	Hivid	NRTI
stavudine or d4T	Zerit	NRTI
lamivudine or 3TC	Epivir	NRTI
abacavir or ABC	Ziagen	NRTI
tenofovir	Viread	NRTI
nevirapine	Viramune	NNRTI
delavirdine	Rescriptor	NNRTI
efavirenz	Sustiva	NNRTI
saquinavir	Invirase, Fortovase	PI
ritonavir	Norvir	PI
indinavir	Crixivan	PI
nelfinavir	Viracept	PI
amprenavir	Agenerase	PI
lopinavir	Kaletra[b]	PI
enfuvirtide	Fuzeon	FI

[a] The drugs are classified into four groups according to their mechanism of action: (1) nucleoside reverse transcriptase inhibitors (NRTIs), (2) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (3) protease inhibitors (PIs), and (4) fusion inhibitors (FIs). [b] In combination with ritonavir.

different steps, i.e., virus−cell adsorption, virus−cell fusion, uncoating, reverse transcription, integration, DNA replication, transcription, translation, budding (assembly/release), and maturation.[13] Twenty years after the identification of HIV-1 as the causative agent for AIDS, an impressive array of antivirals have been developed and marketed (Table 1). All of these drugs can be classified into four groups, i.e., nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), HIV protease inhibitors (PIs), and HIV fusion inhibitors. NRTIs and NNRTIs inhibit RT, but they are targeted at the substrate (dNTP) and allosteric nonsubstrate 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. A HIV fusion inhibitor prevents virus−cell fusion and thus infection of target cells. Combination therapies of at least three antiviral drugs, also known as the highly active antiretroviral therapy (HAART), have resulted in spectacular reductions of plasma virus levels, often leading to a remarkable improvement in quality of life.

Plant Substances Acting on Viral Targets

Important advances were made in the field of plant-derived anti-HIV agents, thereby focusing on their in vitro anti-HIV activity as well as on their mechanism of action. In this chapter, some plant substances with an interesting anti-HIV activity will be discussed according to the viral target(s) with which they interact (Table 2). No attempts are made to cover all plant substances that have been reported to possess anti-HIV activity. For such a review, the reader is referred to refs 5−7.

Virus Entry. HIV is an enveloped virus and its envelope protein complex (Env) controls the key process of viral entry.[14] Env is a complex composed of a transmembrane gp41 subunit and a noncovalently associated surface gp120 subunit. Infection is initiated by the binding of the virion gp120 Env protein to the CD4 molecule present on some T-cells, macrophages, and microglial cells. This interaction induces a conformational change that promotes secondary gp120 binding to the co-receptor CCR5 or CXCR4. Both co-receptors are members of the chemokine receptor family,

Table 2. Potential Anti-HIV Targets for Plant-Derived Substances[a]

compound	entry	RT[b]	integrase	transcription
betulinic acid derivatives	**X**			
calanolides/inophyllums		**X**		
DCQA/DCTA[c] (chicoric acid)	X		X	
flavonoids	X	X	X	X
mannose-specific plant lectins	**X**			
sulfated polysaccharides	**X**			X
trichosanthin, MAP30, GAP31			X	

[a] The main mechanism of action is indicated in bold. [b] RT = reverse transcriptase. [c] DCQA/DCTA = dicaffeoylquinic acid/dicaffeoyltartaric acid.

but CCR5 is the co-receptor for HIV-1 strains that infect macrophages (M-tropic or R5 strains), while CXCR4 is the co-receptor for HIV-1 strains that infect T-cells (T-tropic or X4 strains). Subsequently, gp41 undergoes conformational changes that mediate fusion of the viral membrane with the target cell membrane. HIV entry is therefore a complex process involving multiple protein interactions, each of which can become a potential target for the development of new HIV entry blockers. This strategy has already resulted in the world's first FDA approval of a HIV fusion inhibitor, named T-20 or enfuvirtide. T-20 is a synthetic, 36-amino acid peptide that blocks viral/cellular membrane fusion triggered by gp41 and suppresses viral proliferation.[15] Unfortunately, this peptide fusion inhibitor lacks oral bioavailability, so that two injections per day, which cause important local side effects, are needed. Moreover, the cost of synthesizing large peptides is very high, which excludes the use in developing countries.

Sulfated polysaccharides are well-known and potent inhibitors of HIV-1 and -2 replication in vitro.[16,17] Anti-HIV activity of several plant extracts has been attributed to the presence of these polysaccharides.[18−20] The most interesting ones are of nonplant origin and include heparin (**1**), dextran (**2**) and dextrin sulfate (**3**), pentosan polysulfate (**4**), and mannan sulfate (**5**). They all exhibit the following unique properties: (1) a broad-spectrum antiviral activity against enveloped viruses, such as HIV and HSV; (2) low induction of viral resistance in vitro, (3) inhibition of virus adsorption to the cells; and (4) inhibition of syncytium (giant cell) formation between HIV-infected and normal $CD4^+$ T-cells. Syncytium formation may play an important role in the depletion of $CD4^+$ T-cells in AIDS patients. Sulfated polysaccharides exert their anti-HIV activity by shielding off the positively charged amino acids in the V3 loop of the viral envelope glycoprotein gp120.[17] The V3 loop is necessary for virus attachment to cell surface heparan sulfate, a primary binding site, before more specific binding occurs to the CD4 receptor of $CD4^+$ cells. In a dextran sulfate-resistant HIV-1 strain, it was suggested that the resistance is located in the *env* genome of HIV and specifically in the V3 loop domain.[21] It should be emphasized that sulfated polymers owe their anti-HIV activity to the presence of the sulfated groups, which are responsible for the inhibition of virus−cell binding. Consequently, the sugar backbone is not essential, since anti-HIV activity has also been demonstrated for sulfated polymers, such as poly(vinyl alcohol) sulfate or PVAS.[16]

