

Coumarins as anti-HIV agent and correlation with COVID-19: an overview

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Abstract

Acquired immunodeficiency syndrome (AIDS) is an immunosuppressive disease caused by human immunodeficiency virus (HIV), and leads to infection and malignance, which are life threatening. HIV/AIDS cause numerous deaths in Africa, and is the leading cause of death in India, affecting every fourth living HIV patient. The amount in Southeast Asia is increasing at alarming rate. In this paper, the current issue of COVID-19 and its' possible target of HIV drugs that inhibit entry of the virus into host cell were investigated. Three main groups of coronavirus are surrounded by spike genes, showing that for fusion mechanism, these groups are common mode for attachment. For nearly all disease areas, plant kingdom biodiversity provides a source of new drug candidates. There is a continuous increase in various compounds with anti-HIV activity isolated from natural sources. Various literature and current COVID-19 pandemic situation were considered in the present report. Phase II clinical candidates are calanolide A, a coumarins isolated from *Calophyllum lanigerum*, and two other natural product-derived molecules, such as DSB and 3-hydroxymethyl-4-methyl DCK, with a potential to emerge as HIV therapy drugs. This naturally obtained product with anti-HIV properties was described in the present paper, focusing on current results as anti-HIV agents derived from natural sources.

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Introduction

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome coming mostly from human immunodeficiency virus (HIV) infection that causes long and deep immunosuppression. Since the first case identified in 1981, it is considered as a major, life-threatening problem, and the disease is ranked the highest in spreading in India. Since the start of the epidemic, the virus has infected more than 60 million people. HIV and AIDS are the major reason for increasing death rate in sub-Saharan Africa. Worldwide, the disease is ranked four as the highest in killing. Around

40 million people (37.2 million adults and 2.2 million children) lived with HIV globally by the end of 2004, approximately 22 million of whom had died, according to recent reports of the World Health Organization and UNAIDS. In 2004, 3.1 million adults and children were infected with HIV, with death rate around 2-3 million. The worst affected area is sub-Saharan Africa. In 2004, there were 25.4 million people living affected by HIV/AIDS in the region [1]. In 2004, approximately 1.2 million people in Asia were infected with HIV, and it was estimated that 8.2 million people were living with the disease. Moreover, in 2004, another

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540,000 people have died because of AIDS. HIV has been diversified in India, with a large number of people having an early stage of the disease, and the highest epidemic region in the South. Up to December 2004, 10 of 28 countries and seven union territories with 92% of all nationally reported AIDS cases, had been identified. Maharashtra and Gujarat in the West were the largest, Tamil Nadu and Andhra Pradesh in the South and Northeast were the most numerous parts of the country. In Southern states, Manipur and Nagaland, majority of people infected were injecting drug users. In 2004, the maximum number of AIDS cases has been reported as 96,978, with Tamil Nadu ($n = 44,492$) and Maharashtra ($n = 12,783$). In active reproductive ages, a large number of people (men and women) was infected with HIV, and around half of all those who become infected with HIV were below the age of 25. Potential for infected mothers to transfer the disease to their babies is of great concern [1, 2]. Thus far, HIV was classified mainly into two categories, including HIV-1 and HIV-2. HIV worldwide epidemic is classified as type 1 HIV. This varying virus easily mutates. Many different HIV-1 strains can be grouped and categorized by sub-types (two groups are M and O). At least 10 genetically distinct HIV-1 sub-types are currently known in group M, including sub-types A to J. Group O also contains a separate heterogeneous virus's bunch. HIV-2 is considered as rare and minimally pathogenic, found mainly in West Africa. HIV starts its' host cell infection by binding with host cell CD4 receptor. In many lymphocytes, CD4 appears on the surface, and are tough portion of the immune system of the body. A co-receptor is known to be needed to enter the cell. Entry of HIV into the cell virus is fused with host cell. Virus genetic material releases RNA and is transcribed into DNA reverse. In order to catalyze this viral conversion of RNA into DNA in HIV, there is enzyme known as 'transcriptase'. This viral DNA enters the host cell nucleus, when genetic material of HIV is converted to DNA, and integrates with the cell's genetic material. Integrated enzyme catalyzes the above-mentioned process. With the integration of viral DNA into the host's genetic material, HIV may persist for many years in a latent state. The impediment to eradication or HIV cure is HIV's ability to suppress in typical lately cells that are infected. This is why patients should stay on anti-viral therapy for life, based on current knowledge [3]. Several reviews on natural HIV chemotherapy agents have been previously published. According to their chemical class, some classification appeared in scientific arena. Moreover, discovery and NCI development programs for natural products was based on anti-HIV drugs. In the process of development, a recently published study on various plant materials in accordance with their mechanisms of action as anti-HIV agents has been well accepted by scientific experts [4]. In a recent study, it was found that anti-HIV drug nelfinavir mesylate (viracept) was a potent inhibitor of cell fusion caused by SARS-CoV-2 spike (S) glycoprotein, which would be considered anti-COVID-19. One of significant studies on the relation of HIV and COVID-19 treatment found effective anti-viral modified nucleosides for the development

of HIV-1 reverse-transcriptase inhibitor 'islatravir', modified nucleosides against COVID-19 [5, 6]. However, many anti-viral drugs were identified as potential anti-COVID-19 agents, such as cepharanthine and nelfinavir, and their usage for the combination treatment was investigated [7].

In the current paper, all categories of natural products with anti-HIV activity were shortly discussed, focusing on current example classified by their chemistry. Antiretroviral therapies used in the treatment of AIDS do not eradicate HIV virus, but only prevent the growth of the virus. Final process of HIV virus growth minimizes and decreases the disease progress. Antiretroviral drugs work by targeting on various stages of the virus life cycle and include:

- 1. Nucleoside and nucleotide reverse transcriptase inhibitors.** Mode of action is preventing HIV virus from making new copies of the virus by blocking reverse transcriptase enzyme.
- 2. Non-nucleoside reverse transcriptase inhibitors.** Inhibits HIV replication by preventing reverse transcription and blocking enzyme reverse transcriptase.
- 3. Protease inhibitors.** HIV viruses use the enzyme protease for splitting polypeptides into tiny ones, resulting in formatting new particles of viruses; after inhibition of the enzyme, it continues to replicate, but due to immature virions formation, is not able to infect new cells.
- 4. Fusion inhibitors.** Works by inhibiting combination of HIV envelope and host CD4 cell membrane, therefore stops the entry of HIV into CD4 cells.
- 5. Chemokine receptor antagonists.** It blocks CCR5 co-receptor resulting in prevention of HIV entry into the cell.
- 6. Integrase inhibitors.** They prevent virus to interact with DNA of human cells [8, 9].

