RECENT ADVANCES IN THE DISCOVERY AND DEVELOPMENT OF PLANT-DERIVED NATURAL COUMARINS AND THEIR ANALOGUES AS ANTI HUMAN IMMUNODEFICIENCY VIRUS – TYPE 1 (HIV-1) AGENTS

I. Kostova¹, S. Raleva², P. Genova³, R. Argirova²
Medical University, Faculty of Pharmacy, Dept. of Chemistry, Sofia, Bulgaria¹
National Center of Infectious and Parasitic Diseases, Dept. of Virology, Sofia, Bulgaria²
Sofia University “St. Kliment Ohridski”, Laboratory of Virology, Sofia, Bulgaria ³

ABSTRACT
The acquired immunodeficiency syndrome (AIDS) is a result of human immunodeficiency virus (HIV) infection which leads to severe suppression of immune functions. AIDS is a real threat to the health of mankind, and the search for effective therapies is still of great importance. However, besides the high cost, there are adverse effects and limitations associated with chemotherapy applied. Thus, herbal medicines are frequently used as an alternative therapy by individuals living with HIV. Numerous plant-derived compounds have been evaluated for inhibitory effects on HIV replication, and many coumarins have been found to inhibit different steps in HIV replication cycle. The aim of this review is to summarize research findings for herbal medicines, especially coumarins, which are endowed with the ability to inhibit HIV.

Introduction
In order to combat HIV infection, a colossal amount of money and manpower have been dedicated to search for compounds that can be developed as therapeutic agents. During last two decades, several chemical anti-HIV agents have been developed. However, herbal medicines have also been used by HIV positive individuals as an alternative medical therapy.

The scientific community has approved a number of antiretroviral drugs for clinical use. However, limitations exist - as high cost, decreased sensitivity due to the rapid emergence of resistant strains, adverse effects like lipodystrophy, etc. Thus, more effective and less toxic anti-HIV agents are still needed. In addition, alternative approaches, including herbal therapies, long-term screening of plant extracts, particularly anti-infective or immunomodulating medicinal herbs, and structural modifications of lead compounds, have been attempted. A number of articles discussing anti-HIV activity of herbs (19, 36) suggest...
a variety of chemically disparate molecules, produced by plant species show similar effect. This is the reason plant-derived antiviral agents are prime study candidates.

In the isolation of natural products, it is essential to consider a number of possibilities. First, plants as a source of new antiviral lead compounds should continue to be explored. Second, lead compounds with anti-HIV activity should be developed using modern pharmacological methods to increase activity and decrease toxicity. Finally, herbal medicines and natural products included in combination regimens should be encouraged and continued (36).

The replicative cycle of HIV is comprised of several steps that may be targets for chemotherapy. These steps are: (1) Viral adsorption to the cell membrane; (2) Fusion between the viral envelope and cell membrane, (3) Uncoating of viral nucleocapsid, (4) Reverse transcription of viral RNA to proviral DNA, (5) Integration of proviral DNA to the host cell genome, (6) DNA replication, (7) Transcription of proviral DNA to RNA, (8) Translation of viral mRNA, (9) Maturation of the viral precursor proteins by proteolysis, myristoylation, and glycosylation and (10) Assembly, budding and release of newly formed virions. Step 4 – a key and unique one in replication of retroviruses is catalysed by the virion enzyme reverse transcriptase (RT). Another target of research is step 9, particularly blocking the HIV protease (PR). Therefore, the majority of chemotherapeutic strategies focus on the development of retroviral enzyme inhibitors. Various coumarins have been shown to inhibit, in cell culture, one or more retroviral enzymes.

**Dipyranocoumarins-Calanolides**

(+)-Calanolide A, (+)-[10R, 11S, 12S]-10,11-trans-dihydro-12-hydroxy-6,6,10,11-tetramethyl-4-propyl-2H,6H-benzo[1,2-b:3,4-b':5,6 b'']tripyran-2-one, is a novel nonnucleoside RT inhibitor (NNRTI) with potent activity against HIV-1 (17, 34, 41). The compound was first isolated from a tropical tree (Calophyllum lanigerum) in Malaysia (41). Due to low availability of naturally occurring (+)-calanolide A, a total synthesis of this polycyclic coumarin was developed to provide material for preclinical and clinical research (12, 41).