More than 10 years ago, the in vivo effectiveness of oral and intravenous administration of dextran sulfate to HIV-infected persons was found to be disappointing[22,23] and was correlated with its poor oral bioavailability, short plasma half-life, partial inactivation by plasma components, and

poor cell permeation ability.[16] However, a recent study using agarose gel electrophoresis technique demonstrated that dextran sulfate was absorbed rapidly in humans after oral administration and can be found in plasma, lymphocytes, and urine.[24] In an open phase-I/II dose-escalation study on six AIDS patients, ip administration of dextrin 2-sulfate caused a significant decrease in viral load.[25] In conclusion, one should consider their potential application in the (systemic) prophylaxis of HIV following an accidental needle stick injury or stab wound, i.e., conditions in which AZT has proved inefficacious and/or topical prophylaxis of HIV infection contracted through sexual intercourse.[11] Additionally, the development of drug delivery systems such as liposomes may improve its therapeutic efficacy.

The mannose-specific plant lectins, e.g., from *Cymbidium* hybrid, *Epipactis helleborine*, *Hippeastrum* hybrid, and *Listeria ovata*, and the *N*-acetylglucosamine-specific plant lectins, e.g., from *Urtica dioica*, have been found to inhibit HIV-1 and -2 infection at similar or even lower concentrations than dextran sulfate.[7,26] These plant lectins belong to a large group of bioactive plant proteins bearing a noncatalytic domain that binds irreversibly to specific carbohydrates, normally through a monosaccharide-specific mechanism.[27] They likely interfere with the virus−cell fusion process, and it is assumed that mannose- and *N*-acetylglucosamine-specific plant lectins interact with specific glycosylation sites within the viral glycoproteins gp120 and/or gp41, particularly those sites that are rich in mannose or *N*-acetylglucose.

Recent research on triterpenoids has been focused on oleanolic acid (**6**), betulinic acid (**7**), ursolic acid (**8**), and their derivatives. It was demonstrated that all three triterpenes inhibited HIV-1 protease activity in vitro.[28,29] In a structure−activity relationship study, a large number of betulinic acid derivatives were synthesized, and one compound, named RPR103611 (**9**), was selected for further mechanistic studies.[30,31] RPR103611, a statin derivative, was found to be inactive against HIV-1 protease, RT, and integrase, but it was demonstrated to be a fusion inhibitor.[30] More recently, it was suggested that its antiviral activity was dependent on the stability of the gp120/gp41 complex.[32] For one of the stereoisomers of RPR103611, named IC9564 (**10**), gp120 was proposed as the primary target for its anti-HIV activity.[33] Both compounds appeared to be equally potent in their anti-HIV-1 and antifusion activities. The drug development process of RPR103611 was stopped due to its poor pharmacodynamic properties.[34] Therefore, further research is needed to improve this class of compounds in this regard. In contrast to carbohydrates, flavonoids can inhibit several critical steps of the HIV life cycle. Flavanones with an OH group at position C-3′, such as taxifolin (**11**), inhibit viral protease, RT, CD4/gp120 interaction by binding to the V3 loop of gp120, and bind to nonspecific proteins. Flavanones lacking an OH group at position C-3′, such as aromadendrin (**12**), are more specific in their antiviral activity and inhibit CD4/gp120 interaction, but do not inhibit viral protease or RT.[35] Another example of this nonspecific anti-HIV-1 activity was shown for (−)-epigallocatechin 3-*O*-gallate (**13**), which exhibited a destructive effect of virus particles and post-adsorption entry and inhibited viral protease and RT.[36] Flavonoids are well-known inhibitors of numerous enzymes, and this also applies for enzymes that are essential for HIV replication, such as RT,[37] viral protease,[38] and integrase.[39]

Reverse Transcription. Once HIV enters the cell, the enzymes within the nucleoprotein complex are activated and begin the viral reproductive cycle. The nucleoprotein core of the virus becomes disrupted, and the RNA genome of HIV is transcribed into a double-stranded DNA form by viral RT. The HIV-1-RT controls three consecutive functions, i.e., RNA transcriptase to DNA, degradation of RNA template by RN-ase H, and duplication of the remaining DNA strand. Since RNA-directed DNA synthesis does not occur in uninfected cells, RT activity is considered as one of the most important targets for antiretroviral substances.

One of the most exciting and advanced projects on natural products as potential anti-HIV agents encompasses the 4-propyldipyranocoumarins or calanolides. They were isolated by an anti-HIV bioassay-guided fractionation of an extract of the tropical rainforest tree *Calophyllum lanigerum* Miq. var. *austrocoriaceum*.[40] *Calophyllum* coumarins can be classified into three groups according to the C-4 substituent on the lactone ring of the coumarin: calanolides, inophyllums, and cordatolides are substituted at position C-4 with respectively *n*-propyl, phenyl, and methyl groups.[41] Structure−activity relationship studies revealed the importance of methyl groups at C-10 and C-11 and a hydrogen bond acceptor at C-12 for the anti-HIV-1 activity.[42−44] In the case of calanolides, the C-12 hydroxyl group should be *S* configured, while the C-12 hydroxyl of inophyllums can be either *S* or *R* configured.[45] (+)-Calanolide A (**14**) was about 50 times more active on HIV-1 RT inhibition compared to cordatolides A (**15**) and B (**16**), indicating the importance of the substituent at C-4.[46] Because of its potent activity against HIV-1 RT, its unique sensitivity profile to NNRTI-resistant viruses,[47−49] and its synergistic effect with other anti-HIV drugs,[48,50] (+)-calanolide A was retained as an attractive candidate for therapeutic use.