Natural products as anti-HIV agent

For various conditions, nature has always been the source of drugs. Several plants with anti-HIV properties have been reported. Crude extracts have been bio-actively split into lead molecules to find anti-HIV medication candidates. Substantial progress of studies on products obtained naturally having anti-HIV action over the past decade has been achieved. Several secondary metabolites derived from natural sources demonstrated medium to high HIV action.

Natural products with anti-HIV properties

Coumarins

As non-nucleoside-specific HIV reverse transcriptase inhibitors, coumarins, such as calanolides and inophyllums were identified. These are derived from various Caulophyllum species, the genus found mainly in the Indo-Pacific region, particularly in Malaysia [10]. (1) (+)-calanolide B (2) dihydroderivative, and (-)-7, 8-dihydrocalanolide B, which is taken from fruit and twigs C,

is not present in a single dihydrocalanolide. The cytopathic effects of HIV-1 in T cell lines, both CEM-SS and MT-2, have been significantly inhibited. All categories of calanolides are able to block HIV variants, which are lab-adapted, clinical viral isolates are different classes from A to F, others are isolates of inducing and non-syn-cytium-inducing, with isolate of T-tropical and monocytetropical [11]. In Malaysia, Sarawak MediChem pharmaceutical company along with the National Cancer Institute's exclusive license, works across the world on calanolide group of compounds. For treating HIV, combination therapy of calanolide A was found to be successful in early phase I/II in 48 clinical trials. Results of these clinical trials show an increase in pharmacokinetic effect, safety, and increase in calanolide blood level of human volunteers, therefore, confirming effectiveness of combination therapy of calanolide A. The main reason to use the therapy is no major or serious adverse effect noticed, only minor effects that were previously related to drugs were reported. After positive results of phase I clinical trials, currently, calanolide A passes through phase II clinical trials to evaluate further effects, verify its' long-term anti-HIV function along with other anti-HIV agents, and also to assess its' durability (long-term or not) with other drugs [12].

Sub-categories of cordatolide, such as A (3) and B (4), that are isolated calanolides, structure analogues of *Calophyllum cordato-oblongum*, exhibit strong inhibition against HIV-1 replication in a novel green, fluorescent cell reporter assay [13]. Khellactone coumarins show various biological functions, including anti-HIV, anti-tumor promotion, and anti-platelet aggregation (Figure 1).

Over 50 natural coumarins of khellactone have been discovered so far. Suksdorfian angular pyranocoumarindihydro-seselin-type isolated from *Lomatium suksdorfii* methanol extract, suppressed viral replication in 11 separate HIV-1 H9 lymphocyte cell infections, with an average EC_{50} value of 2.6 mM [14]. It also lower acute HIV-1 infection in mononuclear fresh peripheral blood cells, monocyte/macrophages, and promonocytic cell line 22 U-937 cells [13]. Alterations at 3, 4 yielded 3-R, 4-R-di-O(-)-camphanoyl-(+)-cis-khellactone (6, DCK) with enhanced activity (EC_{50} 0.0004 mM, TI 13677) [15]. Stereo-chemistry effect studies show that R, R isomer was at least 10,000 times more active than any of the remaining three isomers (R, S, S, R, and S, S). Additional tempering resulted in more powerful 4-MeDCK (8) (EC_{50} 1.6' 10⁻⁷ mM, TI > 109) and, currently, 3-hydroxymethyl-4-methyl DCK (9, PA-334B) pre-clinical candidate that are nano-molar in-

