

Current Status of Anti-HIV Agents

V.K. Tandon^a and R.B. Chhor^{b,*}

^aDepartment of Chemistry, University of Lucknow, Lucknow-226001, India, ^bInstitute of Organic Chemistry, University of Regensburg, Regensburg-93053, Germany

Abstract: A large number of chemical enteties, immunogens and topical medications, either synthetic or naturally occuring, against HIV have been brought into focus to combat AIDS epidemic. The three major classes of anti-HIV medications, leading to the development of synthetic drugs worldwide, belong to NRTs, NNRTIs and protease inhibitors. These have been discussed in detail with regard to HIV infected patients. A large number of natural products isolated from various plant species, flora and fauna have been described in detail. Some of these compounds have the potential for development as future drugs for cure of AIDS and could be helpful in replacement of combination therapy presently prevalent for treatment of HIV patients. Proteomics and Genomics are vital cores of biotechnology for prime consideration in lead generation against HIV. The recent aspects of development of combination therapy as well as the development of vaccines for treatment of AIDS, which are of current interest to clinicians, biologists and medicinal chemists also being discussed.

Keywords: NRTIs, NNRTIs, PIs, Combination therapy, Natural Products, Proteomics, Genomics, Vaccine.

I. INTRODUCTION

During the year of 1981, the first case of HIV positive man reported having being involved in homosexuality carried symptoms, considered diagnostic of AIDS was described in Los Angeles and New York. The men had a peculiar type of lung infection called *Pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma. The patients were found to have significantly less count of CD₄ cells. These cells, often referred to as T cells, which help the body fight against infections. Shortly thereafter, this disease was recognised throughout the globe as an epidemic. In 1983, researchers in the United States and France described the virus that causes AIDS, now known as HIV. In 1985, a blood test became available that measures antibodies to HIV, which thereby detects the body's immune response to the HIV. This blood test remains the best method for diagnosis of HIV infection. Recently, tests have become available to look for the same antibodies in the saliva and urine [1].

HIV is present in the biological fluids and secretions of virtually all infected individuals, regardless of whether or not they have symptoms. The spread of HIV can occur when these secretions come in contact with tissues such as those lining the vagina, anal area, mouth, or eyes (the mucosal membranes), or with a break in the skin, such as from a cut or puncture by a needle. The most common ways in which HIV is spreading throughout the world include sexual contact, needle sharing, and transmission from infected mothers to their newborns during pregnancy, labour or breast-feeding. Sexual transmission of HIV has been described from men to men, men to women, women to men, and women to women through vaginal, anal, and oral sex. The best way to avoid sexual transmission is abstinence from

sex until it is certain that both partners in a monogamous relationship are not HIV-infected. Since the HIV antibody test can take upto 6 months to turn positive, both partners would need to test negative 6 months after their last potential exposure to HIV. If abstinence is out of the question, the next best method is the use of latex barriers. This involves placing a condom on the penis as soon as an erection is achieved in order to avoid exposure to pre-ejaculatory and ejaculatory fluids that contain infectious HIV. Concerning oral sex, condoms should be used for fellatio and dental dams for cunnilingus (oral contact with the vaginal area). A dental dam is a piece of latex that prevents vaginal secretions from coming in direct contact with the mouth.

The spread of HIV by exposure to infected blood usually results from sharing needles, as used for intake of drugs. HIV can also be spread by sharing needles for anabolic steroids taken to increase muscle, tattooing, and body piercing. To prevent the spread of HIV, needles should never be shared. At the beginning of the HIV epidemic, many individuals acquired an HIV infection from blood transfusions or blood products, such as those used for haemophiliacs. Currently, however, because blood is tested for antibodies to HIV before transfusion, the risk of acquiring HIV from a blood transfusion considerably goes down.

There is little evidence that HIV can be transferred by casual exposure, as might occur in a household setting. For example, unless there are open sores or blood in the mouth, kissing is generally considered not to be a risk factor for transmitting HIV. This is because saliva, in contrast to genital secretions, has very less concentration of the virus. Still, theoretical risks are associated with the sharing of toothbrushes and shaving razors because they can cause bleeding. Consequently, these items should not be shared with infected persons. Similarly, without sexual exposure or direct contact with blood, there is little if any risk of HIV contagion in the workplace or classroom [2].

*Address correspondence to this author at the Institute of Organic Chemistry, University of Regensburg, Regensburg-93053, Germany; Email: rb_chhor@yahoo.co.in

Now nearly more than 50 million people worldwide have been infected with human immunodeficiency virus (HIV), and more than an estimated 12 to 13 million children have been orphaned by the acquired immune deficiency syndrome (AIDS) epidemic [3]. The natural history of HIV infection continues to provoke the researchers and clinicians to uncover new facts about the virus and develop wide range of new drugs either as a single or combination regimens for patients. The status of anti-HIV treatment is never static but constantly changing directed to meet the cure. Vaccine development is viewed as an essential step in controlling the epidemic but ultimate success is not achieved due to complicated genetic diversity of the virus. Although tremendous efforts have been made in the anti-HIV/AIDS arena, yet daunting challenges remain. This review article presents the current status of Anti-HIV agents.

A. Scope of the Review

It is still to be ascertained whether the people infected with HIV can be cured by the currently available therapies. In fact, individuals who are treated for up to three years and repeatedly undergo test for an undetectable level of the virus in their blood, experience a prompt rebound increase in the number of viral particles when therapy is discontinued. Consequently, the decision to start therapy again must balance the risk of advancement to symptomatic disease against the risks associated with therapy. The risks of therapy include the short- and long-term side effects as well as the possibility that the virus will become resistant to therapy. This resistance then limits the options for future treatment.

A major reason that resistance develops, is the patient's failure to inadvertently follow the prescribed treatment, such as not taking the medications at the correct time. Another factor is the likelihood of suppressing the virus to undetectable levels not as good for patients with lower CD₄ cell counts and higher viral loads. Finally, if the viral load remains detectable on any given regimen, resistance eventually will develop. Thus, if the drugs are used as part of a combination that does not suppress the viral load to undetectable levels, resistance will develop rapidly and the treatment will be rendered ineffective. In practice, HIV becomes resistant to certain other drugs, such as zidovudine (AZT), stavudine (D4T), and protease inhibitors, over months [4]. In addition, resistance to one drug often results in the same reaction to other related drugs, called as cross-resistance. Nevertheless, HIV-infected individuals must realise that antiviral therapy can be very effective. This is the case even in those who have a low CD₄ cell count and advanced disease, as long as drug resistance has not developed.

It would be worth focussing on recent developments in anti-HIV agents as most of the drug regimens suffer from the events of developing resistance against existing combination therapies.

B. Structural and Biological Significance of the Anti-HIV Agents

There are three major classes of anti-HIV drugs categorized as Nucleoside Reverse Transcriptase Inhibitors,

Non-Nucleoside Reverse Transcriptase Inhibitors and Protease Inhibitors. Each of them are equally effective, therefore it is important to review their structural and biological aspects to develop new molecules against HIV mutants.

Nucleoside reverse transcriptase inhibitors circumvent HIV from multiplying by blocking the reverse transcriptase enzyme. This enzyme changes HIV's genetic material (RNA) into DNA. This step has to occur before HIV's genetic code gets combined with an infected cell's own genetic codes. The nucleoside analogues (often called "nukes") mimic the building blocks used by reverse transcriptase to make copies of the HIV genetic material. These fake building blocks disrupt the copying.

Resistance due to mutations occurs at the binding sites of the receptors leading to anti-HIV agents ineffective in due course of therapy. A number of mutations have been characterised and found to be helpful in designing modified agents active against mutants.

For a nucleoside triphosphate-RT complex to be active against M184V RT mutant, the NRTI triphosphates should be able to interact with the active site enzyme residue, such as ARG72 and Tyr115, to maintain their sugar moieties far away from the bulky side chain of Val184. It has been observed by molecular modelling studies that the bulky side chain of Val184 occupies NRI binding site to cause steric hindrance with many NRTIs. In order to be active, NRTIs should be able to avoid the steric hindrance with Val184. AZT, which has 3'-OH equivalent (3'-N₃), shows no cross resistance to M184V RT because 3'-N₃ interacts with NH backbone of Tyr115 to hold down the AZTTP [5].

There are some dioxolane and oxathiolane nucleosides, which have shown promising antiviral activity against AZT- as well as 3TC-resistant mutant RTs. These agents are referred as dioxolane T, DXG, D-FDOC and D-dotC as shown in "Fig. (1)".

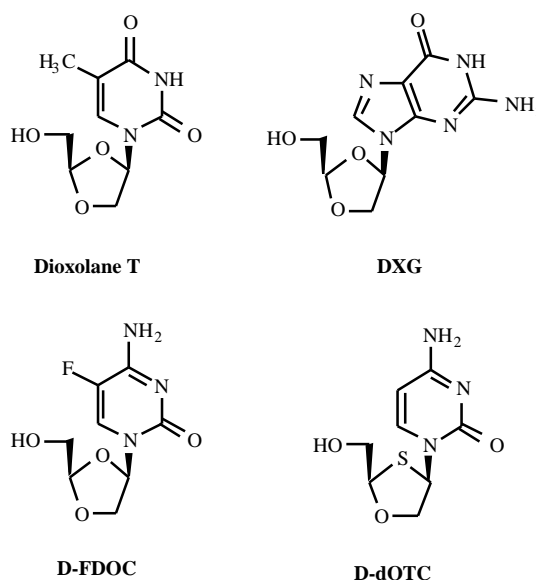


Fig. (1). Dioxolane and Oxathiolane derivatives

A number of dideoxy congeners of the above mentioned nucleosides have also been synthesised, which are known as 2',3'-dideoxy-2',3'-didehydro (d4) nucleosides, found to be active against M184V_{RT} as shown in "Fig. (2)".

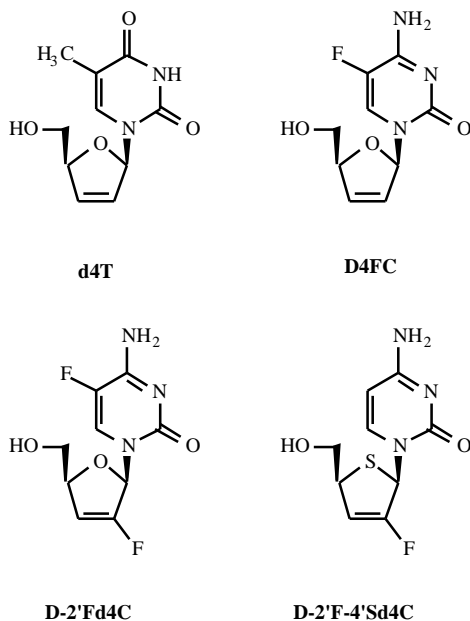


Fig. (2). 2',3'-dideoxy-2',3'-didehydro derivatives

Structure of the NRTIs may contribute to the biological significance of the lead molecules, if they mimic the interaction between the 3'-OH group of the natural substrate and amide backbone of Tyr115 of RT. It can locate their sugar moiety away from the residue 184 to avoid the unfavourable steric hindrance with Val184 in M184V_{RT}.

In the case of dioxolane and oxathiolane nucleoside derivative, the 3'-oxygen atom in the D-dioxolane interacts with either Arg72 or amide backbone of Tyr115, to keep the sugar moiety of the nucleoside away from the Val184 to prevent the unfavourable steric hindrance. Similarly 2',3'-double bond of d4 nucleoside also holds the sugar moiety of NRTI-TP away from Val184 by interacting with the aromatic side chain of Tyr115 [6].

Detailed structural architecture of the RT enzyme in complex with the inhibitor is useful for the promising drug design. A number of NNRTIs complexed with RTenzymes have been reported [7]. Such structural information is the basis for further derivatisation of NNRTIs aimed at maximising binding affinity to RT.

The inhibitor binding site is flexible so as to enable one to design an inhibitor based on the geometry of the binding site, the crystal structures are needed to reflect as many positions as possible. Each reported crystal structure of a RT-NNRTI complex has shown a unique binding pattern specific to one chemical class of inhibitors. DABO and PETT models have been developed and applied to generate RT-NNRTI crystal structures found to be essential in the designing of more potent inhibitors [8].

A number of studies have revealed a common mode of binding for the chemically diverse NNRTIs with their target

site at the HIV-1 RT. The NNRTI-binding site (pocket) is located at about 10 Å distance from the substrate-binding site. The ligands in this pocket are of mainly hydrophobic nature. The most prominent ligands are Y181, Y188, F227, W229, L100, L234, V179 and K103.

Esnouf *et al.* [9] suggested that the NNRTIs binding in the pocket caused a repositioning of the three stranded beta-sheet in the p66 subunit, which contains the catalytic aspartic acid residues 110, 185 and 186. This should inhibit HIV-1 RT by locking the active catalytic site in an inactive conformation. Hsiou *et al.* [10] have published a more convincing model. He suggested that the binding of NNRTIs locks the p66 thumb in a configuration which is more extended compared to DNA-bound RT. These changes may account for the inhibition of polymerisation and the alteration of the cleavage specificity of RNase H by NNRTI.

NNRTI resistance mutations rise rapidly and are most often directly in contact with the NNRTI molecule, and thus are associated with changes in the binding of NNRTIs to RT. For example, primary mutations associated with resistance to nevirapine involve residues K103, V106, V108, Y181, Y188, L100 and G190, which have van der Waals bond with the inhibitors. Mutations of these residues lead to the weakening of the inhibitor binding to RT.

The first RT mutation shown to be associated with and accounts for, HIV-1 resistance to NNRTIs were the Lys103-Asn (K103N) and Tyr181-Cysteine(Y181C) mutations, and have later been observed with virtually all the NNRTIs except for the quinoxalines [11]. Combinations of different RT mutations (i.e. L100I and K103N or K101D and K103N, or K103N and Y181C) are probably required to give rise to high-level resistance to NNRTIs. It is not clear whether such double mutants readily arise *in vivo*, in patients under NNRTI treatment [7], but there is evidence that genetic variants of HIV containing a single mutation and double mutations exist in therapy-naïve HIV-patients [12].