It has become increasingly apparent that the calanolides, including (+)-calanolide A, represent a novel and distinct subgroup of the NNRTI family and that they may be useful in combination therapy with other anti-HIV drugs. The major difference between NNRTIs and (+)-calanolide involves their activities against viruses with Y181C-mutated RT, indicating that both compounds bind to RT in a mechanistically different fashion.[47] Kinetic analysis suggested that (+)-calanolide binds to two sites on the RT.[49] (−)-Calanolide B (costatolide) (**17**) and (−)-dihydrocalanolide B (dihydrocostatolide) (**18**) both showed a sensitivity/resistance profile similar to that of (+)-calanolide A for viruses with Y188H or Y181C amino acid changes in their RT.[48] In an in vitro anti-HIV-1 study, a synergistic three-drug combination for the calanolides was observed with lamivudine and nelfinavir.[50] Synergy with AZT was also demonstrated in a hollow fiber mouse model.[51]

Currently, (+)-calanolide A is being subjected to clinical trials to evaluate its safety and pharmacokinetics in both healthy and HIV-infected volunteers.[52,53] In healthy volunteers, the toxicity of (+)-calanolide A after oral administration was minimal, and the most reported adverse effects were dizziness, oily aftertaste, headache, and nausea.[53] The C_{max} and AUC increased dose-proportionally, indicating linear pharmacokinetics.[52,53] No drug accumulation was seen over the entire dosing period, although (+)-calanolide A did have a relatively long elimination half-life (15−20 h). It must be emphasized that with respect to the pharmacokinetic parameters, the intrasubject variability was high.[53] Sarawak MediChem Pharmaceuticals, a 50/50 joint venture between the State Government of Sarawak (Malaysia) and Woodbridge, IL, based Advanced Life Sciences, will initiate a clinical study to evaluate the calanolide A combination therapy in HIV-infected individuals.

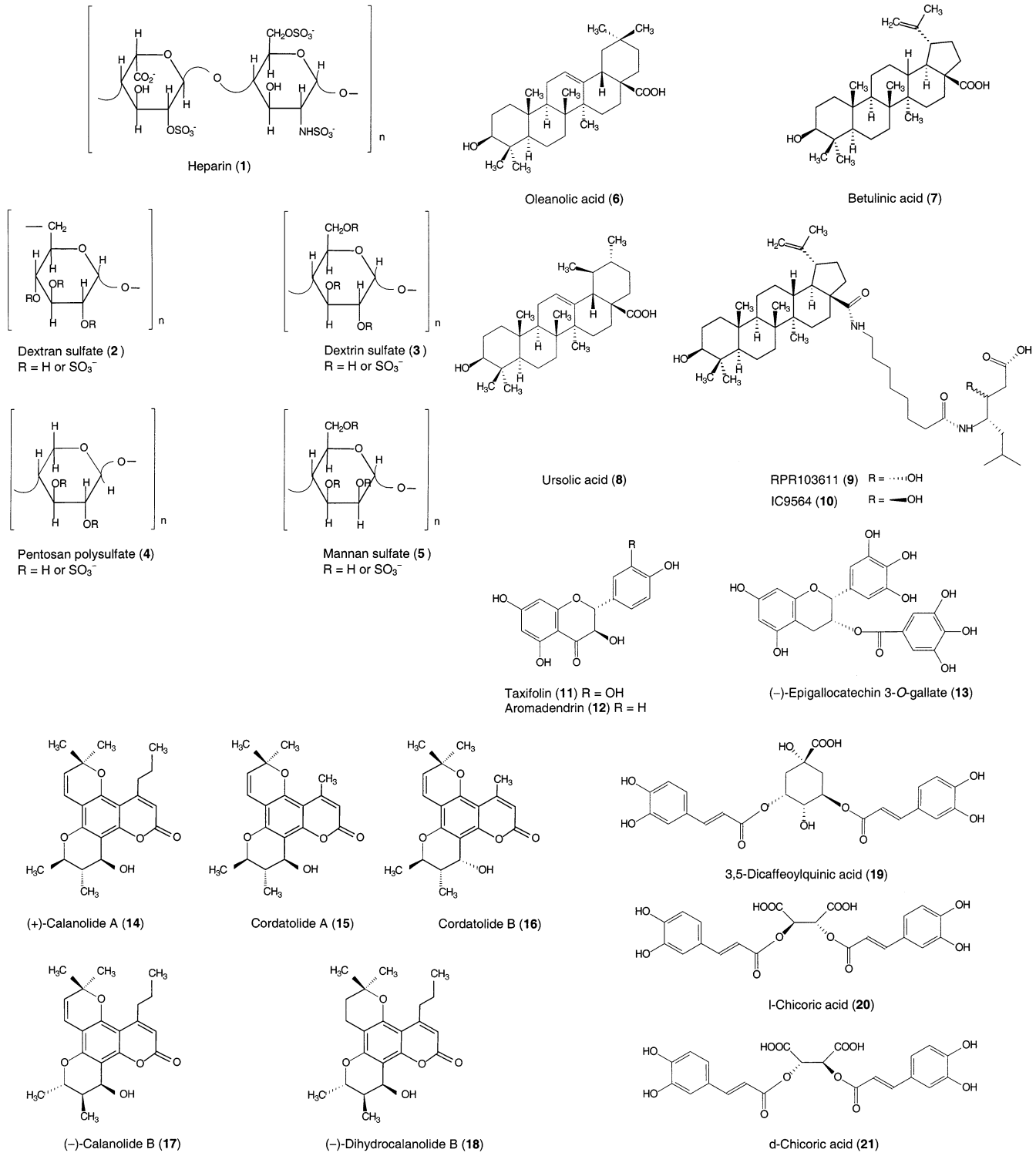

Figure 1. Chemical structures (**1**−**21**) of plant-derived anti-HIV compounds acting on viral targets.