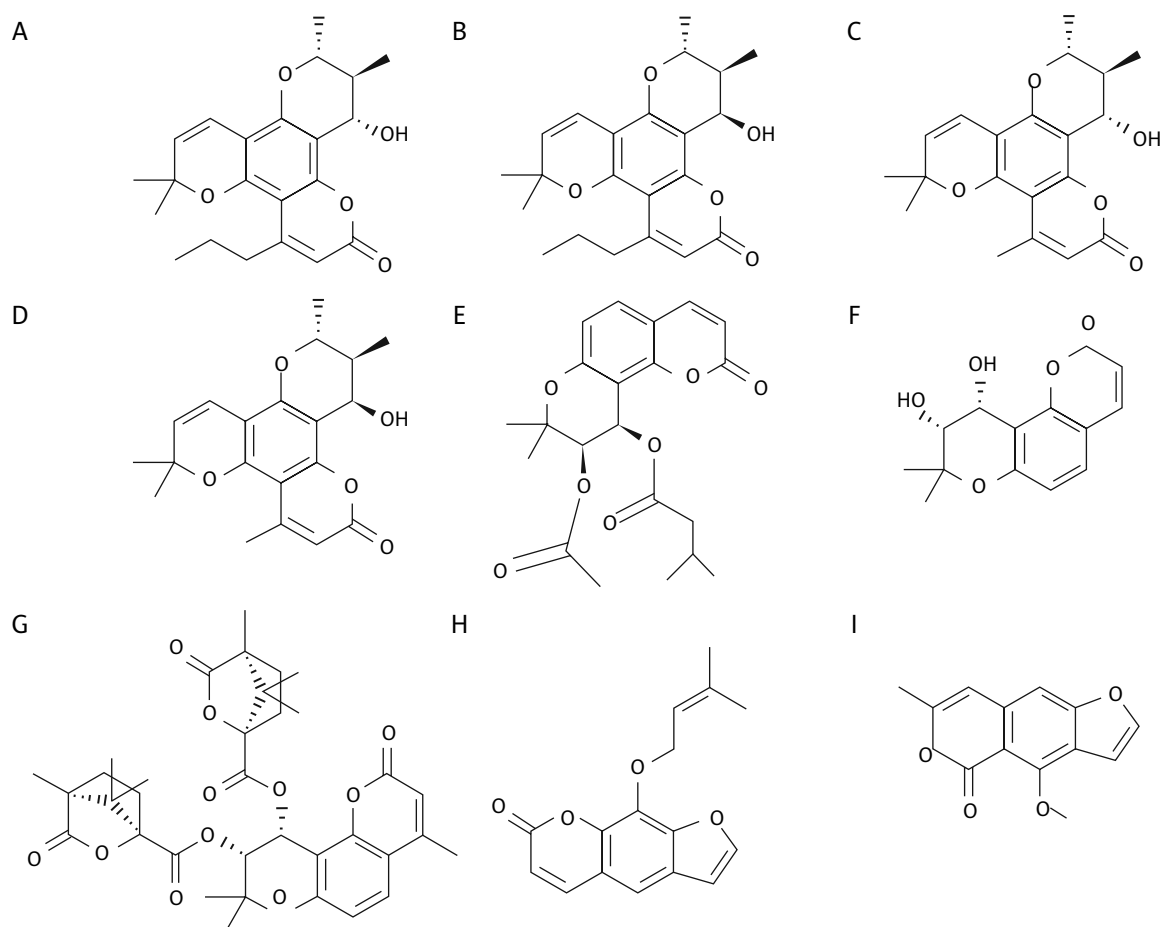


Figure 1. Coumarone derivatives

hibitor for primary and drug-resistant isolates of HIV-1. Oral bio-availability is good in rats and dogs, with plasma half-life 2-3 h in rats. Pre-clinical toxicology study was conducted and showed no or minimum toxicity. Panacos Pharmaceuticals also perform clinical studies, and about to complete pre-clinical phases needed for IND filing [15]. Other derivative, such as furanocoumarin, belongs to *Umbelliferae* family, is dried root methanol extracts of *Ferula sumbul*, which also exhibits strong HIV inhibitory action, with $IC_{50} > 100$ mg/ml, $EC_{50} < 0.10$ mg/ml, and $TI > 1000$. Coriandrin, a coriander *Coriandrum sativum* derived from iso-coumarin, present anti-HIV and other anti-viral activity [16].

Biosynthesis of simple coumarins and their derivatives [17-21]

Simple coumarins are biogenetically classified from shikimic acid, via cinnamic acid. Synthesis of process by

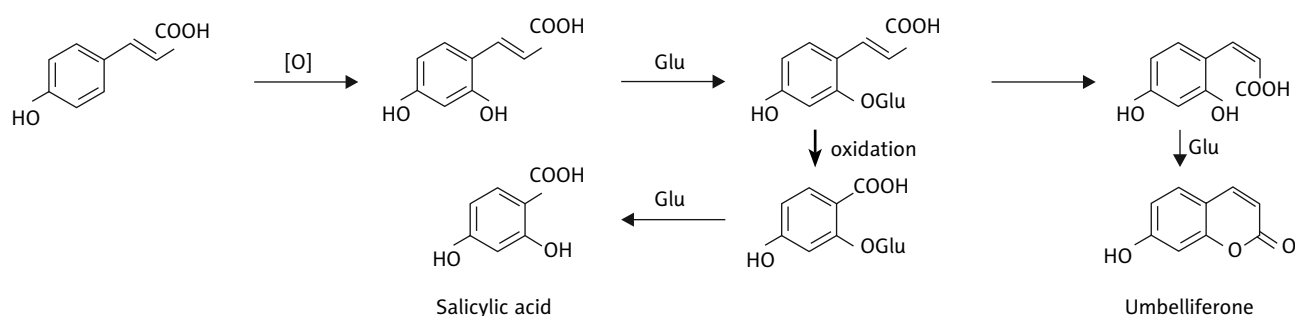


Figure 2. Biosynthesis of simple coumarins [21]

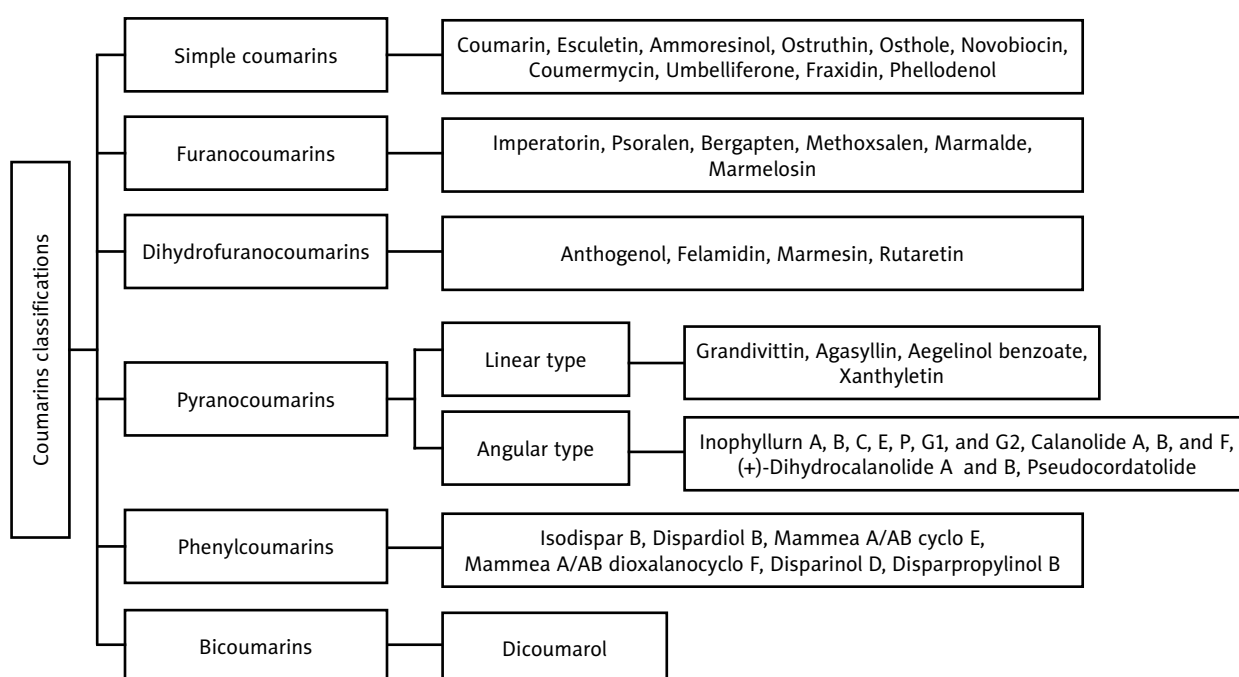


Figure 3. Principal types of coumarins isolated from plants [24]

the help of taking C-2 hydroxylation that produces a break, i.e. β -oxidation from the side chain or chain isomerization and subsequent lactonization, generating umbelliferone (Figure 2).

Classification of coumarins [22, 23]

There are four main coumarin sub-types, including simple coumarins, furanocoumarins, pyranocoumarins, and pyrone-substituted coumarin. Further classification in a diagrammatic way is presented in Figure 3.

Structure-activity relationships of synthetic coumarins as HIV-1 inhibitors

Coumarins are strong antioxidants and present chain breaking property. They show variable biochemical and

pharmacological actions, few of them consisting of a particular member of compounds belonging to this group, and specifically having effects on cellular systems of numbers of mammalian [24, 25]. Number of types of substitution occur in basic structure of coumarin due to their structure variability, so they are responsible for influencing biological activity [26]. SAR activity of derivative of coumarin mainly calanolides can be explained as below.