HIV infects cells and directs the cellular machinery to make viral proteins and RNA. Several of the proteins are synthesised in one continuous chain (polyprotein). The polyprotein is cleaved into smaller chains which can then assemble to form new virus particles. The cleavage, which takes place at specific sites on the polyprotein, is carried out by the virus protease enzyme. If it is possible to block the activity of the protease the synthesis of new virus can be prevented.

There are numerous proteases throughout all living organisms: digestive enzymes and blood clotting factors are just two examples in humans. The catalytic mechanism provides a way to classify proteases into three large families. Aspartic proteases contain two aspartic acid side chains which participate in cleavage of the peptide bond. Serine proteases use a serine-histidine-aspartate triad. Metalloproteases use a metal ion (e.g., Zn⁺⁺) to help in catalyzing proteolysis.

Some proteases are fairly non-specific, and others have very high specificity. In HIV protease, some sites are essentially non-existent and the other sites are specific for large hydrophobic side chains such as phenylalanine and proline. Therefore, in designing an inhibitor, there should be

substituents which look like these side chains. Another consideration in designing an inhibitor is that the inhibitor should not be hydrolysed by the protease, so amide bonds should be avoided. In fact, in the central part of the inhibitor, it is useful to mimic the transition state or intermediate in the hydrolysis reaction. This is because enzymes work by facilitating the development of the transition state being on the pathway from substrate to product. They do this in part by binding more favourably to the transition state than to either substrate or product [13].

It was known from work on renin, another aspartic protease that replacement of the peptide C=O with a hydroxy (-OH) group makes a good transition state mimic. But renin has different specificity sites than HIV protease, so known renin inhibitors were not very good HIV protease inhibitors [14].

The structures of the inhibitors should include hydrogen bonding interactions in the active site as well as hydrophobic interactions in the specificity sites. One particularly important feature, which is routinely observed in crystal structures of HIV protease is that the inhibitors bound to the active site which is due to the presence of a tightly bound water molecule near the top of the active site. It forms two hydrogen bonds from water hydrogens to oxygen atoms on the inhibitor (in the natural function of the protease, it forms hydrogen bonds to two peptide backbone C=O oxygen atoms). In turn, the water oxygen atom forms two hydrogen bonds to peptide backbone N-H atoms of the protease [15].

Currently available HIV protease inhibitors suffer due to certain limitations. Viruses are notorious for their ability to develop resistance to drugs. Some of the available inhibitors are too rapidly metabolised or too poorly absorbed, so that large doses must be used. This is expensive, and it increases the likelihood of adverse side effects. This is one of the reasons why combination therapy is being preferred now a days.

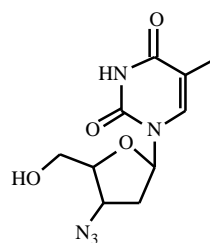
II. ANTI-HIV MEDICATIONS - A GLOBAL PHARMACEUTICAL SURVEY

Virtually all the given compounds described in Table 1 are being currently used, or in a clinical phase, for the treatment of HIV infections, belong to the following classes:

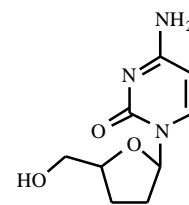
There are 16 synthetic leads developed worldwide as potent anti-HIV agents. Out of them, eight belong to the NRTIs, [few of them are shown in "Fig. (3)"] three to the NNRTIs and five to the protease inhibitors as shown in the Table 1.

Table 1. List of Synthetic anti-HIV Agents

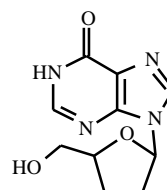
No.	NRTIs	NNRTIs	PIs
1	Zidovudine	Nevirapine	Saquinavir
2	Zalcitabine	Delaviradine	Ritonavir
3	Didanosine	Efavirenz	Indinavir
4	Stavudine		Nelfinavir
5	Lamivudine		Amprnavir
6	Abacabir		
7	Emtricitabine		
8	Viread		



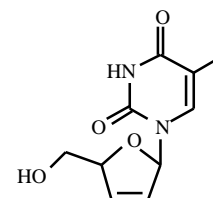
Zidovudine (AZT), Trade name : Combivir, (Glaxo Wellcome Nc.)



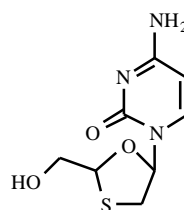
Zalcitabine, Trade name : HIVID, (Hoffmann-La Roche NJ, USA)



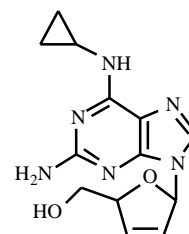
Didanosine, Trade name : VIDEX, (Bristol-Myers Squibb Princeton, NJ)



Stavudine, Trade name : Zerit, (Bristol-Myers Squibb Princeton, NJ)



Lamivudine, Trade name : Epivir, (a.Glaxo Wellcome Nc., b. BioChem Pharma, Quebec)

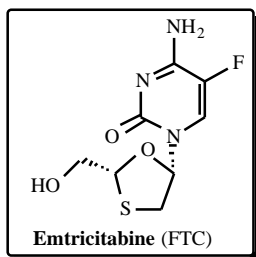


Abacabir, Trade name : Ziagen, (Glaxo Wellcome Nc.)

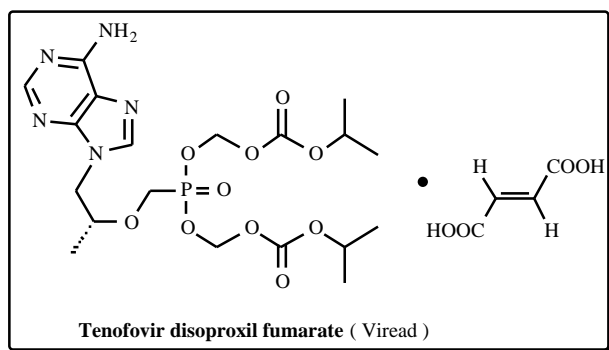
Fig. (3). Nucleoside Reverse Transcriptase Inhibitors

Since last two years, emtricitabine was seeking approval from FDA after rigorous clinical trials. Recently, it has been approved by FDA for the treatment of treatment-naïve and treatment-experienced HIV patients. Emtriva (emtricitabine) is indicated in combination with other antiretroviral agents for the treatment of HIV infection in adults. This indication is based on the analyses of plasma HIV RNA levels and CD₄ cell counts in two Phase III clinical trials of Emtriva of 48 weeks duration. The drug works by inhibiting reverse transcriptase. By interfering with this process, which is a key step to the replication of HIV, Emtriva helps to lower the amount of HIV, or "viral load," in a patient's body and in turn helps to increase the number of immune system cells. Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness [16].

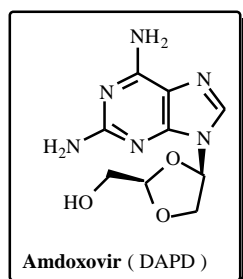
Recent results with tenofovir disoproxil fumarate (Viread) studies may allow once-daily HAART regimen for the monotherapy, which resembles that of protease inhibitor monotherapy in its ability to reduce plasma HIV-1 RNA levels [17]. Tenofovir disoproxil fumarate (a prodrug of tenofovir) is an acyclic nucleoside phosphonate diester



analogue of adenosine monophosphate. Tenofovir disoproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination [18].

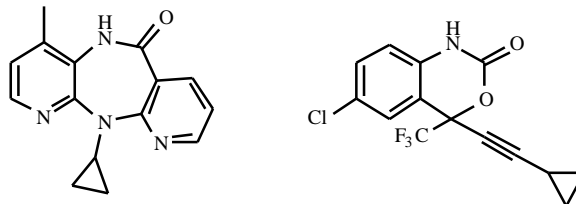


Combivir, a two drug combination has been developed by Glaxo Smith Kline against HIV infection. It is a combination of zidovudine and lamivudine. Three drugs combination, trizivir has also been developed by Glaxo Smith Kline which comprises abacavir, zidovudine and lamivudine.



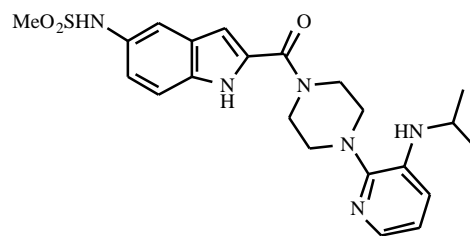
Amdoxovir is still seeking FDA approval and currently in clinical trials. It is an anti-HIV medication, which falls under the category of HIV medicines, NRTIs. Amdoxovir prevents HIV by entering the nucleus of the healthy T-cells. This prevents the cells from producing new virus and decreases the amount of virus in the body. Recent studies revealed that the amdoxovir will only be effective if used in combination with other drugs, including another NRTI and at least one or NNRTIs [19].

Nevirapine [20], delvaridine [21] and efavirenz [22] have been in market, while emivirine (MKC-442) is yet to be approved by FDA, as shown in "Fig. (4)".



Nevirapine, Trade name :
Viramune, (Boehringer Ingelheim
Pharmaceuticals, ridgefield, CT)

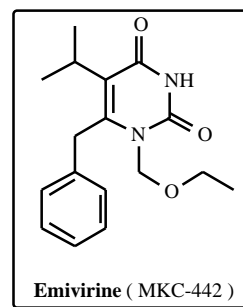
Efavirenz, Trade name :
Sustiva, (Du pont
Pharmaceuticals, Wilmington)



Delvaridine, Trade name :
Rescriptor, (Agouron
Pharmaceuticals a Pfizer company
California)

Fig. (4). Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Emivirine, NNRTI, which is being developed by Triangle pharmaceuticals and Mitsubishi chemicals and its phase II/III studies show that the oral bioavailability in rats dosed at 50 mg / kg was 18.4%. The maximal plasma concentration (C_{max}) 10.3 μM, reached within 15 minutes following oral administration. In rats, LD₅₀ was greater than 2000 mg / kg and no significant toxicity was observed in rats dosed orally at 100 mg / kg / day for two weeks [23].



Protease inhibitors used in combination with RT inhibitors represent the most effective anti-HIV therapies developed till to date. Several studies have reported that combination therapies reduce HIV viral load to undetectable levels for sustained periods of time in up to 90% of patients. The use of RT inhibitors not only results in synergism but also substantially reduces the likelihood of protease or multiple-resistant HIV strains developing.

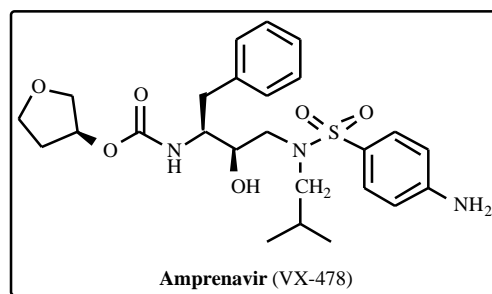
The development of protease inhibitors has been facilitated by the determination of a three dimensional structure of the HIV protease based on the extensive knowledge gained from other aspartyl proteases and their inhibitors, such as renin, and the identification of the HIV protease cleavage sites (Tyr | Pro, Phe | Pro, Leu | Ala, Met | Met, Phe | Tyr, Phe | Leu, and Leu | Phe).

Four protease inhibitors, saquinavir, ritonavir, indinavir and nelfinavir have already been approved and several others are in the late stages of clinical development [24] as shown in "Fig. (5)". Current efforts are underway to develop simpler compounds with higher bioavailability and less susceptibility to viral resistance.

On September 30, 1999 Kissei Pharmaceutical Co., Ltd., launched a fifth protease inhibitor, Prozei (amprenavir) [25], an orally administered HIV protease inhibitor, in Japan. Prozei was approved in mid-September '99 through a new fast-track evaluation process instituted by the Japanese Ministry of Health and Welfare for innovative HIV therapies. Prozei (known outside of Japan by the trade name Agenerase) was discovered by Vertex Pharmaceuticals Incorporation and has been developed in Japan by Kissei.

Prozei is indicated in combination with other antiretroviral agents for the treatment of HIV infection. Prozei has a long half-life, allowing the drug to be administered twice daily as dose. Prozei can be taken with or

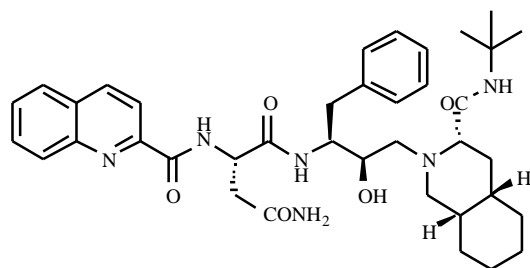
without food, but it is recommended that the drug should not be taken alongwith fat rich meal.



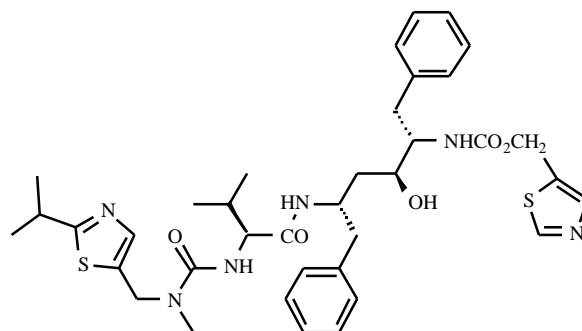
In the United States, Vertex's partner Glaxo Wellcome received accelerated approval for Agenerase from the FDA in April 1999. Agenerase is being marketed in the United States by Glaxo Wellcome and will be co-promoted with Vertex.

