Abstract. Acquired immune deficiency syndrome (AIDS) caused by infection with the human immunodeficiency virus (HIV) represents a major health problem worldwide, especially in developing countries. Combinational anti-HIV therapy has largely improved patients’ lives, but is not widely available in developing countries. Moreover, drug resistance is a main problem during HIV treatment. Therefore, there is a continuous need for new drugs effective against otherwise drug-resistant HIV strains. Chemical compounds from natural sources provide a large variety of potential anti-HIV compounds. The most promising natural products are discussed in this review.

Even though the number of new human immunodeficiency virus (HIV) infections is decreasing, acquired immune deficiency syndrome (AIDS) is still one of the leading causes of deaths worldwide. In 2007, an estimated 33.2 million people globally were infected with AIDS. The virus represents a major threat in developing countries, where over 95% of all infected people worldwide are living (1). In Africa, AIDS remains the main cause of death. Sub-Saharan Africa is most rigorously affected, with over two-thirds of the population infected with HIV. Even though there is still no cure for AIDS, advances in HIV treatment have improved patients’ quality of life and prolonged their lives (2). However, the high cost of treatment is a major problem in developing countries. Additionally, there are several limitations to standard HIV therapy such as drug resistance and dose-limiting side-effects. Therefore, medicinal plants and their chemical constituents with activity against HIV have come into the focus of virus research.

HIV and Standard Anti-HIV Therapy

HIV infects CD4+ T-lymphocytes, resulting in their depletion and in subsequent immunodeficiency. The CD4 molecule was the first and principal retroviral receptor identified (4). Binding of the virus to the CD4 receptor via gp120 represents the initial step of host cell infection. However, for membrane fusion and virus entry, additional binding of gp120 to a co-receptor is necessary. M-tropic viruses generally use the CCR5 chemokine co-receptor (“R5” virus), while T-tropic viruses usually bind to the chemokine CXCR4 receptor (“X4” virus). Receptor binding induces a conformational change of the gp120 protein and subsequently a change in the gp41 protein, the latter being responsible for fusion of the virus with the host cell membrane (5). Following membrane fusion, the viral core is released into the host cytoplasm. The virus mRNA is then reverse transcribed into cDNA by the viral enzyme reverse transcriptase (RT). This enzyme also synthesizes the complementary DNA strand and exerts RNAse activity. The double-stranded DNA is translocated into the nucleus, where its integration into the host genome is catalyzed by the viral enzyme integrase (IN) (6). Inside the host genome, the so-called provirus can remain latent for a long period of time. Once transcribed, both spliced and
Drug-resistant mutations arise and accumulate frequently in the HIV genome. This is mainly due to two reasons: (a) the lack of proof-reading activity of the RT, resulting in a high mutation rate during virus replication (about $3.4 \times 10^{-5}$ mutations per bp per replication cycle) (12) and (b) the high replication rate of HIV (13). All current anti-HIV treatments eventually lead to drug-resistant mutants, which is one of the main reasons for therapy failure (14). As drug resistance is a major problem in anti-HIV therapy, mutations leading to resistance have been widely investigated and mutation profiles for the currently approved drugs have been developed. For a comprehensive review of mutation profiles, the reader is referred to Johnson et al. (15). Moreover, cross resistance to drugs of the same class is a major problem, as illustrated by thymidine analogue-associated mutations (TAMs). TAMs are selected by thymidine analogues such as zidovudine and stavudine and lead to cross-resistance to all currently approved NRTIs (16, 17). In NNRTI-associated mutations, cross-resistance to drugs of the same class frequently emerges by the simple occurrence of only one mutation at codon 103 or 166, respectively.

Three or more mutations are often required for PI-resistant virus particles, which is not exclusively found in the gp120 gene (enfuvirtide) (9) or block the CCR5 co-receptor (maraviroc) (10). The latter drug is only active against R5 viruses as X4 viruses bind to the CXCR4 co-receptor (maraviroc). The first IN inhibitor, raltegravir, was only recently approved by the FDA in October 2007 (11).