Integration. Integrase is the only enzyme other than RT and protease that is encoded by HIV-1. Integrase catalyzes the integration of viral DNA into host DNA in two steps: 3′-processing and strand transfer. First, integrase cleaves the last two nucleotides from each 3′-end of the linear viral DNA. Second, the subsequent DNA strand transfer reaction involves the nucleophilic attack of these 3′-ends on host chromosomal DNA. Since integrase-negative mutants of HIV do not produce infectious virus particles and no cellular homologue of HIV integrase has been described, integrase is considered as an attractive target. However, in contrast to RT and protease, not a single integrase inhibitor has yet entered the anti-HIV drug market. This challenge is related to the structural and functional complexity of the integration process and the limitations of the available in vitro assays.[54]

Recently, there was considerable debate about the actual anti-HIV-1 mechanism of dicaffeoylquinic acids (DCQAs) and dicaffeoyltartaric acids (DCTAs). One research group suggested that those biphenolic depsides targeted the HIV-1 integrase.[55−57] In enzymatic assays, the DCQAs, such as 3,5-dicaffeoylquinic acid (**19**), and DCTAs, such as l-chicoric acid (**20**), demonstrated a 10- to 100-fold higher preference for inhibition of HIV integrase than of HIV RT.[55]

Of all the bis-catechols tested, 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 independent of divalent cations.[56] A HIV-1 mutant containing a single glycine-to-serine substitution at position 140 of integrase displayed resistance to l-chicoric acid, indicating that the compound is likely to interact at residues near the catalytic triad in the integrase active site.[57] However, this proposed mechanism of action was recently questioned.[58] HIV strains that were made 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 virus strains that were resistant toward polyanionic compounds, such as dextran sulfate.[58] Consequently, the primary anti-HIV target of l-chicoric acid and its analogues would be the envelope glycoprotein gp120. Nevertheless, a great number of DCQA and DCTA analogues were synthesized and evaluated as HIV-1 integrase inhibitors.[59−63] Structure−activity relationship studies on these synthesized compounds demonstrated that l-chicoric acid and D-chicoric acid (**21**) exhibited similar anti-HIV-1 integrase activity, and removal of one or both of its carboxylic groups did not result in a significantly lower integrase inhibitory activity.[61] The bis-catechol moieties were essential to obtain a high inhibitory activity of integrase,[62] but blockage of these catechol groups through conversion to tetraacetate esters had only a minor negative effect on the inhibition of HIV integrase.[61]

Another group of plant compounds thought to act as an integrase inhibitor are the ribosome-inactivating proteins (RIPs).[27] RIPs are RNA *N*-glycosidases that inactivate ribosomes through a site-specific deadenylation of the large ribosomal RNA.[64] Recently, MAP30, a plant protein of 30 kDa isolated from *Momordica charantia* L., received a lot of attention because of its potent antitumor and anti-HIV potential.[65,66] Moreover, MAP30 toxicity is specific to tumor-transformed or HIV-infected cells, while it shows no adverse effects on normal cells. Besides its RNA *N*-glycosidase activity, it was also shown that MAP30 acts like a DNA glycosylase/apurinic lyase.[67] The latter activity 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. Consequently, this newly discovered DNA glycosylase/apurinic lyase activity of MAP30[67] and other RIPs[68] suggested that RIPs have anti-HIV activity independent of their ribosome inactivation activity. This was confirmed in a study where endopeptidase digestion of MAP30 and GAP31 resulted in the generation of peptide fragments with full antiviral and antitumor activity.[69] In addition, these fragments remained fully active in HIV integrase inhibition and HIV-LTR topological inactivation, but not ribosome inactivation. This study demonstrated clearly that the antiviral and antitumor activities of MAP30 and GAP31 are independent of their ribosome inactivation activity.

Trichosanthin, which is a single-chain RIP isolated from the root tubers of *Trichosanthes kirilowii* Maxim and is used as a traditional Chinese medicine to induce abortion and to treat choriocarcinoma in the People's Republic of China, was the first RIP found to inhibit HIV in vitro.[70] During clinical trials with trichosanthin, mild to severe anaphylactic side effects were encountered.[71] To reduce the antigenicity of trichosanthin, the last seven *C*-terminal amino acid residues of trichosanthin were deleted, resulting in a 2.7-fold decrease in antigenicity, but also a 10-fold reduction in in vitro ribosome-inactivation activity.[72] Several plant RIPs, including agrostin, gelonin, luffin, α-momorcharin, *β*-momorcharin, saporin, and trichosanthin, were examined for their ability to inhibit HIV-1 replication.[73] All these RIPs exhibited a very weak suppressive effect on HIV-1 RT and HIV-1 protease, while, with the exception of agrostin, all the RIPs strongly inhibited HIV-1 integrase. Some recent studies indicate that the anti-HIV activity of trichosanthin is not entirely dependent on its ribosome-inactivating activity.[74,75] However, whether interference with integrase is the key mechanism for the anti-HIV activity of RIPs remains to be determined.

Transcription. After integration of viral DNA into the host genome, HIV-1 DNA transcription is low until levels of its trans-activator protein Tat increase. Tat, which is an 86-amino acid polypeptide, acts by binding to the trans-activation response element (TAR) at the 5′-end of the viral mRNA, increasing thereby the elongation capacity of host RNA polymerase.[76] The Tat protein can be released from HIV-1-infected cells and enter new cells in an active form, where it may stimulate the transcriptional activity of HIV-1 long terminal repeat (LTR).[77]

Pentosan polysulfate (**4**)[78] and heparin (**1**)[79] may also exhibit anti-HIV-1 activity through inhibition of Tat activity, while selective 2-*O*-, 6-*O*-, or *N*-desulfation of heparin prevented Tat−heparin interaction. A series of sulfated derivatives of dextrin were also able to inhibit HIV-1 Tat, whereas the unsulfated dextrin did not inhibit HIV-1 Tat.[80] This is not surprising, because of the highly cationic character of the Tat basic domain.