Reverse transcriptase inhibitors

Synthetic calanolides

Calanolides inhibit reverse transcriptase, from HIV-1 type. Calanolide is extracted from *Calophyllum lanigerum* with their seven related compounds. For testing structure-activity relationship of trans-10, 11-dimethyldihydropyran-12-ol ring (designated ring C), an analogue of structure were made and estimated from complete cell cytopathicity assay (XTT) method. Results showed that there is lower in the action by removing 10-methyl group, and exhibits only one epimer presenting anti-HIV action. There are some coumarins, such as calanolide and inophyllums that indicate inhibition, particularly for non-nucleoside inhibitors of HIV reverse transcriptase. This is obtained from various species of *Caulophyllum* from *Clusiaceae* family, species found in the Indo-Pacific region, specifical-

ly Malaysia. (+)-Calanolide A (15), (-)-Calanolide B (16), and its' dihydro-derivative, (-)-7, 8-dihydrocalanolide B derived from fruits and twigs of *C. Calanolide A* biological study and research on enzyme kinetic demonstrated its' anti-HIV activity. Calanolide A shows strong action against viral that is isolated with Y181C amino acid mutation in reverse transcriptase of HIV-1. In vitro study with other drugs for nucleoside analogues protease inhibitors and NNRTIs, showed enhancement in anti-HIV function, which concluded that it have synergistic action on anti-HIV action. Calanolide A, an anti-HIV agent (+/-) synthesis is completed in five steps: initial steps from phloroglucinol, having Pechmann reaction, Friedel-Crafts acylation, chromenylation with 4,4-dimethoxy-2-methylbutan-2-ol, cyclization, and Luche reduction. In a study on natural products, it was stated that (+)-calanolide A exhibits action as anti-HIV, while (-)-calanolide A not having any such action. Additionally, an *in vitro* study was performed on anti-viral action against HIV for derivative of coumarine (+)-, (-)-, and (+/-)-12-oxocalanolide A (Figure 4), and found inhibitors of HIV-1 reverse transcriptase showing strong action against number of viruses chosen for resistance to other HIV-1 non-nucleoside RT inhibitors. This is the first study to report information that calanolide analogue is also able to inhibit such activity [27, 28].

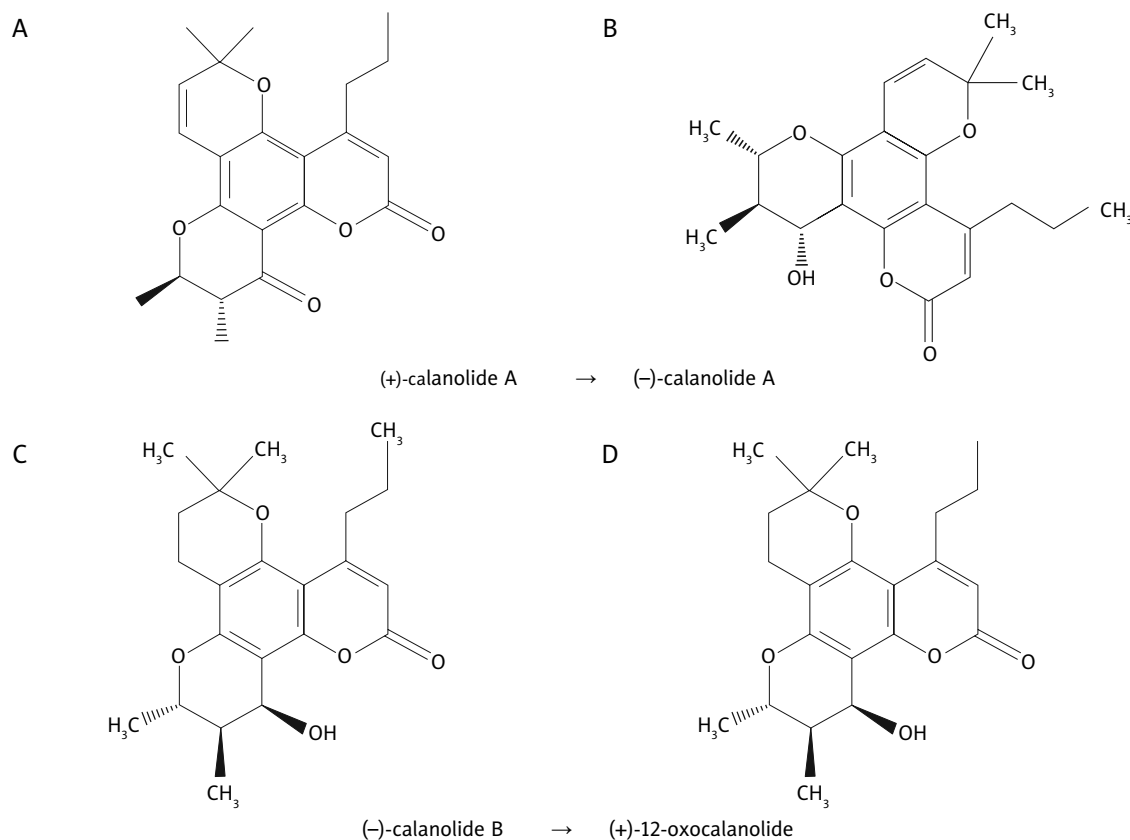


Figure 4. Coumarin derivative, i.e., calanolides

Other anti-HIV coumarins

Protease inhibitor

Retroviral aspartyl protease, also known as 'HIV-1 protease', is a main factor in HIV life span, virus by which AIDS occurs. Many studies were investigated this enzyme, and found significant enzyme for targeting of various studied in medicine. Auto-dock software program was used to optimize HIV-1 protease enzyme computationally using docking methods, and for evaluation of energy, Monte-Carlo simulation was applied [29, 30]. HIV protease activity inhibitions cause improper cleavage for components of proteins, showing non-effective HIV virions. Protease inhibitor is a choice for targeting researches due to its' property of disrupting HIV capability of replicating and infecting cells (Figure 5) [31-33].

Integrase inhibitor

Numerous HIV-1 integrase inhibitors are under investigation, including compounds having two aryl units separated by a central linker, with bis-catechols and coumarins. A natural tetrameric coumarin is rich in anti-integrase activity and have clinical advantages. Other coumarin compounds, i.e., bis-coumarin, is linked with a phenyl ring and increased anti-integrase activity. Oligodeoxynucleotide conjugated to coumarins, changes the rate of polymerization by inhibiting or slightly activating, followed by inhibition that depends on the concentration. When chain terminator 30 ddT was added, ligand-oligodeoxynucleotide complex was easily converted to a strong inhibitor [34-38].