Previous in vitro studies have demonstrated the protective activity of (+)-calanolide A to established cell lines and primary human cells against a wide variety of HIV-1 isolates including syncytium-inducing (SI), non-syncytium-inducing (NSI) viruses, T-cell tropic and monocyte-macrophage tropic (M-tropic) viruses (1, 4, 7, 17, 41). No activity was detected against HIV-2 or simian immunodeficiency virus (SIV). Direct cytotoxicity of (+)-calanolide A was found at concentrations approximately 100 to 200 times higher than IC50 in all cell lines tested (2, 5, 8, 41). The structure-activity relationships of (+)-calanolide A and cross-resistance evaluation are thoroughly discussed in (2, 3, 5, 6).

Hizi A. et al. (16) studying the effect of calanolide A on HIV replication, has found that the compound specifically inhibited DNA polymerase activity of HIV-1 RT but had no effect on RNase H activity. No similar effect has been observed on HIV-2 RT.

(+)-Calanolide A, a dipyranocoumarin from Calophyllum lanigerum var. austrocoiriaceum, and a closely related compound, (-)-calanolide B, isolated from Calophyllum teysmannii var. inophylloide, represent a distinct class of nonnucleoside HIV-1 specific RT inhibitor under development (26, 27, 31). National Cancer Institute (NCI) repository specimens of total 315 organic extracts from 31 taxa of Calophyllum were analyzed for related pyranocoumarins and their anti-HIV effect (21).

Eight new coumarin compounds were isolated from Calophyllum lanigerum (17). The structures of calanolide A, 12-acetoxy-calanolide A, 12-methoxycalanolide A,
Caalanolide B, 12-methoxycalanolide B, caalanolide C and related derivatives were solved by extensive spectroscopic analyses. The absolute stereochemistry of caalanolide A and caalanolide B was established. Caalanolides A and B were found protective against HIV-1 replication and cytopathicity, but were inactive against HIV-2. Caalanolide A was active against a number of HIV-1 resistant strains emerging after nucleoside and non-nucleoside RT inhibitors.

The study by Currens M.J. et al. (7) focused on further characterization of selective antiviral activity and mechanism of action of caalanolide A. The compound inhibited replication of laboratory strains of HIV-1, as well as promonocytotropic and lymphocytotropic clinical isolates, even of drug-resistant strains. It has been established that caalanolide A acted similarly to the known RT inhibitor 2', 3'-dideoxy- cytidine. Biochemical mechanism of RT inhibition by caalanolide A included a binding near the active site of the enzyme and interference with dNTP. So, caalanolide A inhibited HIV-1 RT similarly to nevirapine but with distinguishing mechanism. At certain concentrations, caalanolide A binds HIV-1 RT in exclusive way with respect to both the pyrophosphate analogue, phosphonoformic acid and the acyclic nucleoside analogue 1-ethoxymethyl-5-ethyl-6-phenylthio-2-thioracil. This indicates that caalanolide A shares binding domains with both phosphonoformic acid and 1-ethoxymethyl-5-ethyl-6-phenylthio-2-thioracil, presumably reflecting that it interacts with RT near both the pyrophosphate binding site and the active site of the enzyme.

Synergy has been demonstrated between (+)-caalanolide A and a number of other antiretroviral agents, including NRTI's, NNRTI's, and PI's in vitro (26, 41). (+)-Caalanolide A remains active against virus isolates with zidovudine (AZT) and 3TC resistant mutations (1, 4, 26, 41). This is a unique and promising feature of the compound (5). Anti-HIV action of caalanolides in combination with other HIV-1-inhibitors was studied by Robert W. et al. (29).

The toxicity of (+)-caalanolide A in a number of animal species, including mice, rats, and dogs, has been studied (12). Toxicities associated with oral administration of (+)-caalanolide A for up to 28 days in animals were gastric irritation with consequent hyperplasia and edema in rats. (+)-Caalanolide A did not produce teratogenic effects when administered to rats during gestation.

In vitro studies indicate that metabolism is qualitatively similar in rats, dogs, monkeys, and humans, with four to seven main metabolites produced (24). CYP3A4 is the primary isoform of P450 that metabolizes (+)-caalanolide A, although CYP2C may be involved as a minor isoform. Animal studies have shown that compound-related radioactivity distributes into both the brain and the lymph after oral administration, while after intravenous administration the radioactivity accumulates in the brain. These studies indicate that (+)-caalanolide A crosses the blood-brain barrier and may be preferentially distributed in the lymphatic system.