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Natural Products
A vast number of natural products from medicinal plants such as terpenoids, coumarins, alkaloids, polyphenols, tannins and flavonoids, have been shown to possess anti-HIV activity. Some of these natural products have already entered clinical trials. In this review, we focused on the most promising small molecules (Figure 1); macromolecules, such as proteins and polysaccharides, were not considered. Criteria for inclusion of natural products or their derivates were: (a) an effective concentration (EC)50 value below 1 μM and known
mechanism of action and/or (b) promising in vivo activity or clinical trial data. For further details, the reader is referred to several recent reviews (18-21).

Betulinic acid (1) is a triterpene isolated from Syzygium claviflorum and many other plants. Betulinic acid was shown to inhibit HIV replication in vitro with an EC\textsubscript{50} of 1.4 μM (22). Modifications of betulinic acid lead to even more potent derivatives. Structural modifications at the C3 position of betulinic acid showed that an ester group at this position is important for anti-HIV activity (21). The most promising of these derivatives, 3-O-(3'3'-dimethylsuccinyl)betulinic acid (DSB, bevirimat, PA-457) exhibits remarkable anti-HIV activity with an EC\textsubscript{50} value of 0.35 nM (21). Bevirimat also showed promising reduction of viral load and a promising safety profile in several clinical studies (23). A double-blind, randomized phase IIa study showed significant reduction of the viral load in HIV-infected patients treated with bevirimat compared to a placebo group when administered orally (in solution) and daily for ten days at a dose of 100 or 200 mg. Viral load decreased by about 90%. Preliminary results of a currently ongoing phase IIb study also showed a significant overall viral load reduction in drug-resistant patients failing standard HIV therapy. However, reduction of viral load was less than expected. A reduction of virus load was observed in bevirimat-treated patients by application of a daily tablet dose of 400 mg compared to placebo. The bevirimat plasma concentrations were also lower than expected, which may be due to galenic properties of the tablets used (23).

Anti-HIV activity of bevirimat is mediated through maturation inhibition. Maturation of the virus involves cleavage of the Gag and GagPol precursor proteins into the mature Gag proteins and the viral enzymes. Bevirimat was shown to block cleavage of the capsid protein precursor (p25) into the mature capsid protein (p24). This is a unique mechanism of action compared to any other approved HIV drug so far. This might explain why bevirimat is also active against strains which are resistant to other currently approved drugs (23, 24).

Various calanolides, isolated from Calophyllum langerium reveal anti-HIV-1 activity (25). The most promising compound, calanolide A (2), is an NNRTI with EC\textsubscript{50} values between 0.02 and 0.5 μM. In contrast to currently approved NNRTIs, which inhibit RT non-competitively, calanolide A’s mechanism of RT inhibition is at least partly competitive (26). Calanolide A interacts with RT at two sites: the active site and presumably the pyrophosphate-binding site of the enzyme (27). The compound is active against a broad range of HIV-1 strains, including otherwise drug-resistant variants (28). Phase 1a clinical trials with calanolide A have been performed and have shown the compound to be safe and well tolerated at oral doses between 200 and 800 mg (28). A phase Ib study in HIV-infected, therapy-naïve patients showed a significant mean viral load reduction with oral dosage of 600 mg calanolide A bid (twice daily) (29). Phase II studies are ongoing (21). Making it an even more interesting candidate for therapeutic use, calanolide A also showed synergistic effects with other anti-HIV drugs (30).

(→)Epigallocatechin gallate (EGCG, 3) is a flavonoid isolated from the green tea plant Camellia sinensis. EGCG inhibits several steps in the HIV life cycle such as post-adsorption entry and protease kinetics (31). Most remarkable is its IC\textsubscript{50} value of 0.01-0.02 μg/ml for inhibition of the viral RT (32). Moreover, EGCG binds with high affinity to CD4+ T-cells and inhibits binding of gp120 to the CD4+ cells at a physiologically relevant concentration of 0.2 μM (33). Besides antiviral activities, EGCG also has antiinflammatory promoting, antiinflammatory and antioxidative activities (31).

Glycyrrhizin (GL, 4), a terpenoid from the roots of Glycyrrhiza glabra, improved the CD4+ T-cell count in AIDS patients (34). The CD4+ T-cell count was significantly higher (243.6/1) in a group treated with glycyrrhizin and HAART compared to a group of patients receiving HAART only (170.8). In vitro, GL had an IC\textsubscript{50} of 0.15 μM (18). Its action seems to be mediated through inhibition of virus binding to the host cell and inhibition of protein kinase C (PKC) (35). Additionally, GL has affinity to HIV surface proteins (36).