Carbohydrates

Carbohydrates from different natural sources are known to present anti-viral activity. Number of sulphate polysac-

charides consisting of envelope glycoprotein gp120 on the surface is deactivated by binding HIV. The new particular inhibitor of REV protein/RRE RNA with IC50 value of 3.3 mM, Niruriside, is derived from methanolic extract of *Phyllanthusniruri* L. leaves in dry state. The designated *Thuja* polysaccharide g-fraction showed activity of reverse transcriptase of HIV-1, isolated from *Thuja occidentalis* (*Cupressaceae* family) [39].

Peptides

Chassalia parvifolia belonging to *Rubiaceae* family, is a rich source of cyclic peptides with anti-HIV properties [40, 41] as well as *Leonia cymosa* and *circin*, small macrocyclic peptides, cycloviols isolated from tropical plants. Amino acid cyclic polypeptide palicourein obtained from organic extract of *Palicourea condensata* tree on isolation, belongs to *Rubiaceae* family and capable of inhibiting scytopathic effects of HIV-1RF infection of CEM-SS cells *in vitro* [42].

Proteins

Proteins, which particularly interact with eukaryotic protein translation (PRIs), are ribosome-inactivating proteins (RIPs). RIPs are common in atmosphere, but mostly present in plants, bacteria, and mushrooms. Their physical and cellular functions vary greatly. RIPs obtained from plant sources are used in traditional Chinese medicine, and some of the reporting clinical effects of these plants may be attributed to RIPs [43]. Cells that got infected in either acute or chronically manner, such as lymphocyte and mononuclear phagocytes cells, trichosanthin, b-momorcharin, and lmomorcharin, are able to inhibit the replication of HIV. Other inhibition initiated by trichosanthin is H9 and CEM HIV replication [44].

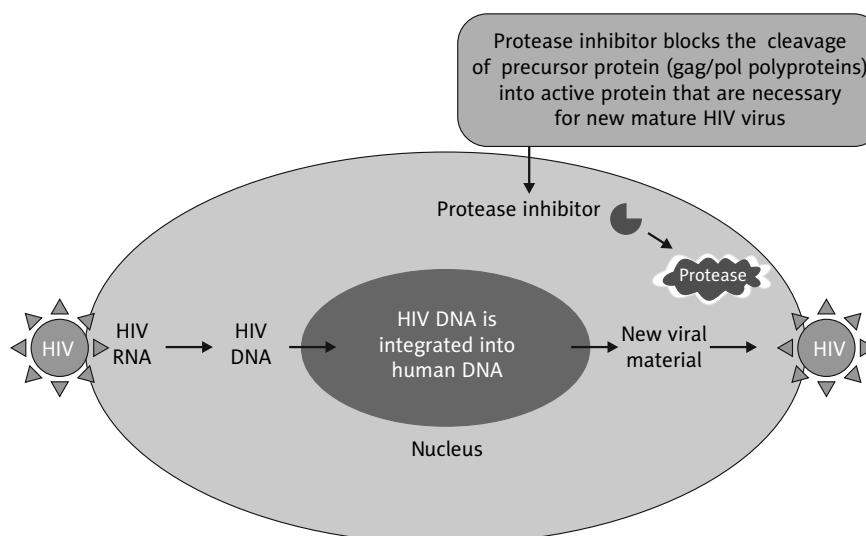


Figure 5. HIV protease inhibition [34]