The studies by Newman R. A. et al. (23) were undertaken to compare the relative pharmacokinetic parameters and bioavailability of (+)-caalanolide A and (+)-dihydrocaalanolide A. The pharmacokinetic data obtained suggest that selection of the dihydro- derivative might be a reasonable choice for preclinical development and Phase I clinical evaluation. During the pharmacokinetic studies of caalanolide A (34) no acute serious or life-threatening adverse experiences were seen. In fact, almost all previously described adverse effects were of minimal clinical significance and without consequence. Taken together, the favorable safety profile, long half-life, and increased plasma concentrations in humans allow twice-daily dosing of this novel anti-HIV agent. The favorable safety
profile and pharmacokinetics of (+)-calanolide A has led to further clinical development.

Costatolides

In an effort to identify an adequate and sustainable natural source of the recently described anti-HIV drug development candidate calanolide A, Fuller, R.W. et al. (14) undertook chemical and biological studies of the latex exuded from trees of the genus Calophyllum. Although they found that calanolide A was not present in latex from the original source species, C. lanigerum var. austrocoriaceum, they observed that a related coumarin, costatolide, was abundant in latex of C. teysmannii var. inophylloide. Costatolide is currently being evaluated as a possible alternative to calanolide A for drug development (20).

Two isomers of calanolide A, (-)-calanolide B (costatolide) and (-)-dihydrocalanolide B (dihydrocostatolide), possess antiviral properties similar to those of calanolide A (4, 14). Each of these three compounds has properties of NNRTIs. The calanolide analogues, however, exhibit enhanced antiviral activity against drug-resistant viruses after NNRTI treatment. Costatolide and dihydrocostatolide are highly effective inhibitors of clinical strains, including those representing various HIV-1 clades, SIs, NSIs, T- and M-tropic isolates. Similar to calanolide A, decreased activities of both isomers were observed against viruses with amino acid changes at codons 100, 103, 139, and 188 in the genome, although costatolide exhibited a smaller loss of activity against most NNRTI-resistant isolates. Each of the three stereoisomers might interact differently with the RT, despite their high degree of structural similarity. In assays with combinations of anti-HIV agents, costatolide exhibited synergy with these anti-HIV agents. The calanolide isomers represent a novel and distinct subgroup of the NNRTI family, and should be evaluated further for therapeutic use (35).

Inophyllums

The major constituents of the most active Calophyllum species were examined (28). Inophyllums are novel NNRTIs isolated from the plant Calophyllum inophyllum and identified through an enzyme screening program and (33). They were active against HIV-1 in cell culture.

Searching for novel inhibitors of HIV-1 RT, it has been shown that the acetone extract of the giant African snail, Achatina fulica is active (24). Inophyllums B and P inhibited HIV-1 RT and both were active against HIV-1 in cell culture. Closely related inophyllums A, C, D, and E, including calophyllic acids, were significantly less active or totally inactive, indicating certain structural requirements in the chromanol ring. Altogether, 11 compounds of the inophyllum class were isolated from C. inophyllum and were described together with the structure-activity relationships of these novel anti-HIV compounds.

The seeds of Calophyllum cerasiferum Vesque (Family-Clusiaceae) and Calophyllum inophyllum Linn. (Family-Clusiaceae) contain several known coumarins, among them the potent HIV-1 RT inhibitors costatolide and inophyllum P. Calophyllum cerasiferum contained (-)-calanolide B as major coumarin constituent thus providing a renewable source of this compound (32).

Pyranocoumarins-Cordatolides

During a chemotaxonomic survey of Calophyllum extracts available in the National Cancer Institute’s natural product repository, four new pyranocoumarins were isolated from extracts of C. lanigerum var. austrocoriaceum and C. teysmannii var. inophylloide (King.) P. F. Stevens (Clusiaceae). The structure and anti-HIV activity of calanolide E2, cordatolide E, pseudocordatolide C and calanolide F, along with a simple prenylated coumarin precursor, were described (22). Soulattrolide, a coumarin isolated from Calophyllum teysman-
nii latex, was found a potent inhibitor of HIV-1 RT, while calanone and the ketone were inactive (15). Inhibition was remarkably specific, with no activity toward HIV-2 RT, avian myelocytomatosis virus (AMV) RT, RNA polymerase, or DNA polymerases (25).