A Japanese research group demonstrated that tannic acid repressed 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced HIV promoter activity and found a putative tannic acid-responsive element between positions 133 and 104 of the HIV promoter.[81] Furthermore, they evaluated a number of natural compounds related to tannins for their HIV promoter suppression effects.[82] This structure−activity relationship study revealed that 3-phenylcoumarins, isoflavones, and chalcones suppressed TPA-induced HIV promoter activity more effectively than tannic acid, while flavonoids and acetogenins had no suppressive effect.

Plant Substances Acting on Cellular Targets

As discussed above, plant substances can act as virus-specific inhibitors, targeting the standard viral targets, such as RT and integrase. In general, they possess a high antiviral activity and a low cytotoxicity, and thus a high selectivity index. The main disadvantages of virus-specific inhibitors are their narrow antiviral spectrum and their property to induce drug-resistance.[83] In contrast, inhibitors targeting cellular factors that affect viral replication exhibit a broad antiviral spectrum and a low risk for drug-resistance. A major concern is that the target is critical for some cellular functions, whereby long-term modulation could cause mechanism-based toxicity.[84] In this review, plant substances acting on these cellular targets will be discussed, but without dealing with immunomodulating plant substances.

Inhibitors of Cellular Transcription Factors (NF-*κ*B and Sp1). The transcription factor NF-*κ*B has been shown to play a determinant role in inducing genes involved in a wide range of diseases, including asthma, atherosclerosis, rheumatoid arthritis, and AIDS.[85] NF-*κ*B is typically a heterodimer that consists of p65 (Rel A) and p50 monomeric proteins. NF-*κ*B may be various heterodimers or homodimers. The mammalian NF-*κ*B/Rel family includes five members: p65 or RelA, RelB, c-Rel,

NF-κB1 (p50/p105), and NF-κB2 (p52/p100).[86] They are all characterized by a conserved stretch of 300 amino acids, called the Rel homology domain (RHD). This domain is essential for dimerization and binding to DNA, but it also serves as interaction surface for the inhibitory protein IκB. The IκB protein family includes IκBα, IκB*β*, IκB*γ*/p105, IκB*δ*/p100, IκB*ϵ*, and Bcl3. The IκB proteins bind with different affinities and specificities to NF-κB dimers. Consequently, besides different NF-κB dimers in a specific cell type, there are also a large number of combinations between IκB and NF-κB dimers, illustrating the sophistication of the system.[87]

In unstimulated cells, NF-κB dimers are retained in the cytoplasm in an inactive form as a consequence of their association with IκB. After cell stimulation, NF-κB is released from IκB and migrates into the nucleus, where it induces gene expression after DNA binding. Potent inducers of NF-κB include IL-1, tumor necrosis factor (TNF), bacterial lipopolysaccharides, viruses such as HIV-1, reactive oxygen species (ROS), and UV- and *γ*-irradiation.[88]

The HIV-1 LTR contains two NF-κB binding sites, followed by three tandem Sp1-binding sites and a TATA box sequence.[89] Activation of HIV-1 expression via the NF-κB pathway is a well-characterized aspect of viral regulation. Furthermore, cooperative interaction between NF-κB and other elements, especially Sp1, may be required for full proviral expression.[88] Therefore, compounds that interact with NF-κB and/or Sp1 may become promising anti-HIV-1 compounds. This review will focus only on plant-derived inhibitors of NF-κB having an additional anti-HIV activity. For a detailed review on NF-κB modulators from natural sources the reader is referred to the excellent review article by Bremner and Heinrich.[90] The most-studied group of plant-derived NF-κB inhibitors are the sesquiterpene lactones.[90] However, to the best of our knowledge, no potent anti-HIV activity up to now has been reported for these sesquiterpene lactones.

The biscoclaurine alkaloid, cepharanthine (**22**), isolated from *Stephania cepharantha* Kayata, possesses antiinflammatory, antiallergic, and immunomodulatory activities in vivo.[91,92] Since several inflammatory cytokines affect the progression and pathogenesis of HIV-1 infection, the inhibitory effects of cepharanthine on TNF-α and TPA-induced HIV-1 replication in chronically infected monocytic and T lymphocytic cell lines were evaluated. It was found that cepharanthine is a potent inhibitor of HIV-1 in the monocytic cell line, but not in the T lymphocytic cell line. Also cepharanthine suppressed HIV-1 LTR-driven gene expression through inhibition of NF-κB activation.[93]

Curcumin (**23**), which is a yellow pigment isolated from the rhizome of the well-known spice *Curcuma longa* L., exhibits a variety of pharmacological effects, including anti-inflammatory and antiretroviral activities. Curcumin has been reported to prevent HIV-1 replication by inhibiting HIV-1 integrase[94] and protease.[95] However, this inhibition occurs only at high and thus irrelevant concentrations in vivo. In contrast, curcumin is a potent inhibitor of TNF-induced NF-κB activation.[96] Similar results were obtained for the caffeic acid phenethyl ester (CAPE) (**24**), which is an active component of some forms of propolis from honeybee hives. It has antiviral, anticarcinogenic, anti-inflammatory, and immunomodulatory properties. CAPE specifically inhibited NF-κB activation induced by different agents, such as TNF, phorbol ester, H_2O_2, okadaic acid, and ceramide.[97] Synthesis of structural analogues showed that the 5,6-dihydroxy form was the most potent inhibitor, whereas the 6,7-dihydroxy variant was the least active.[97]

The plant lignan 3′-*O*-methylnordihydroguaiaretic acid (**25**), isolated from *Larrea tridentata* L., was able to suppress HIV-1 replication by blocking the promoter activity of HIV-1 LTR.[98] Gel mobility-shift studies revealed that it did not affect NF-κB binding but did strongly inhibit Sp1 binding.