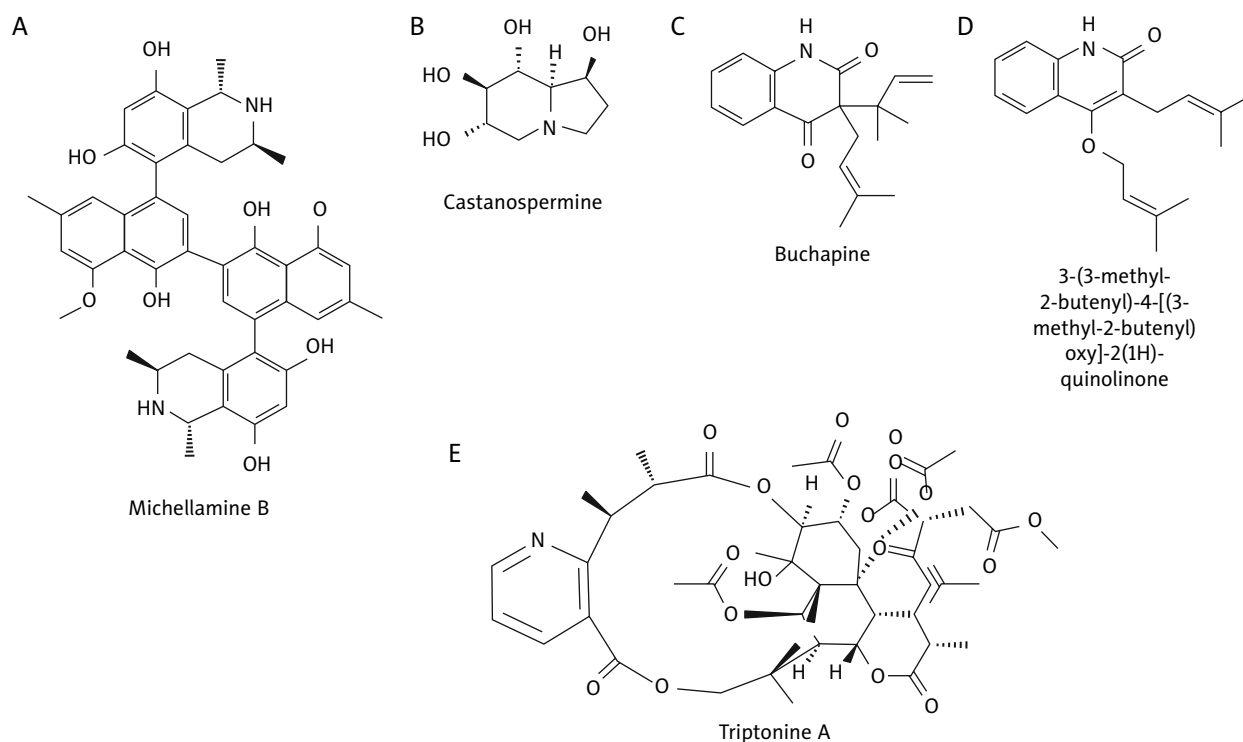


Figure 6. Anti-HIV alkaloids from plants

Alkaloids

Various types of alkaloids are rich in HIV-inhibiting property. Alkaloid dimmers, such as Michell-amines include naphthylisoquinoline alkaloid atropisomerically obtained from *Ancistrocladus korupensis* leaves extract. It is capable of inhibiting reverse transcriptase and in further steps initiates the inhibition of cell fusion and syncytium formation [33]. Michell-amine B (1) reacts in both ways in either early or late stages of HIV life cycle. *Castanospermum australe* belongs to *Fabaceae* family, and is found in Australia's natural rainforests in Eastern and Northern region, consisting of major essential alkaloid called tetrahydroxyindolizidine castanospermine (2) that is capable of inhibiting HIV replication and formation by HIV envelope-induced syncytium. Glycosidase inhibitory activity has also been reported [45]. Bucapine (3), two isoprene units of quinolinone isolating eodiaroxburghiana, and a native plant in Southeast Asia and Australia isolating eodiaroxburghiana, protected CEM-SS cells from HIV-1 cytopathic effects *in vitro* [46]. Tripterygiumhypoglaucum and T are isolated from sesquiterpene pyridine, triptonin A (5), triptonin B, and hypoglaunin B (Figure 6). With a therapeutic index 11 of more than 1000, Wilfordii showed a strong *in vitro* anti-HIV activity. An additional alkaloid cepharanthine exist, which has an anti-allergic, anti-inflammatory, and immune-modulatory properties can block HIV-1 replication strongly [47]. Cell-based test showed specific anti-HIV property in nitidine isolated from roots of *Tod-*

dalie asiatica (*Rutaceae* family). HIV transcriptase inhibitor activity was also reported [48]. *Buchenavia capitata* from *Combretaceae* family is a rich source of o-demethylbuchenavianine, a piperidine flavone-rated alkaloid exhibits property in cell-based, anti-cancer, and anti-HIV screens [48]. Harminewas were found to be inhibiting HIV replication in lymphocytic H9 cells, isolated by *Symplocos setchuensis*. N-butylharmin was the strongest having EC₅₀ value of 0.037 mM, and a therapeutic index 15 of 210 among its' 28 derivatives. The potent anti-HIV activity (EC₅₀ being 0.26 mg/ml) of 1-methoxycanthinone isolated from *Leitneria floridana* was investigated [49].

Flavonoids

Reportedly, flavonoids have a various biological functions and strong antioxidant activity. Free radical generation is related to damage of numerous cells and tissues, i.e., cell death, apoptosis, and tissue necrosis due to various diseases. Productivity of ROS in healthy individuals is balanced with a protection system for antioxidants [50]. Oxidative stress is due to misbalancing of the production and inactivation of reactive oxygen species [51].

Various conditions, such as cancer, Parkinson's disease, and AIDS affect oxidative stress. In addition, increased level of lipid peroxidation product (malondialdehyde) leads to damage to oxidative DNA, such as 8-hydroxyguanine in HIV-positive people. Anti-viral activity was observed in culture of cells and experimental animal studies on different fla-

vonoids against multiple viruses [52]. HIV inhibitory action of the XTT-based, whole-cell screen was shown by prenylation flavonoids, 6, 8-diprenylkaempferol, and 6, 8-diprenylaromadendrin of *Monotes africanus* extract. *Acer okamotoanum* (*Acercaceae* family) has anti-HIV-1 integrase activity, with IC50 values of 18.1 ± 1.3 and 24.2 ± 6.6 mg/ml, respectively [53]. Quercetin 3-O-(2 net-galloyl) and α -L-arabinopyranose and flavonoid gaullet ester are obtained by isolation from ethanolic extract. An *in vitro* test showed strong inhibitory polymerase of HIV-1 RTase in bi-flavonoids, robust flavones, and hinoki-flavones isolated in twigs and leaf extracts of *Rhussucedanea* (*Anacardiaceae* family) [54]. Another bi-flavonoid, wikstrol B, was found to show good activity with respect to HIV-1 *in vitro* studies in extracts of *Wikstroemia indica* roots (family *Thymelaeaceae*). Plants of genus *Erythrin* have been reported useful for HIV-inhibitory pterocarpan and iso-flavonoids [55] (Figure 7).

Lignans

HIV-1 inhibitor, HIV-1-compounded cytopathic activity, virum production p24 antigen, and reverse transcriptase in non-toxic C8166 lymphocytes have been shown in xanthohumol, prenylchalcone recently isolated from hops (*Humulus lupulus*). It has been shown that a number of lignans have anti-viral activities. Isolated from *Anogeissusa cuminata*, dibenzylbutadiene lignans, anolignan A, and anolignan B showed HIV-1 inhibitory activity [56]. Compounds anolignan A and anolignan B works as synergic property accord-

ing to reported studies. Anolignan A exhibits 60.4 mg/ml of IC50 in comparison to 1,073 mg/ml of HIV-1 RTase exhibited by anolignan B. When combination of anolignan A and anolignan B was tested in variant ratios, this property was noticed in increasing manner. Anolignan A also showed activity with an IC50 of 106 mg/ml against a drug-resistant form of HIV-1-RTase [57]. Isolated from *Ipomoea cairica* and *Arctium lappa*, dibenzylbutyrolactone-type lignanolide (-) arctigenin showed anti-HIV action basically due to inhibition of HIV-proviral DNA and not related to HIV-1 RTase interference. Demethoxyepiexcelsin from methanol extract of leaves and branches of *Litsea verticillata* (*Lauraceae* family) demonstrates strong anti-HIV property, while (+)-epiexcelsin did not show anti-HIV activity [58]. Phyllamyricin B and its' lactone retrojusticidin B isolated from *Phyllanthus myrtifolius*/P. *urinaria* chloroform extract (*Euphorbiaceae* family) showed strong HIV-RTase inhibition [59]. Among *Kadsura*-isolated lignans (-), gomisin was observed to be the strongest HIV replication inhibitor (EC50 0.006 mg/ml; TI 600). Insulated from *Kadsura coccinea*, Kadsulingnan M presented anti-HIV action *in vitro* [60].

Globoidnan A, a lignan isolated by bioassay-guided fractionation from methanol extract of *Eucalyptus globoidea* buds, recently inhibited the combined HIV integrase 3-processing and strand-transfer activity [61]. Ethanol extract of *Terminalia bellerica* fruit rind from *Combretaceae* family, is widely used plant in India for traditional medicine purposes. Plant also yield edanolignan B and lignans with significant *in-vitro* anti-HIV property [62].