Twigs of Calophyllum cordato-oblongum were shown to contain a new pyranocoumarin, the methyl ether of cordatolide B, three reported pyranocoumarins, cordatoblongic acid, friedelin, canophyllol and taraxerol. Buds of this species contained large quantities of pyranocoumarins and a small amount of sitosterol. This observation indicates that the coumarin-synthesizing tissues are mainly located at the nonwoody young plant parts of C. cordato-oblongum (11). Cordatolide A and B and a number of coumarins, xanthones and chromene acids from different Calophyllum species of Sri Lanka, as well as both cordatolide A and B were active against the HIV-1 RT, but in different way under differing conditions (9, 10).

**Pyranocoumarins - Suksdorfin and DCK**

Suksdorfin, isolated from the fruit of Loematium suksdorfii, was found to inhibit HIV-1 replication in T cell line, H9, and to be suppressive in acute HIV-1 infections of PBMC, monocyte/macrophages and promonocytic cell line, U937 (18). Combinations of suksdorfin and NRTIs ddl and ddc demonstrated statistically synergy in inhibiting HIV-1 replication, which was not the case with AZT. Comparison of the structure and activity of suksdorfin with those of ten related compounds indicated that dihydroseselin type of pyranocoumarin possessing a 4'-isovaleryl group was important for increased anti-HIV activity of suksdorfin.

Lee K.H. et al. (19) have found that Chinese herbal medicines and subsequent structural modification of discovered leads could provide new, effective, and less toxic drug candidates. Two series of compounds demonstrated anti-HIV effect: the triterpene derivatives DSB and DSD, developed from betulinic acid, isolated from Syzygium claviflorum and the coumarin derivative DCK, developed from suksdorfin. As already mentioned, the pyranocoumarin suksdorfin [(3'R,4'R)-3'acetoxy-4'-(isovaleryloxy)-3',4'-dihydroseselin] inhibited HIV replication. Changing the type and stereochemistry of the 3' and 4' acyl groups led to an extremely potent compound, 3',4'-di-O-(-)-camphonyl-(+) -cis-khellactone (DCK), with 366-fold more potent inhibitory activity and 11-fold higher selectivity than AZT. Different syntheses and structure-activity relationship studies were followed to obtain extremely potent isomeric methoxy and methyl substituted DCK analogs. 4-Methyl-3' and 4'-di-O-(-)-camphyll-(+) -cis-khelthiolactone exhibited extremely potent anti-HIV activity.

**Other natural coumarins**

A new coumarin identified as 5-hydroxy-6-methoxy-7-(3-methyl-but-2-enyloxy)-2H-1-benzopyran-2-one (isoobtusitin) was isolated from Psiadia dentata (13). This compound showed weak activity against HIV.

The methanol extract of the dried aerial parts of Prangos tschimganica gave three new coumarin derivatives and 30 known coumarin derivatives (30). Their structures were established and some of the isolated compounds showed anti-HIV activity.

Dicoumarol was capable of inhibiting HIV-1 RT, alpha-glucosidase, beta-glucosidase and beta-glucuronidase (37).

Five new constituents including a flavonoid, artemisidin A, and four coumarins, artemicapins A, B, C and D, together with 70 known compounds were isolated from the aerial part of Artemisia capillaris and characterized. Among them, three compounds demonstrated significant activity against HIV in H9 cells (38).

The methanol extract of dried roots of Ferula sumbul afforded two furanocou-
marin esters, fesumtuorin A, B, one bicoumarin, fesumtuorin C, five spirobicoumarins, fesumtuorin D, E, F, G and H, along with 19 known coumarins. Some of the isolated compounds showed anti-HIV activity and weak inhibition of cytokine release (42).

Conclusions
Natural plant-derived products continue to be excellent source of new drug candidates with anti-HIV effect. Although the history of herbal medicines dates back thousands of years, herb-drug interactions should not be overlooked. Attention should be paid to adverse effects and toxicity due to drug-drug interaction in HIV-infected persons. Thus, the search for effective and less toxic agents of single structure is still developing. A promising approach is to modify novel, lead compounds derived from plants. There is considerable evidence that Sukudorfin, Calanolides and other plant-derived coumarins are important lead compounds for the development of antiviral and/or virucidal anti-HIV drugs. At present, the herbal kingdom should be explored for discovery and development of anti-HIV agents and these investigations should be encouraged and continued.

REFERENCES
21. McKeel T.C., Covington C.D., Fuller R.W.,