Inhibitors of Cytokine Production (TNF-α and IL-6). As HIV directly infects cells of the immune system, triggering a strong immune response, and HIV-infected cells produce an enormous amount of virions, HIV infection leads to chronic activation of the immune system.[99] This activation is intimately linked to cytokine secretion, by which several HIV-inducing cytokines, such as TNF-α, IL-1*β*, and IL-6, are overexpressed in lymphoid tissues of HIV-1-positive individuals.[100] As discussed before, TNF-α is a potent inducer of HIV replication through NF-κB activation. Compounds that block these HIV-inducing cytokines may inhibit HIV replication. However, it must be emphasized that the level of HIV replication in patients reflects a balance between stimulatory and inhibitory cytokines. Consequently, therapeutic strategies affecting cytokines and their receptors should be applied with great caution and oversimplification must be avoided.[99] Nevertheless, these new strategies can have a realistic potential and should therefore be further investigated.

Potential targets for modulation of TNF production and function by natural products can be classified into three groups: (1) inhibition of TNF production and secretion; (2) TNF receptor antagonism; and (3) inhibition of TNF function through modulation of its signal transduction pathway(s).[101] Except for the TNF receptor antagonist activity, the two other activities are mainly associated with NF-κB activation. Other targets for natural products to inhibit TNF production are kinase enzymes and the cAMP system. A recent study on citrus flavonoids demonstrated that several polymethoxylated flavonoids inhibited the release of TNF-α from monocytes in vitro.[102] Elevation of cAMP through inhibition of cAMP cleavage by phosphodiesterase enzymes was proposed as the mechanism of action, although other effects could not be ruled out.[102] Since protein tyrosine kinase is essential for the activation of NF-κB by various agents, kinase inhibitors can inhibit TNF release. This strategy was demonstrated both in vitro and in vivo for the protein tyrosine kinase inhibitor genistein[103] (**26**) and the nonselective kinase inhibitor staurosporine[104] (**27**).

Antioxidants. Reactive oxygen species (ROS) is a collective term for oxygen-derived species, namely, oxygen radicals, such as $O_2^{\bullet -}$ and NO•, and certain nonradicals, such as H_2O_2 and $^1O_2$, that are oxidizing agents and/or easily converted into radicals.[105] A free radical is defined as any species capable of independent existence and containing one or more unpaired electrons. All aerobic organisms possess an antioxidant defense system to protect against ROS-mediated injury. In healthy individuals, the production of ROS is balanced with the antioxidant defense system. Oxidative stress results from the imbalance between ROS production and inactivation. Oxidative stress has been implicated in a wide variety of human disorders, such as cancer, Parkinson's disease, and AIDS.[105] An increasing number of studies supports the theory that oxidative stress is involved in the progression of HIV disease.[106−108] Tissue levels of antioxidants, including reduced glutathione (GSH), ascorbic acid, α-tocopherol, and selenium have been shown to be depleted in HIV-positive persons.[109] Furthermore, increased levels of products of lipid peroxidation, such as malondialdehyde,[106] and of

