Anti-HIV natural products

Inder Pal Singh*, Sandip B. Bharate and K. K. Bhutani

Department of Natural Products, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), is an immunosuppressive disease that results in life-threatening opportunistic infections and malignancies. Despite continuous advances made in antiretroviral therapy, AIDS has become the leading cause of death in Africa and fourth worldwide; the number of people with HIV is increasing at an alarming rate in India and Southeast Asia. Biodiversity of the plant kingdom has always provided a source of new drug candidates for almost all disease areas. The number of compounds exhibiting anti-HIV activity and isolated from natural sources is increasing steadily. Calanolide A, a coumarin isolated from *Callophyllum lanigerum* and two other natural product-derived molecules, DSB and 3-hydroxymethyl-4-methyl DCK are phase II clinical candidates, with potential to come up as drugs for treatment of HIV infection. Here, the natural products possessing anti-HIV activity have been discussed, with main focus on recent outcomes from natural sources as anti-HIV agents.

Keywords: AIDS, anti-HIV natural products, HIV.

ACQUIRED immunodeficiency syndrome (AIDS) is a clinical syndrome that is the result of infection with human immunodeficiency virus (HIV), which causes profound immunosuppression. It has been a serious, life-threatening health problem since the first case was identified in 1981 and is the most quickly spreading disease of the century. Since the epidemic began, more than 60 million people have been infected with the virus. HIV/AIDS is now the leading cause of death in Sub-Saharan Africa. Worldwide, it is the fourth biggest killer. According to recent reports of WHO and UNAIDS, at the end of 2004, an estimated 40 million people (37.2 million adults and 2.2 million children) globally were living with HIV, out of which about 22 million had died. The most affected is Sub-Saharan Africa, where 3.1 million adults and children became infected with HIV during the year 2004 and 2.3 million died in 2004. By the end of 2004, the total number of people living with HIV/AIDS in the region has reached 25.4 million[1]. Around 1.2 million people in Asia acquired HIV in 2004, bringing the number of people living with HIV to an estimated 8.2 million. A further 540,000 people are estimated to have died of AIDS in 2004. The spread of HIV in India has been diverse, with much of India having a low rate of infection and the epidemic being most extreme in the southern states. As of December 2004, 92% of all nationally reported AIDS cases has been found in 10 of the 28 states and 7 union territories. The greatest numbers were in Maharashtra and Gujarat in the west; Tamil Nadu and Andhra Pradesh in the south; and Manipur in the Northeast. In the southern states, the infections are mostly due to heterosexual contact, while infections are mainly found amongst injecting drug-users in Manipur and Nagaland. The maximum number of AIDS cases has been reported in Tamil Nadu (44,492) followed by Maharashtra (12,783) out of 96,978 AIDS cases in year 2004. A very high proportion of men and women infected with HIV virus are in their active reproductive ages and around half of the people who acquire HIV become infected before they turn 25. Of greater concern is the possibility of infected mothers transferring the disease to their babies[1,2].

Two major types of HIV have been identified so far, HIV-1 and HIV-2. HIV-1 is the cause of the worldwide epidemic and is most commonly referred to as HIV. It is a highly variable virus, which mutates readily. There are many different strains of HIV-1, which can be classified according to groups and subtypes; there are two groups, M and O. Within group M, there are currently known to be at least ten genetically distinct subtypes of HIV-1. These are subtypes A to J. In addition, Group O contains another distinct group of heterogeneous viruses. HIV-2 is much less pathogenic and occurs rarely; it is found mostly in West Africa[3].

HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. CD4 is present on the surface of many lymphocytes, which are a critical part of the body's immune system. It is now known that a co-receptor is needed for HIV to enter the cell. Following fusion of the virus with the host cell, HIV enters the cell. The genetic material of the virus, which is RNA, is released and undergoes reverse transcription into DNA. An enzyme in HIV called reverse transcriptase is necessary to catalyse this conversion of viral RNA into DNA. Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the cell. The enzyme integrase catalyses this process. Once the viral DNA is integrated into the genetic material of the host, it is possible that HIV may persist in a latent state for many years. This ability of HIV to persist in certain latently infected cells is the major barrier to eradication or cure of HIV. For this reason, based on current knowledge, patients must remain on anti-viral therapy for life[3].

*For correspondence. (e-mail: ipsingh@niper.ac.in)

Several reviews on the natural products for chemotherapy of HIV infection have been published earlier[4–7]. Matthee *et al.*[4] reviewed naturally occurring HIV reverse transcriptase inhibitors. Jung *et al.*[5] discussed anti-HIV agents according to their chemical classes. Yang *et al.*[6] reviewed natural products-based anti-HIV drug discovery and development facilitated by NCI development programme. Recently, Cos *et al.*[7] reviewed different plant substances as anti-HIV agents according to their mechanism of action. In the present review, we have attempted to cover briefly all major classes of natural products exhibiting anti-HIV activity, with emphasis on recent examples categorized according to their chemical nature.

Anti-HIV natural products

Nature has always provided a source of drugs for various ailments. A number of medicinal plants have been reported to have anti-HIV properties. The bioactivity-guided fractionation of crude extracts has provided lead molecules for discovery of anti-HIV drug candidates. Over the past decade, substantial progress has been made in research on the natural products possessing anti-HIV activity. A variety of secondary metabolites obtained from natural origin showed moderate to good anti-HIV activity. Natural products possessing anti-HIV potential are enlisted along with their activity in Table 1.

Natural products from plants

Alkaloids: A variety of alkaloids have been found to possess HIV-inhibitory activity. Michellamines are atropisomeric naphthylisoquinoline alkaloid dimers isolated from leaves of *Ancistrocladus korupensis* (family Ancistrocladaceae), a plant native to the Korup National Park in Cameroon's southwest Province. Michellamine B (**1**) acts both at an early stage of the HIV life cycle by inhibiting reverse transcriptase as well as at later stages by inhibiting cellular fusion and syncytium formation[8]. A tetrahydroxyindolizidine alkaloid, castanospermine (**2**) isolated from *Castanospermum australe* (family Fabaceae), a plant that occurs naturally in the rainforests of eastern and northern Australia showed inhibition of HIV replication and syncytium formation induced by the envelope glycoprotein of HIV. It has also been reported to have glycosidase inhibitory activity[9]. Buchapine (**3**), a quinolinone containing two isoprene units and its structural isomer 3-(3-methyl-2-butenyl)-4-[(3-methyl-2-butenyl) oxy]-2(1H)-quinolinone (**4**) isolated from *Eodia roxburghiana*, a plant indigenous to Southeast Asia and Australia, protected CEM-SS cells from the cytopathic effects of HIV-1 *in vitro*[10]. Sesquiterpene pyridine alkaloids, triptonine A (**5**), triptonine B (**6**) and hypoglaunine B (**7**) isolated from *Tripterygium hypoglaucum* and *T. wilfordii* exhibited potent *in vitro* anti-HIV activity with a therapeutic index[11] of more than 1000.

FK-3000 (**8**), a morphine-related compound obtained from methanolic extract of root tubers of *Stephania cepharantha* (family Menispermaceae[12]), inhibited the cytopathic effects of HIV-1 on MT-4 cells at 7.8 μg/ml. Another alkaloid, cepharanthine (**9**) isolated from the same plant, has been reported to have antiallergic, anti-inflammatory and immunomodulatory activity and also can potently inhibit HIV-1 replication[12]. Nitidine (**10**), isolated from roots of *Toddalia asiatica* (family Rutaceae), showed significant anti-HIV activity in the cell-based assay. It is also reported to have HIV-reverse transcriptase inhibitory activity[13]. *O*-Demethyl-buchenavianine (**11**), a piperidine-flavone-related alkaloid isolated from *Buchenavia capitata* (family Combretaceae), showed activity in both anti-HIV and anti-cancer cell-based screens[14]. Harmine (**12**) isolated from *Symplocos setchuensis* was found to inhibit HIV replication in H9 lymphocyte cells. Amongst its 28 derivatives, *N*-butylharmine (**13**) was found to be most potent with EC_{50} of 0.037 μM and therapeutic index[15] of 210. 1-Methoxy canthinone (**14**) isolated from *Leitneria floridana*, showed potent anti-HIV activity (EC_{50} is 0.26 μg/ml)[16].

Coumarins: Coumarins such as calanolides and inophyllums have been established as non-nucleoside-specific inhibitors of HIV reverse transcriptase. These are obtained from various species of *Callophyllum* (family Clusiaceae), the genus primarily found in the Indo-Pacific region, particularly Malaysia[17]. (+)-Calanolide A (**15**), (–)-calanolide B (**16**) and its dihydro-derivative, (–)-7,8-dihydrocalanolide B isolated from the fruits and twigs of *C. lanigerum*, significantly inhibited the cytopathic effects of HIV-1 in T-cell lines, including both CEM-SS cells and MT-2 cells[18]. All three calanolides inhibited the laboratory-adapted HIV-1 variants, the clinical viral isolates, inclusive of the diverse clades (A–F), syncytium-inducing and non-syncytium-inducing isolates, and T-tropic and monocyte-tropic isolates[18].

Sarawak MediChem Pharmaceuticals, Malaysia has the exclusive worldwide license to the calanolide class of compounds from the National Cancer Institute. They have successfully completed early phase I/II 48-subject clinical trial of calanolide A in combination therapy for HIV, which evaluated the effect of therapy on pharmacokinetic enhancement and safety. Results of the trial confirmed that the combination therapy was effective in increasing the blood levels of calanolide in human volunteers. Additionally, no serious adverse events were noted in any subjects and the small number of adverse events observed was similar to those previously associated with the drug. Calanolide A is currently in phase II clinical trials, focused on assessment of its long-term anti-HIV activity in combination with other anti-HIV agents and an assessment of the long-term durability of such drug combinations[19]. Cordatolide A (**17**) and B (**18**), structural analogues of calanolides isolated from *Callophyllum cordato-oblongum* showed potent inhibitory activity against HIV-1 replication in a novel green fluorescent protein-based reporter cell assay[20].

Table 1. Anti-HIV natural products

Natural product	Source	Anti-HIV activity	Reference
Alkaloids			
Batzelladines A (**102**)	*Batzella* sp.	10 μM[a,l]	110
Batzelladines B (**103**)	*Batzella* sp.	25 μM[a,l]	110
Buchapine (**3**)	*Eodia roxburghiana*	0.94 μM[b,h]	10
Castanospermine (**2**)	*Castanospermum australe*	> 10 μg/ml[b,d,i,k]	9
Cepharanthine (**9**)	*Stephania cepharantha*	*[d]	12
Crambescidin 826 (**100**)	Sponge *Monanchora* sp.	1–3 μM[a,j]	109
Dehydrocrambine A (**101**)	Sponge *Monanchora* sp.	~35 μM[a,j]	109
O-Demethyl-buchenavianine (**11**)	*Buchenavia capitata*	*	14
FK-3000 (**8**)	*Stephania cepharantha*	7.8 μg/ml[h]	12
Harmine (**12**)	*Symplocos setchuensis*	*[d]	15
Hypoglaunine B (**7**)	*Tripterygium hypoglaucum*	0.1 μg/ml[b]	11
3-(3-Methyl-2-butenyl)-4-[(3-methyl-2-butenyl) oxy]-2 (1H)-quinolinone (**4**)	*Euodia roxburghiana*	1.64 μM[b,h]	10
1-Methoxy canthinone (**14**)	*Leitneria floridana*	0.26 μg/ml	16
Michellamine B (**1**)	*Ancistrocladus korupensis*	1 μM[b,e,j,k]	8
Nitidine (**10**)	*Toddalia asiatica*	14 μM[b,e]	13
Trikendiol (**104**)	*Trikentrion loeve*	2 μg/ml[a,h]	111
Triptonine A (**5**)	*Trypterigium hypoglaucum*	2.54 μg/ml[b]	11
Triptonine B (**6**)	*Trypterigium hypoglaucum*	< 0.1 μg/ml[b]	11
Coumarins			
(+)-Calanolide A (**15**)	*Callophyllum lanigerum*	0.2 μM[b,h,k]	18
(–)-Calanolide B (**16**)	*C. lanigerum*	0.2 μM[b,h,k]	18
Cordatolide A (**17**)	*C. cordato-oblongum*	19.3 μM[a,d]	20
Cordatolide B (**18**)	*C. cordato-oblongum*	11.7 μM[a,d]	20
Coriandrin (**24**)	*Coriandrum sativum*	*	24
(–)-7,8-Dihydrocalanolide B	*Callophyllum lanigerum*	0.1 μM[b]	18
Imperatorin (**23**)	*Ferula sumbul*	100 μg/ml[a]	25
Suksdorfin (**19**)	*Lomatium suksdorfii*	2.6 μM[b,d]	22
Flavonoids			
6,8-Diprenylaromadendrin (**25**)	*Monotes africanus*	*	26
6,8-Diprenylkaempferol (**26**)	*M. africanus*	*	26
Hinokiflavone (**29**)	*Rhus succedanea*	65 μM[a,e]	28
Quercetin 3-*O*-(2′-galloyl) *a*-L-arbinopyranose (**27**)	*Acer okamotoanum*	18.1 μg/ml[b,g]	27
Robustaflavone (**28**)	*R. succedanea*	65 μM[a,e]	28
Wikstrol B (**30**)	*Wikstroemia indica*	*	29
Xanthohumol (**31**)	*Humulus lupulus*	*[h]	31
Lignans			
Anolignan A (**32**)	*Anogeissus acuminata*	60.4 μg/ml[a,e]	33
Anolignan B (**33**)	*A. acuminata*	1072 μg/ml[a,e]	33
(–)-Arctigenin (**34**)	*Arctium lappa*	*[m]	34
(+)-5′-Demethoxyepiexcelsin (**35**)	*Litsea verticillata*	42.7 μM[a]	35
Globoidnan A (**40**)	*Eucalyptus globoidea*	0.64 μM[a,g]	39
Gomisin (**38**)	*Kadsura interior*	0.006 μg/ml[b]	37
Kadsulingnan M (**39**)	*Kadsura coccinea*	119 μM[a]	38
Phyllamyricin B (**36**)	*Phyllanthus myrtifolius*	*[e]	36
Retrojusticidin B (**37**)	*P. myrtifolius*	*[e]	36
Phenolics			
8-C-Ascorbyl (–)-epigallocatechin (**44**)	Green and black tea	4 μg/ml[b]	43
Balanocarpol (**60**)	*Hopea malibato*	*	57
Caffeic acid tetramer salts (**50**–**52**)	*Arnebia eucbroma*	1.5–4.0 μg/ml[b,d]	48
Camellia tannin H	*Camellia japonica*	0.9 μM[a]	50
Calceolarioside B (**56**)	*Fraxinus sieboldiana*	0.1 μg/ml[a,l]	54
Corilagin (**41**)	*Chamaesyce hyssopifolia*	20 μM[a,e]	41
Diprenylated bibenzyl (**61**)	*Glycyrrhiza lepidota*	*	58
Guttiferone A(**49**)	*Symphonia globulifera*	8 μM[b,h]	47

Contd…

Table 1. (*Contd…*)