Fusion Inhibitors

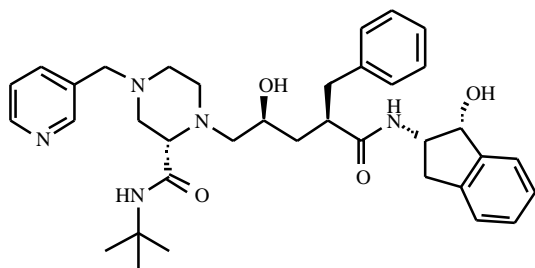
Tremendous studies have been performed in recent years to understand fusion process of HIV to the host cell and the mechanistic insight gained from these studies has led to the formulation of exciting new approaches for therapeutic intervention. Eventually some peptide based drugs have been emerged and termed as fusion inhibitors. These fusion



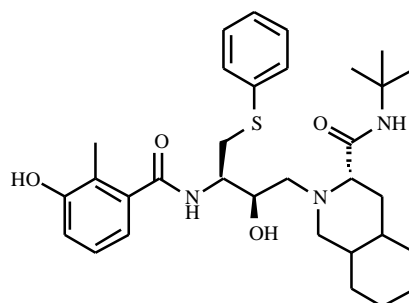
Saquinavir, Trade name : Fortovase,
(Hoffmann-La, Roche, NJ, USA)



Ritonavir, Trade name : Norvir,
(Abbott Laboratories)



Indinavir, Trade name : Crixivan,
(Merck NJ, USA)



Nelfinavir, Trade name :
Viracept, (Agouron Pharmaceuticals
a Pfizer company, California)

Fig. (5). Protease Inhibitors (PIs).

inhibitors act by freezing a transient structural intermediate of the HIV fusion process, thus blocking an essential step in viral entry.

Fusion inhibitors act on the target, HIV gp41, a molecule on the virus' surface that use to change its shape in order to facilitate the fusion of virus to the host cell and inject the viral genetic material inside host cell.

By directly binding to gp41, enfuvirtide (Fuzeon, formerly T-20) [26], [27] and its congener T-1249 [28] are able to powerfully block the fusion process.

Clinical studies data reveal that eighty percent of patients under investigation receiving a Fuzeon-based anti-HIV drug regimen achieved undetectable levels of the virus at 24 weeks maintained this response at 48 weeks.

Ac-YTSLIHLIEESQNQKEKNEQELLELDKWASLWNWF-NH₂

MW 4491.92

T-20

It has also been reported that 37 percent of heavily treatment-experienced patients treated with a Fuzeon-based combination maintained at least a 90 percent reduction in blood levels of HIV at 48 weeks, vs. 17 percent of patients on a regimen without Fuzeon. Fuzeon have been co-developed by Roche and Trimeris and was granted accelerated approval by FDA in March 2003, and it became the first and only approved fusion inhibitor for the treatment of HIV.

Ac-WQEWQKITA LLEQAQIQQE KNEYELQKLD KWASLWEWF-NH₂

MW 5036.61

T-1249

Phase I clinical study data of the second generation fusion inhibitor T-1249 shown that the 53 patients who were participating in Phase II or Phase III studies of Fuzeon for 10 days and exhibited HIV RNA levels between 5000 and 500,000 copies/mL at two consecutive clinical visits. Patients in the study discontinued Fuzeon and added T-1249 to an unchanged individualised anti-HIV drug regimen. At day 11, 73 percent of patients demonstrated a greater reduction in HIV RNA.

Safety evaluations revealed no serious adverse effects related to T-1249. The most frequent side effects were joint pain (4%), diarrhoea (4%), fatigue (4%), muscle pain (4%) and fever (4%). The next step in the development of T-1249 will be Phase II studies, which are projected to begin in coming years.

III. HOW MEDICINAL CHEMISTRY IS HELPFUL TO FIGHT AGAINST HIV INFECTION - TO MEET THERAPEUTICAL REQUIREMENTS IN THE 21ST CENTURY

The effective treatment of patients infected with AIDS to a great extent relies on the ability of medicinal chemists to

discover new drugs. Medicinal chemists have the opportunity not only to advance the frontiers of science but also to see that their research directly contributes in alleviating many of the diseases afflicting mankind.

Recent advances in a number of disciplines have created exciting new opportunities for medicinal chemists. Synthetic organic chemistry has progressed over the last several decades to a level where molecules of great complexity can be efficiently constructed. Large computer databases of chemical reactions and biomedical information enable researchers using search engines to quickly identify literature which is crucial to their work. These enormous facilities permit medicinal chemists to simultaneously keep abreast of developments in organic synthesis and the various biomedical disciplines important for carrying out research at the cutting edge of contemporary medicinal chemistry. X-ray crystallography and NMR spectroscopy are providing an ever increasing quantity and quality of structural information on enzymes, DNA, receptors and other biological macromolecules.

This information, alongwith a variety of recently developed computer-based molecular modelling capabilities allows medicinal chemists to design new drugs in a much more rational fashion than could be accomplished before. Molecular biology has also provided the capability to produce larger quantities of pure enzymes and receptors as well as new ways to study the biological function of these macromolecules. These capabilities are being used to identify enzymes and receptors which enable medicinal chemists to target new anti-HIV drugs. All these combined developments provide unprecedented opportunities for medicinal chemists. The drug discovery which is a heart core of medicinal chemistry, is a long and complex process. It takes many years of dedicated research in biological sciences combined with cutting-edge medicinal chemistry to evolve new ideas and generate new chemical entities and drugs. The procedure is tightly governed by national and international rules. The clinical compounds have to be designed not only to bind the receptor for which they are designed, but also have to be stable, non-toxic, and able to be absorbed into the blood stream and to cross from the blood plasma into the infected blood cell. To fulfill all these requirements, new chemical entities have to pass through laboratory and animal testing prior to clinical trials before these are approved as a drug.

International rules and regulations governing research involving genetic manipulation are even more stringent because of the uncertainty about the long-term effect of this type of research on the human body. This adds to the cost of the research at all stages, from basic research to application. Eventually, the medicinal chemists have developed a number of formulations to meet the therapeutical requirements against HIV in the 21st century.

A. Bio-organic Aspects of Molecules from Nature

A large number of anti-HIV compounds are still to be isolated from the natural herbs, shrubs, flora and fauna. Researchers are trying hard to unearth them as their natural diversity is the major hurdle although a number of products from natural origin have been investigated against various

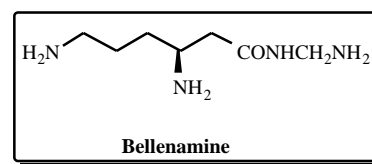
strains of HIV, particularly against virus entry, viral adsorption, virus-cell fusion, reverse transcription, proviral DNA integration, viral transcription, viral translation, virus release, viral assembly, budding, and maturation. Recently reported anti-HIV natural products have been summarised as described below:

1. Avarol, Avarone, Psychotrine and Phloroglucinol Derivatives

Red Sea sponge *Dysidea cinerea* was found to be a source of Avarol and avarone derivatives [29], the alkaloids psychotrine and O-methylpsychotrine (isolated from ipecac, the dried rhizome and root of *Cephaelis ipecacuanha* [30] and phloroglucinol derivatives such as mallotojaponin, from the pericarps of *Mallotus japonicus* "Fig. (6)" [31], have all been reported primarily to inhibit the reverse transcriptase (RT) activity of HIV-1, noncompetitively with respect to the natural substrate (dNTP).

2. Bellenamine

Bellenamine, (R)-3,6-diamino-N-(aminomethyl) hexanamide is produced by *Streptomyces nashvillensis* [32]. It was found to inhibit HIV-1 infection at an IC_{50} of 0.62 mg / mL. Similar to the well-established glycosylation inhibitors, castanospermine and 1-deoxynojirimycin; bellenamine inhibits the secondary spread of HIV. Mechanism of action is unknown and yet to be elucidated.

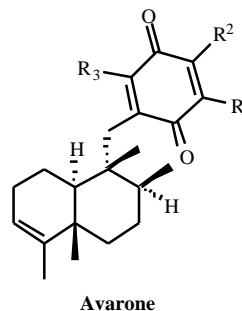
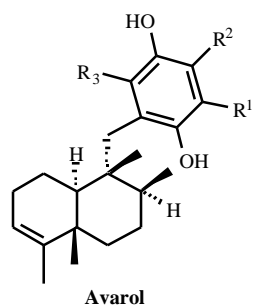


3. Baicalin

Baicalin monohydrate (TJN-151) is a flavonoid (5,6,7-trihydroxyflavone-7-O- β -D-glucopyranoside uronic acid monohydrate), isolated from *Scutellariae radix*. It inhibits HIV-1 replication in peripheral blood mononuclear cells at an IC_{50} of 0.5 mg / mL. Baicalin also inhibits HIV-1 reverse transcriptase (RT), but not human DNA polymerases and (DNA polymerase being slightly inhibited), hence the anti-HIV-1 effect of baicalin can be attributed even if partly, to the inhibition of HIV-1 RT [33]. No inhibition of virus adsorption was noted, which contrasts with the anti-HIV activity of other flavonoids [34], that have been claimed to inhibit HIV adsorption through an (irreversible) interaction with the glycoprotein gp120.

4. Betulinic Acids

Betulinic acid and platanic acid "Fig. (7)", the triterpenoids isolated from *Syzygium claviflorum*, exhibit inhibitory activity against HIV-1 replication in H_9 lymphocyte cells at an IC_{50} of 1.4 and 6.5 mM, respectively [35]. Hydrogenation of betulinic acid yielded



Avarone E; $R^1 = H, R^2 = OMe, R^3 = OH$

Avarone F; $R^1 = H, R^2 = H, R^3 = OH$

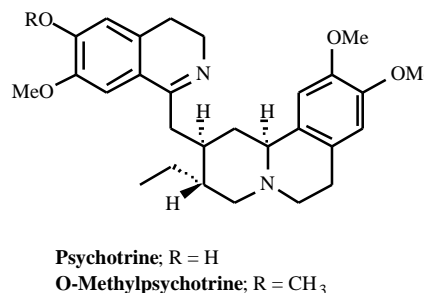
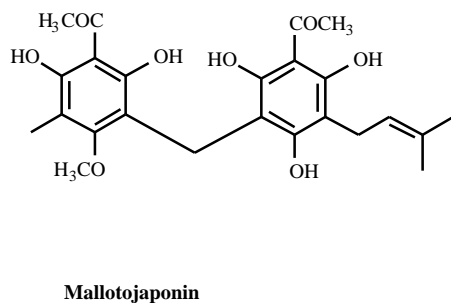
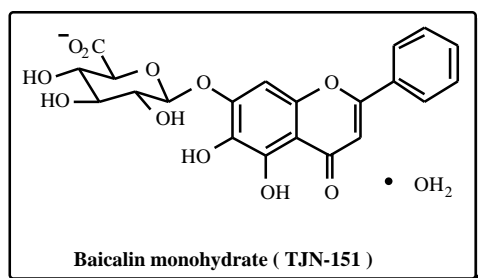


Fig. (6). Avarol, Avarone, Psychotrine and Phloroglucinol derivatives



dihydrobetulinic acid, which showed an IC_{50} of 0.9 mM. Incorporation of a 3,3-dimethylsuccinyl group at the C3 hydroxyl group of betulinic acid and dihydrobetulinic acid significantly increased the anti-HIV activity of betulinic acid and dihydrobetulinic acid IC_{50} : < 0.35 nM. This is a remarkably high potency and selectivity ever reported for betulinic acids. Therefore, it can be considered as an attractive lead compound for the design and development of HIV-cell fusion inhibitors.

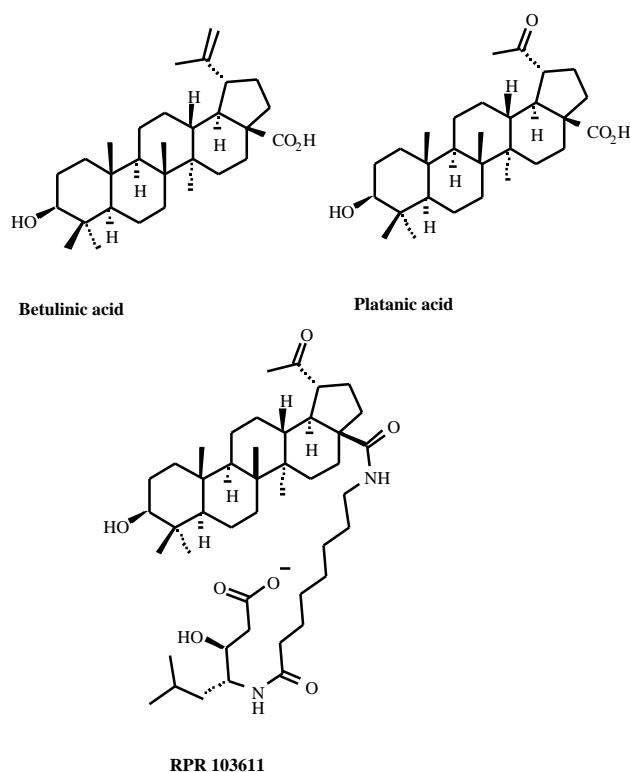


Fig. (7). Betulinic acid and their derivatives.

5. Cyanovirin-N

Cyanovirin-N, 11-kDa protein "Fig. (8)", has been isolated from the cyanobacterium *Nostoc ellipsosporum*. It is effective against both laboratory strains and primary isolates of HIV-1 and HIV-2 at an order of nanomolar concentration. Additionally, Cyanovirin-N aborts cell-to-cell fusion and transmission of HIV-1 infection [36]. The antiviral activity of cyanovirin-N has been attributed to a tight binding of the compound to the viral envelope glycoprotein gp120, which

must result in a reduced infectivity of the virus as well as reduced capacity of virus-infected cells to fuse with uninfected cells. Cyanovirin-N is supposed to be promising for the development of a topical formulation in the prevention of genital HIV transmission.

(H₂N)-Leu-Gly-Lys-Phe-Ser-Gln-Thr-Cys-Asn-Ser-Ala-Ile-Gln-Gly-Ser-Val-Leu-Thr-Ser-Thr-Cys-Glu-Arg-Thr-Asn-Gly-Gly-Trp-Asn-Thr-Ser-Ser-Ile-Asp-Leu-Asn-Ser-Val-Ile-Glu-Asn-Val-Asp-Gly-Ser-Leu-Lys-Trp-Gln-Pro-Ser-Asn-Phe-Ile-Glu-Thr-Cy-Arg-Asn-Thr-Gln-Leu-Ala-Gly-Ser-Ser-Glu-Leu-Ala-Ala-Glu-Cys-Lys-Thr-Arg-Ala-Gln-Gln-Phe-Val-Ser-Thr-Lys-Ile-Asn-Leu-Asp-Asp-His-Ile-Ala-Asn-Ile-Asp-Gly-Thr-Leu-Lys-Tyr-Glu(COOH)

Fig. (8). Amino acid sequence of Cyanovirin-N.