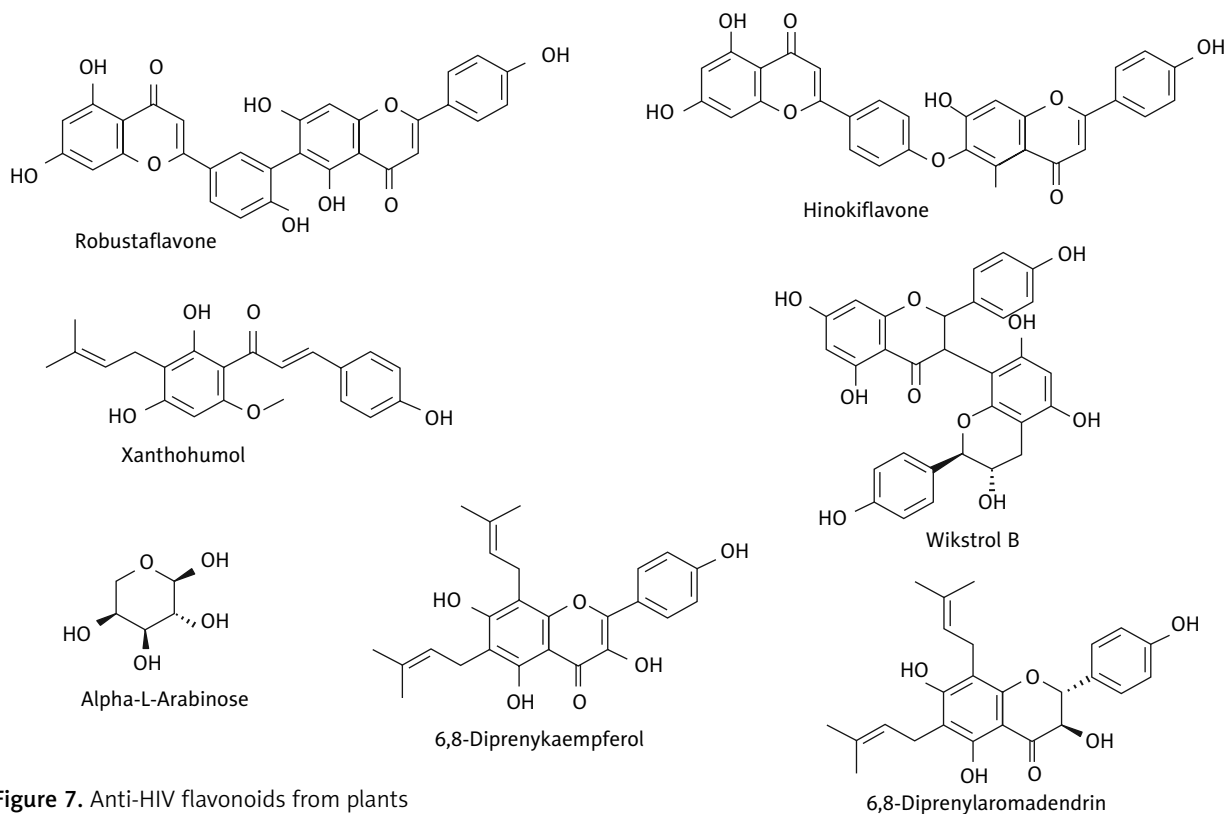


Figure 7. Anti-HIV flavonoids from plants

Xanthones

Swertia franchetiana plant, consisting of swertifranche-side as its' discovered flavonone-xanthoneglucoside elements present HIV-1-RTase inhibition property. Its' mode of action has been identified as related to DNA binding, and described inhibiting many other polymerases, including DNA polymerase [63]. *Maclura tinctoria* (*Moraceae* family), with prenylated xanthone B, has moderate anti-HIV activities [64, 65].

Anti-viral and COVID-19 correlation

COVID-19 is currently a major issue, and studies were performed worldwide to find a potential treatment. For experimenting, anti-viral drugs become the first choice for researchers to test. Few countries suggested these drugs as preventive medication for their frontline workers. From numerous studies and trials, it was confirmed that HIV patients who are on effective antiretroviral therapy are at very low risk of infecting COVID-19, or with very minor symptoms if COVID-19-infected. According to Cao *et al.* who studied lopinavir and ritonavir in patients hospitalized with severe COVID-19, antiretroviral drug treatment used in HIV was found in activity of orally administered lopinavir/ritonavir for SARS-CoV-2 infection. Patients were divided into two groups; in the first group, a combination of drugs was administered, and other group was treated with standard care. Better clinical improvement was observed on 14th day for those on lopinavir and ritonavir comparing with patients under standard care, but due to severe adverse effects of lopinavir/ritonavir, its' administration was stopped in 13 patients (13.8%). This needs further studies, which might help patients with severe illness, confirming possibility of treatments' benefit [66]. According to previous literature review, the use of anti-viral drugs is a motivation to treat COVID-19 infection, which is a viral disease [67]. Cai *et al.* evaluated favipiravir for COVID-19 patients, and found that favipiravir present significant good effects in treating COVID-19 in relation to progress of the disease and viral clearance comparing with lopinavir and ritonavir. Favipiravir, an anti-viral drug is a pro-drug, and is a novel RNA-dependent RNA polymerase inhibitor [68]. Antivirals, i.e. broad spectrum or other compounds show activity against SARS-CoV or MERS-CoV, which are now considered choice of drugs for treatment of infection due to COVID-19 [69]. Proofs from clinical studies demonstrate that SARS-CoV-2 is developing new mutations, and might be capable of escaping anti-viral drugs [70]. Lim *et al.* reported that by giving anti-viral drug, such as lopinavir or ritonavir, the effect of β -coronavirus viral load was decreased, and due to coronavirus titers, its' minimal presence was not detectable. Lim *et al.* observed that administration of anti-viral drugs of lopinavir/ritonavir started to decrease the β -coronavirus viral load. However, the effect of lopinavir/ritonavir is not clear, as its' lowers the load of SARS-CoV-2, because of naturally healing process rather than lopinavir/ritonavir administration; therefore, further research in this field are needed [71].

Future prospects

The validated target of HIV is utilized for the identification of virtual lead for COVID-19, due to closed likeness of both the targets. The gp41 has been found highly similar with the S2 protein of coronavirus, and targeting this protein would inhibit the interaction of cells and viruses. Binding interactions of the molecule's similar target of HIV and COVID-19 could be beneficial and can be developed as a broad spectrum ant-viral drug. The reasonable factor for the fusion proteins of SARS-CoV revealed as highly similar to gp41 from human immunodeficiency virus [72], hemagglutinin (HA) from influenza [73], and fusion protein of paramyxovirus [74]. Three main groups of coronavirus are surrounded entirely by spike genes, stating that for fusion mechanism, these groups are common mode for attachment. Various studies investigated cryo-EM and molecular docking for complete spike structure and active site of SARS-CoV [75, 76].