Natural product	Source	Anti-HIV activity	Reference
1,3,4,6-Tetra-*O*-galloyl-*b*-D-glucopyranose (**42**)	*Chamaesyce hyssopifolia*	80 μM[a,e]	41
Laxifloranone (**55**)	*Marila laxiflora*	*[h]	53
Mallotojaponin (**54**)	*Mallotus japonicus*	*[e]	52
Macrocarpals (**53**)	*Eucalyptus globulus*	5.3 μM[a,e]	51
Peltatol A (**62**)	*Pothomorphe peltata*	8 μM[b]	59
Repandusinic acid (**43**)	*Phyllanthus niruri*	*[e]	42
Theasinensin D (**45**)	*Thea sinensis*	8 μg/ml[b]	44
Vismiaphenone D (**48**)	*Vismia cayennensis*	11 μg/ml[b]	46
Quinones			
Conocurvone (**63**)	*Conospermum incurvum*	0.02 μM[b,h]	61
Hypericin (**64**)	*Hypericum perforatum*	*[e]	62
Saponins			
Actein (**65**)	*Cimicifuga racemosa*	0.375 mg/ml[b]	63
Escins	*Aesculus chinensis*	*	65
Saponin B1	Soybean seeds	0.5 μg/ml[d,j]	64
Terpenes/sterols			
Andrographolide (**92**)	*Andrographis paniculata*	*[d,k]	86
Artemisinin (**86**)	*Artemisia annua*	100 μM[a]	81
Betulinic acid (**66**)	*Syzygium claviflorum*	13 μM[a]	66
Celasdin B (**78**)	*Celastrus hindsii*	0.8 μM[b,d]	74
Clathsterol (**107**)	*Clathria* sp.	10 μM[a,e]	114
Clausenolide-1-ethyl ether (**94**)	*Clausena excavate*	> 20 μg/ml[k]	90
Cyanthiwigin B (**110**)	*Myrmekioderma styx*	42.1 μM[b]	6
Cytosporic acid (**121**)	Fungus *Cytospora* sp.	20 μM[a,g]	133
12-Deoxyphorbol-13-(3*E*,5*E*-decadienoate) (**91**)	*Excoecaria agallocha*	6 nM[a,e]	85
Dihydrobetulinic acid (**69**)	*S. claviflorum*	13 μM[a]	66
16*b*,17-Dihydroxy-ent-kauran-19-oic acid (**87**)	*Annona squamosa*	0. 8 μg/ml[b]	82
Garcisaterpene A (**81**)	*Garcinia speciosa*	5.8 μg/ml[b,e,k]	77
Garcisaterpene B (**82**)	*G. speciosa*	37 μg/ml[b,e,k]	77
Halistanol sulphate G (**108**)	*Pseudoaxinissa digitata*	3 μM[b,h]	115
Halistanol sulphate H (**109**)	*P. digitata*	6 μM[b,h]	115
Haplosamates A (**105**)	*Xestospongia* sp.	50 μg/ml[a,g]	113
Haplosamates B (**106**)	*Xestospongia* sp.	15 μg/ml[a,g]	113
1*b*-Hydroxymaprounic 3-*p*-hydroxybenzoate (**76**)	*Maprounea africana*	3.7 μM[a,e]	73
Lancilactone C (**84**)	*Kadsura lancilimba*	1.4 μg/ml[b,d]	79
Linearol (**88**) and analogues	*Sideritis akmanii*	0.1–3.11 μg/ml[b,d]	83
Limonin (**95**)	*Citrus* spp.	60 μM[b,f]	91
Nigranoic acid (**83**)	*Schisandra sphaerandra*	*[e]	78
Nomilin (**96**)	*Citrus* spp.	52 μM[b,f]	91
Nortripterifordin (**93**)	*Tripterygium wilfordii*	25 nM[b,d]	87
Maslinic acid (**74**)	*Geum japonicum*	17.9 μg/ml[a,f]	71
Moronic acid (**75**)	*Myrceugenia euosma*	< 0.1 μg/ml[b]	72
Oleanolic acid (**68**)	*S. claviflorum*	21.8 μg/ml[a,d]	68
Oxygenated triterpenes (e.g. Ganoderic acid-*a* (**79**))	*Ganoderma lucidum*	0.17–0.23 μM[a,f,h]	75
Prostratin (**89**)	*Homalanthus nutans*	< 0.132 μM[b]	6
Shinjulactone C (**85**)	*Allanthus altissima*	10.6 μM[b]	80
Suberosol (**80**)	*Polyalthia suberosa*	3 μg/ml[a,d]	76
12-*O*-Tetradecanoyl phorbol-13-acetate (**90**)	*Croton tiglium*	0.48 ng/ml[a ,h]	84
Ursolic acid (**73**)	*Crataegus pinatifida*	8 μM[a,f]	70
Uvaol (**72**)	*C. pinatifida*	5.5 μM[a,f]	70
Xanthones			
Swertifrancheside (**97**)	*Swertia franchetiana*	43 μM[a,e]	92

Contd…

Table 1. (*Contd…*)

Natural product	Source	Anti-HIV activity	Reference
Macluraxanthone B (**98**)	*Maclura tinctoria*	*	93
Carbohydrates			
Galactan sulphate	*Aghaedhlella tenera*	0.6–0.4 μg/ml[a,h]	118
Niruriside (**99**)	*Phyllanthus niruri*	3.3 μM[a]	94
Rhamnan sulphate	*Monostroma latissimum*	*	120
Sulpahated xylomannan	*Nothogenia fastigiata*	13.7 μg/ml[a,d]	121
Peptides			
Callipeltin A	*Callipelta*	0.01 μg/ml[c]	122
Circulins	*Chassalia parvifolia*	0.5 μM[b]	96
Complestatin A	*Streptomyces* spp.	200 nM[d]	124
Cycloviolins	*Leonia cymosa*	*	97
Microspinosamide	*Sidonops microspinosa*	0.2 μg/ml[b,h]	121
Palicourein	*Palicourea condensate*	1.5 μM[a], 0.1 μM[b,h]	98
Siamycins	*Streptomyces*	*[k]	127
[Ile7] surfactin	*Bacillus subtilis natto*	20 μM[b,h]	132
[Leu7] surfactin	*B. s. natto*	14 μM[b,h]	132
Proteins			
Cyanovirin-N	*Nostoc ellipsosporum*	*	124
Trichosanthin	*Trichosanthes kirilowii*	*[d,k]	99
GAP31	*Gelonium multiflorum*	0.2–0.3 nM[b,g]	102
MAP30	*Momordica charantia*	0.2-0.3 nM[b,d,e,k]	100
MRK29	*M. charantia*	18 μg/ml[a,e]	101
Myrianthus holstii lectin (MHL)	*Myrianthus holstii*	150 nM[b]	103
TAP29	*Trichosanthes kirilowii*	0.2–0.3 nM[b,d,e,k]	100

[a]IC_{50}, [b]EC_{50}, [c]ED_{50}, Inhibitory activity against: [d]HIV-1 replication, [e]HIV RTase, [f]HIV protease, [g]HIV integrase, [h]HIV-induced cytopathic effects, [i]Glycosylation, [j]Cellular fusion, [k]Syncytium formation, [l]Binding of HIV to surface of T-cells, [m]HIV proviral DNA.
*IC_{50}/EC_{50}/ED_{50} not available.

Khellactone coumarins have shown a number of biological activities such as anti-HIV, anti-tumour promoting and anti-platelet aggregation[21]. So far, more than 50 natural khellactone coumarins have been discovered. Suksdorfin (**19**), a dihydroseselin-type angular pyranocoumarin isolated from methanol extract of *Lomatium suksdorfii*, suppressed viral replication in eleven separate acute HIV-1 infections of H9 lymphocyte cells with an average EC_{50} value of 2.6 μM. It also suppressed acute HIV-1 infections in fresh peripheral blood mononuclear cells, monocyte/macrophages and U-937 cells, a promonocytic cell line[22]. Modifications at 3′,4′-position yielded 3′-*R*,4′-*R*-di-*O*-(–)-camphanoyl-(+)-*cis*-khellactone (**20**, DCK), which showed improved activity (EC_{50} 0.0004 μM, TI 136719). Studies on the effect of stereochemistry showed that the *R*,*R* isomer was at least 10,000 times more active than any of the other three (*R*,*S*, *S*,*R* and *S*,*S*) isomers. Further modifications led to more potent 4-MeDCK (**21**) (EC_{50} 1.6×10^{-7} μM, TI > 10^9) and then recently, to the preclinical candidate 3-hydroxymethyl-4-methyl DCK (**22**, PA-334B), which is a nanomolar inhibitor of both primary clinical and drug-resistant HIV-1 isolates. It is orally bioavailable in rats and dogs, with a plasma half-life of 2–3 h in rats. In preclinical toxicology studies, minimal toxicities were found. Panacos Pharmaceuticals has nearly completed the required preclinical studies for IND filing[23,24].

A furanocoumarin, imperatorin (**23**), obtained from methanolic extracts of dried roots of *Ferula sumbul* (family Umbelliferae)[25] showed HIV inhibitory activity with IC_{50} >100 μg/ml, EC_{50} < 0.10 μg/ml and TI > 1000. Coriandrin (**24**), an isocoumarin isolated from the coriander *Coriandrum sativum*, possessed anti-HIV and other antiviral activities[24].

Flavonoids: These have been reported to possess a number of biological activities and are well known for their antioxidant properties. Many cell and tissue damages such as cell death, apoptosis, tissue necrosis, which are responsible for a number of diseases, are associated with free-radical generation. In healthy individuals, the production of reactive oxygen species is balanced with the antioxidant defence system. Oxidative stress results from the imbalance between reactive oxygen species production and inactivation. Oxidative stress has been implicated in a variety of disorders such as cancer, Parkinson's disease and AIDS. Furthermore, increased levels of products of lipid peroxidation such as malondialdehyde and of oxidative DNA damage such as 8-hydroxyguanine were observed in HIV-positive

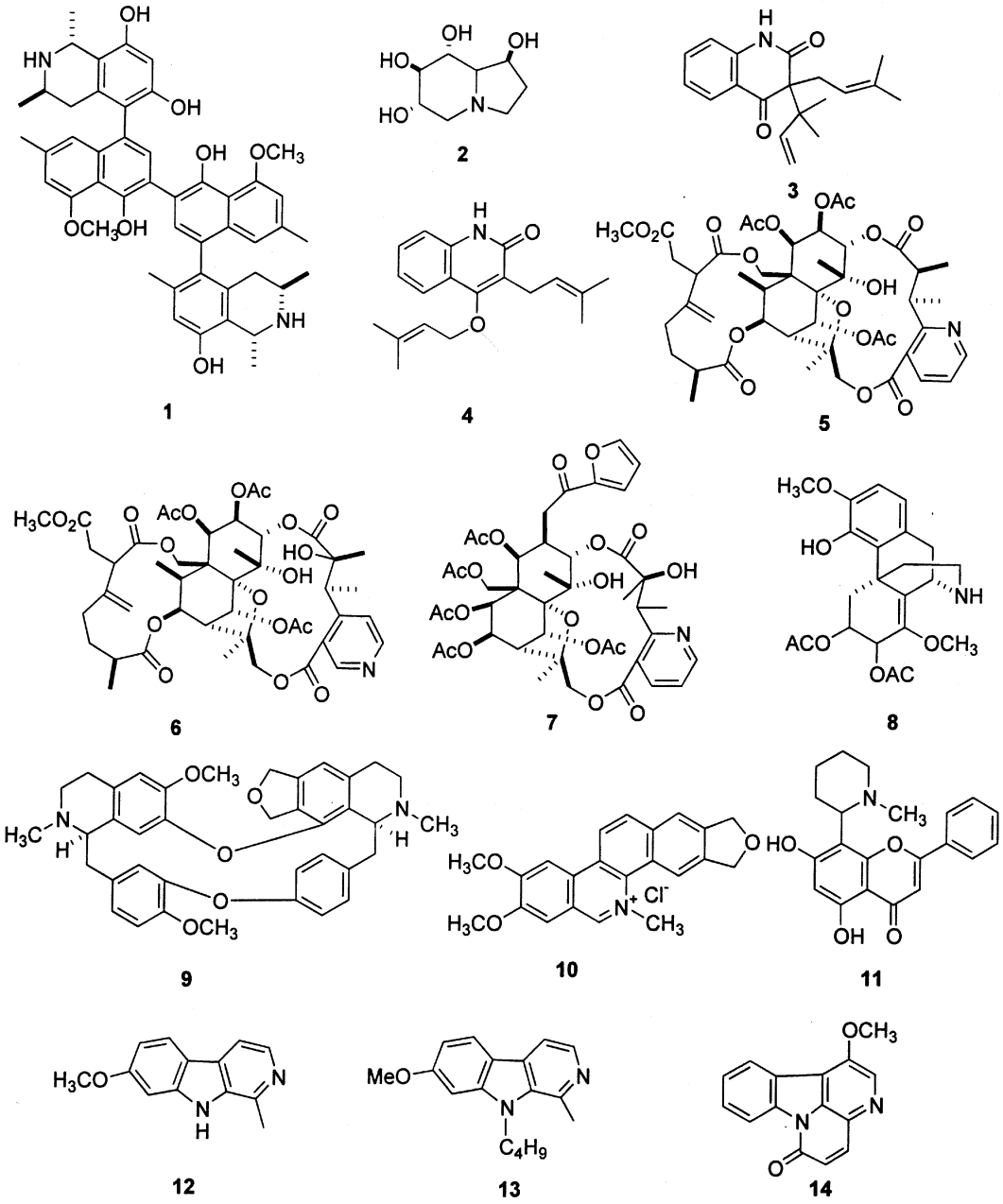


Figure 1. Anti-HIV alkaloids from plants.

persons. The antiviral activity of various flavonoids against several viruses in cell cultures and in animal models has been demonstrated. Prenylated flavonoids, 6,8-diprenylaromadendrin (**25**) and 6,8-diprenylkaempferol (**26**) isolated from the extract of *Monotes africanus* exhibited HIV-inhibitory activity in the XTT-based, whole-cell screen[26]. Quercetin 3-*O*-(2′-galloyl) *a*-L-arbinopyranose (**27**) and flavonoid gallate ester isolated from ethanolic extract of *Acer okamotoanum* (family Aceraceae), possessed anti-HIV-1 integrase activity with IC_{50} values of 18.1 ± 1.3 and 24.2 ± 6.6 μg/ml respectively[27]. Biflavonoids, robustaflavone (**28**) and hinokiflavone (**29**) isolated from methanolic extracts of twigs and leaves of *Rhus succedanea* (family Anacardiaceae), showed strong inhibition of the polymerase of HIV-1 RTase in *in vitro* assay[28]. Another biflavonoid, wikstrol B (**30**) obtained from extracts of roots of *Wikstroemia indica* (family Thymelaeaceae), showed good activity against HIV-1 in *in vitro* studies[29]. HIV-inhibitory pterocarpans and isoflavonoids have been reported from plants of genus *Erythrina*[30]. Xanthohumol (**31**), a prenylchalcone recently isolated from hops *Humulus lupulus*, has shown HIV-1 inhibitory activity as well as HIV-1-induced cytopathic effects, production of viral p24 antigen and reverse transcriptase in C8166 lymphocytes at non-toxic concentration[31].

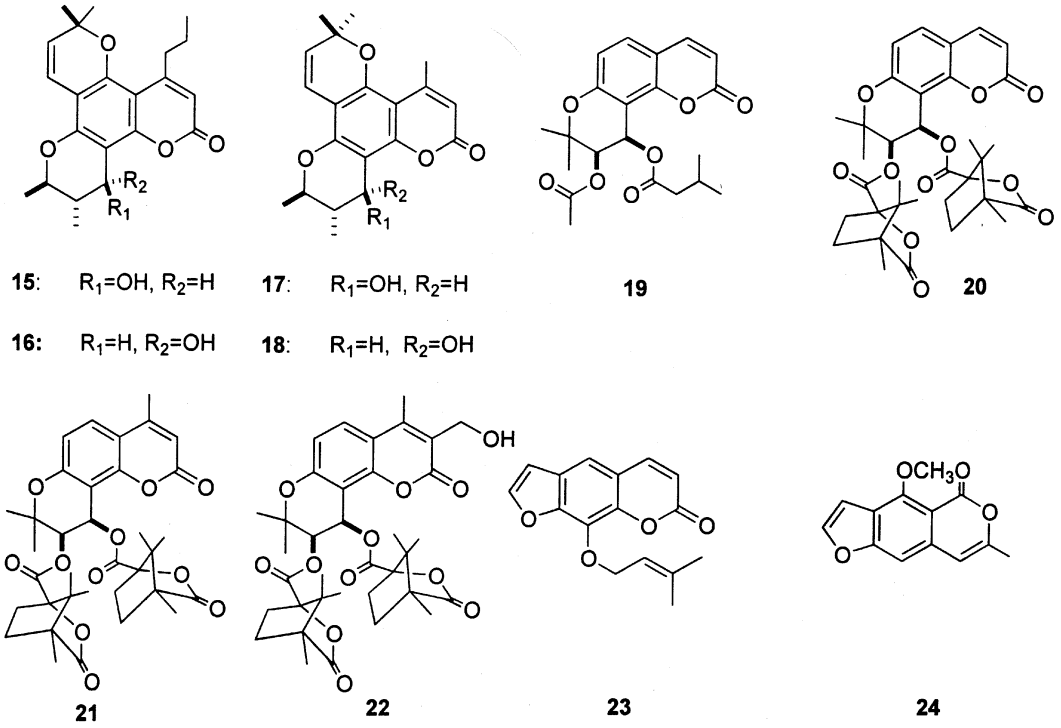


Figure 2. Anti-HIV coumarins from plants.