6. Cyclosporins

Among the cyclosporins, a fermentation product of the fungus *Tolypocladium niveum*, particularly the nonimmunosuppressive derivative SDZ NIM811 or [N-Melle4]-cyclosporin, "Fig. (9)" [37], a cyclic undecapeptide, in which the N-methyl-L-leucine of cyclosporin A is replaced by N-methyl-L-isoleucine, exhibits potent anti-HIV-1 activity. SDZ NIM811 interferes at two stages of the viral replication cycle: (i) translocation of the preintegration complex (PIC) from the cytoplasm to the nucleus and (ii) production of infectious virus particles [38]. Immunosuppressive activity is not correlated with anti-HIV-1 activity of cyclosporins. Instead, the cyclophilin A, the major cellular receptor protein of cyclosporins, is a prerequisite for HIV inhibition. Cyclophilins possess peptidyl-prolyl cis-trans isomerase activity that is involved in protein folding, and specifically bind to the HIV-1 capsid gag polyprotein p55. This association is blocked in the presence of cyclosporin A as well as SDZ NIM811 [39]. Thus, inhibition of HIV-1 replication by cyclosporins correlates with their ability to disrupt the gag-cyclophilin A interaction [40]. Significant amounts of cyclophilin A were found to be associated with virus particles propagated in primary T-cells: SDZ NIM811 caused a strong reduction in the amount of incorporated cyclophilin A [41], thereby reducing the formation of infectious virus particles from chronically infected cells [42]. Gag proteins not only play a role in virus assembly but also in the nuclear localisation of the preintegration complex (PIC) and, accordingly, SDZ NIM811 also inhibits integration of proviral DNA into cellular DNA [43]. Various nonimmunosuppressive analogues of cyclosporin A have been found to inhibit HIV replication [44]. They constitute a promising class of lead compounds for the treatment of HIV infection.

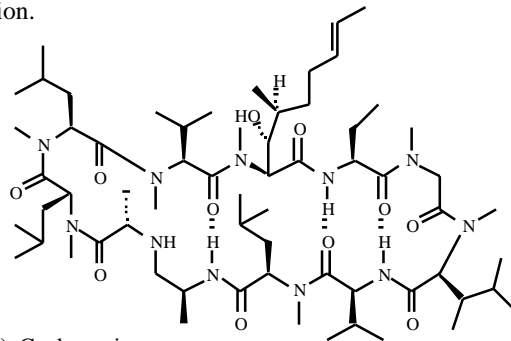


Fig. (9). Cyclosporins.

7. Curcumin and their Analogues

Curcumin, "Fig. (10)" corresponds to the yellow pigment in turmeric (*Curcuma longa*). It is widely used as a spice, food colouring and preservative. Curcumin has been reported to inhibit HIV-1 integrase at an IC_{50} of 40 μ M [45]. Dicafeoylquinic acids, "Fig. (10)" inhibit HIV-1 integrase at submicromolar concentrations [46]. The dicafeoylquinic and L-chicoric acids "Fig. (10)", were reported to inhibit HIV-1 integrase at concentrations ranging from 0.06-0.66 μ M; and HIV-1 replication in cell cultures at 1-4 μ M [47]. Curcumin analogues such as dicafeoylmethane and rosmarinic acid both inhibit activities of integrase with IC_{50} values below 10 μ M [48]. In follow-up studies, dicafeoylquinic acids (DCQAs) and dicafeoyltartaric acids (DCTAs) were found to inhibit HIV-1 integrase at concentrations between 0.15 and 0.84 μ M, and HIV replication at concentrations between 2 and 12 μ M; no inhibition of gp120 binding to CD₄ was noted at concentrations up to 80 μ M. Likewise, no inhibition of reverse transcription or RNase H was noted, and it was concluded that the DCQAs and DCTAs act as specific integrase inhibitors, and that their activity against integrase is consistent with their observed anti-HIV activity in cell cultures [49]. The integrase would be an excellent target for combination chemotherapy of HIV infection was further ascertained by combination experiments where L-chicoric acid, the putative integrase inhibitor, was combined with a protease inhibitor (AG1350) and zidovudine [50]. Arguing for an integrase-targeted action was the finding that introduction of the mutant integrase containing a single Gly-Ser substitution at position 140 into the native, L-chicoric acid sensitive virus was found to be sufficient to confer resistance to L-chicoric acid [51]. However, this finding could not be supported by evidence. Although L-chicoric acid was able to inhibit HIV integrase in an oligonucleotide driven assay, integrase carrying the G140S mutation was inhibited to the same extent as the wild type integrase. Furthermore, HIV-1 strains that were made resistant to L-chicoric acid, contained several mutations in the V₂, V₃, and V₄ loop regions of the envelope glycoprotein gp120, but not in the integrase and, as could be expected from a compound targeted at virus-cell binding, L-chicoric acid did not prove inhibitory effect to virus strains that were resistant towards polyanionic substances.

8. Calanolides and Inophyllums

Calophyllum lanigerum [52] and the Malaysian tree, *Calophyllum inophyllum*, [53] are two main sources of calanolides and inophyllums respectively. Both compounds as shown in "Fig. (11)" were found to inhibit HIV-1 replication at an IC_{50} of approximately 0.1 μ M and 1 μ M. These compounds can be considered as NNRTIs, as they are primarily active against HIV-1 RT, but differ from the synthetic NNRTIs in their HIV sensitivity and resistance profile. Several structural analogues of (+)-calanolide-A have been prepared [54]. Two isomers of calanolide-A, (-)-calanolide-B (costatolide) and (-)-dihydrocalanolide-B (dihydrocostatolide) possess antiviral properties similar to those of calanolide A. The calanolide analogues exhibit 10-fold enhanced antiviral activity against drug-resistant viruses that bear one of the most prevalent NNRTI resistance RT

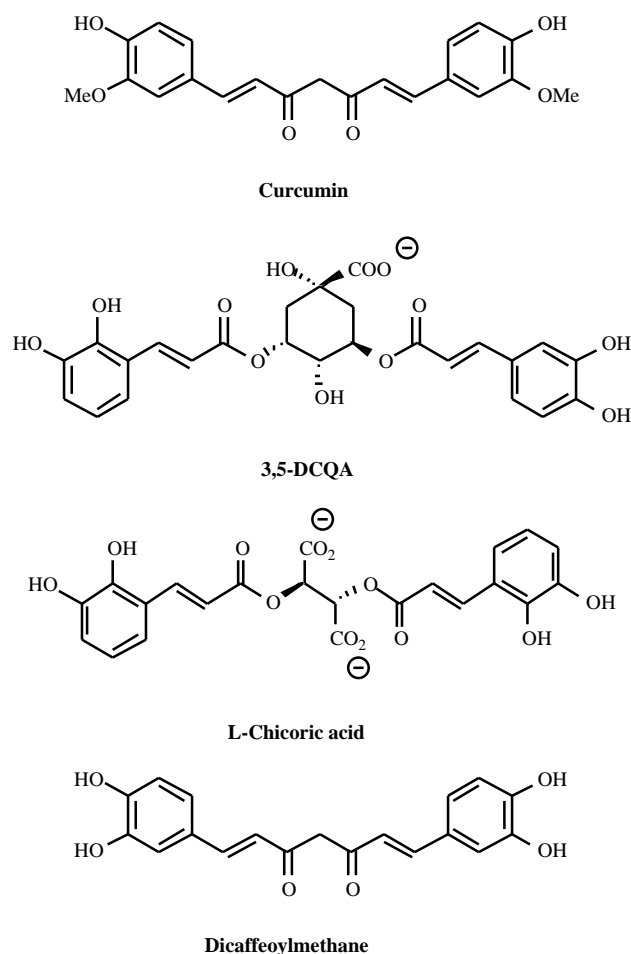


Fig. (10). Curcumin and their analogues.

mutations (Y181C); in turn, the calanolide analogues lead to the selection of drug-resistant virus strains carrying the T139I, L100I, Y188H or L187F mutations in their RT. The calanolide isomers represent a novel and distinct subgroup of the NNRTI family; they are taken for further evaluation for their therapeutic potential in combination with other anti-HIV agents [55].

9. Equisetin

Equisetin, has been isolated from the fungus *Fusarium heterosporum*. It is HIV integrase inhibitor. The enantiomeric homologue of equisetin, and two additional analogues, a novel decalin derivative, integric acid, and oteromycin were isolated from *Phoma species*. Equisetin inhibits 3'-end processing and strand transfer at an IC_{50} of approximately 10 μ M. Equisetin and related compounds also inhibit disintegration catalysed by either full-length enzyme or the truncated integrase core domain. These compounds also inhibit strand transfer reactions catalysed by stable complexes assembled *in vitro* and integration reactions catalysed by pre-integration complexes isolated from HIV-1-infected cells [56]. Whether these fungal metabolites also inhibit HIV-1 replication within the cells, due to inhibition of integration, remains to be determined.

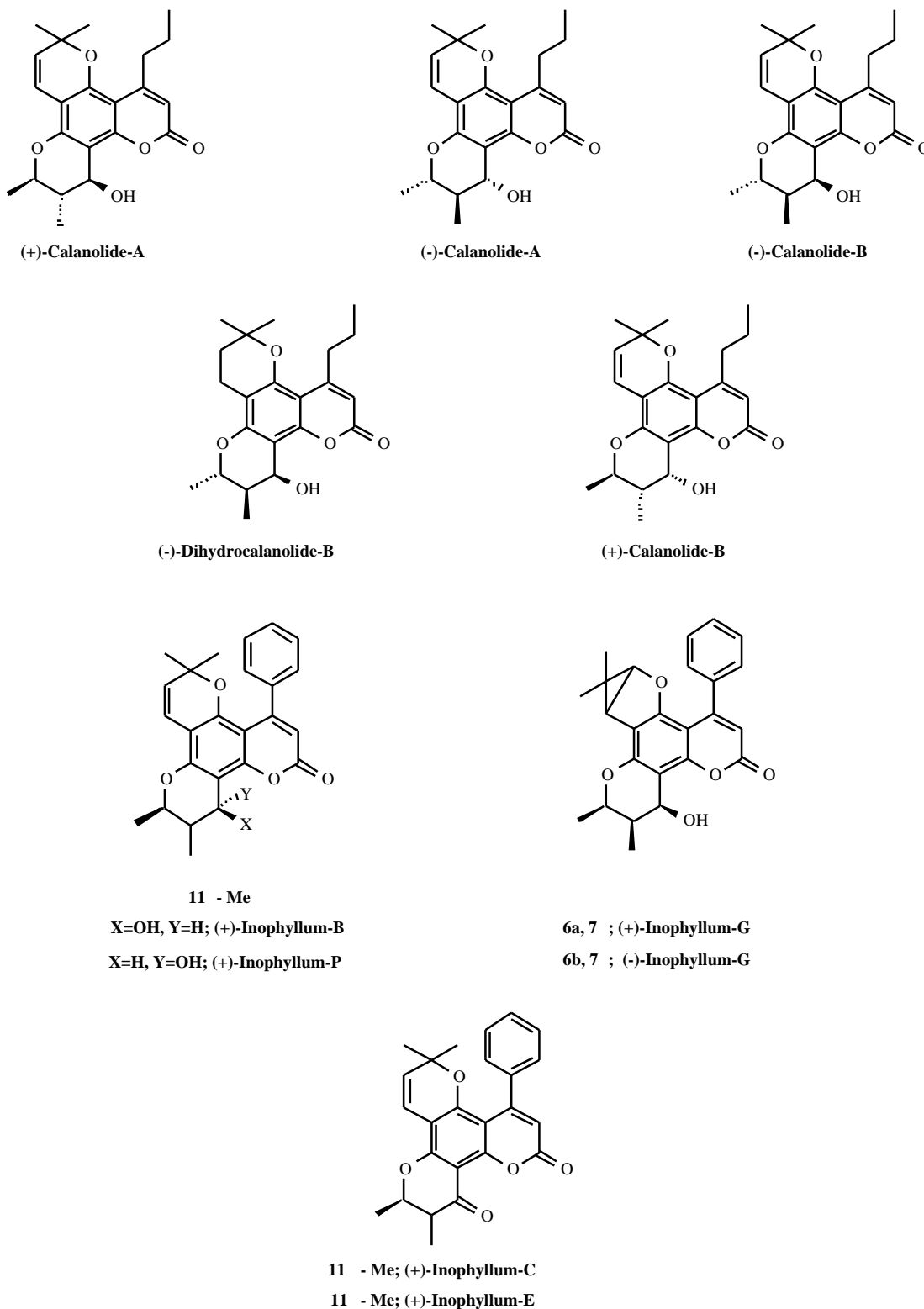
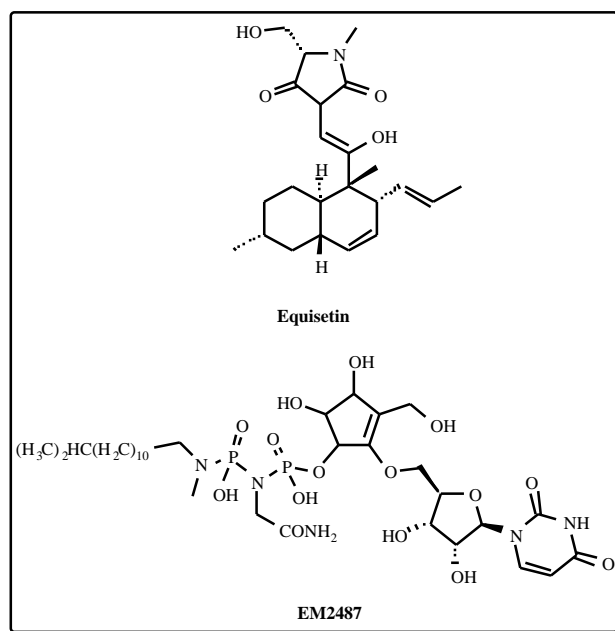


Fig. (11). Calanolides and Inophyllums.