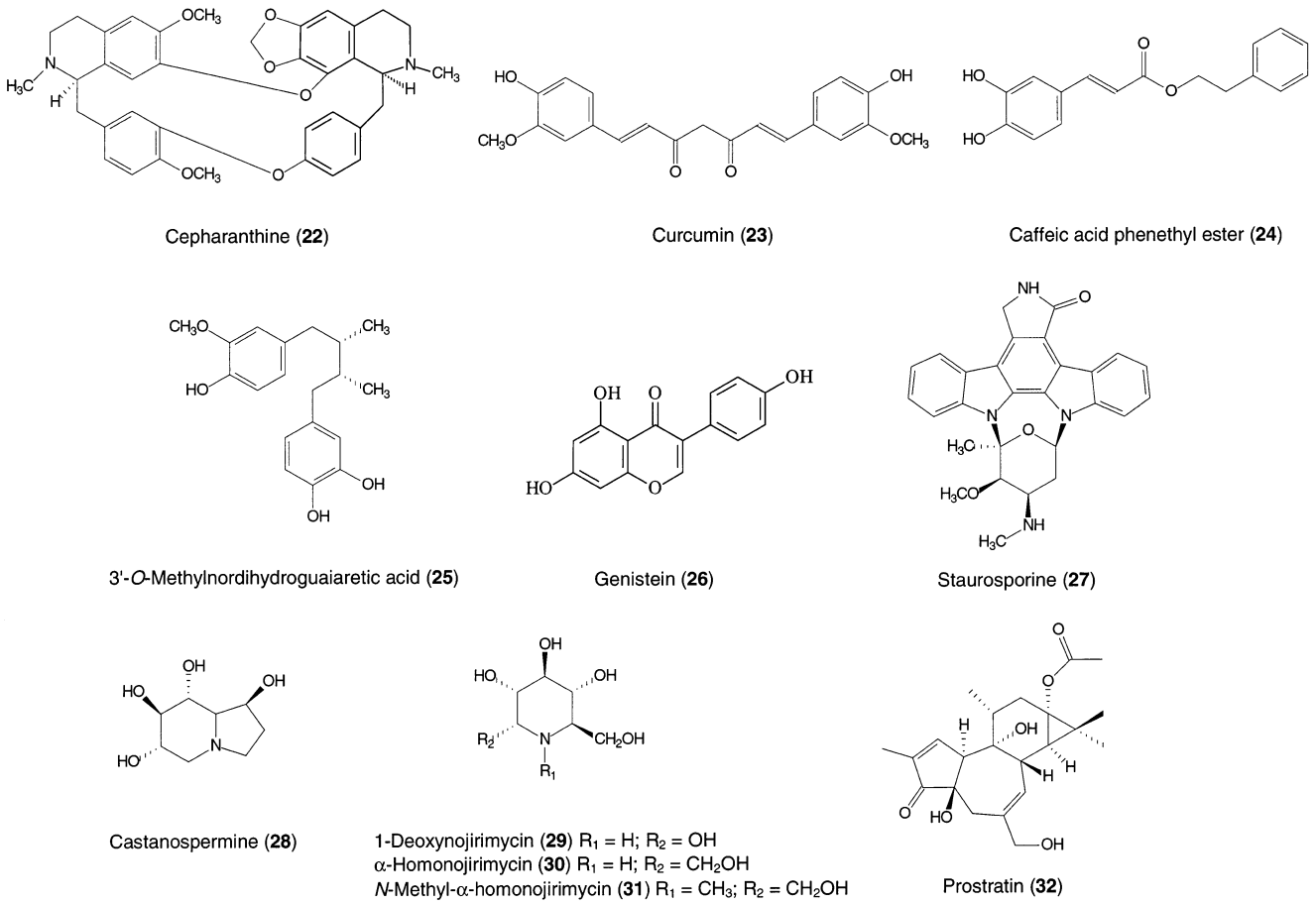

Figure 2. Chemical structures (**22**−**32**) of plant-derived anti-HIV compounds acting on cellular targets.

oxidative DNA damage, such as 8-hydroxyguanine,[110] were observed in HIV-positive persons.

The role of oxidative stress in HIV disease appears to be quite broad and may involve alterations in viral replication, immune function, and apoptosis. The nuclear transcription factor NF-κB, which plays a critical role in HIV-1 replication by activation of HIV-1 transcription, is activated by ROS and inhibited by antioxidants. The antioxidant GSH is a major intracellular thiol and is thought to inhibit NF-κB activation.[111] Apoptosis or programmed cell death may contribute to the depletion of $CD4^+$ T-cells in HIV-positive individuals.[112] Apoptosis is a form of programmed cell death that is characterized by cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation, and formation of apoptotic bodies. Hydrogen peroxide and TNF-α have been shown to induce apoptosis in HIV-infected cells in vitro, while antioxidants such as *N*-acetylcysteine (NAC) were shown to inhibit apoptosis.[113,114]

Despite the compelling evidence on the presence of oxidative stress in HIV-infected individuals, investigations on the role of plant-derived antioxidants, such as flavonoids and proanthocyanidins, on the progression of HIV disease are still scarce. It is evident that more studies are needed to evaluate their role in HIV infection.

Polyphenolic compounds, such as flavonoids[115] and proanthocyanidins,[116] are well-known for their antioxidant properties, but may have limited in vivo potential by their relatively low oral availability. In a recent study by our research group, several well-known plant-derived antioxidants were evaluated for their in vitro antioxidant profile, and procyanidin C1 was classified as the most promising antioxidant.[117] In addition, several other studies on proanthocyanidins reported interesting apoptotic-modulating and NF-κB-inhibiting activities.[116] Although proanthocyanidins are known to bind nonspecifically to proteins, a selective activity was demonstrated on NF-κB-dependent gene expression, indicating the importance of degree of polymerization in determining their activity. Furthermore, a limited number of studies suggested that the oral absorption of proanthocyanidins is limited to those with a low polymerization degree and/or their metabolites. These results question the general statement that proanthocyanidins are nonspecific inhibitors that cannot be absorbed and should therefore be removed during plant extraction.

In summary, oxidative stress caused by HIV infection may accelerate the progression of HIV disease. However, this may not imply that administration of antioxidants could slow the progression of HIV disease for several reasons. First, an important, but often neglected prerequisite to evaluate the in vivo effect of antioxidants is the pharmacological monitoring of oxidative stress. Second, excessive antioxidant protection could lean over the balance from oxidative stress to "oxidative deficit". The latter is also deleterious, since low levels of ROS might have beneficial biological effects, e.g., in phagocytosis. Third, ROS, antioxidants, transcription factors, and cytokines are part of a large human defense network that behaves like a black box, but which is essential for human life. For all those reasons, controlled clinical trials in which oxidative stress is measured and softened by administering antioxidants will be needed to determine the clinical significance of oxidative stress on HIV disease progression. For example, a dietary intervention with antioxidants could be an inexpensive

contributing intervention to the existing HIV treatment strategies (HAART).