Lignans: A number of lignans have been shown to possess antiviral activities[32]. Dibenzylbutadiene lignans, anolignan A (**32**) and anolignan B (**33**) isolated from *Anogeissus acuminata*, showed HIV-1 RTase inhibitory activity. Compounds **32** and **33** are reported to act by synergistic effect. Compound **32** showed an IC_{50} of 60.4 μg/ml compared to 1073 μg/ml shown by **33** for HIV-1 RTase. This activity was greatly increased when a mixture of **32** and **33** was tested in different ratios. Compound **32** also showed activity against a drug-resistant form of HIV-1 RTase[33] with an IC_{50} of 106 μg/ml. Dibenzylbutyrolactone-type lignanolide, (–)-arctigenin (**34**) isolated from *Ipomoea cairica* and *Arctium lappa* showed anti-HIV activity that was primarily due to inhibition of HIV proviral DNA and not related to interference with HIV-1 RTase[34]. (+)-5′-Demethoxyepiexcelsin (**35**) obtained from methanolic extract of leaves and twigs of *Litsea verticillata* (family Lauraceae) showed good anti-HIV activity while (+)-epiexcelsin was devoid of anti-HIV activity[35]. Phyllamyricin B (**36**) and its lactone retrojusticidin B (**37**) isolated from chloroform extract of *Phyllanthus myrtifolius*/*P. urinaria* (family Euphorbiaceae), demonstrated strong inhibition of HIV-RTase[36]. Amongst the lignans isolated from *Kadsura interior* (–) gomisin (**38**) has been found to be the most potent (EC_{50} 0.006 μg/ml; TI 600) inhibitor of HIV replication[37]. Kadsulingnan M (**39**) isolated from *Kadsura coccinea* showed an anti-HIV activity *in vitro*[38]. Recently, globoidnan A (**40**), a lignan isolated from the methanol extract of buds of *Eucalyptus globoidea* by bioassay-guided fractionation, inhibited the combined 3′-processing and strand-transfer activity of HIV integrase[39]. The ethanolic extract of the fruit rind of *Terminalia bellerica* (family Combretaceae), one of the commonly used plants in the Indian traditional systems of medicine, also yielded **33** and other lignans, which possessed demonstrable anti-HIV activity *in vitro*[40].

Phenolics: Several of the virucidal plant compounds are tannins or related phenolic substances, which are often responsible for the virucidal effects in several viral systems. In general, polyphenols act by associating with proteins of viral particles and/or host cell surfaces, resulting in reduction or prevention of viral adsorption. Several hydrolysable tannins such as chebulagic acid, punicalin and punicalagin from *Terminalia chebula* show anti-HIV activity. A dimeric, hydrolysable tannin, cornusin A isolated from fruits of *Cornus oficinalis* (family Cornaceae) inhibited RTase from avian myeloblastosis virus. Corilagin (**41**) and 1,3,4,6-tetra-*O*-galloyl-*b*-D-glucopyranose (**42**) isolated from *Chamaesyce hyssopifolia* inhibited HIV-RTase[41].

Repandusinic acid (**43**) isolated from an aqueous extract of *Phyllanthus niruri* (family Euphorbiaceae) inhibited HIV-1 RTase[42]. 8-*C*-ascorbyl (–)-epigallocatechin (**44**) showed potent anti-HIV activity with an adequate therapeutic index value[43] of 9.5. Theasinensin D (**45**) exhibited moderate anti-HIV activity[44]. Gossypol (**46**) and 1,1′-dideoxygossylic acid (**47**), yellow pigments from the cotton plant, are also

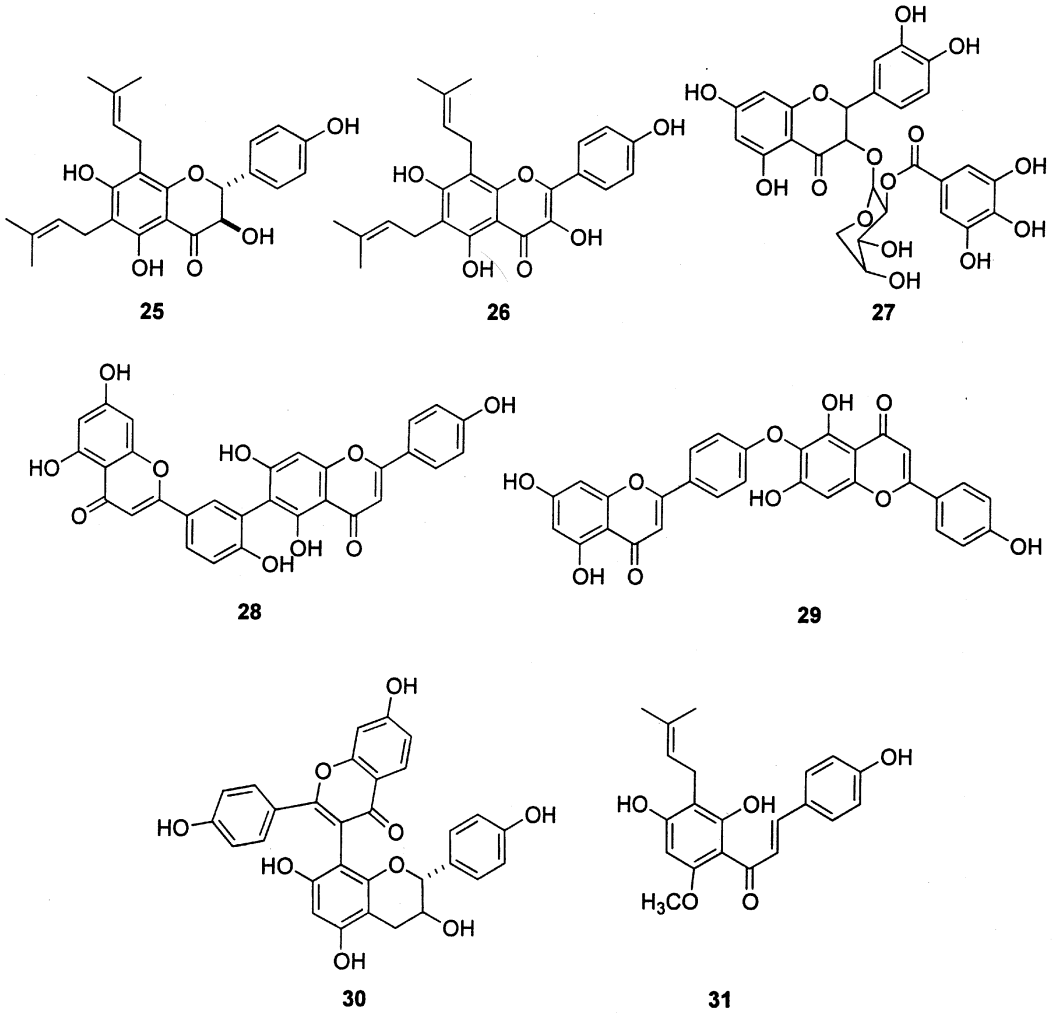


Figure 3. Anti-HIV flavonoids from plants.

reported to have anti-HIV activities[45]. Vismiaphenone D (**48**) isolated from *Vismia cayennensis* exhibited activity in the primary anti-HIV screens[46], while guttiferone A (**49**), isolated from *Symphonia globulifera* (family Guttiferae), provided cytoprotection of CEM-SS cells from HIV-1 infection[47].

Monosodium and monopotassium salts (**50**–**52**) of isomeric caffeic acid tetramer isolated from the aqueous acetone extract of *Arnebia eucbroma* (Boraginaceae) by bioactivity-guided fractionation, showed potent inhibitory activity against HIV replication in acutely infected H9 cells with EC_{50} values of 2.8, 4.0, and 1.5 μg/ml, respectively; their TI values[48] were 19.6, 12.5 and 33.3 respectively. 1,3,4,5-tetra-*O*-galloylquinic acid isolated from the stem bark of the *Lepidobotrys staudti* (family Lepidobotryaceae), showed significant anti-HIV activity. It protected CEM-SS cells from cytopathic effects of HIV-1_{RF}. Gallic acid and galloyl glucoses isolated from *Terminalia chebula* (family Combretaceae) exhibited HIV integrase inhibitory activity[49] and camellia-tannin H isolated from the pericarp of *Camellia japonica* showed a potent HIV-1 protease inhibitory activity[50].

Phloroglucinol derivatives are also known to possess HIV reverse transcriptase inhibitory activity and other activities such as antimalarial, antifouling, antibacterial and EBV inhibitory. A number of macrocarpals (A–E) isolated from *Eucalyptus globulus* possessed anti-HIV RTase inhibitory activity, with IC_{50} ranging from 5 to 12 μM. Amongst these, macrocarpal B (**53**) was found to be most potent[51] with IC_{50} of 5.3 μM. Macrocarpals have isopentyl phloroglucinol moiety joined to various sesquiterpenes such as aromadendrane and eudesman. Mallotojaponin (**54**), a dimeric phloroglucinol derivative isolated from the pericarps of *Mallotus japonicus*, inhibited HIV-1 RTase noncompetitively with respect to the natural substrate[52]. Another phloroglucinol derivative, laxifloranone (**55**) isolated from *Marila laxiflora*, showed moderate inhibition of the cytopathic effects of *in vitro* HIV infection[53]. Phenylethanoid glycoside, calceolarioside B (**56**) isolated from *n*-butanol fraction of *Fraxinus sieboldiana* var. *an-*

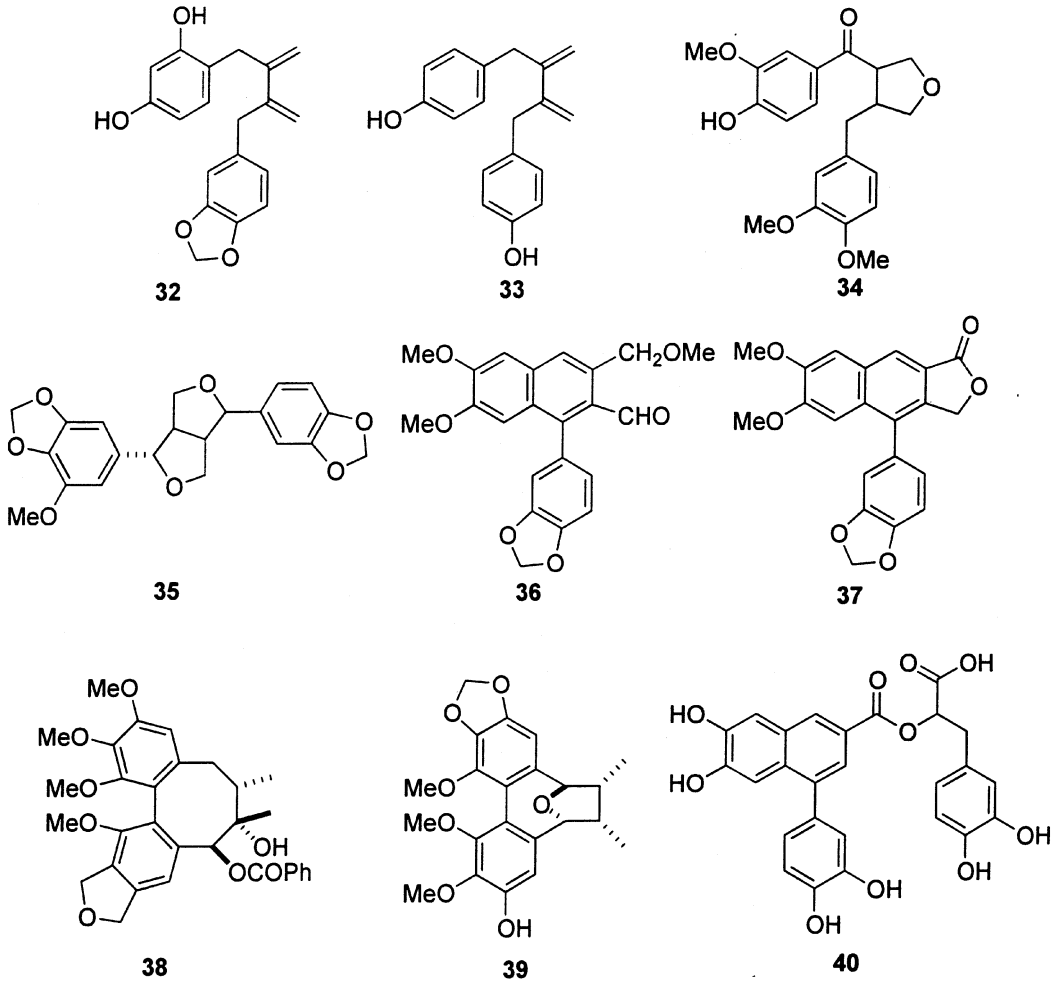


Figure 4. Anti-HIV lignans from plants.

gustata[54], showed moderate binding affinity on HIV gp41. The curcuminoids isolated from ethyl acetate extract of rhizomes of *Curcuma longa* showed modest HIV-1 and HIV-2 protease inhibitory activity[55]. Bergenin (**57**), norbergenin (**58**) and methyl norbergenin (**59**) isolated from methanolic extract of the aerial parts of *Ardisia japonica* (family Myrisinaceae) showed moderate *in vitro* anti-HIV activity[56]. Balanocarpol (**60**), hydroxylated stilbene compound isolated from *Hopea malibato* (family Dipterocarpaceae) exhibited modest HIV inhibitory activity in the antiviral assay[57]. Diprenylated bibenzyl (**61**) isolated from *Glycyrrhiza lepidota* (family Fabaceae) showed moderate activity in the US NCI *in vitro* anti-HIV-1 bioassay[58]. Prenylated catechol dimer, the peltatol A (**62**) isolated from *Pothomorphe peltata* (family Piperaceae) demonstrated strong anti-HIV activity[59].

Quinones: Several naphthoquinones such as 1,4-naphthoquinone, vitamin K_3, juglone and plumbagin showed HIV-inhibitory activity[60]. A trimeric naphthoquinone, conocurvone (**63**) isolated from *Conospermum incurvum* (family Proteaceae) showed potent anti-HIV activity. It was shown to act by a novel mechanism different from any of the earlier known mechanisms, the inhibitory action occurring in the late phase of viral replication cycle. Conocurvone added 48 h after infection, protected T-cells from cytopathogenic effect of HIV-1. It has been under development by the Australian company, AMRAD[61]. A polycyclic aromatic dianthroquinone, hypericin (**64**) obtained from *Hypericum perforatum* showed activity against non-human retroviruses as well as human retroviruses in lymphocytes. It has also inhibited HIV-1 RTase[62].

Saponins: Actein (**65**), a tetracyclic triterpenoid saponin isolated from the rhizome of *Cimicifuga racemosa* (black cohosh), showed potent anti-HIV activity[63]. Soybean saponins isolated from soybean seeds inhibited HIV-1 replication in MT-4 cells. They possess narrow therapeutic index and did not inhibit HIV-1 RTase. One of the saponins (B1) inhibits HIV-induced cell fusion in MOLT-4 cells[64]. Escins, the triterpenoid saponin mixture extracted from the seeds of *Aesculus chinensis* (family Hippocastanaceae), was found to show moderate anti-HIV-1 protease activity[65].

Terpenes: Betulinic acid (**66**), platanic acid (**67**) and oleanolic acid (**68)** isolated from the leaves of *Syzigium claviflorum*, exhibited anti-HIV activity in H9 lymphocyte cell. Betulinic acid demonstrated an anti-HIV activity with an EC_{50} value of 1.4 μM and an IC_{50} value of 13 μM. Dihydrobetulinic acid (**69**) showed EC_{50} and IC50 values of 0.9 and 13 μM respectively[66]. Modification of betulinic acid and dihydrobetulinic acids has successfully increased anti-HIV potency. Esterification at C-3 hydroxyl resulted in more potent compounds with tremendously improved TI values. 3-*O*-(3,3′-dimethylsuccinyl) betulinic acid (**70**, DSB, PA-457) had an $EC_{50} < 3.5 \times 10^{-4}$ μM and TI > 20,000)[67]. DSB (PA-457), which was discovered by Panacos scientists, works by a mechanism different from that of any approved drug or other drugs under development, by blocking a key step in the processing of a viral core protein called capsid.