10. EM2487

EM2487 isolated from *Streptomyces species*, proved inhibitory to HIV-1 replication in both acutely and chronically infected cells. Its EC₅₀ for acute HIV-1 infection

in peripheral blood mononuclear cells was 0.27 mM; its CC₅₀ was found to be 13.3 mM. The anti-HIV activity of EM2487 could be ascribed to an inhibitory effect at the transcriptional level; more specifically, to interference with the Tat-mediated transactivation process [57].



11. Flavonoids

The flavonoid chrysin "Fig. (12)" has been shown to inhibit casein kinase II (CKII), a cellular protein that may regulate HIV-1 transcription by phosphorylating cellular proteins involved in the HIV-1 transcription transactivation process [58]. Its mechanism of action is independent of the nuclear factor κ B-driven transcription pathway [59]. Thus, flavonoids interfere with HIV-1 transcription, and hence prevent HIV expression in latently infected cells [60]. Their specificity and usefulness as HIV transcription inhibitors remain to be assessed.

A series of flavonoids evaluated for their anti-HIV potential, (-)-epicatechin and (-)-epicatechin-3-O-gallate (from *Detarium microcarpum*) were found to block HIV infection through an irreversible interaction with the glycoprotein gp120 [61], IC_{50} for HIV replication in C8166 cells, 2 and 1 mg/mL, respectively, as compared to a CC_{50} of >100 mg/mL. (-)-Epicatechin gallate and (-)-epigallocatechin gallate, two components isolated from the tea plant *Camellia sinensis*, were described as inhibitors of reverse transcriptase and cellular RNA and DNA polymerases [62]. They may thus be able to interact at multiple targets of the HIV replicative cycle.

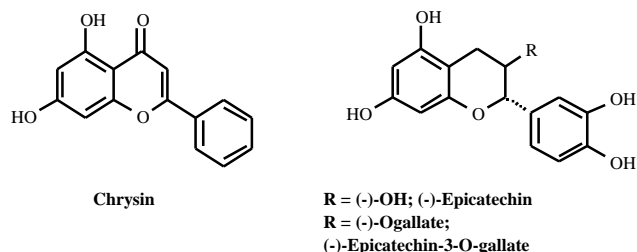
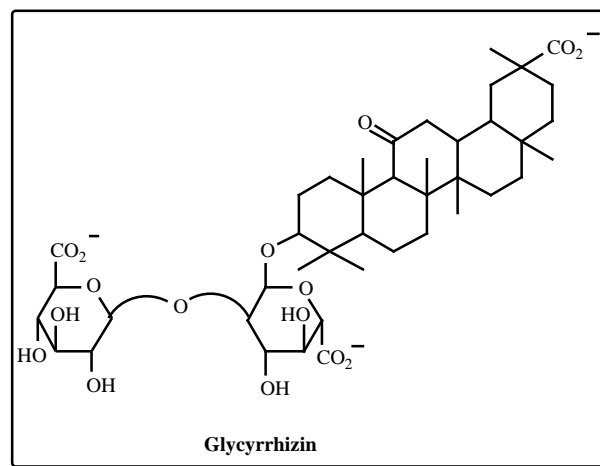


Fig. (12). Flavonoids.

12. Glycyrrhizin

Glycyrrhizin isolated from licorice root (*Glycyrrhiza radix*), has been known for some time as an antiviral agent,

its IC_{50} for HIV-1(IIIB) in MT-4 cells being 0.15 mM. The mechanism of action of glycyrrhizin may partially be attributed interference with virus-cell binding [63]. However, the site of interaction of glycyrrhizin at the envelope glycoprotein has not been further characterised.



13. Hypericin and Pseudohypericin

The aromatic polycyclic diones hypericin and pseudohypericin, which are present in plants of the family *Hypericum* (*Saint Johnswort*), "Fig. (13)", have been accredited with anti-HIV activity [64] since more than a decade. They would possess the capacity to directly inactivate HIV virions as well as interfere with their assembly [65]. Hypericin has received continued interest as a potential therapeutic agent [66] and more recently, was found to interact with preintegration complexes and thus affect the proviral DNA integration process [67].

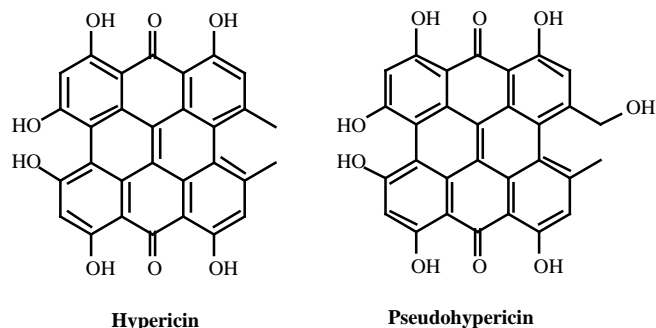
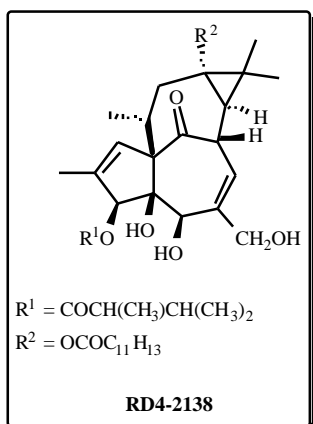


Fig. (13). Polycyclic diones.

14. Ingenol Derivatives

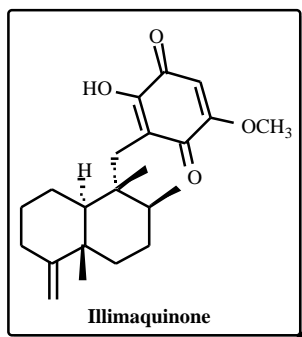
Ingenol derivatives isolated from Kansui, the dried roots of *Euphorbia kansui* have a variety of biological activities, including anti-HIV activity, due to inhibition of virus adsorption to the host cells [68]. Among the ingenol derivatives, 13-hydroxyingenol-3-(2,3-dimethylbutanoate)-13-dodecanoate (RD4-2138) proved to be exquisitely potent (IC_{50} : 0.07 nM) against HIV-1(IIIB strain) in MT-4 cells [69]. The mechanism of anti-HIV action of RD4-2138 could at least in part be attributed to down regulation of the CD_4 receptor for gp120, thus accounting for the inhibitory activity

of this class of compounds on virus adsorption. Although ingenol derivatives are highly potent inhibitors of HIV-1 replication in acutely infected cells, it was recently shown [70] that some ingenol derivatives enhance HIV-1 replication in chronically infected cells, whether or not through activation of the nuclear factor κ B and protein kinase C.



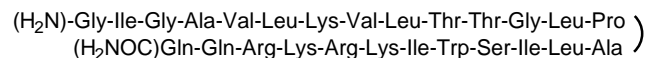
15. Illimaquinone

Illimaquinone, [71] isolated from a Red Sea sponge, i.e., *Smenospongia*, inhibits the RNase H activity, associated with the HIV-1 reverse transcriptase (RT), at a concentration of 5-10 mg/mL, while not being active against the RNA dependent DNA polymerase and DNA-dependent DNA polymerase activities of the enzyme at a concentration of 50 mg/mL [72]. Since the activity of illimaquinone against HIV replication in cell culture was not assessed, the findings remain inconclusive.



16. Melittin

Melittin, occurs in bee venom and has recently been found capable of suppressing HIV-1 gene expression. It is a 26-amino acid amphipathic α -helical peptide. Melittin inhibits HIV-1 infection in both acutely and persistently infected T-lymphoma (KE37/1) and fibroblastoid (LC5) cells at an IC50 of 0.5-1.5 mM; this effect is mediated by a direct suppressive action on the HIV long terminal repeat [73]. Antimicrobial peptides such as melittin (honey bees), cecropin (moths), and magainin (frogs) may thus inhibit cell-associated HIV-1 production at the transcription level. Their clinical usefulness remains to be ascertained.



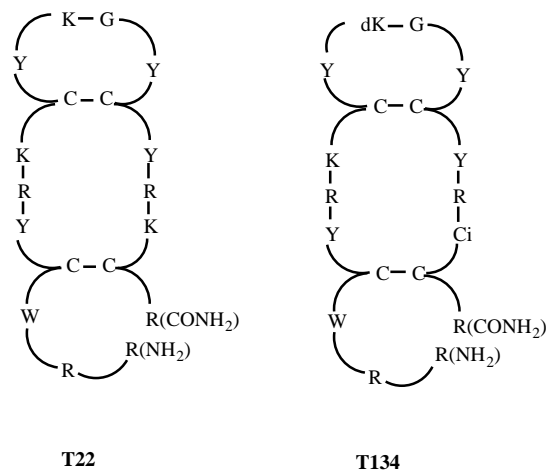
Melittin

17. Mannose-specific Plant Lectins

A number of mannose-specific agglutinins from *Galanthus nivalis*, *Hippeastrum hybrid*, *Narcissus pseudonarcissus*, *Listera ovata*, *Cymbidium hybrid*, *Epipactis helleborine*, and the N-acetylglucosamine specific lectin from *Urtica dioica*, have been found to inhibit HIV-1 and HIV-2 infection at similar concentrations as dextran sulfate (IC50: 0.2-0.6 mg/mL) or even lower (IC50: 0.04-0.08 mg/mL). Compared to sulfated polysaccharides, the plant lectins, also exhibit activity against various enveloped viruses other than HIV, i.e., HSV-1, HSV-2, CMV, RSV, and influenza virus [74]. Plant lectins would primarily interfere with the virus-cell fusion process. The mode of action has yet to be ascertained.

18. Polyphemusins

The polypeptides tachypleusins and polyphemusins are highly abundant in haemocyte debris of the horseshoe crabs *Tachypleus tridentatus* and *Limulus polyphemus*. An analogue of one of these polypeptides, T22 or [Tyr-5,12,Lys-7] polyphemusins II was found to inhibit HIV infection at an IC50 of 8 ng/mL CC50; 54 mg/ml. It is a synthetic peptide that consists of 18 amino acid residues, "Fig. (14)". A post-binding process, corresponding to virus-cell fusion, has been suggested as the target for the anti-HIV activity of T22 [75]. SAR studies have implicated to the importance of the Arg residues in the anti-HIV activity of T22 [76]. During further studies the compound was shown to act as a specific antagonist of CXCR4, the coreceptor used by T-tropic (X4) HIV-1 strains to enter the cells [77]. This antagonism towards the CXCR4 coreceptor can be attributed to the polyargininic character of T22. New T22 derivatives with lower cytotoxicity have been synthesised [78], and one of these T22 derivatives, T134, was shown to block HIV-1 infection with a better selectivity index than T22 [79].



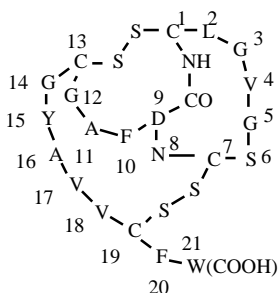
Polyphemusins II

Fig. (14). Polyphemusins.

19. Siamycins

Three varieties of siamycin have been described, from *Streptomyces*. These are siamycin I (BMY-29304), siamycin II (RP 71955, BMY 29303) and NP-06 (FR 901724). They differ from one another only at position 4 (Val or Ile) or 17

(again, Val or Ile), which should not make big difference in terms of conformational or functional properties. The siamycins have been found to inhibit HIV infection *in vitro*; they exhibit a strong inhibitory effect on syncytium formation while only weakly inhibiting virus-cell binding. The most likely target for the mode of action of siamycins is the HIV envelope glycoprotein gp41 [80]. Thus, siamycins can be assumed to interfere with the fusogenic activity of gp41. The exact mechanism of action, their clinical potential and pharmacokinetics are yet to be ascertained.



Siamycin I = **BMY-29304**

Siamycin II = **RP71955 = BMY-29303**

NP-06 = **FR901724**

20. Sulfated Polysaccharides

Sulfated polysaccharides, such as dextran sulfate, pentosan polysulfate, and heparin "Fig. (15)", as well as sulfated polysaccharides have been isolated from sea algae [81]. These have long been recognised as effective anti-HIV agents. The sulfated galactan from the red seaweed *Aghardhiella tenera* inhibits HIV-1 and HIV-2 infection at a concentration that is 10-fold higher than the IC₅₀ required for a dextran sulfate to inhibit these viruses (IC₅₀: 0.5 and 0.05 mg/mL for HIV-1 and HIV-2, respectively) [82]. Their development as anti-HIV drug candidates was not taken up further due to their less potential compared to other drug candidates.

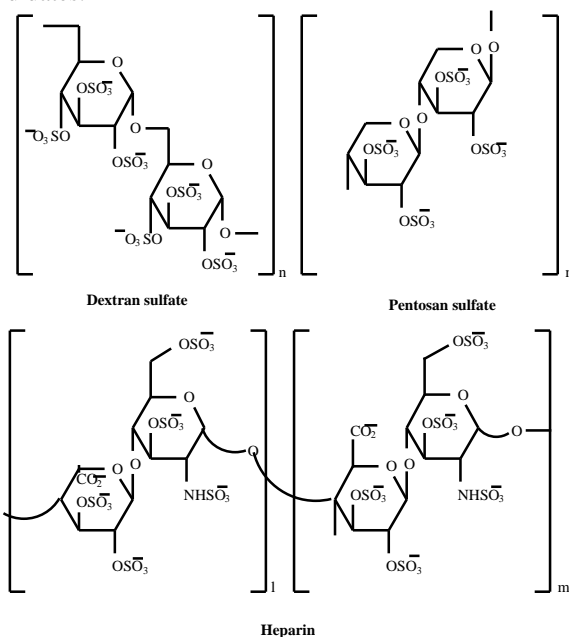


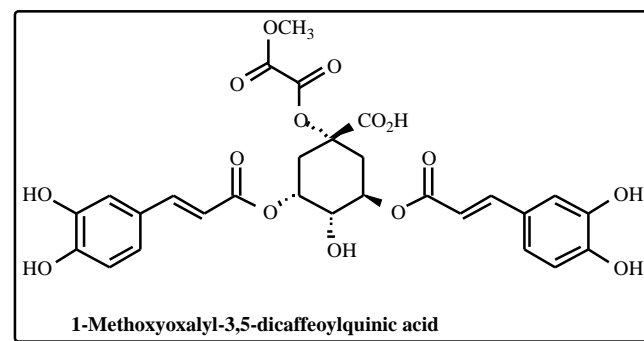
Fig. (15). Sulfated polysaccharides.