Andrographolide (5) is a diterpenoid lactone isolated from Andrographis paniculata. In a phase I clinical trial, andrographolide caused a significant rise in CD4+ T-cells from 405 cells/ml to 501 cells/ml in HIV-positive patients at a dosage of 10 mg/kg (7). However, the viral load did not decline significantly in patients and the trial was discontinued due to severe side-effects. Side-effects such as headache, fatigue and altered sense of taste were mild to moderate, but one HIV-positive individual developed an anaphylactic reaction during the clinical trial. Andrographolide probably acts through inhibition of the dysregulation of the cell cycle in HIV infected cells.

Polycitona A (6), an aromatic alkaloid isolated from marine Polycitor sp., inhibits RT of wild-type and some resistant HIV-1 strains as well as of HIV-2 strains. Polycitona A has a low IC\textsubscript{50} of 245 nM for RNA-directed DNA polymerase function of RT and an IC\textsubscript{50} of 470 nM for DNA-directed DNA-polymerase function. The mechanism for inhibition of DNA-polymerase activity of HIV-1 RT has been investigated (38). These studies proposed polycitona A to be an allosteric RT inhibitor which interferes with DNA primer extension and also inhibits formation of the RT-DNA complex. Notably, polycitona A has five hydroxyl groups, which seem to be important for its inhibitory activity. Inhibition of the RT-DNA complex formation is an anti-HIV mechanism not used by any currently approved drug. Therefore, polycitona A is an interesting model compound for drug development.

Geriannin (7) was isolated from Phyllanthus amarus. Geraniin inhibits HIV-1 replication in MT-4 cells (EC\textsubscript{50}=0.22 μM). This natural product acts through two
different mechanisms. First, it acts as a competitive RT inhibitor. Second, it inhibits viral uptake. This makes geraniin interesting for therapeutic use, since inhibiting the viral life cycle at two different sites presumably slows down the emergence of resistant mutants. Additionally, geraniin has also been reported to inhibit several viral strains resistant to currently approved NRTIs and NNRTIs (39).

3'R,4'R-di-O-(-)-camphanyloyl-(-)-cis-khellactone (DCK) (8), a derivative of suksdorfin, a pyranocoumarin isolated from Lomatium suksdorfii, demonstrated promising anti-HIV activity in vitro (EC₅₀ of 0.049 μM) (40). Several modifications of DCK were performed to further increase potency and bioavailability (40). In silico studies, investigating the binding mode of DCK analogs, suggested a mechanism of action that is similar to other RT inhibitors (41). Previous studies, however, did not show inhibition of viral enzymes or RT and proposed that DCK inhibits HIV at a point after virus entry and before integration into the host DNA (40).

**Conclusion**

The swift emergence of resistant mutants, an enormous problem during HIV treatment, limits the use of currently approved drugs and continuously calls for new anti-HIV drugs. Access to conventional drugs is rather limited in developing countries, even though a vast majority of HIV-infected people live there. Natural products and their derivatives provide an excellent source for new anti-HIV drugs. Since many natural products are present in medicinal plants, which can be abundantly found in developing countries, a straightforward idea might be to propose administration of natural anti-HIV products in forms of tea or by direct consumption of the plant by patients in these countries. However, there are several limitations to this kind of application. First, concentration of the compound may not be high enough for its anti-HIV activity in a tea extract or even in the plant itself. Second, bioavailability may not be sufficient after oral uptake and serum concentrations may be too low for anti-viral effects. Remarkably, suboptimal concentrations can facilitate the emergence of drug-resistant virus strains, which should be avoided under any circumstances. Moreover, some of the most promising compounds are derivatives of natural products. Hence chemical modifications are sometimes necessary for improved antiviral activity.

Out of the innumerable compounds reported in the literature as revealing anti-HIV activity, this review points out the most promising natural products, some of which show unique ways of action. A few of them have already been investigated in clinical trials. The vast majority of natural compounds have, however, only been analyzed in vitro leaving the treasure of potential novel HIV drugs from natural origin to be unearthed in the future.

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