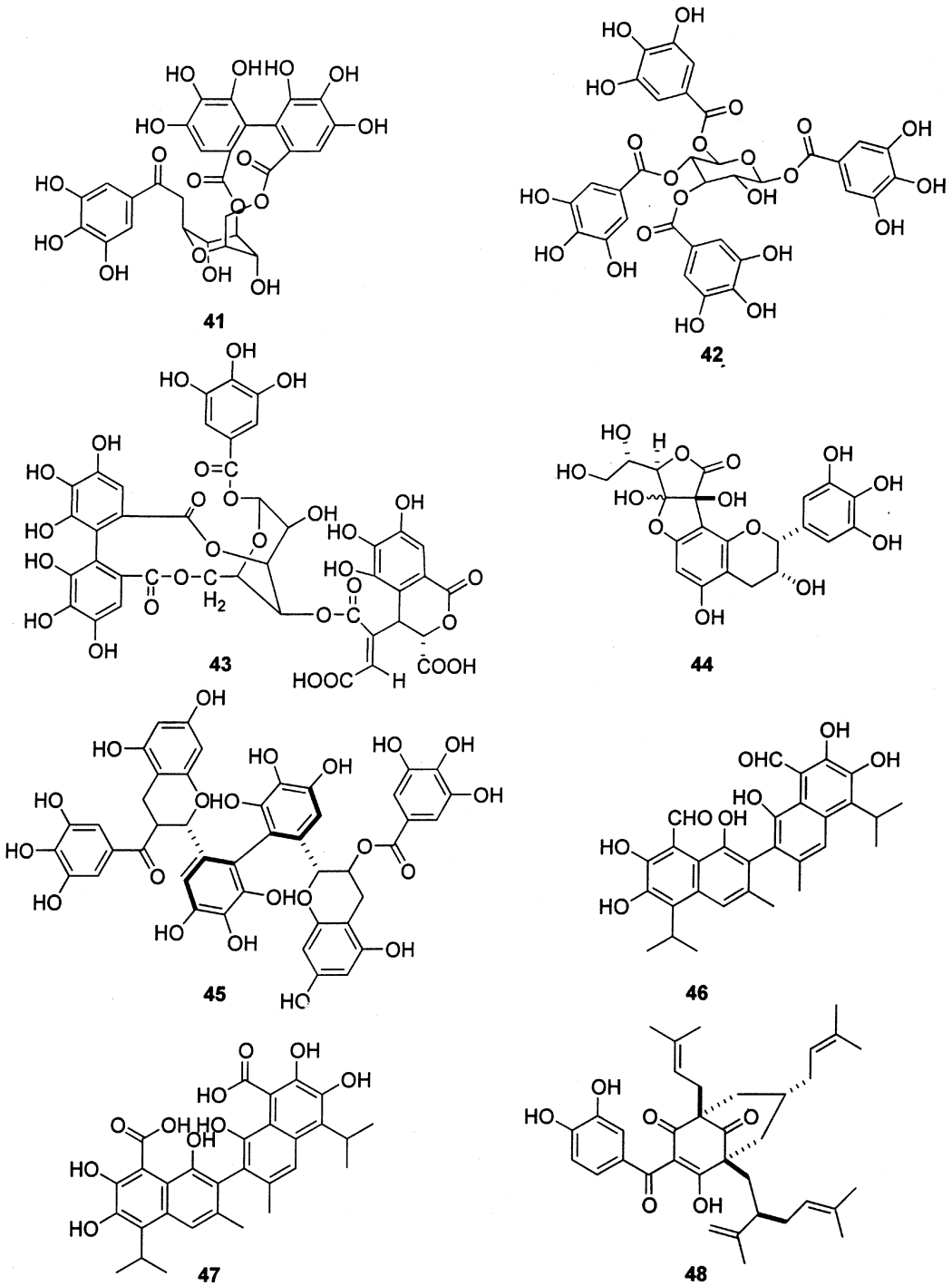


Figure 5. (*contd…*)

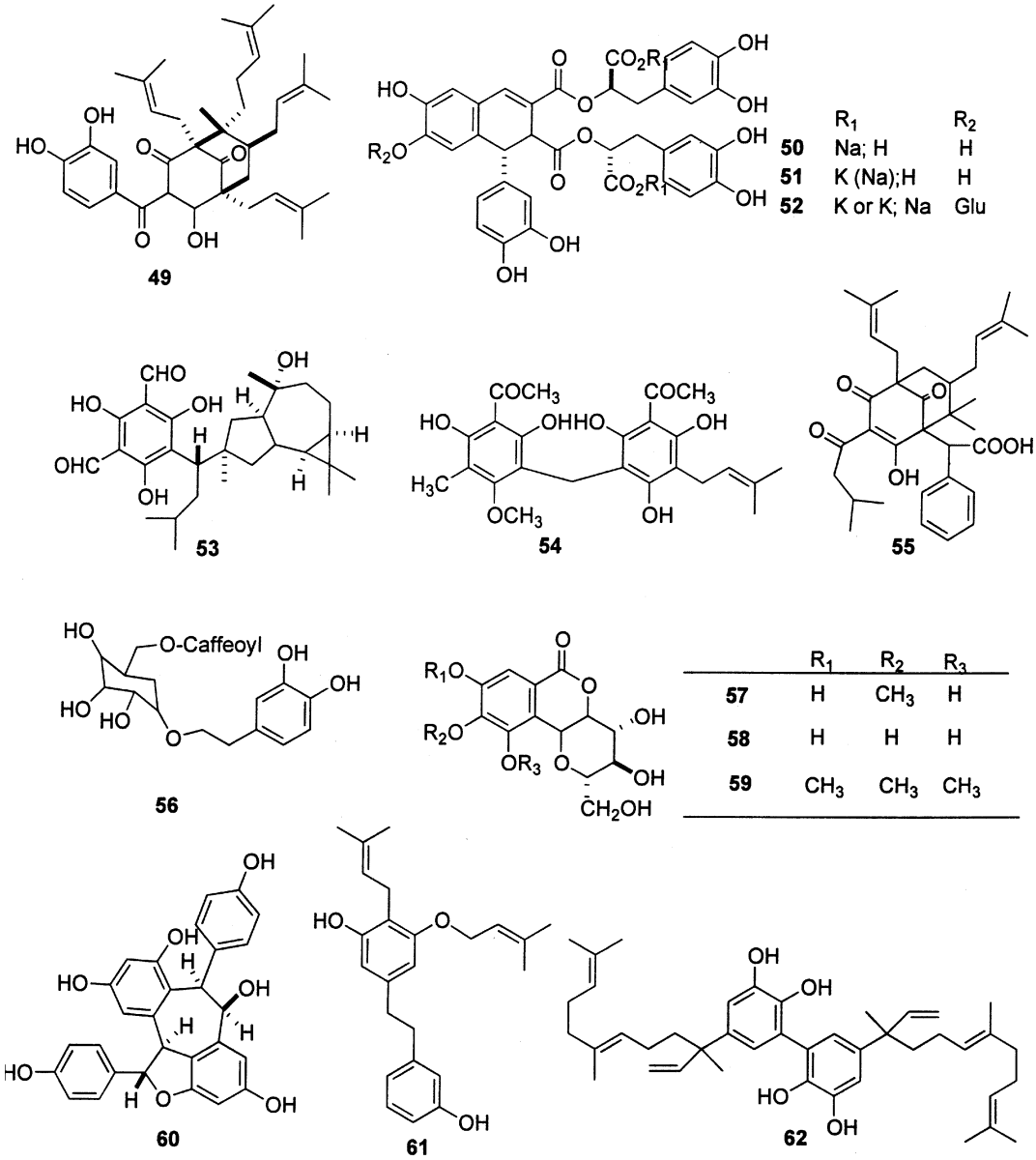


Figure 5. Anti-HIV phenolics from plants.

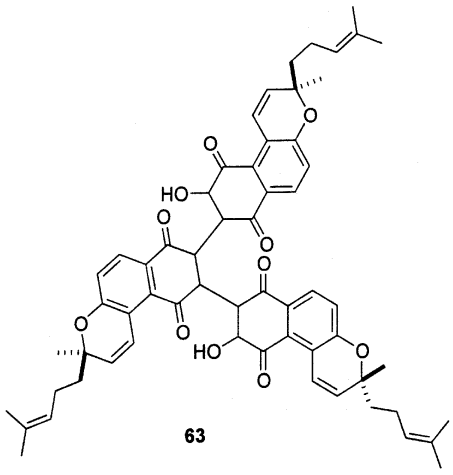
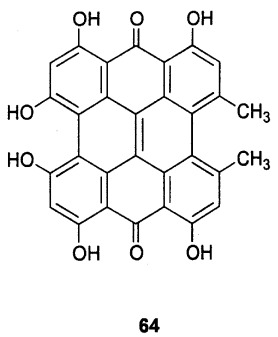


Figure 6. Anti-HIV quinones from plants.



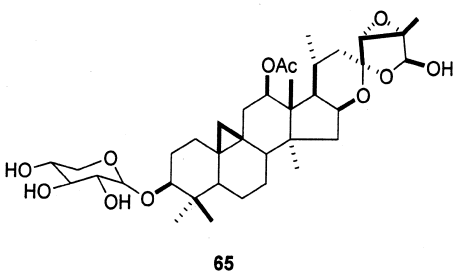


Figure 7. Anti-HIV saponin from plants.

Preclinical studies have shown that PA-457 retains full activity against drug-resistant virus, is effective in an animal model of HIV infection and should be suitable for use in combination therapy with other drugs. Recently, Panacos announced positive results from a phase I/II clinical trial in HIV-infected patients. Following a single oral dose of PA-457, significant reduction in viral load from baseline, of up to approximately 0.7 $\log_{10}$, was seen in patients receiving higher dose levels. The company also recently completed a phase Ib clinical trial of PA-457, administered orally once a day for 10 days to uninfected volunteers. The drug candidate was well-tolerated and plasma concentrations of PA-457 reached levels significantly greater than those predicted to provide a therapeutic benefit in HIV-infected patients. Recently, Panacos Pharmaceuticals has started phase II clinical studies[23] of PA-457.

Oleanolic acid isolated from methanolic extract of wood of *Xanthoceras sorbifolia* (family Sapindaceae), inhibited HIV-1 replication in acutely infected H9 cells with an EC_{50} value of 1.7 μg/ml, and inhibited H9 cell growth with an IC_{50} value of 21.8 μg/ml (TI 12.8)[68]. Like betulinic acid, esterification at C-3 hydroxyl of oleanolic acid resulted in 3-

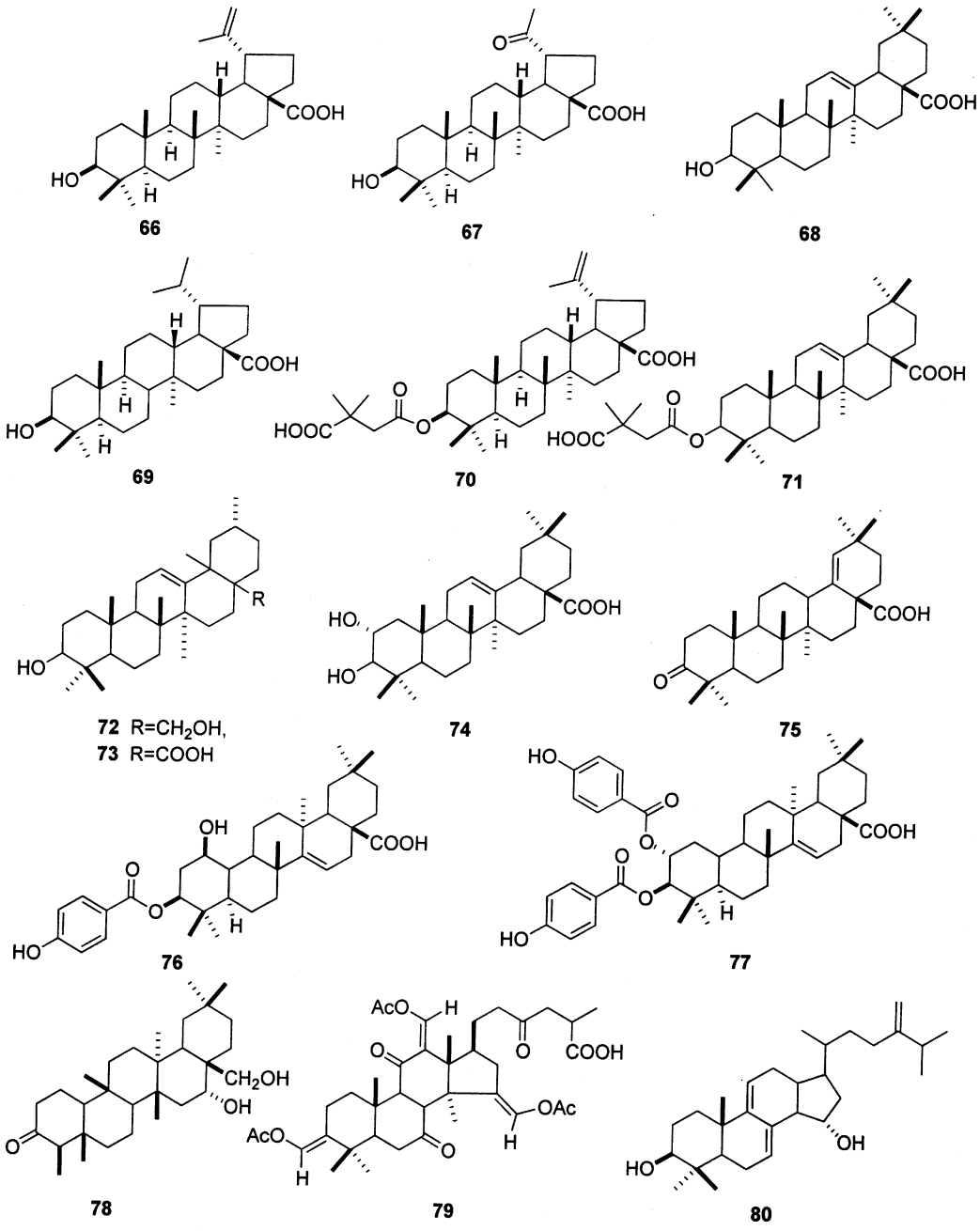


Figure 8. (*contd*…)

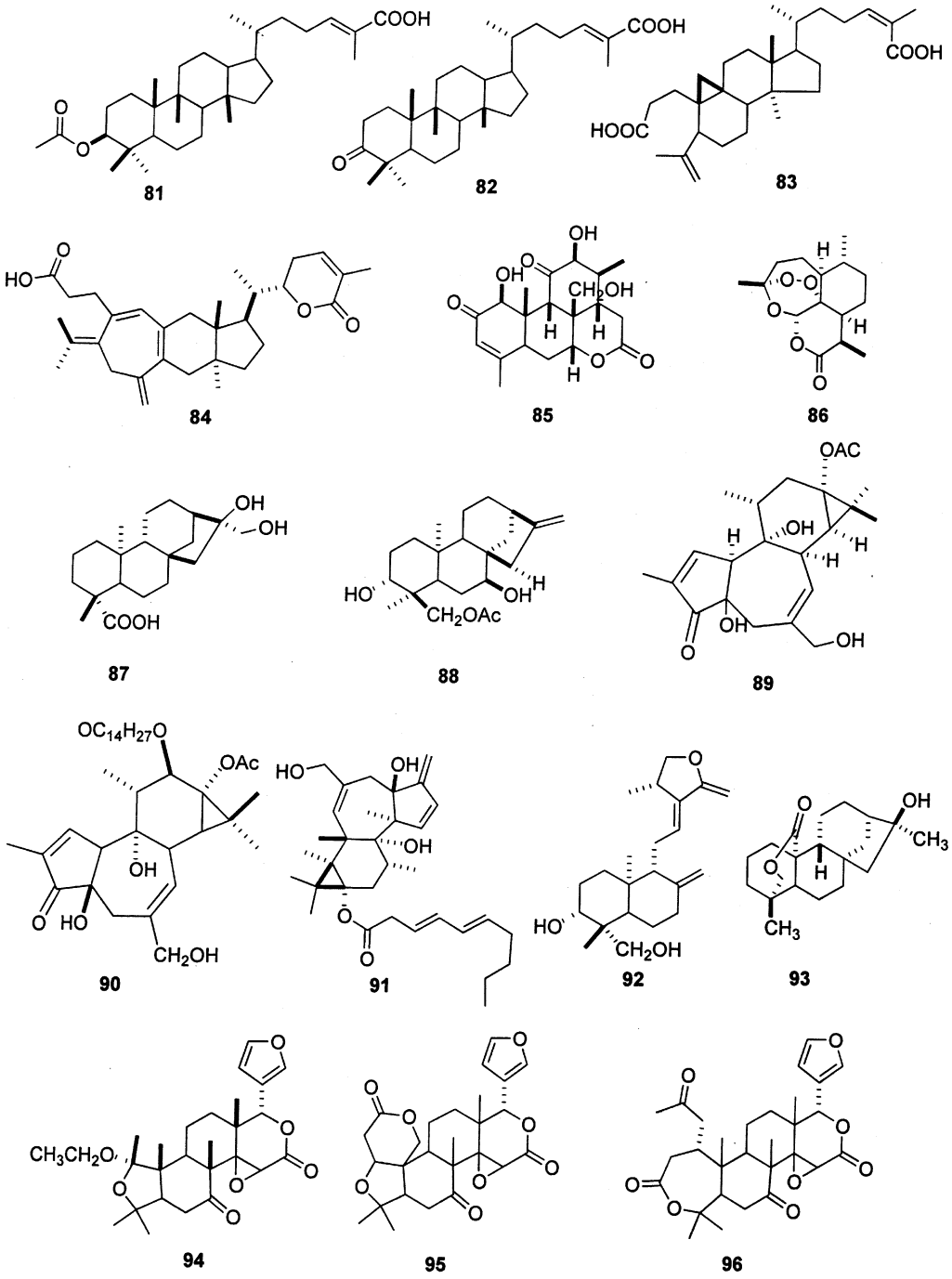


Figure 8. Anti-HIV terpenes from plants.