21. Trichosanthins

A series of proteins [i.e., -trichosanthin (-TCS)] isolated from the root tubers of *Trichosanthes kirilowii*; MAP30 isolated from the seeds and fruits of the bitter melon *Momordica charantia*; GAP31 from *Euphorbiaceae himalaya* seeds (*Gelonium multiflorum*) and DAP30 and DAP32 from carnation leaves (*Dianthus caryophyllus*) [83] have been described to inhibit HIV infection, although, at noncytotoxic concentrations [84]. These compounds referred as RIPs (ribosome-inactivating proteins) are known to block eukaryotic protein synthesis through inactivation of the ribosomes. RIPs inactivate ribosomes by cleaving the adenine N-glycosidic bond at position 4324 of 28S rRNA. In addition to this RNA N-glycosidase activity, RIPs, such as MAP30, also act as DNA glycosylase/apurinic (ap)lyases, thus explaining the apparent ability of MAP30 to inhibit HIV-1 integrase and irreversibly relax supercoiled DNA. RIPs, and MAP30, both have potential to develop as anti-HIV agents [85].

22. Dicafeoylquinic Acid Derivative

Cynarin (isolated from artichoke, *Cynara scolymus*), its isomeric form 3,5-dicafeoylquinic acid has shown significant anti-HIV activity. An analogue of dicafeoylquinic acid, the 1-methoxyoxalyl- 3,5-dicafeoylquinic acid (1-MO-3,5-DCQA), was reported to show strong inhibitory action against HIV-1 integrase [86]. ED₅₀ value of this compound was found 4 μg/mL in the cell-based anti-HIV assay.



Thus, the dicafeoylquinic acid isomers may contribute a lead compound as anti-HIV agents through anti-integrase drug research.

23. Quinolone Derivatives

Several species of the genus *Euodia* have been used in folk medicine by indigenous people in Australia and Southeast Asia.

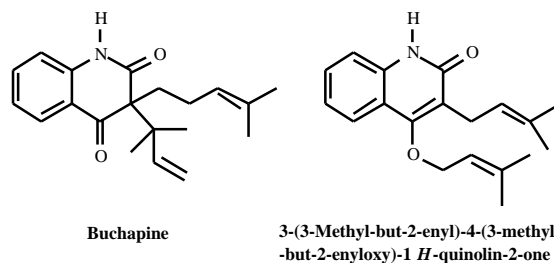


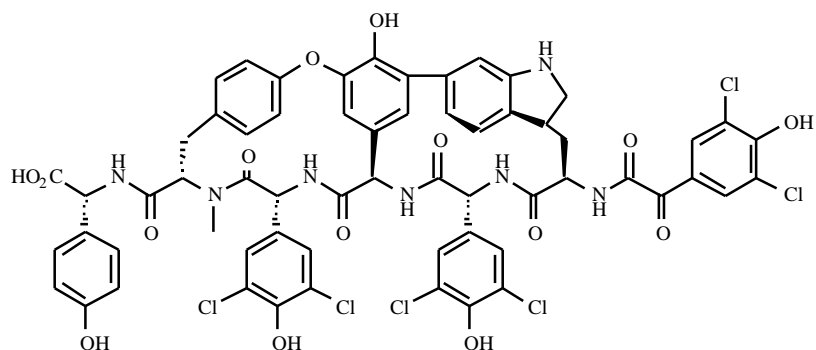
Fig. (16). Quinolone derivative.

Buchapine and 3-(3-methyl-but-2-enyl)-4-(3-methyl-but-2-enyloxy)-1*H*-quinolin-2-one, "Fig. (16)", extracted from *Euodia roxburghiana* Benth (Rutaceae) and collected in Thailand, showed significant anti-HIV activity in the cell-based assay, with EC₅₀ values of 0.94 and 1.64 μM, respectively. These quinolones also inhibited HIV-1 reverse transcriptase activity in a cell-free assay, with IC₅₀ values of 10 and 8 μM, respectively [87]. These compounds may serve as useful leads for NNRTI drug design since it has also been

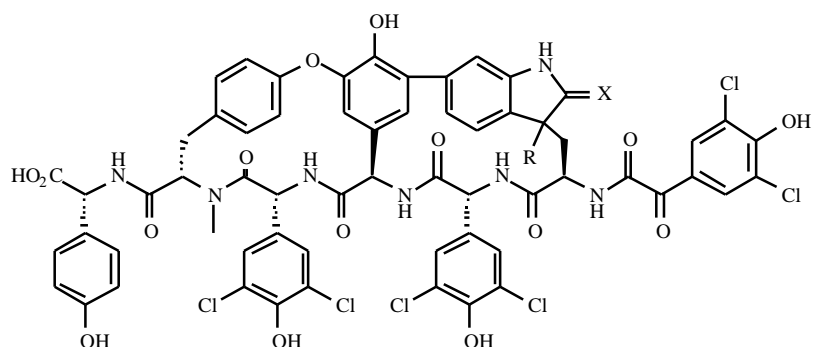
reported that similar compounds isolated from marine sponges exhibit anti-HIV RT activity [88].

24. Complestatin Derivatives

Complestatin derivatives shown in "Fig. (17)" have been isolated from *Streptomyces* sp. as a potent inhibitor of HIV-1 integrase [89]. Isocomplestatin inhibited *in vitro* HIV-1 integrase coupled and strand transfer activities with IC₅₀ values of 0.2 and 4.0 μM, respectively. Chloropectin I,



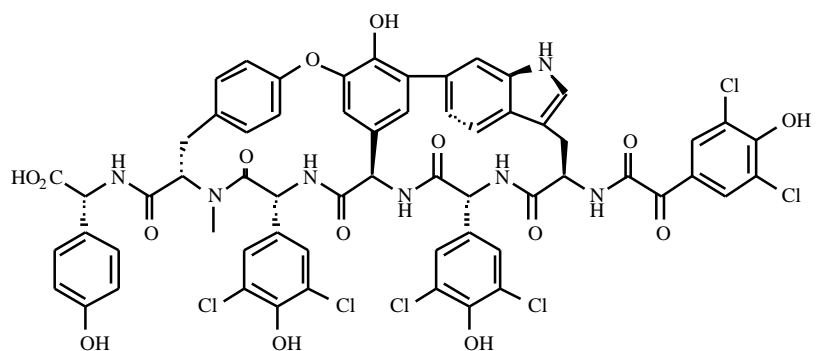
Isocomplestatin



R = H; X = H, H; Complestatin

R = H; X = O; Complestatin A

R = OH; X = O; Complestatin B



Chloropectin I

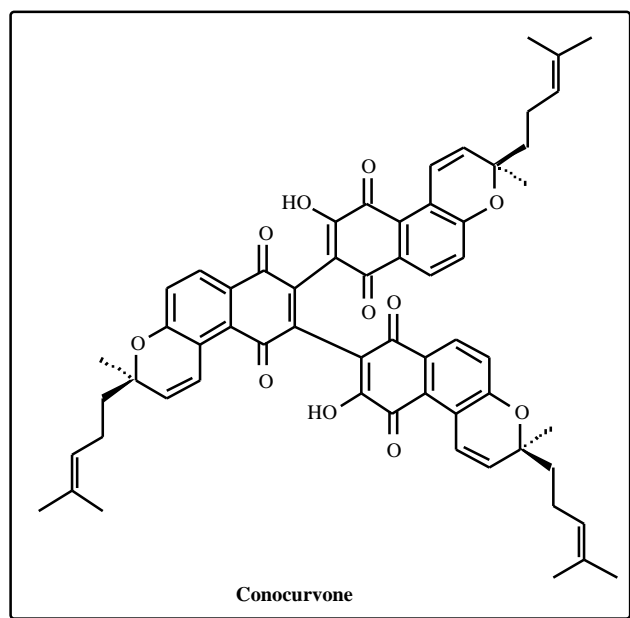
Fig. (17). Complestatin derivatives.

complestatin-A, and complestatin-B were less active than isocomplestatin and exhibited IC_{50} values of 0.4-1.7 and 5-12.5 μM in the coupled and strand transfer assays, respectively. Isocomplestatin inhibits preintegration complexes from HIV-1 infected cells with an IC_{50} comparable to that observed for inhibition of strand transfer using complexes assembled on the viral donor DNA with recombinantly expressed integrase.

The observation that isocomplestatin is active both in strand transfer and against HIV-1 preintegration complexes suggests that this compound may be an alternative for designing novel strand transfer inhibitors. Isocomplestatin inhibits the integrase protein from several related retroviruses. Isocomplestatin inhibits recombinant feline immunodeficiency virus with an IC_{50} value of 0.5 μM and was a more potent inhibitor of recombinant simian immunodeficiency virus, with an IC_{50} value of 0.1 μM [90].

25. Conocurvone

Trimeric naphthoquinone, conocurvone was isolated from Organic extracts of most of the parts of the Western Australian plant *Conospermum incurvum* Lindley (Proteaceae) except root. It showed potent anti-HIV activity [91]. The mechanism of action was not reported and it was ascertained that its inhibitory action occurs in the late phase of the viral replication cycle. Conocurvone was able to protect T-cells from cytopathic effect of HIV-1 even when added after 48 h of injection. Structure-activity studies showed that the trimeric central core of the compound is critical for antiviral activity.

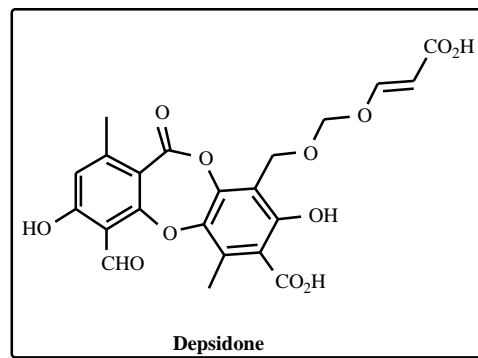


This compound is under drug development by the Australian company AMRAD, working in collaboration with Western Australian Organisations [92].

26. Depsidone

Depsides and depsidones were isolated from *Lichens*. These have been reported to inhibit HIV integrase. Depsidone exhibited significant anti-HIV activity in the cell-

based assay (EC_{50} at 8.4 μM) and a correspondingly strong anti-integrase activity ($IC_{50} = 4.9 \mu\text{M}$ for 3'-processing and 4.6 μM for strand transfer) [93].



Likewise, the therapeutic index for depsidone was found to be low, and no further development was warranted.

27. Verticillatol and Epiexcelsin derivatives

The eudesmane sesquiterpenoid, verticillatol as well as (+)-5'-demethoxyepiexcelsin and (+)-epiexcelsin, were isolated from *Litsea verticillata* Hance as shown in "Fig. (18)". (+)-5'-Demethoxyepiexcelsin showed moderate anti-HIV activity with an IC_{50} value of 16.4 $\mu\text{g/ml}$ (42.7 μM), while the lignan (+)-epiexcelsin was inactive up to a concentration of 20 $\mu\text{g/ml}$ (48.3 μM).

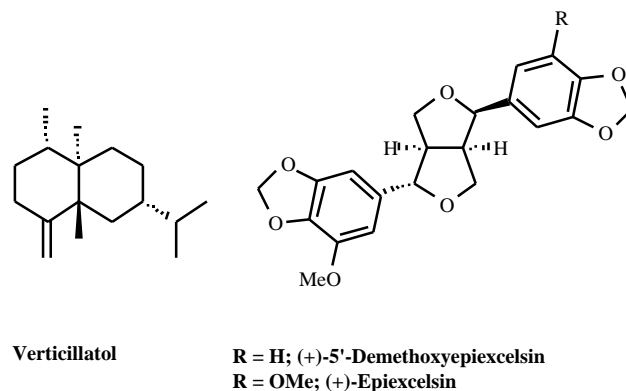
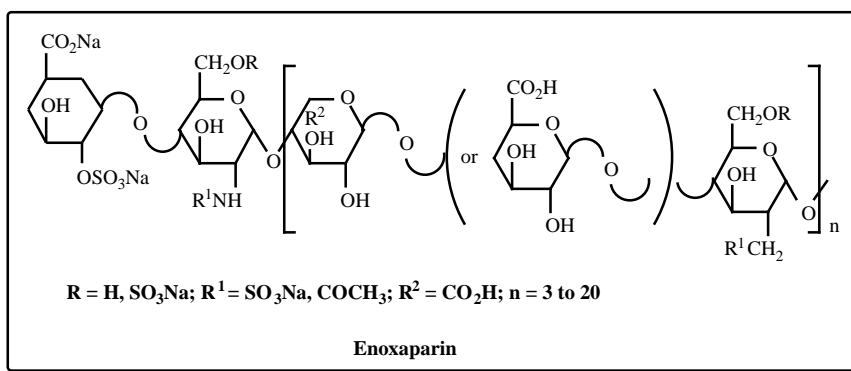


Fig. (18). Verticillatol and Epiexcelsin derivatives

Verticillatol demonstrated weak activity with an IC_{50} value of 34.5 $\mu\text{g/ml}$ while being devoid of cytotoxicity at 20 $\mu\text{g/ml}$ [94], therefore they have not been taken for further developments.

28. Enoxaparin

Enoxaparin in the primary antiviral screening, as well as in subsequent detailed mechanistic studies, was found to protect human T cells, both CEM-SS and MT-4, as well as peripheral blood leukocytes, against the cytopathogenic effect of a number of HIV-1 variants. It showed a range of inhibitory activities against HIV-1 wild-type IIIb and RF strains, HIV-2, and a pyridinone-resistant strain, A-17. Enoxaparin exhibited an EC_{50} value in the range of 0.2-1.6 μM against HIV-1 and exhibited little or no apparent toxicity. The IC_{50} for enoxaparin ranged from 56 to 180 μM



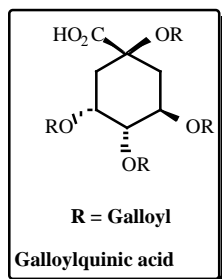
when tested in CEM cells, MT-4 lymphocytes, or peripheral blood lymphocytes (PBLs).