Fusion inhibitors

Infusion of the virus to cells creates an opportunity to take resources from the host cell to grow inside and transfuse outside from the host cell to enhance viral load in the host body. In view of that, the treatment strategy should emphasize entry or infusion, which would rarely disturb essential physiology of the host cell, and would avoid the most concern of anti-viral drug. Arrival of the virus inside the host cell compromises cytoplasmic transport systems to move sites of replication within cytosol for RNA viruses and nucleus for DNA viruses. Replication of the genetic material starts with the final step of viral entry, which proliferates the genome to get in the shape through the transfusion from the host cell. Anti-viral therapies as an infusion inhibitors/entry blockers have been a keen target; moreover, many research scientists revealed various targets, including S protein with RBD_eACE2 blockers, S cleavage inhibitors, fusion core blockers, neutralizing antibodies, protease inhibitors, S protein inhibitors, and small interfering RNAs. Among them is US FDA-approved (2003) enfuvirtide as entry blocker of HIV-1 virus with the gp41-derived peptide (INN) [77].

Structural similarity between HIV-1 gp41 and SARS-CoV S2 proteins

Zhang *et al.* (2004) reported the same structural features of HIV-1 gp41 and SARS-CoV S2 proteins through 3D modelling structure, such as S2 sub-unit, and compared these models with a core structure of gp41 of HIV-1. They observed that SARS-CoV S2 and gp41 shares similar two α helices, and stating that the two viruses could follow an analogous membrane fusion mechanism. Ligand-binding test demonstrated that the two inhibitors GGL and D-peptide from HIV-1 gp41 might serve as inhibitors for SARS-CoV entry. Therefore, the gp41 of HIV-1 target is used for docking and molecular interaction purpose. Genomic resemblance of both the targets has an advantage that both viruses could be blocked from entry point.

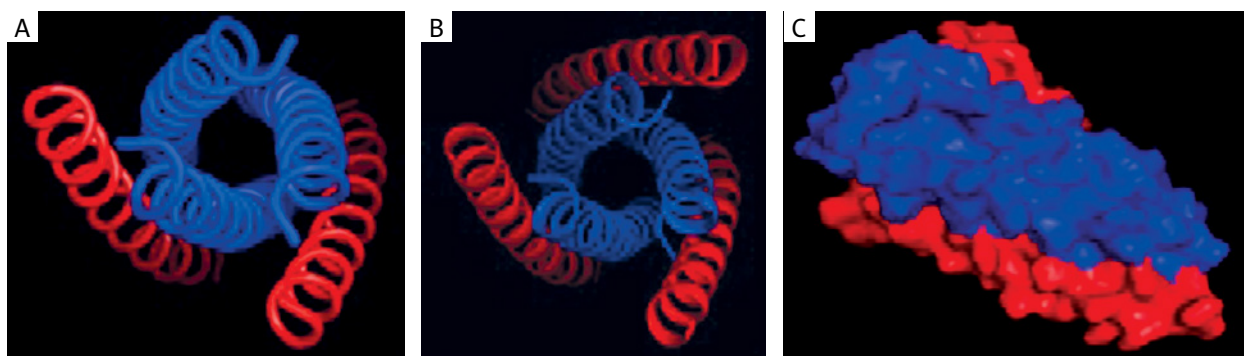


Figure 8. Removal of C-34 helix to expose hydrophobic area

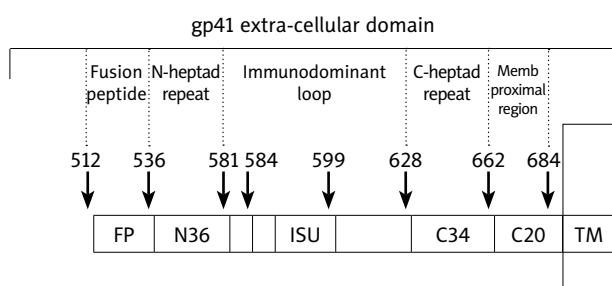


Figure 9. HIV-1 gp41 extra-cellular domain showing functional regions of gp41 (aa 512-684)

gp41 protein

Similarity between human immunodeficiency virus structure of gp41 and coronavirus is well-validated through various crystal and in-silico studies. The structure is composed of N36/C34 complex that is a six-stranded helical bundle. The middle portion of this bundle belongs to a parallel, trimeric coiled-coil of three N36 helices wrapped in a gradual left-handed superhelix. Three C34 helices wrap anti-parallelly to the N36 helices in a left-handed side around the outside of the central coiled-coil trimer. The arrangement in helices is in such a manner that anticipated to be based on sequence analysis and bio-physical studies [78,79]. Complete dimensions of this complex consists of a cylinder measuring 35 Å in diameter and 55 Å in height. Every groove on the surface of the N36 trimer has a specifically deep cavity. This cavity is large (16 Å long, 7 Å wide, and 5-6 Å deep) [80]. By removing C34-helix from the bundle of helix (six), docking investigation was performed and hydrophobic region of gp41 was observed (Figure 8).

Upper right

Molecular surface view of the exposed hydrophobic pocket is visible in such a way that red color on outer are C34 helices, blue color inside are N36 helices, on upper left portion shows one C34 helix removed resulting in exposure of hydrophobic pocket, and lower molecular surface shows hydrophobic pocket.

gp41 structure and functional domains

The gp41 can be defined as a composite transmembrane protein consisting of numerous well-defined domains. Hydrophobic fusion domain belongs to N-terminal end of the protein [81]. Follows the N- and C-terminal heptad repeat (HR) portion forms to fusion pore formation [82, 83]. The N- and C-terminal heptads replicate regions connected by an immune dominant loop region that plays a major role in fusion. A study of active site also confirms the presence of N-36 and C-34 sites for catalytic action (Figure 9) [84].

Conclusions

The present study investigated various plant materials as an anti-HIV or anti-viral agents, which have been well-received by scientific experts. Among the plants, coumarins is one of the potent natural agent against HIV consisting large number of biologically active compounds, and is being used traditionally for thousands of years. Coumarins have been used as an antioxidant, anti-proliferative, and anti-HIV agents. There are number of coumarins derivatives comprising specific content, which shows strong anti-HIV activity and have other therapeutic potential. Therefore, the structure-activity relationship of strong anti-HIV coumarins derivatives is not easy in terms of development of drugs against HIV. Designing of a new anti-AIDS drugs lead to structure of various synthetics in coumarins nucleus, present in number of lead molecules. The specific advantage of these molecules could be because of their additional beneficiary effect on COVID-19 as fusion inhibitors. There are numerous methods, such as molecular modelling for developing new anti-HIV agents. The future of anti-HIV drug will support the anti-COVID drug, because of high similarity in the mechanism of the virus.

Conflict of interest

The authors declare no conflict of interest.

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