oxotirucalla-7,24-dien-21-oic acid (**71**) with improved activity (EC_{50} 0.0039 μg/ml, TI 3750). It also inhibited[69] HIV protease with an IC_{50} value of 10 μg/ml. Uvaol (**72**) and ursolic acid (**73**) isolated from the methanolic extract of leaves of *Crataegus pinatifida* (family Rosaceae)[70], showed potent inhibitory activity against HIV-1 protease at concentration of 100 μg/ml. Maslinic acid (**74**) isolated from *Geum japonicum*[71], showed potent inhibitory activity against HIV-1 protease at a concentration of 17.9 μg/ml. Moronic acid (**75**) isolated from *Myrceugenia euosma* (family Myrtaceae), showed significant anti-HIV activity with therapeutic index[72] of more than 186. Pentacyclic triterpenes, 1*b*-hydroxymaprounic 3-*p*-hydroxybenzoate (**76**), and 2*a*-hydroxymaprounic acid 2,3-*bis*-*p*-hydroxybenzoate (**77**) isolated from the roots of *Maprounea africana* Muell.-Arg. (Euphorbiaceae)[73], inhibited HIV-1 RTase with an IC_{50} value of 3.7 μM. Celasdin B (**78**) isolated from ethanolic extract of *Celastrus hindsii* (family Celas-

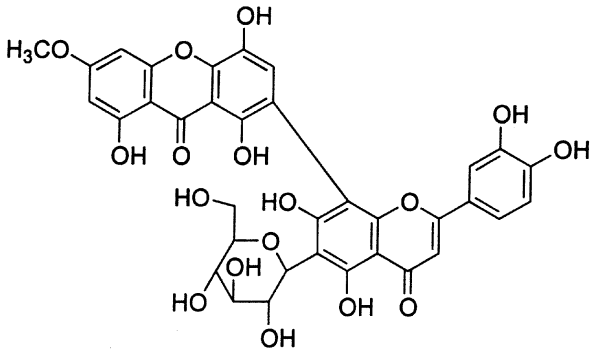
traceae), exhibited anti-HIV replication activity in H9 lymphocyte cells *in vitro*[74]. Oxygenated triterpenes, such as ganoderic acid-*a* (**79**), ganoderiol F, ganodermontriol, ganoderic acid B, ganoderiol B, and ganoderic acid C1 isolated from methanolic extracts of *Ganoderma lucidum* (family Polyporaceae), were found to inhibit HIV-1 induced cytopathic effects in MT-4 cells and also possessed HIV-1 protease inhibitory activity[75]. Lanostane-type triterpene, suberosol (**80**) isolated from ethanolic extract of the stems and leaves of *Polyalthia suberosa* (family Annonaceae) showed anti-HIV replication activity in H9 lymphocyte cells[76]. The protostanes, garcisaterpenes A (**81**) and C (**82**) isolated from ethyl acetate extract of bark and stems of *Garcinia speciosa*, showed significant inhibitory activities against HIV-1 RTase and in the syncytium assay[77]. A ring-secocycloartene triterpenoid, nigranoic acid (**83**) isolated from the stems of *Schisandra sphaerandra*[78], inhibited HIV-1 RTase and HIV-2 RTase. Triterpene lactone, lancilactone C (**84**) isolated from stems and roots of *Kadsura lancilimba*, also possessed inhibitory activity against HIV replication in H9 lymphocytes[79].

Shinjulactone C (**85**), possessing unusual structure isolated from *Brucea javanica* and *Brucea antidysenterica*[80], showed anti-HIV activity with therapeutic index of more than 25. Well-known antimalarial sesquiterpene lactone, artemisinin (**86**), isolated from *Artemisia anuua* L.[81] showed anti-HIV activity with an IC_{50} of 100 μM and EC_{50} of 100 μM.

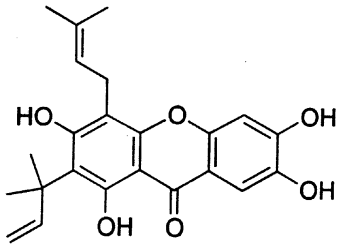
Kaurane diterpenoid, 16*b*,17-dihydroxy-*ent*-kauran-19-oic acid (**87**) isolated from methanolic extracts of the fresh fruits of *Annona squamosa* L. (Annonaceae), significantly inhibited HIV with an EC_{50} value of 0.8 μg/ml (TI > 5)[82]. Linearol (**88**), an *ent*-kaurane diterpenoid isolated from *Sideritis akmanii* and its semisynthetic derivatives showed significant anti-HIV activity against HIV-1 replication in H9 lymphocyte cells[83]. Phorbol ester, prostratin **(89)** isolated from *Homalanthus nutans* (family Euphorbiaceae), showed potent HIV inhibitory property. Another phorbol diester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA; **90**) isolated from methanolic extract of *Croton tiglium* (family Euphorbiaceae) inhibited HIV-1-induced cytopathic effects[84]. 12-Deoxyphorbol 13-(3*E*, 5*E*-decadienoate) (**91**), isolated from leaves and stems of *Excoecaria agallocha* inhibited HIV-1 RTase[85].

Diterpene lactone, andrographolide (**92**) isolated from *Andrographis paniculata* inhibited HIV-infected cells from arresting in G2 phase in which viral replication is optimal. It has also been reported to inhibit cell-to-cell transmission, viral replication and syncytia formation in HIV-infected cells[86]. Another diterpene lactone, nortriptterifordin (**93**) isolated from *Tripterygium wilfordii* inhibited HIV replication in H9 lymphocytes[87]. Diterpenes from *Homolanthus acuminatus* and *Chrysobalanus icaco* have shown HIV-inhibitory activity in *in vitro* screening[88]. Glycyrrhizin from licorice root has shown anti-HIV-1 activity in MT-4 cells[89]. A limonoid, clausenolide-1-ethyl ether (**94**) isolated from ethanol extract of rhizomes of *Clausena excavata* (family Rutaceae), exhibited HIV inhibitory activity in 1A2 cell line in syncytium assay[90]. Limonin (**95**) and nomilin (**96**) isolated from *Citrus* spp. (family Rutaceae) exhibited anti-HIV-1 activity in different cell-based assays. A dose-dependent inhibition of viral replication was observed in PMBC isolated from healthy donors and infected with HIV-1 strain after incubation with limonin and nomilin. These also inhibited the production of HIV-1 p-24 antigen in infected monocytes/macrophages. The mechanism of anti-HIV-1 effect of limonoids was found to be inhibition of HIV-1 protease[91].

Xanthones: Swertifrancheside (**97**), a flavonone–xanthone glucoside isolated from *Swertia franchetiana* was found to inhibit HIV-1 RTase. Its mode of action was found to be related to its binding with DNA, and may explain why it is also an inhibitor of several other polymerases, including DNA polymerase, and thus not a selective HIV-1 RTase inhibitor[92]. The prenylated xanthone, macluraxanthone B

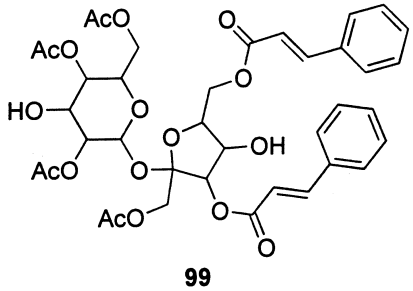


97



98

Figure 9. Anti-HIV xanthones from plants.



99

Figure 10. Anti-HIV carbohydrate from plants.

(**98**) isolated from *Maclura tinctoria* (family Moraceae) exhibited moderate anti-HIV activity[93].

Carbohydrates: The antiviral activity of carbohydrates extracted from various natural sources has been known since long time. Several sulphated polysaccharides were shown to inactivate HIV by binding with the surface envelope glycoprotein gp120. Niruriside (**99**), isolated from the methanolic extract of dried leaves of *Phyllanthus niruri* L.[94], is a novel specific inhibitor of REV protein/RRE RNA with an IC_{50} value of 3.3 μM. A polysaccharide fraction isolated from *Thuja occidentalis* (family Cuppressaceae), designated *Thuja* polysaccharide *g*-fraction exhibited HIV-1 reverse transcriptase activity[95].

Peptides: Small macrocyclic peptides, cycloviolins isolated from tropical plant *Leonia cymosa* and circulins, a group of cyclic peptides isolated from *Chassalia parvifolia* (family Rubiaceae), exhibited anti-HIV activity[96,97]. Palicourein, a 37 amino acid cyclic polypeptide, isolated from organic extract of the tropical tree *Palicourea condensata* (family Rubiaceae), inhibits the *in vitro* cytopathic effects of HIV-1_{RF} infection of CEM-SS cells[98].

Proteins: Ribosome inactivating proteins (RIPs) are those that specifically interfere with eukaryotic protein translation. RIPs are widely distributed in nature but are found predominantly in plants, bacteria and fungi. They vary greatly in their physical properties and cellular effects. Many of the plants from which RIPs are isolated are used medicinally in traditional Chinese medicine and the RIPs may account for some of the reported clinical efficacies of these plants[99]. Trichosanthin, *b*-momorcharin and L-momorcharin inhibited HIV replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. Trichosanthin also inhibited HIV replication in H9 and CEM-SS cells, and syncytium formation between infected H9 cells and uninfected Sup-T1 cells[99].

Anti-HIV proteins, MAP30, TAP29 isolated from *Momordica charantia* seeds and *Trichosanthes kirilowii* tubers, elicited a dose-dependent inhibition of cell-free HIV-1 infection and replication. Viral-associated reverse transcriptase activity in HIV-1-infected H9 cells was also inhibited in conjunction with a suppression of syncytium formation in the CD4-positive, syncytium-sensitive, Leu3a-sensitive T-cell line CEM-SS and viral core protein p24 expression[100]. MRK29, a Thai bitter gourd protein isolated from *Momordica charantia* inhibited reverse transcriptase[101]. GAP31 isolated from *Gelonium multiflorum* inhibited HIV-1 integrase[102]. Saporin and luffin, also exhibited anti-HIV integrase activity[99]. *Myrianthus holstii* lectin (MHL), a 9284-Da, cysteine-rich protein isolated from aqueous extract of *M. holstii* (family Moraceae)[103], showed anti-HIV activity with an EC_{50} value of 150 nM.

Miscellaneous: The methanolic extracts of *Crinum asiaticum*[104], *Tetrapteris macrocarpa*[105], 50% hydroalcoholic extract of *Hysopp officinalis*, aqueous extract of *Dittrichia viscose*[106], *Jatropha curca, Chamaesyce hyssopifolia, Cordia spinescens, Hyptis lantanifolia*[105] and extracts of *Tuberosa lignose, Sanguisorba minor magnolii* exhibited anti-HIV activity. The acetone fraction of *Combretum paniculatum* and the methanolic fraction of *Dodonaea angustifolia* showed selective inhibition of HIV-1 replication with selectivity indices of 6.4 and 4.9, and afforded cell protection of viral-induced cytopathic effect of 100 and 99% respectively[107]. The hydroalcoholic extract of *Derris scandens* increased the natural killer cell activity in HIV infected individuals[108].

Natural products from marine organisms

In a relatively short span of three decades, marine organisms have yielded an array of structures exhibiting a range of biological activities. Several marine natural products have shown anti-HIV activity.

Alkaloids: Sponge-derived polycyclic guanidine alkaloids exhibit diverse biological activities, including cytotoxicity towards cancer cell lines and antifungal, antimicrobial and antiviral activities. Crambescidin 826 (**100**) and dehydrocrambine A (**101**) inhibited HIV-1 envelope-mediated fusion *in vitro*[109]. Other polycyclic guanidine alkaloids, batzelladines A (**102**) and B (**103**) isolated from the ethanol extracts of a bright red Caribbean sponge of genus *Batzella* were active in the cell-based assay that measures the binding of gp120 to CD4-positive T-cells[110]. Trikendiol (**104**), an unusual red pigment, isolated from the sponge *Trikentrion loeve* Carter was found to be active in a CEM-4 HIV-1 infection assay, as measured by inhibition of cytopathogenic effect of the virus[111]. Manzamine alkaloids, ent-12,34-oxamanzamine E, ent-12,34-oxamanzamine F and 12,34-oxamanzamine A isolated from sponge, showed activity against AIDS OI-pathogens (e.g. *Cryptosporidium parvum* and *Toxoplasma gondii*)[112].

Sterols: Marine sponges are known to produce a variety of interesting and unconventional steroids among which polyoxygenated steroids have received greatest attention due to remarkable biological and pharmacological activities. In particular, sulphated steroids have been examined for their potential as inhibitors of HIV. Haplosamates A (**105**) and B (**106**), sulphated sterols isolated from Philippine sponge *Xestospongia* sp. inhibited HIV integrase[113], while another sulphated sterol, clathsterol (**107**) isolated from red sea sponge, *Clathria* sp. inhibited HIV-1 RTase[114]. Halistanol sulphates G (**108**) and H (**109**) isolated from marine sponge *Pseudoaxinissa digitata* were cytoprotective against HIV-1 in the NCI primary anti-HIV screen[115].

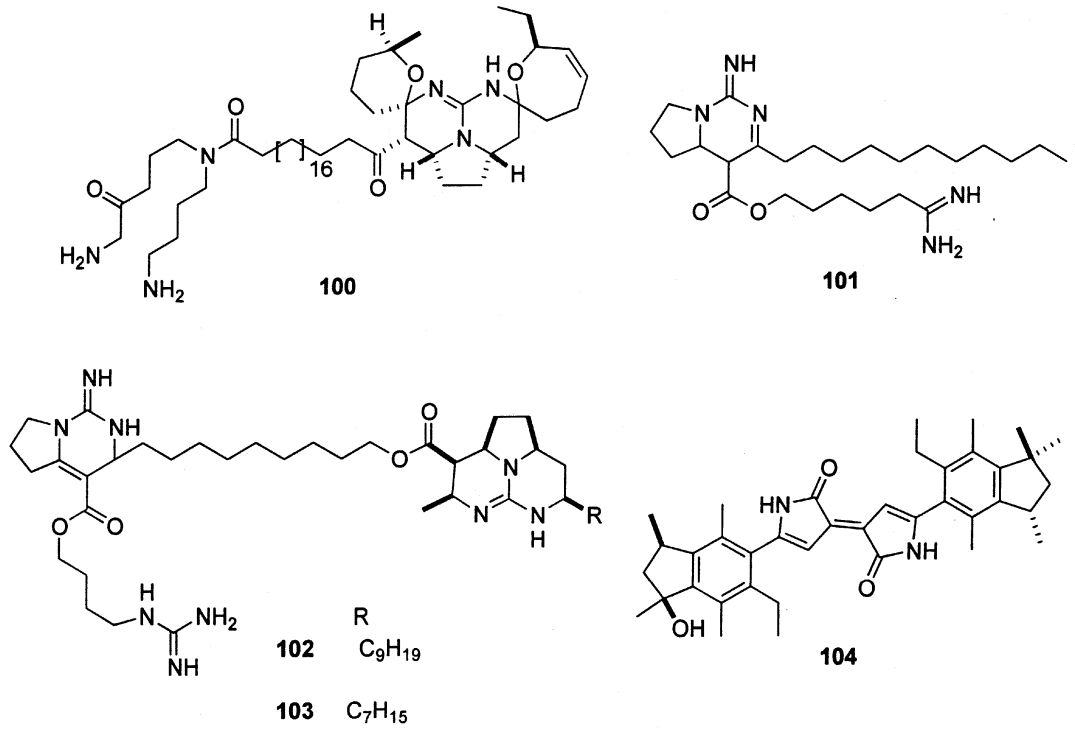


Figure 11. Anti-HIV alkaloids of marine origin.