Cellular Enzymes. α-Glucosidase inhibitors inhibit the glycosylation of gp160 within the endoplasmic reticulum, thereby preventing the formation of infectious virus particles.[118] A number of alkaloids including indolizidines, such as castanospermine (**28**), and piperidines, such as 1-deoxynojirimycin (**29**), are capable of inhibiting N-linked oligosaccharide processing and are also found to inhibit HIV replication.[119,120] Since there is a good correlation between anti-HIV potency and α-glucosidase I inhibitory activity, it is presumed that their anti-HIV activity is related to their inhibition of α-glucosidase I.[121] In a recent study, a series of natural epimers of α-homonojirimycin and its *N*-alkylated derivatives have been isolated and synthesized and tested for their inhibitory activity on both HIV and α-glucosidase I.[122] Interestingly, it was demonstrated that α-homonojirimycin (**30**) and *N*-methyl-α-homonojirimycin (**31**) were more potent inhibitors of α-glucosidase I than 1-deoxynojirimycin or castanospermine, but only the two latter compounds showed a significant anti-HIV-1 activity. These results indicate that inhibition of HIV by glucosidase inhibitors can be due to factors other than simply inhibition of α-glucosidase I. A further study suggested that 1-deoxynojirimycin blocked HIV envelope glycoprotein-mediated membrane fusion at the CXCR4 binding step.[123]

Another important enzyme for HIV replication is casein kinase II, which is a cAMP-, cGMP-, and Ca^{2+}/phospholipid-independent serine/threonine protein kinase that phosphorylates several HIV-1 structural proteins. Several anti-HIV-1 flavonoids, including quercetin, chrysin, and (−)-epigallocatechin gallate, showed inhibitory activity against casein kinase II.[124−126] However, further research is needed to fully understand the biological significance of casein kinase II in HIV-1 replication and its inhibition by flavonoids.

Others. From the Samoan medicinal plant *Homolanthus nutans*, prostratin (**32**), which is a member of the class of phorbol esters, was isolated. Prostratin inhibited in vitro HIV-1 replication, but unlike many phorbol esters, it showed no tumor-promoting activity. It did not exert its anti-HIV activity through inhibition of the viral enzymes RT and protease, nor did it inhibit the binding of gp120 to CD4.[127] It was suggested that the anti-HIV-1 activity of prostratin involves an interaction with protein kinase C. However, recent research is mainly focused on its ability to activate HIV-1 replication in latently infected cells.[128,129] Despite HAART, viral reservoirs can persist, resulting in a viral rebound when HAART is ceased. In a recent study, prostratin induced HIV-1 expression within cells of patients following HAART. The final goal of this potential inductive adjuvant therapy for HAART is to eliminate persistent viral reservoirs in HIV-infected persons. In another study, 12-deoxyphorbol 13-phenylacetate, which is a non-tumor-promoting phorbol ester isolated from *Euphorbia poissonii*, induced HIV-1 gene expression in latently infected T-cells at concentrations 20- to 40-fold lower than prostratin.[130] Notwithstanding these promising data, further research is needed to evaluate in humans its efficacy and safety.

Conclusions

Despite the protracted efforts to stop the AIDS epidemic, AIDS has become the leading cause of death in Africa and the fourth one worldwide. The current treatment options rely on a combination therapy of at least three antivirals. These small molecular weight chemical molecules are targeted at two viral enzymes (RT and protease) and the virus−cell fusion process. The main problem of the current drugs is their diminishing effectiveness as the virus develops resistance.

Therefore, as shown in this review, many research groups are exploring the biodiversity of the plant kingdom to find new and better anti-HIV drugs. However, no plant substances or even semisynthetic plant-derived molecules have yet been approved for the treatment of HIV infections. In other disease areas, plant-derived molecules have reached the market. For example, the antimalarial artemisinins, originally isolated from *Artemisia annua* L., are now available to the patient as artemether and artesunate.[131] Furthermore, artemisinins have served as a template for the synthesis of new trioxane antimalarials.[132]

As for chemical compounds, the obstacles for plant-derived molecules to pass through preclinical and clinical trials and to market as an anti-HIV drug are numerous. A potent in vitro antiviral activity and a low cytotoxicity, thus a high selectivity index, may not guarantee an in vivo successful compound. First, due to the chronic nature of the disease, safety evaluation and tolerability are very important in assessing the therapeutic potential of new antiretrovirals. Second, pharmacokinetic studies providing information on the absorption, distribution, metabolism, and excretion have hampered the further development of several chemical and plant-derived compounds. In the anticipation that current treatment therapies with their high pill burden would be applicable in the future, parenteral administration could never be used due to difficulties in patient compliance, especially in developing countries. For example, the clinical application of dextran sulfate is hampered by its low oral bioavailability. In addition to the classical problems of pure chemical entities in drug development, there are also some obstacles specific to whole plant and partially purified plant extracts. A principal requirement is the need to guarantee consistent supplies of high-quality standardized plant extracts. In that respect, particular attention should be given to impurities or contaminants, such as pesticides, heavy metals, pathogenic microbes, and aflatoxins. Batch-to-batch consistency and specification are primary requirements within the currently existing regulatory guidelines on natural products. At present, there is still a lack of adequate guidance on the batch consistence requirement and acceptance criteria. Adapting this regulatory issue to complex mixtures would already contribute a lot to move forward promising herbal medicinal products into the development pipeline.

Despite all these setbacks and obstacles, research on anti-HIV plant substances should undoubtedly be encouraged. First, only 5−15% of the approximately 250 000 higher plants has been systematically investigated for the presence of bioactive compounds.[3] Second, plant compounds can also serve as lead compounds for chemical modification in order to increase their therapeutic potential. For example, several betulinic acid derivatives were synthesized and showed a significantly higher selectivity index. Third, due to their amazing structural diversity, they may support the discovery and/or validation of new drug targets.

Besides the viral targets, research on host cellular factors that affect HIV-1 replication has recently gained a lot of momentum. Progress has been made on determining their role in HIV-induced pathogenesis. In contrast to compounds acting on viral targets, they would have the advantage of exhibiting a broad antiviral activity and, more importantly, a low risk for drug-resistance. A major concern may be their mechanism-based toxicity. As cellular factors are linked to each other, therapeutic strategies affecting

these cellular factors may not be simple and should be applied with considerable caution. Nevertheless, these new strategies can have a realistic potential and should therefore be further investigated. Since several plant substances are known to modulate these cellular factors, they can certainly contribute to this research.

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