Mechanistically, enoxaparin interacts with viral gp120 and competes with the binding of gp120 to the CD₄ recognition site on the T-lymphocyte cell surface, as suggested by the stoichiometric relationship observed between the number of CD₄-gp120 interactions and the concentration of enoxaparin required to prevent these interactions [95].

29. Galloylquinic Acid

1,3,4,5-Tetra-*O*-galloylquinic acid was isolated from the stem bark of the monotypic plant *Lepidobotrys staudtii* Engl. (Lepidobotryaceae), inhabitant of Cameroon. It has demonstrated significant anti-HIV activity. It protected CEM-SS cells from the cytopathic effects of HIV-1_{RF} at an EC₅₀ value of 0.5 μM, but was much less effective against HIV-2_{ROD} with an EC₅₀ of 10 mM in the cell-based assay.

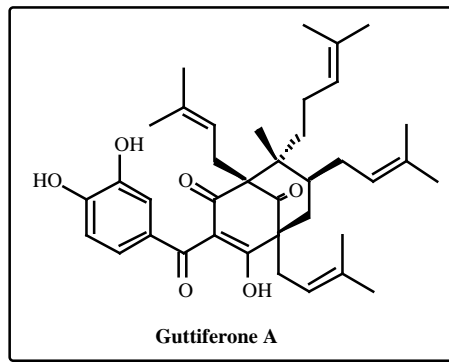
The same compound was also reported to inhibit recombinant reverse transcriptases of HIV-1 and HIV-2 at IC₅₀ values of about 0.8 μM. Unfortunately, cellular DNA polymerases and were also affected [96], therefore it may not be considered as chemical entity for further developments.



30. Guttiferone A

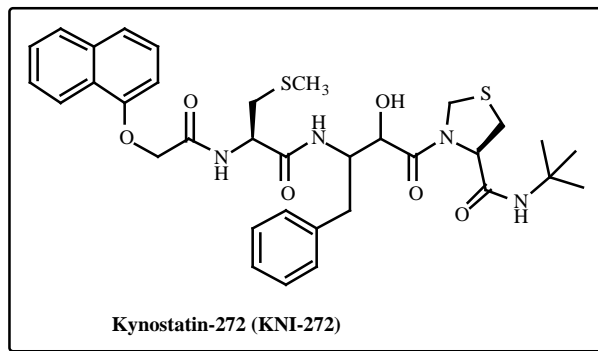
Guttiferone A was isolated from *Symphonia globulifera* (Clusiaceae). It is a native plant of Tanzania [97] and a member of the Clusiaceae (Guttiferae) family. Altogether, extracts of four different members of the Clusiaceae, *S. globulifera*, *Clusia rosea*, *Garcinia livingstonei*, and *G. ovalifolia* have been found to show anti-HIV activity in the cell-based screening assay [98].

Guttiferone A as an active agent, provided cytoprotection of CEM-SS cells from HIV-1 infection at EC₅₀ values of 1-10 μM holds great promise for further developments.



31. Kynostatins

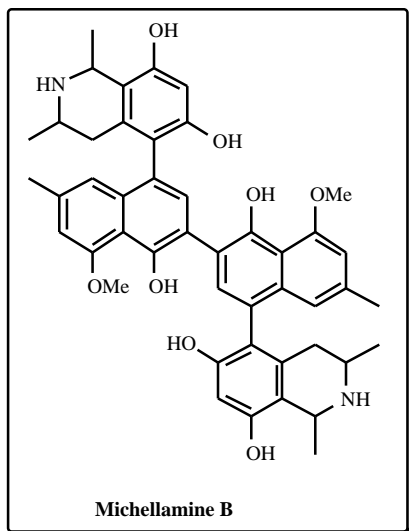
Kynostatin-272 (KNI-272), a protease inhibitor of microbial origin was advanced by National Cancer Institute, USA. KNI-272, as well as its analogue KNI-227, were found to be highly potent against a spectrum of HIV-1 strains and HIV-2 at EC₅₀ concentrations of 0.02-0.1 μM when tested *in vitro* [99]. KNI-272 inhibition was also highly synergistic with AZT or with other NNRTIs in HIV-1 infected CEM-2 cells. Moreover, resistant HIV variants emerged in HIV-1-infected tissue culture exposed continuously to KNI-272 for 9 months, whereas treatment with the currently available Ritonavir generally resulted in resistant mutants in 1 month. In another study with KNI-227, no drug-resistant variants emerged in the HIV-1-infected cell cultures exposed to the drug until after 55 passages conducted over a period exceeding 1 year [100]. KNI-272 has entered into clinical trials and results of a 12-week Phase I trial on 37 patients with AIDS or symptomatic HIV infection treated with an escalating dose of KNI-272 *via* oral administration.



Treated patients tolerated the treatment well and showed a continuous decrease in plasma HIV RNA copies throughout the 7-8 weeks of treatment that shows immense scope of further clinical study of KNI-272 to optimize its use in combination therapy [101].

32. Michellamine B

The anti-HIV alkaloid michellamine B, is an alkaloid isolated from the leaves of *Ancistrocladus korupensis* (Ancistrocladaceae). It is a plant native to Cameroon's Southwest Province. Michellamine B inhibits HIV-1 during the early phase of viral infection of T-lymphocytes. It was also noted that it inhibited HIV-2 in MT-2 cells equally well [102].



Although the mechanism of action remain viable for michellamine B. Collective consideration of its inhibitory characteristics, range of activity, and the difficulty in cell membrane penetration indicates that it most probably acts at the cell surface. The clinical development of michellamine B was not pursued due to the unacceptable neural toxicity in preclinical studies.

33. Nitidine and Magnoflorine

The alkaloid nitidine as shown in "Fig. (19)", was isolated from the roots of *Toddalia asiatica* (Rutaceae) [103]. It is a plant native to India, China, Taiwan, and Japan. Nitidine showed significant anti-HIV activity in the cell-based assay ($EC_{50} = 14 \mu\text{M}$; $TI_{50} = 3$) and was reported earlier to inhibit HIV reverse transcriptase [104].

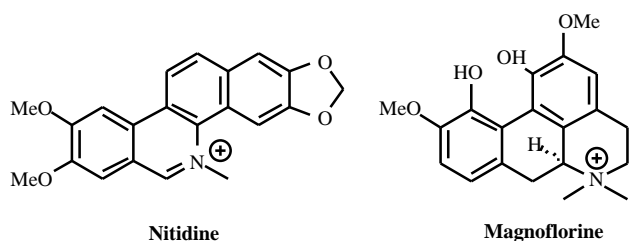
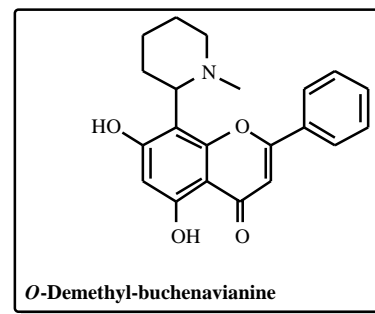


Fig. (19). Nitidine and Magnoflorine

An aporphine alkaloid, magnoflorine "Fig. (19)", isolated from same plant showed significant anti-HIV activity.

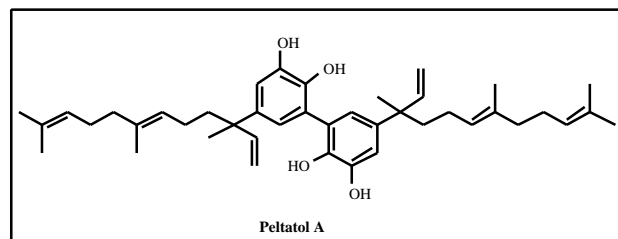
34. Buchenavianine

The piperidine derived alkaloid, *O*-demethyl-buchenavianine, isolated from *Buchenavia capitata* (Vahl) Eichl. (Combretaceae) [105] collected in the Dominican Republic, showed significant activity against HIV-1_{RF} ($EC_{50} = 0.26 \mu\text{M}$; $TI_{50} = 3$).



35. Peltatol A

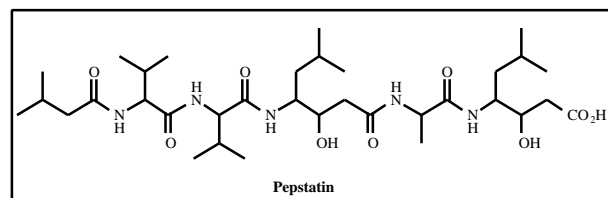
Extracts prepared from *Pothomorphe peltata* (Piperaceae), demonstrated strong anti-HIV activity, and bioassay-guided fractionation yielded several prenylated catechol dimers, the peltatols, as the active agents.



In primary Anti-HIV-1 screening, it showed to have EC_{50} 8.0 mM and TI_{50} value 4 [106]. Therefore further development of the peltatols is required.

36. Pepstatin

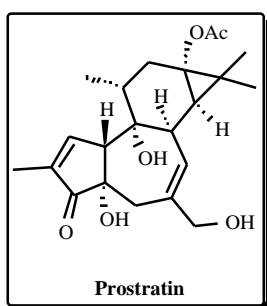
Pepstatin A, a metabolite of *Streptomyces testaceus* and *Streptomyces argentolus* var. *toyonakensis*, was shown to inhibit HIV-1 protease activity at a K_i value of 1.1 mM also known as aspartyl protease inhibitor, whose anti-HIV activity varied over a wide concentration range. An averaged EC_{50} value of 51.8 μM together with a TI_{50} value of >1.4 was estimated. In recent confirmatory retests, pepstatin A demonstrated an EC_{50} value of 69 and 150 μM when tested against CEM cells infected with the HIV-1_{RF} strain and with the Rojo clinical strain of HIV, respectively.



This suggests that pepstatin A is able to penetrate the cell membrane, albeit at high concentrations [107]. Structurally, pepstatin A is a small pentapeptide with a unique hydroxyamino acid, that sterically blocks the active site of HIV-1 protease to make it ineffective.

37. Prostratin

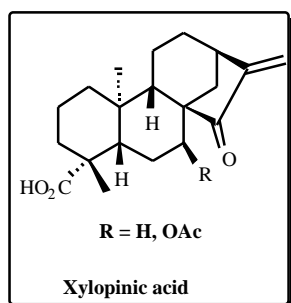
The phorbol ester, prostratin, isolated from stem bark of *Homoalanthus nutans* (Forster) Pax (Euphorbiaceae), is an active agent (EC_{50} against HIV-1 = 0.132 μM and TI_{50} = 250) [108]. When added to T-cells or cells in tissue culture, prostratin exhibited typical cytostatic effects on the growth kinetics of the cells. Its range of anti-HIV activity spanned across certain HIV strains such as HIV-1_{RF}, HIV-1_{IIB}, HIV-2, and certain drug-resistant HIV-1 variants in the appropriate host cells, CEMSS cells and C8166 cells. Prostratin was able to provide complete cytoprotection even when added to HIV-infected cultures at as late as 24-30 h post-infection, but it failed to prevent viral replication. Its potency and extent of cytoprotection, however, are reported to depend on the HIV strain and the host cell type involved. In two latently infected cell lines, prostratin was reported to activate viral expression, in contrast, it failed to activate viral expression in cell lines chronically infected with HIV.



It has been found that HIV viral particles that stayed dormant in the host's tissue and, hence, escaped detection, could be responsible for the onset of eventual, sudden viremic crises. Compounds like prostratin may be able to stimulate the emergence of such dormant viral particles for exposure to effective anti-HIV chemotherapy, such as HAART [109].

38. Xylopinic acid

Xylopinic acid, isolated from aqueous extract of fruits of a *Xylopia* sp. (Annonaceae), collected from Peru, is a new diterpene isolated as the active agent. It protected CEM-SS cells from the cytopathic effects of HIV-1 infection at an EC_{50} of 0.9 μM [110].



This new diterpene, xylopinic acid, could serve as a lead for anti-HIV drug development.

B. Proteomics and Genomics in the Lead Generation Against HIV

The functions and interactions of the genes are dealt with a study of particular branch of biological science, termed as genomics, while proteomics is defined as the study of all the proteins expressed by the genome. Genomics and proteomics are now a core of modern biotechnology and are key to a wide range of research areas in the biological sciences including bioorganic/medicinal chemistry and medical biotechnology. The sequencing of the human genome and the rapid emergence of high-throughput genomic and proteomic techniques are resulting in a number of new drug targets such as extracellular receptors, ion channels, transporters, intracellular second messengers, transcription factors and chromosomal DNA itself. The genome and the proteome are linked between a complex pathway of transcription and translation, which principally involves mRNA processing, protein folding and post-translational modifications. Both genomics and proteomics incorporate areas of biotechnology, bioinformatics and biology by utilising a multitude of methods and techniques to study gene and protein expression profiles of normal cells or infected cells and eventually whole biological systems. The pace of drug discovery has been influenced by sequencing the three billion base pairs of the human genome. Along with many other scientific aspects, this presented us with a list of all possible drug targets. Unfortunately, however, these valuable entities are supposed to be scattered amongst the genes we expect to find. The challenge will now be to find the small percentage of genes encoding proteins that are both tractable drug targets, and that influence disease susceptibility or are involved in pathological processes.