Terpenes: Cyanthiwigin B (**110**), a diterpene isolated from Jamaican sponge *Myrmekioderma styx* exhibited activity against HIV-1 and cembrane diterpenoids, lobohedleolides exhibited moderate anti-HIV inhibitory activity in cell-based *in vitro* anti-HIV assay[116]. Avarone (**111**) and avarol (**112**) isolated from the sponge *Dysidea avara* showed anti-HIV activity, but controlled studies failed to confirm their utility in treatment of HIV-infection. Illimaquinone (**113**) isolated from red sea sponge *Smenospongia* sp. inhibited specifically RNase H[117].

Carbohydrates: Several sulphated polysaccharides and other polyanionic materials were shown to be inhibitors of the replication of HIV-1 *in vitro*, activity of which has been responsible for the presence of polyanionic charges[5]. A galactan sulphate, isolated from an aqueous extract of the red seaweed *Aghardhiella tenera*, inhibited the cytopathic effect of HIV-1 and HIV-2 in MT-4 cells with IC_{50} values of 0.6–0.4 and 0.5–0.3 μg/ml respectively[118]. Sulphated water-soluble polysaccharides such as agarocolloids and carageenans from gametic, carposporic and tetrasporic stages of the Mediterranean gametic and tetrasporic galactans were found to be active against HIV. These inhibit HIV replication in cell culture without any toxicity to the host cells. Maximal antiviral effect involves the presence of polysaccharides after or during infection, but not before infection. This time of action suggests an inhibition at an early step of HIV infection[119]. Rhamnan sulphate, a natural sulphated polysaccharide isolated from Chlorophyta, *Monostroma latissimum* is a potent antiviral substance[120] against HSV-1, HCMV and HIV-1. A sulphated xylomannan, obtained from the water extracts of the red seaweed *Nothogenia fastigiata*, was found to inhibit efficiently the replication of HSV-1 and several other viruses. Another sulphated xylomannan was only weakly active against HIV-1 and HIV-2 with an IC_{50} value of 13.7 and 13.4 μg/ml respectively[121].

Peptides: A cyclic depsidecapeptide, callipeltin A was isolated from a shallow water sponge of the genus *Callipelta*, collected in the waters of New Caledonia. Callipeltin A showed cytotoxicity at CD_{50} of 0.29 μg/ml, and ED_{50} of 0.01 μg/ml, giving a selectivity index of 29 (SI ratio CD_{50}/ED_{50})[122]. Microspinosamide, a new cyclic depsipeptide incorporating 13 amino acid residues, was isolated from extracts of an Indonesian collection of the marine sponge *Sidonops microspinosa*. It is the first naturally occurring peptide to contain a *b*-hydroxy-*p*-bromophenylalanine residue. It inhibited the cytopathic effect of HIV-1 infection in an XTT-based *in vitro* assay[123].

Proteins: Cyanovirin-N is a 11-kDa protein that has been isolated from the cyanobacterium (blue-green alga) *Nostoc ellipsosporum.* At low nanomolar concentrations, it irreversibly inactivates both laboratory strains and primary isolates of HIV-1 and HIV-2; in addition, it aborts cell-to-cell fusion and transmission of HIV infection[124].

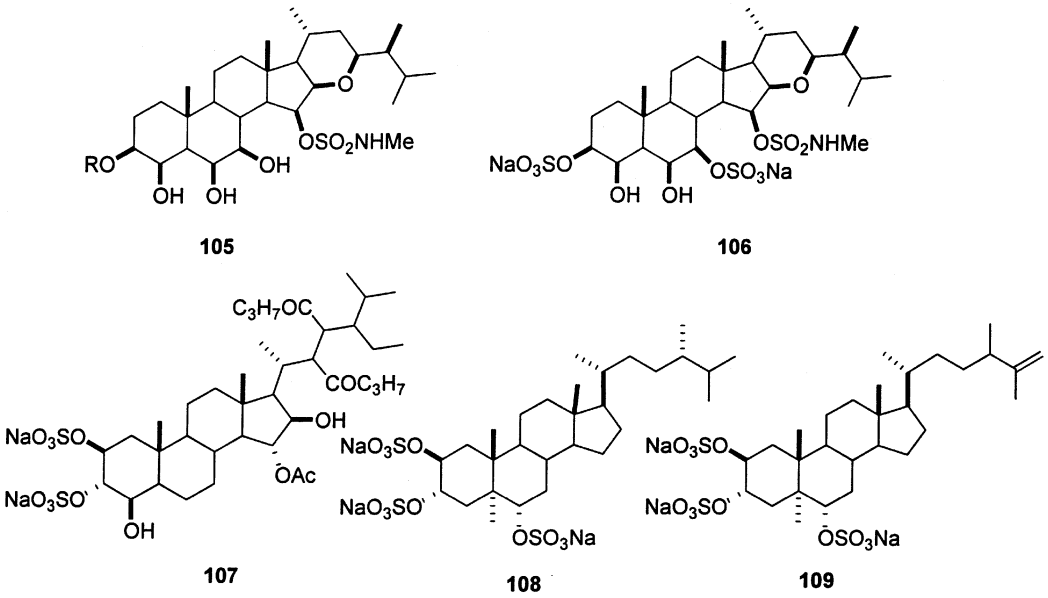


Figure 12. Anti-HIV sterols of marine origin.

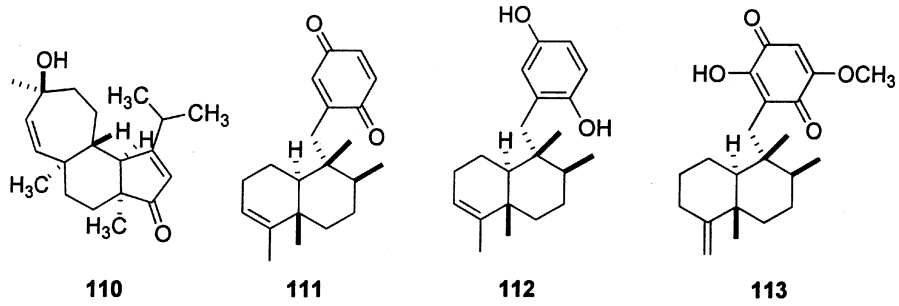


Figure 13. Anti-HIV terpenes of marine origin.

Natural products from microbial origin

Tat is a small HIV protein essential for both viral replication and progression of HIV disease. Durhamycin A isolated from the methyl ethyl ketone extract of fermentation broth of *Actinoplanes durhamensis*, was found to be a potent inhibitor of tat transactivation (IC_{50} 4.8 nM)[125]. Complestatin A and B, isocomplestatin and chloropeptin isolated from the fermentation broth of *Streptomyces* sp. MA7234 showed HIV inhibitory activities in coupled 3′-end processing/strand transfer (200 nM), strand transfer (4 μM) and HIV-1 replication (200 nM) in virus-infected cells[126]. Siamycins, polypeptides isolated from *Streptomyces* were found to inhibit HIV infection *in vitro*. They exert a strong inhibitory effect on syncytium formation, while only weakly inhibiting virus cell-binding[127]. Equisetin, obtained from the fungus *Fusarium heterosporum*, inhibits 3′-end processing and strand transfer at an IC_{50} of approximately 10 μM. It also inhibits strand-transfer reactions catalysed by pre-integration complexes isolated from HIV-infected cells. Whether it inhibits HIV-1 replication within the cells due to inhibition of integration, remains to be determined[128]. Integrastatins A (**114**) and B (**115**), isolated from an endophytic fungus *Ascochyta* sp., possess a novel [6/6/6/6]-ring system and are racemic despite having two asymmetric centres. These compounds inhibited the strand-transfer reaction of HIV-1 integrase[129] with IC_{50} value of 1.1–2.5 μM. Integracins A–C (**116–118**) are three novel dimeric alkyl aromatic inhibitors of HIV-1 integrase, discovered from the screening of fungal extracts using an *in vitro* assay. These compounds inhibit both coupled and strand-transfer activity of HIV-1 integrase with IC_{50} values of 3.2–6.1 and 17–88 μM respectively[130]. Integracides A and B, 4,4-dimethylergosterone triterpenoid sulphated esters isolated from the fermentation broth of a *Fusarium* sp. exhibited significant inhibitory activity against strand-transfer reaction of HIV-1 integrase[131]. [Ile7]surfactin (**119**) and [Leu7]surfactin (**120**) isolated from *Bacillus subtilis natto* are cyclic depsipeptides which contain a *b*-hydroxy fatty acid and seven amino acids. They exhibited moderate anti-HIV activities in XTT formazan assay for HIV-1 cytopathic effects[132]. Cytosporic acid (**121**) isolated from fermentation broth of filamentous

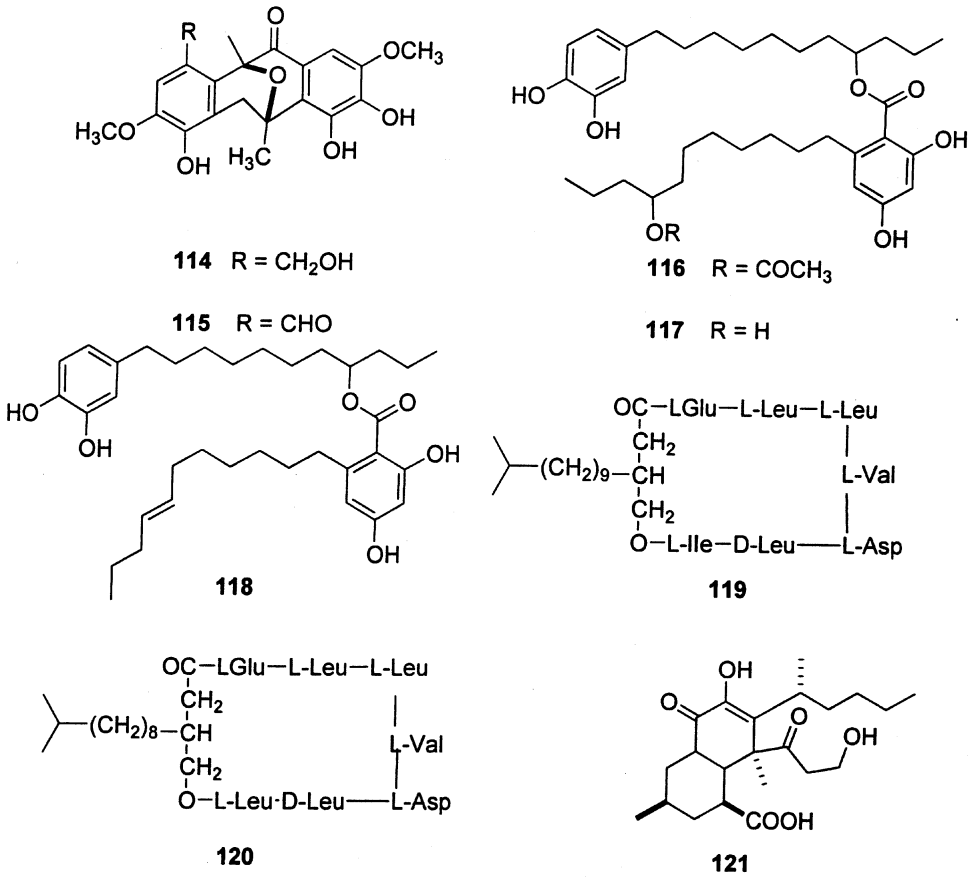


Figure 14. Anti-HIV natural products of microbial origin.

fungus *Cytospora* sp. inhibited strand-transfer reaction of HIV-1 integrase[133].

Miscellaneous

Bovine milk proteins: Bovine milk contains a number of proteins such as lactoferrin, lactoperoxidase, glycolactin, angiogenin-1, lactogenin, *a*-lactalbumin, lactoglobulin and casein. These proteins inhibited HIV-1 reverse transcriptase, protease and integrase to different extents. Lactoferrin strongly inhibited HIV-1 reverse transcriptase, but only slightly inhibited HIV-1 protease and integrase. On the other hand, *a*-lactalbumin, *b*-lactoglobulin and casein inhibited HIV-1 protease and integrase to an appreciable extent, but did not inhibit HIV-1 reverse transcriptase. Glycolactin and angiogenin-1 suppressed the activity of HIV-1 reverse transcriptase to a moderate extent, but more powerfully inhibited HIV-1 protease and integrase. In comparison with other milk proteins, glycolactin has been reported to have strong inhibitory activity against HIV-1 protease and integrase and a moderate inhibitory activity against HIV-1 reverse transcriptase. Lactogenin was a strong inhibitor of HIV-1 integrase, a moderate inhibitor of HIV-1 reverse transcriptase and a weak inhibitor of HIV-1 protease[134].

Leguminous antifungal proteins: Antifungal proteins are defence molecules produced by diverse organisms, including plants and invertebrate animals. A variety of antifungal proteins were isolated from seeds of leguminous plants, including french bean, cowpea, field bean, mung bean, peanut and red kidney bean. The cowpea *b*-antifungal protein was found to have high potency in inhibiting HIV-1 protease and HIV-1 integrase, while cowpea *a*-antifungal protein was potent in inhibiting HIV-1 reverse transcriptase and HIV-1 integrase. Peanut antifungal protein had high inhibitory activity against HIV-1 integrase and an intermediate potency in inhibiting HIV-1 reverse transcriptase and HIV-1 protease. French bean thaumatin-like protein has low HIV-1 protease inhibitory activity and red kidney bean lectin weakly inhibited HIV-1 integrase. Antifungal proteins from field bean and mung bean had an intermediate potency in inhibiting HIV-1 protease and integrase. However, mung bean antifungal protein was not capable of inhibiting HIV-1 reverse transcriptase. The above findings indicate that nearly all leguminous antifungal proteins examined were able to inhibit HIV-1 reverse transcriptase, protease and integrase to some extent[135].

Lysozyme: This is a potent AIDS-fighting protein naturally found in tears, saliva and urine of pregnant women, and could help in more effective treatments for AIDS.

Lysozyme breaks down the AIDS virus. It has been suspected that lysozyme works with another anti-AIDS protein found in urine, called ribonuclease to break down the genetic material of the HIV and prevent the virus from replicating. These proteins are promising anti-AIDS agents and likely will be well tolerated by the body, causing few side effects. These findings may help explain why AIDS is not transmitted through saliva. Lysozymes found in mother's breast milk, white blood cells and chicken egg white also show powerful anti-AIDS activity. Ribonuclease from the pancrease of cows also possesses anti-HIV properties[136].

Conclusion

In a decade of extensive research, great progress has been achieved in the discovery of potent anti-HIV agents from nature. A number of natural products have been used as lead compounds because of their specific activity and low toxicity. Many of them have potential to interfere with particular viral target, which can result in mechanisms of action complementary to those of existing antiviral drugs. Although no plant-derived drug is currently in clinical use to treat AIDS, promising activities have been shown by three natural product(s)/natural product-derived candidates in preclinical and early clinical trials. Sarawak MediChem Pharmaceuticals currently started phase II clinical trials of calanolide A for assessment of long-term anti-HIV activity of calanolide A in combination with other anti-HIV agents and an assessment of the long-term durability of such drug combinations. Another two lead molecules which are licensed to Panacos Pharmaceuticals, 3-hydroxymethyl-4-methyl DCK (PA-334B) and DSB (PA-457), have also successfully completed preclinical studies. Recently, Panacos has started phase II clinical studies of PA-457. These three clinical candidates have the potential to come up as drugs for treatment of HIV infection.

1. *AIDS Epidemic Update – 2004*, UNAIDS/WHO (http://www.unaids.org)
2. Coffin, J. M., HIV population dynamics *in vivo*: Implications for genetic variation, pathogenesis, and therapy. *Science*, 1995, **267**, 483–488.
3. De Clercq, E., Antiviral therapy for human immunodeficiency virus infections. *Clin. Microbiol. Rev.*, 1995, **8**, 200–239.
4. Matthee, G., Wright, A. D. and Konig, G. M., HIV reverse transcriptase inhibitors of natural origin. *Planta Med.*, 1999, **65**, 493–506.
5. Jung, M., Lee, S., Kim, H. and Kim, H., Recent studies on natural products as anti-HIV agents. *Curr. Med. Chem.*, 2000, **7**, 649–661.
6. Yang, S. S., Cragg, G. M., Newman, D. J. and Bader, J. P., Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program. *J. Nat. Prod.*, 2001, **64**, 265–277.
7. Cos, P., Mees, L., Berghe, D. V., Hermans, N., Pieters, L. and Vlietinck, A., Plant substances as anti-HIV agents selected according to their putative mechanism of action. *J. Nat. Prod.*, 2004, **67**, 284–293.
8. Manfredi, K. P., Blunt, J. W., Cardellina, J. H. I., McMahon, J. B., Pannell, L. K., Cragg, G. M. and Boyd, M. R., Novel alkaloids from the tropical plant *Ancistrocladus abbreviatus* inhibit cell killing by HIV-1 and HIV-2. *J. Med. Chem.*, 1991, **34**, 3402–3405.
9. Karpas, Y. A., Fleet, G. W. J. and Dwek, R. A., Amino sugar derivatives as potential anti-HIV agents. *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 9229.
10. McMormick, J. L., McKee, T. C., Cardellino, J. H. and Boyd, M. R., HIV inhibitory natural products. Quinoline alkaloids from *Euodia roxburghiana*. *J. Nat. Prod.*, 1996, **59**, 469–471.
11. Duan, H., Takaishi, Y., Imakura, Y., Jia, Y., Li, D., Cosentino, L. M. and Lee, K. H., Sesquiterpene alkaloids from *Tripterigium hypoglaucum* and *Tripterygium wilfordii*: A new class of potent anti-HIV agents. *J. Nat. Prod.*, 2000, **63**, 357–361.
12. Ma, C. M., Nakamura, N., Miyashiro, H., Hattori, M., Komatsu, K., Kawahata, T. and Otake, T., Screening of Chinese and Mongolian herbal drugs for anti human immunodeficiency virus type-1 (HIV-1) activity. *Phytother. Res.*, 2002, **16**, 186–189.
13. Tan, G. T., Pezzuto, J. M., Kinghorn, A. D. and Hughes, S. H., Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *J. Nat. Prod.*, 1991, **54**, 143–154.
14. Beutler, J. A., Cardellina, J. H., McMahon, J. B., Boyd, M. R., Kashman, Y. and Cragg, G. M., Anti-HIV and cytotoxic alkaloids from *Buchenavia capitata*. *J. Nat. Prod.*, 1992, **55**, 207–213.
15. Ishida, J., Wang, H. K., Oyama, M., Cosentino, M. L., Hu, C. Q. and Lee, K. H., Anti-AIDS agents 46. Anti-HIV activity of harman, an anti-HIV principle from *Symplocos setchuensis*, and its derivatives. *J. Nat. Prod.*, 2001, **64**, 958–960.
16. Xu, Z. *et al.*, Anti-HIV agents and antitumor agents. Two new sesquiterpenes, leitneridanins A and B and the cytotoxic and anti-HIV principles from *Leineria floridana*. *J. Nat. Prod.*, 2000, **63**, 1712–1715.
17. McKee, T. C., Covington, C. D. and Fuller, R., Pyranocoumarins from species of the genus *Callophyllum*: A chemotaxanomic study of extracts in the National Cancer Institute Collection. *J. Nat. Prod.*, 1998, **61**, 1252–1256.
18. Buckheit, R. W. *et al.*, Unique anti-human immunodeficiency virus activities of the non-nucleoside reverse transcriptase inhibitors calanolide A, costatolide, and dihydrocostatolide. *Antimicrob. Agents Chemother.*, 1999, **43**, 1827–1834.
19. Sarawak MediChem Pharmaceuticals Inc, Woodridge, IL, USA (http://www.sarawak-medichem.com).
20. Dharmaratne, H. R. W., Tan, G. T., Marasinghe, G. P. K. and Pezzuto, J. M., Inhibition of HIV-1 reverse transcriptase and HIV-1 replication by *Callophyllum* coumarins and xanthones. *Planta Med.*, 2002, **68**, 86–87.
21. Tsai, I. L., Wun, M. F., Teng, C. M., Ishikawa, S. and Chen, I. S., Anti-platelet aggregation constituents from formosan *Toddalia asiatica*. *Phytochemistry*, 1998, **48**, 1377–1382.
22. Huang, L., Kashiwada, Y., Cosentino, L. M., Fan, S. and Lee, K.-H., 3′,4′-Di-*O*-(–)-camphanoyl-(+)-*cis*-khellactone and related compounds: A new class of potent anti-HIV agents. *Bioorg. Med. Chem. Lett.*, 1994, **4**, 593–598.
23. Panacos Pharmaceuticals Inc, Gaithersburg, Maryland, USA (http://www.panacos.com).
24. Yu, D., Suzuki, M., Morris-Natschke, S. L. and Lee, K. H., Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med. Res. Rev.*, 2003, **23**, 322–345.
25. Zhou, P., Takaishi, Y. and Duan, H., Coumarins and bicoumarins from *Ferula sumbul*: Anti-HIV activity and inhibition of cytokine release. *Phytochemistry*, 2000, **53**, 689–697.
26. Meragelman, K. M., McKee, T. C. and Boyd, M. R., Anti-HIV prenylated flavonoids from *Monotes africanus*. *J. Nat. Prod.*, 2001, **64**, 546–548.
27. Kim, H. J., Woo, E. R. and Shin, C. G., A new flavonol glycoside gallate ester from *Acer okamotoanum* and its inhibitory activity

against human immunodeficiency virus-1 (HIV-1) integrase. *J. Nat. Prod.*, 1998, **61**, 145–148.
28. Lin, Y. M., Anderson, H., Flavin, M. T. and Pai, Y. S. H., *In vitro* anti-HIV activity of biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. *J. Nat. Prod.*, 1997, **60**, 884–888.
29. Hu, K., Kobayashi, H. and Dong, A., antifungal, antimitotic and anti-HIV-1 agents from the roots of *Wikstroemia indica*. *Planta Med.*, 2000, **66**, 564–567.
30. McKee, T. C., Covington, C. D. and Fuller, R., Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine and microbial organisms. *J. Nat. Prod.*, 1998, **60**, 431–436.
31. Wang, Q., Ding, Z. H., Liu, J. K. and Zheng, Y. T., Xanthohumol, a novel anti-HIV-1 agent purified from hops *Humulus lupulus*. *Antiviral Res.*, 2004, **64**, 189–194.
32. Charlton, J. L., Antiviral activity of lignans. *J. Nat. Prod.*, 1998, **61**, 1447–1451.
33. Rimando, A. M., Pezzuto, J. M., Fransworth, N. R., Santisuk, T., Reutrakul, V. and Kawanishi, K., New lignans from *Anogeissus acuminata* with HIV-1 reverse transcriptase inhibitory activity. *J. Nat. Prod.*, 1994, **57**, 904–996.
34. Eich, E., Pertz, H., Kaloga, M., Schulz, J., Fesen, M. R., Mazumder, A. and Pommier, Y., (–)-Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type-1 integrase. *J. Med. Chem.*, 1996, **39**, 86–95.
35. Hoang, V. D. *et al.*, Natural anti-HIV agents-Part-I: (+)-Demethoxyepiexcelsin and verticillatol from *Litsea verticillata*. *Phytochemistry*, 2002, **59**, 325–329.
36. Liul, K. C. S. C., Lin, M. T., Lee, S. S., Chiou, J. F., Ren, S. and Lien, E., Antiviral tannins from two *Phyllanthus* species. *Planta Med.*, 1999, **65**, 43–46.
37. Chen, D. F. *et al.*, Anti-AIDS agents – XXVI. Structure–activity correlations of gomisin-G-related anti-HIV lignans from *Kadsura interior* and of related synthetic analogues. *Bioorg. Med. Chem.*, 1997, **5**, 1715–1723.
38. Liu, J. S. and Li, L., Kadsulignans L–N, three dibenzocyclooctadiene lignans from *Kadsura coccinea*. *Phytochemistry*, 1995, **38**, 241–245.
39. Ovenden, S. B. P. *et al.*, Globoidnan A: A lignan from *Eucalyptus globoidea* inhibits HIV integrase. *Phytochemistry*, 2004, **65**, 3255–3259.
40. Valsaraj, R. *et al.*, New anti-HIV-1, antimalarial, and antifungal compounds from *Terminalia bellerica*. *J. Nat. Prod.*, 1997, **60**, 739–742.
41. Lim, Y. A., Mei, M. C., Kusumoto, I. T., Miyashiro, H. and Hattori, M., HIV-1 reverse transcriptase inhibitory principles from *Chamaesyce hyssopifolia*. *Phytother. Res.*, 1997, **11**, 22–27.
42. Ogata, T., Higuchi, S., Mochida, H., Matsumoto, A., Kato, T., Endo, A. K. and Kaji, H., HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. *AIDS Res. Hum. Retroviruses*, 1992, 1937–1944.
43. Nonaka, G., Sakai, R. and Nishioka, I., Hydrolyasable tannins and proanthocyanidins from green tea. *Phytochemistry*, 1984, **23**, 1753–1755.
44. Hashimoto, F., Kashiwada, Y., Nonaka, G., Nohara, T., Cosentino, L. M. and Lee, K. H., Evaluation of tea polyphenols as anti-HIV agents. *Bioorg. Med. Chem. Lett.*, 1996, **6**, 695.
45. Royer, R. E., Deck, L. M., Jagt, T. J. V., Martinez, F. J., Mills, R. G., Stephen, A. Y. and Jagt, D. L. V., Synthesis and anti-HIV activity of 1,1′-dideoxygossypol and related compounds. *J. Med. Chem.*, 1995, **38**, 2427–2432.
46. Fuller, R. W., Westergaard, C. K., Collins, J. W., J. H., C. and Boyd, M. R., Vismiaphenones D–G, new prenylated benzophenones from *Vismia cayennensis*. *J. Nat. Prod.*, 1999, **62**, 67–69.
47. Gustafson, K. R. *et al.*, The guttiferones, HIV-inhibitory benzophenones from *Symphonia globulifera*, *Garcinia livingstonei*, *Garcinia ovalifolia* and *Clusia rosea*. *Tetrahedron*, 1992, **48**, 10093–10102.
48. Kashiwada, Y. *et al.*, Anti-AIDS agents. Sodium and potassium salts of caffeic acid triterpenes from *Arnebia euchroma* as anti-HIV agents. *J. Nat. Prod.*, 1995, **58**, 392–400.
49. Ahn, M. J. *et al.*, Inhibition of HIV-1 integrase by galloyl glucoses from *Terminalia chebula* and flavonol glucoside gallates from *Euphorbia pekinensis*. *Planta Med.*, 2002, **68**, 454–457.
50. Park, J. C. *et al.*, Inhibitory effects of Korean medicinal plants and camellia tannin H from *Camellia japonica* on human immunodeficiency virus type-1 protease. *Phytother. Res.*, 2002, **16**, 422–426.
51. Nishizawa, M., Emura, M., Kan, Y., Yamada, H., Ogawa, K. and Hamanaka, N., Macrocarpals: HIV-RTase inhibitors of *Eucalyptus globulus*. *Tetrahedron Lett.*, 1992, **33**, 2983–2986.
52. Nakane, H., Arisawa, M., Fujita, A., Koshimura, S. and Ono, K., Inhibition of HIV reverse transcriptase activity by some phloroglucinol derivatives. *FEBS Lett.*, 1991, **286**, 83–85.
53. Bokesch, H. D., Growiess, A., McKee, T. C. and Boyd, M. R., Laxifloranone, a new phloroglucinol derivative from *Marila laxiflora*. *J. Nat. Prod.*, 1999, **62**, 1197–1199.
54. Kim, H. J., Yu, Y. G., Park, H. and Lee, Y. S., HIV gp41-binding phenolic components from *Fraxinus sieboldiana* var. *angustata*. *Planta Med.*, 2002, **68**, 1034–1036.
55. Roth, G. N., Chandra, A. and Nair, M. G., Novel bioactivities of *Curcuma longa* constituents. *J. Nat. Prod.*, 1998, **61**, 542–545.
56. Piancente, S., Pizza, C., Tommasi, N. D. and Mahmood, N., Constituents of *Ardisia japonica* and their *in vitro* anti-HIV activity. *J. Nat. Prod.*, 1996, **59**, 565–569.
57. Dai, J. R., Hallok, Y. F. and Cardellina, J. H., HIV-inhibitory and cytotoxic oligostilbenes from the leaves of *Hopea malibato*. *J. Nat. Prod.*, 1998, **61**, 351–353.
58. Manfredi, K. P., Vallurupalli, V. and Demidova, M., Isolation of an anti-HIV diprenylated bibenzyl from *Glycyrrhiza leptipoda*. *Phytochemistry*, 2001, **58**, 153–157.
59. Gustafson, K. R., II, J. H. C., McMahon, J. B., Gulakowski, R. J., Pannell, L. K., Cragg, G. M. and Boyd, M. R., HIV inhibitory natural products. 6. The peltatols, novel HIV-inhibitory catechol derivatives from *Pothomorphe peltata*. *J. Org. Chem.*, 1992, **57**, 2809–2811.
60. Min, B. S., Miyashiro, H. and Hattori, M., Inhibitory effects of quinones on RNase H activity associated with HIV-1 reverse transcriptase. *Phytother. Res.*, 2002, **16**, S57–S62.
61. Decosterd, L. A. *et al.*, HIV inhibitory natural products. 11. Structure, absolute stereochemistry, and synthesis of conocurvone, a potent, novel HIV-inhibitory naphthoquinone trimer from a *Conospermum* sp. *J. Am. Chem. Soc.*, 1993, **115**, 6673–6679.
62. Higuchi, H., Mori, K. and Kato, A., Antiretoviral activities of anthraquinones and their inhibitory effects on reverse transcription. *Antiviral Res.*, 1991, **15**, 205.
63. Sakurai, N. *et al.*, Anti-HIV agents. Actein, an anti-HIV principle from rhizome of *Cimicifuga racemosa* (black cohosh), and the anti-HIV activity of related saponins. *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1329–1332.
64. Nakamura, M., Kunimoto, S. and Takahashi, Y., Inhibitory effects of polyethers on human immunodeficiency virus replication. *Antimicrob. Agents Chemother.*, 1992, **36**, 492.
65. Yang, X. W., Zhao, J. and Cui, Y. X., Anti-HIV-1 protease triterpenoid saponins from the seeds of *Aesculus chinensis*. *J. Nat. Prod.*, 1999, **62**, 1510–1513.
66. Fujoka, T. and Kashiwada, Y., Anti-AIDS agents. Betulinic acid and platanic acid as anti-HIV principles from *Syzygium claviflorum* and the anti-HIV activity of structurally related triterpenoids. *J. Nat. Prod.*, 1994, **57**, 243–247.
67. Kashiwada, Y., Hashimoto, F., Cosentino, L. M., Chen, C. H., Garrett, P. E. and Lee, K. H., Betulinic acid and dihydrobetulinic

acid derivatives as potent anti-HIV agents. *J. Med. Chem.*, 1996, **39**, 1016–1017.
68. Kashiwada, Y. *et al.*, Anti-AIDS agents. Anti-HIV activity of oleanolic acid, pomolic acid and structurally related tritepenoids. *J. Nat. Prod.*, 1998, **61**, 1090.
69. Ma, C. M., Nakamura, N. and Hattori, M., Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. *J. Nat. Prod.*, 2000, **63**, 238–242.
70. Min, B. S., Jung, H. J. and Lee, J. S., Inhibitory effect of triterpenes from *Crataegus pinatifida* on HIV-1 protease. *Planta Med.*, 1999, **65**, 374–375.
71. Xu, H.-X., Zeng, F.-Q., Wan, M. and Sim, K.-Y., Anti-HIV triterpene acids from *Geum japonicum*. *J. Nat. Prod.*, 1996, **59**, 643–645.
72. Ito, J., Chang, F.-R., Wang, H.-K., Park, Y. K., Ikegaki, M., Kilgore, N. and Lee, K.-H., Anti-AIDS agents. Anti-HIV activity of moronic acid derivatives and the new melliferone related triterpenoid isolated from Brazilian Propolis. *J. Nat. Prod.*, 2001, **64**, 1278–1281.
73. Pengsuparp, T., Cai, L., Fong, H. H. S., Kinghorn, A. D., Pezzuto, J. M., Wani, M. C. and Wall, M. E., Pentacyclic triterpenes derived from *Maprounea africana* are potent inhibitors of HIV-1 reverse transcriptase. *J. Nat. Prod.*, 1994, **57**, 415–418.
74. Kuo, Y. H. and Kuo, L. M. Y., Antitumour and anti-AIDS triterpenoids from *Celastrus hindsii*. *Phytochemistry*, 1997, **44**, 1275–1281.
75. Mekkawy, S. E., Meselhy, M. R. and Nakamura, N., Anti-HIV-1 and anti-HIV-1 protease substances from *Ganoderma lucidum*. *Phytochemistry*, 1998, **49**, 1651–1657.
76. Li, H. Y., Sun, N. J., Kashiwada, Y. and Sun, L., Anti-AIDS agents. Suberosol, a new C31 lanostane type triterpene and anti-HIV principle from *Polyalthia suberosa*. *J. Nat. Prod.*, 1993, **56**, 1130–1133.
77. Rukachaisirikul, V. *et al.*, Anti-HIV-1 protostane triterpenes and digeranylbenzophenone from trunk bark and stems of *Garcinia speciosa*. *Planta Med.*, 2003, **69**, 1141–1146.
78. Sun, H.-D. *et al.*, Nigranoic acid, a triterpenoid from *Schisandra sphaerandra* that inhibits HIV-1 reverse transcriptase. *J. Nat. Prod.*, 1996, **59**, 525–527.
79. Chen, D. F., Zang, S. X. and Wang, H. K., Novel anti-HIV lancilactone C and related triterpenes from *Kadsura lancilimba*. *J. Nat. Prod.*, 1999, **62**, 94–97.
80. Sun, I.-C. *et al.*, Anti-AIDS agents. Synthesis and structure–activity relationships of betulin derivatives as anti-HIV agents. *J. Med. Chem.*, 1998, **41**, 4648–4657.
81. Jung, M. and Schinazi, R. F., Synthesis and *in vitro* anti-human immunodeficiency virus activity of artemisinin (Qinghaousu)-related trioxanes. *Bioorg. Med. Chem. Lett.*, 1994, **4**, 931–934.
82. Wu, Y. C., Hung, Y. C., Chang, F. R., Cosentino, M., Wang, H. K. and Lee, K. H., Identification of *ent*-16,17-dihydroxykauran-19-oic acid as an anti-HIV principle and isolation of the new diterpenoids, annosquamosins A and B from *Annona squamosa*. *J. Nat. Prod.*, 1996, **59**, 635–637.
83. Bruno, M., Rosselli, S., Pibiri, I., Kilgore, N. and Lee, K. H., Anti-HIV agents from the *ent*-kaurane diterpenoid linearol. *J. Nat. Prod.*, 2002, **65**, 1594–1597.
84. Mekkawy, S. E., Meselhy, M. R. and Nakamura, N., Anti-HIV phorbol esters from the seeds of *Croton tiglium*. *Phytochemistry*, 2000, **53**, 457–464.
85. Erickson, K. L., Beutler, J. A., Cardellina, J. H., McMahon, J. B., Newman, D. J. and Boyd, M. R., A novel phorbol ester from *Excoecaria agallocha*. *J. Nat. Prod.*, 1995, **58**, 769.
86. Calabrese, C. *et al.*, A phase I trial of andrographolide in HIV-positive patients and normal volunteers. *Phytother. Res.*, 2000, **14**, 333–338.
87. Duan, H., Takaishi, Y., Bando, M., Kido, M., Imakura, Y. and Lee, K. H., Novel sesquiterpene esters with alkaloid and monoterpene and related compounds from *Tripterygium hypoglaucum*: A new class of potent anti-HIV agents. *Tetrahedron Lett.*, 1999, **40**, 2969.
88. Gustafson, K. R. *et al.*, HIV inhibitory natural products. Diterpenes from *Homolanthus acuminatus* and *Chrysobalanus icaco*. *Tetrahedron*, 1991, **47**, 4547–4554.
89. Ito, M. *et al.*, Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus. *Antiviral Res.*, 1988, **10**, 289–298.
90. Sunthitikawinsakul, A. *et al.*, Anti-HIV-1 limonoid: First isolation from *Clausena excavata*. *Phytother. Res.*, 2003, **17**, 1101–1103.
91. Battinelli, L., Mengoni, F., Lichtner, M., Mazzanti, G., Saija, A., Mastroianni, C. M. and Vullo, V., Effect of limonin and nomilin on HIV-1 replication on infected human mononuclear cells. *Planta Med.*, 2003, **69**, 910–913.
92. Wang, J. N., Hou, C. Y., Liu, Y. L., Lin, L. Z., Gil, R. R. and Cordell, G. A., Swertifrancheside, an HIV-reverse transcriptase inhibitor and the first flavone-xanthone dimer from *Swertia franchetiana*. *J. Nat. Prod.*, 1994, **57**, 211–217.
93. Groweiss, A., Cardellina, J. H. and Boyd, M. R., HIV inhibitory prenylated xanthones and flavones from *Maclura tinctoria*. *J. Nat. Prod.*, 2000, **63**, 1537–1539.
94. Qian-Cutrone, J. *et al.*, Niruriside, a new HIV REV/RRE binding inhibitor from *Phyllanthus niruri*. *J. Nat. Prod.*, 1996, **59**, 196–199.
95. Offergeld, R., Reinecker, C., Gumz, E., Schrum, S., Treiker, R., Neth, R. D. and Gohla, S. H., Mitogenic activity of high molecular polysaccharide fractions isolated from the cuppressaceae *Thuja occidentalis* L. enhanced cytokine production by thyapolysaccharide, g-fraction (TPSg). *Leukemia*, 1992, **6**, 189s–191s.
96. Gustafson, K. R. *et al.*, Circulins A and B. Novel human immunodeficiency virus (HIV)-inhibitory macrocyclic peptides from the tropical tree *Chassalia parvifolia*. *J. Am. Chem. Soc.*, 1994, **116**, 9337–9338.
97. Hallock, Y. F. *et al.*, Cycloviolins A–D, anti-HIV macrocyclic peptides from *Leonia cymosa*. *J. Org. Chem.*, 2000, **65**, 124–128.
98. Bokesch, H. R., Pannell, L. K. and Cochran, P. K., A novel anti-HIV macrocyclic peptide from *Palicourea condensata*. *J. Nat. Prod.*, 2001, **64**, 249–250.
99. Au, T. K., Collins, R. A., Lam, T. L., Ng, T. B., Fong, W. P. and Wan, D. C. C., The plant ribosome inactivating proteins luffin and saporin are potent inhibitors of HIV-1 integrase. *FEBS Lett.*, 2000, **471**, 169–172.
100. Lee-Huang, S., Huang, P. L., Chen, H.-C., Huang, P. L., Bourinbaiar, A., Huang, H. I. And Kung, H.-F., Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. *Gene*, 1995, **161**, 151–156.
101. Jiratchariyakul, W. *et al.*, HIV inhibitor from Thai bitter gourd. *Planta Med.*, 2001, **67**, 350–353.
102. Lee-Huang, S. *et al.*, A new class of anti-HIV agents: GAP31, DAPs 30 and 32. *FEBS Lett.*, 1991, **291**, 139–144.
103. Charan, R. D., Munro, H. G. and R. O'Keefe, B., Isolation and characterisation of *Myrianthus holstii* lectin, a potent HIV-1 inhibitory protein from the plant *Myrianthus holstii*. *J. Nat. Prod.*, 2000, **63**, 1170–1174.
104. Min, B. S., Kim, Y. H., Tomiyama, M., Nakamura, N., Miyashiro, H., Otake, T. and Hattori, M., Inhibitory effects of Korean plants on HIV-1 activities. *Phytother. Res.*, 2001, **15**, 481–486.
105. Matsuse, I. T., Lim, Y. A., Hattori, M., Correa, M. and Gupta, M. P., A search for anti-viral properties in Panamanian medicinal plants. The effects on HIV and its essential enzymes. *J. Ethnopharmacol.*, 1999, **64**, 15–22.
106. Beyoda, L. S., Palomino, S. S., Abad, M. J., Bermejo, P. and Alcami, J., Screening of selected plant extracts for *in vitro* inhibitory activity on human immunodeficiency virus. *Phytother. Res.*, 2002, **16**, 550–554.
107. Asres, K., Bucar, F., Kartnig, T., Witvrouw, M., Pannecouque, C. and Clercq, E. D., Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnomedically selected Ethiopian medicinal plants. *Phytother. Res.*, 2001, **15**, 62–69.

108. Sriwanthana, B. and Chavalittumrong, P., *In vitro* effect of *Derris scandens* on normal lymphocyte proliferation and its activities on natural killer cells in normals and HIV-1 infected patients. *J. Ethnopharmacol.*, 2001, **76**, 125–129.
109. Chang, L. C., Whittaker, N. F. and Bewley, C. A., Crambescidin 826 and dehydrocrambine A: New polycyclic guanidine alkaloids from the marine sponge *Monanchora* sp. that inhibit HIV-1 fusion. *J. Nat. Prod.*, 2003, **66**, 1490–1494.
110. Patil, A. D. *et al.*, Novel alkaloids from the sponge *Batzella* sp. Inhibitors of HIV gp-120 human CD4 binding. *J. Org. Chem.*, 1995, **60**, 1182–1188.
111. Loukaci, A. and Guyot, M., Trikendiol, an unusual red pigment from the sponge *Trikentrion loeve*, anti-HIV-1 metabolite. *Tetrahedron. Lett.*, 1994, **35**, 6869–6872.
112. Yousaf, M. *et al.*, 12,34-Oxamanzamines, novel biocatalytic and natural products from manzamine producing Indo-Pacific sponges. *Tetrahedron*, 2002, **58**, 7397–7402.
113. Qureshi, A. and Faullmer, D. J., Haplosamates A and B: New steroidal sulfamate esters from two hapiosclerid sponges. *Tetrahedron*, 1999, **55**, 8323–8330.
114. Rudi, A., Yoseif, T., Loya, S., Hizi, A., Schleyer, M. and Kashman, Y., Clathsterol, a novel anti-HIV-1 RTase sulphated sterol from the sponge *Clathria* species. *J. Nat. Prod.*, 2001, **64**, 1451–1453.
115. Bifulco, G., Bruno, I., Minale, L. and Riccio, R., Novel HIV inhibitory halistanol sulphates F–H from a marine sponge, *Pseudoaxinissa digitata*. *J. Nat. Prod.*, 1994, **57**, 164–167.
116. Rashid, M. A., Gustafson, K. R. and Boyed, M. R., HIV-inhibitory derivatives from a Philippines collection of the soft coral *Lobophytum* species. *J. Nat. Prod.*, 2000, **63**, 531–533.
117. Schroder, H. C., Begin, M. E., Klocking, R., Matthes, E., Sarma, A. S., Gai, M. and Muller, W. E. G., Avarol restores the altered prostaglandin and leukotriene metabolism in monocytes infected with human immunodeficiency virus type 1. *Virus Res.*, 1991, **21**, 213–223.
118. Amornrut, C. *et al.*, A new sulphated galactan from clams with anti-HIV activity. *Carbohydr. Res.*, 1999, **321**, 121–127.
119. Haslin, C., Lahaye, M. and Pellegrini, M., *In vitro* anti-HIV activity of sulphated cell-wall polysaccharides from gametic, carposporic and tetrasporic stages of the Mediterranean red alga *Asparagopsis armata*. *Planta Med.*, 2001, **67**, 301–305.
120. Lee, J. B., Hayashi, K. and Hayashi, T., Antiviral activities against HSV-1, HCMV and HIV-1 of Rhamnan sulphate from *Monostroma latissimum*. *Planta Med.*, 1999, **65**, 439–441.
121. Damonte, E. *et al.*, Antiviral activity of a sulphated polysaccharide from the red seaweed *Nothogenia fastigiata*. *Biochem. Pharm.*, 1994, **47**, 2187.
122. Zampella, A., D'Auria, M. V., Paloma, L. G., Casapullo, A., Minale, L., Debitus, C. and Henin, Y., Callipeltin A, an anti-HIV cyclic depsipeptide from the new caledonian Lithiastida sponge *Callipelta* sp. *J. Am. Chem. Soc.*, 1996, **118**, 6202–6209.
123. Rashid, M. A., Gustafson, K. R. and Cartner, L. K., Microspinosamide, a new HIV inhibitory cyclic depsipeptide from the marine sponge *Sidonops microspinosa*. *J. Nat. Prod.*, 2001, **64**, 117–121.
124. Boyd, M. R. *et al.*, Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: Potential applications to microbicide development. *Antimicrob. Agents Chemother.*, 1997, **41**, 1521–1530.
125. Jayasurya, H. *et al.*, Durhamycin A, a potent inhibitor of HIV tat transactivation. *J. Nat. Prod.*, 2002, **65**, 1091–1095.
126. Singh, S. B. *et al.*, The complestatins as HIV-1 integrase inhibitors. Efficient isolation, structure elucidation and inhibitory activities of isocomplestatin I, new complestatin A and B, and acid hydrolysis products of chloropeptin I. *J. Nat. Prod.*, 2001, **64**, 874–882.
127. Chokekijchai, S. *et al.*, NP-06: A novel anti-human immunodeficiency virus polypeptide produced by a *Streptomyces* species. *Antimicrob. Agents Chemother.*, 1995, **39**, 2345–2347.
128. Hazuda, D. *et al.*, Isolation and characterisation of novel human immunodeficiency virus integrase inhibitors from fungal metabolites. *Antiviral Chem. Chemother.*, 1999, **10**, 63–70.
129. Singh, S. B., Zink, D. L., Quamina, D. S., Pelaez, F., Teran, A., Felock, P. and Hazuda, D. J., Integrastatins: Structure and HIV-1 integrase inhibitory activities of two novel racemic tetracyclic aromatic heterocycles produced by two fungal species. *Tetrahedron Lett.*, 2002, **43**, 2351–2354.
130. Singh, S. B. *et al.*, Discovery, structure and HIV-1 integrase inhibitory activities of integracins, novel dimeric alkyl aromatics from *Cytonaema* sp. *Tetrahedron Lett.*, 2002, **43**, 1617–1620.
131. Singh, S. B. *et al.*, Integracides: Tetracyclic triterpenoid inhibitors of HIV-1 integrase produced by *Fusarium* sp. *Bioorg. Med. Chem.*, 2003, **11**, 1577–1582.
132. Itokawa, H., Miyashita, T., Morita, H., Takeya, K., Hirano, T., Homma, M. and Oka, K., Structural and conformational studies of [Ile7] and [Leu7]surfactins from *Bacillus subtilis* natto. *Chem. Pharm. Bull.*, 1994, **42**, 604.
133. Jayasurya, H., Guan, Z., Polishook, J. D., Dombrowski, A. W., Felock, P. J., Hazuda, D. J. and Singh, S. B., Isolation, structure and HIV-1 integrase inhibitory activity of cytosporic acid, a fungal metabolite produced by a *Cytospora* sp. *J. Nat. Prod.*, 2003, **66**, 551–553.
134. Ng, T. B., Au, T. K., Lam, T. L., Ye, X. Y. and Wan, C. C., Inhibition of human immunodeficiency virus type 1 reverse transcriptase, protease and integrase by bovine milk proteins. *Life Sci.*, 2001, **69**, 2217–2223.
135. Ng, T. B., Au, T. K., Lam, T. L., Ye, X. Y. and Wan, C. C., Inhibitory effects of antifungal proteins on human immunodeficiency virus type 1 reverse transcriptase, protease and integrase. *Life Sci.*, 2002, **70**, 927–935.
136. Lee-Huang, S., Huang, P. L., Sun, Y., Kung, H., Blithe, D. L. and Chen, H. C., Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 2678–2688.

ACKNOWLEDGEMENT. We thank Prof. P. Rama Rao, Director, NIPER, Punjab for support.

Received 17 February 2005; revised accepted 16 May 2005