Understanding the genetic basis of disease is likely to provide novel approaches to allow more effective treatment of established disease, but will also ultimately provide fundamental insights that may facilitate the prediction and prevention of these conditions. Most genomics-based studies focussed on mining of genomic databases to identify novel members of known drug target families, and the transcriptional analysis of such genes in various tissues throughout the body. The goal of such research is to identify molecular targets that are differentially expressed in diseased tissues but are absent in other tissues throughout the body. Furthermore, attractive drug targets may not necessarily be over-expressed in diseased tissue, the activity of many molecular targets, such as ion channels and G protein coupled receptors, are not regulated at the level of gene or protein expression and do not demonstrate an enhanced expression in disease. To understand how genetic studies can be used to validate and prioritise drug targets, it is important to mention that polymorphism in genes is common and that such polymorphisms can contribute towards the development of disease. It is now thought that a SNP (Single Nucleotide Polymorphism) occurs in every 300-500 base pairs (at a frequency of at least 5 per cent in the population) and, on average, each gene contains one functional SNP in its coding region that is predicted to change the structure, and possibly function of the gene product [111]. A gene may also have

SNPs in its promoter region that may affect the level of expression in tissues throughout the body, while SNPs in intronic regions may control alternative splicing of mRNA. It is these alternative forms of genes or alleles that, in combination with other genetic or environmental risk factors, trigger the onset of disease. There are already a few examples where genetic studies have highlighted a drug target linked to an important disease pathway. In some cases, the findings are unexpected - such as when the studies demonstrated that certain individuals who were resistant to HIV infection carried a loss-of function due to mutation in the chemokine receptor, CCR-5 [112]. This led to the discovery that CCR-5 is a co-receptor for the HIV virus, and highlighted an exciting approach towards anti-HIV research.

Since the complete genome of HIV is sequenced therefore, it is possible to predict how HIV destroys the immunity. A study based on genomics pointed out that a human gene named ATR normally protects the system by preventing the replication of cells damaged by radiation or toxic chemicals. This particular gene used to be involved with a gene in the AIDS virus and turns it into a weapon that prevents reproduction of white blood cells, leaving HIV patients vulnerable to deadly infections, so called syndrome. It is known that an HIV gene named vpr led to the depletion of white blood cells named CD₄ lymphocytes. The new study suggests vpr does that by activating the ATR gene, which is found in white blood cells of human beings.

The ATR gene's normal job is to detect genetic damage to cells caused by radiation, toxic chemicals and chemotherapy, and to stop the damaged cells from replicating until they can repair themselves. Researchers found evidence that the vpr gene, one of nine genes in the AIDS virus, exploits this normal repair process to stop vital white blood cells from replicating, thus disabling the immune system. The study opens the possibility of treating AIDS-related immune-system damage with medicines that prevent the human ATR gene from being activated by HIV's vpr gene [113].

Recent advances in structural genomics not only help us to understand protein functions but also have a big impact on the pharmaceutical industry. Recently, the use of protein structural information in drug discovery research has matured, and it is now used at all levels, ranging from genomics-derived target identification and selection to the final design of suitable drug candidates. Now these techniques became more powerful due to the developments in combinatorial chemistry and appears as a tool to define how structural genomics and combinatorial chemistry can be used in rational drug design. The optimisation of multiple factors in developing potent inhibitors for the reverse transcriptase enzyme of human immunodeficiency virus (HIV), as potential drugs to prevent the development of AIDS. HIV RT is vital for replication of the virus and is therefore rightly considered as a primary target for developing drugs. On the basis of crystal structures of HIV RT and its ligand-bound forms, two primary types of target site were identified: the dNTP-binding site (used when RT carries out its polymerase function) and the non-nucleoside RT-inhibiting site. Non-nucleoside RT-inhibitors were found suitable especially for disrupting HIV RT function. NNRTI

targeted drugs use to be chemically very diverse and should not compete with nucleotide-binding substrates, while inhibiting RT in nanomolar concentrations. To identify the most potent NNRTI drugs, molecular modelling has been used to calculate the interaction energy of a potential substrate bound at the non-nucleoside RT inhibiting site, which consists of a hydrophobic pocket. It turns out that the lower energy conformations usually produce more potent drug. Selected targets are synthesised and then assessed through antiviral screening experiments. This procedure allowed faster design of a number of powerful NNRTI drugs. The most successful candidates, such as TMC120 and TMC125, appear to have low toxicity (in the micromolar range) and high selectivity. Monotherapy experiments with each of these produce up to 30-fold drop in the virus population *in vitro* [114].

C. Recent Developments in Combination Therapy for Treatment of AIDS

Till to date, the FDA has approved 47 drugs for the treatment of HIV/AIDS, including 19 antiretroviral drugs for use singly or in combination therapy. Highly Active Anti-Retroviral Therapy (HAART) regimens utilizing protease inhibitors are now emerged as a major factor in reducing the number of AIDS deaths from the mid-1990s to the present. It has been identified as a pipeline of 52 drugs for HIV infection and AIDS, including extended release formulations, immune modulators, fusion inhibitors, vaccines and topical microbicides and drugs targeting post treatment issues like dementia, lipodystrophy and other complications of AIDS.

The most common initial regimens consist of two nucleoside analogues, combined with either a PI, an NNRTI or a third nucleoside analogue. The combination of two nucleoside analogues and a PI is supported by data from randomised studies with clinical endpoints [115]. These regimens, however, often involve a considerable pill burden and relatively frequent side effects, which makes compliance difficulties.

NNRTs with two nucleoside analogues regimen is found to have advantages as they are effective with low pill burden and good tolerability. However, the significantly emerging disadvantage is the rapid development of cross resistance. Most explored nucleoside analogues combination is Trizivir (AZT, 3TC and abacavir) but studies have shown that in patients with a high viral load, efficacy was inferior to PI combinations [116].

Zerit [117], an extended-release preparation of stavudine, which is a nucleoside reverse transcriptase inhibitor (NRTI) has been introduced by Bristol-Myers Squibb. The one-capsule, once daily formulation should improve quality of life and compliance relative to the FDA-approved immediate-release formulation of Zerit, with similar virologic activity. In a recent phase III clinical studies Zerit with efavirenz and lamivudine, showed that 80% of the 392 patients in the treatment arm containing Zerit extended release achieved viral load suppression below 400 copies/mL after 48 weeks of treatment, compared with 75% of the 391 patients in the arm containing Zerit immediate release. More than half of patients in each treatment arm reduced viral load to fewer than 50 copies/mL, and adverse events were similar

in both groups. Coviracil (emtricitabine) is another NRTI developed by Triangle Pharmaceuticals, has potent selective activity against both hepatitis B virus (HBV) and HIV. In a 48-week, double-blind, placebo-controlled phase III trial FTC-301, [The FTC 301 study is a randomised, double-blind, double dummy comparative study of FTC (Emtricitabine) vs d4T with a backbone of didanosine and efavirenz in 571 treatment naive individuals] comparing once-a-day coviracil to immediate-release Zerit given twice daily, each combined with efavirenz and Videx EC, interim analyses of safety and efficacy led to unblinding the trial and offering coviracil to all 571 subjects for further studies. Patients with coviracil in the arm had significant improvements in immunologic function, and 87% had persistent virologic response through six months.

Capravirine [118], a second-generation NNRTI which has been shown in several studies to retain activity against HIV that has developed some NNRTI-resistant mutations, including K103N [119] is being developed by Pfizer. Agouron Pharmaceuticals launched capravirine in the market and believes that it may offer unique benefits to HIV

infected patients and therefore remains committed to its clinical development and to additional toxicology studies.

One of the problems with available protease inhibitors (PI) is their tendency to increase blood lipid concentrations against that atazanavir [120], a PI as shown in "Fig. (20)" formerly called BMS-232632 is being developed by Bristol-Myers Squibb. A randomised trial in 467 patients at 51 sites suggests that concentrations of total cholesterol, LDL cholesterol and triglycerides do not increase significantly in treatment naive patients taking a HAART regimen including atazanavir, and that the drug is effective, safe and well tolerated compared to nelfinavir. Other advantages include once daily dosing and fewer gastrointestinal effects, but the drug is more likely than nelfinavir to induce jaundice.

Unlike currently approved antiretroviral drugs, which target viral enzymes involved in replication, the fusion inhibitors are designed to block HIV from fusing with a host cell before the virus begins replication within the cell. Trimeris has developed a fusion inhibitor Fuzeon (enfuvirtide; formerly T-20) [121] and received fast track designation from the FDA. Data from two large,

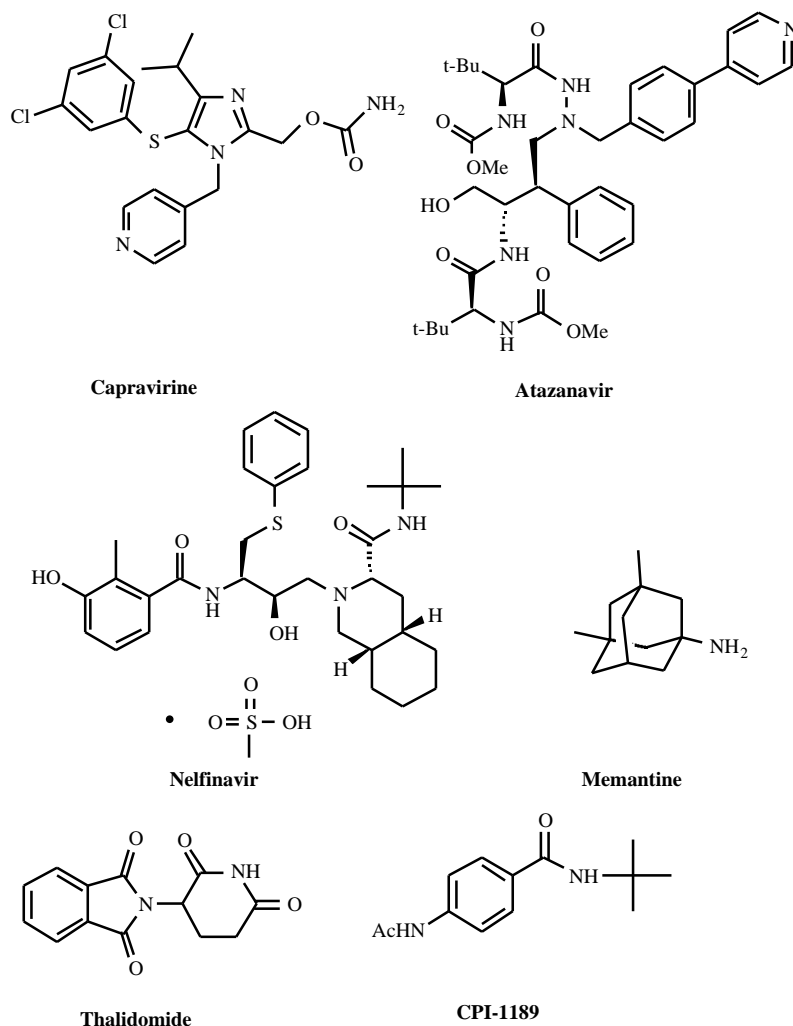


Fig. (20). Drug Candidates recently involved in combination therapy and post treatment issues

international phase III trials suggest that patients on combination therapy with Fuzeon are twice as likely to achieve undetectable blood levels of HIV and a significant increase in immune cells at 24 weeks compared with patients who received combination therapy without Fuzeon.

PRO 542 (PRO 542, also known as CD₄-IgG₂, is a novel fusion protein that incorporates the HIV-binding region of the human cell surface receptor for HIV into a human antibody molecule) [122] from Progenics Pharmaceuticals is a novel fusion protein shown to reduce viral load by 60% to 80% in selected patients. Multi-dose phase II studies are being performed for PRO 542 as salvage therapy for HIV-infected patients refractory to currently available antiretroviral medications. Rather than targeting the HIV virus directly, other option is that the modulators can boost the ability of the immune system to pause its spread. These include Celgene's drug Thalomid (thalidomide) [123], Chiron's product IL-2 Proleukin (aldesleukin) [124], a recombinant form of interleukin-2 Alferon N injection (interferon -n3), a human leukocyte derived natural interferon, and WF10, designed by Dimethaid Research to enhance macrophage function and to thereby eliminate reservoirs of HIV-infected CD₄ cells and macrophages. All these drugs are in phase III clinical studies. WF10 [125], is a chemically stabilised chloride matrix called tetrachlorodecaoxygen (TDCO). Studies suggest that WF10, administered intravenously, is safe in patients with HIV that may be especially useful in moderate to advanced AIDS patients. By promoting phagocytosis and reducing macrophage production of TNF- α , WF10 may slow AIDS progression and reduce the likelihood of opportunistic infections.

Remune (HIV-1 immunogen) [126] is a promising adjunctive therapy using inactivated virus to restore HIV specific immune responses, with positive effects on viral load and on counts of CD4 helper T lymphocytes have been supported by phase II trial studies performed by Immune Response Corporation.

Another topical approach to prevent HIV infection is the spermicidal microbicide BufferGel (polyacrylic acid) which is in phase III development by ReProtect. By increasing the acidity of semen, this product tends to kill sperm and to inactivate pathogens including HIV, HPV, herpes, syphilis and gonorrhoea [127]. Data on the anti-HIV activity of BufferGel are yet to appear.

There are several post HIV treatment issues to be handled, for that a number of drugs have been developed as shown in "Fig. (20)".

Lipodystrophy syndrome is one of the complications associated with HAART, for which EG005, a drug that reduces cellular levels of Angiotensin II and thereby increases mitochondrial efficiency has been developed by Ark Therapeutics.

Drugs targeting AIDS-related dementia for which phase II testing is completed include CPI-1189 from Centaur Pharmaceuticals. While memantine from Forest Laboratories.

CPI 1189 (prevents apoptosis and reduces glial fibrillary acidic protein immunostaining in a TNF- α infusion model

for AIDS dementia complex) [128], an oral drug that potentially inhibits neuroinflammation, has produced significant cognitive improvements in AIDS dementia in double-blind, placebo- controlled clinical studies lasting five months.

Memantine, which modulates N-methyl-D-aspartate (NMDA) receptor activity has been used to treat other types of dementia, with rapid and lasting improvement in cognitive, psychological, social and motor impairments and further studies are being carried out on the basis of animal models [129].

Drugs identified in the pipeline may continue to improve the outlook for AIDS patients, however, significant progress in HAART has reduced the number of AIDS deaths in recent years. New antiretroviral drugs and their combination regimens may improve tolerability and compliance. Emergence of fusion proteins, which prevent HIV entry into the cell may prove as one of the major tools in the management of the disease. Additionally immune modulators work synergistically in order to reduce the viral load as well as immunogens are supposed to improve immunity.

IV. DEVELOPMENTAL STAGES OF ANTI-HIV VACCINES

The worldwide epidemic of AIDS and mutations in HIV plead the scientists for the development of an effective vaccine. Vaccines stimulate the body's immune system to provide protection against infection or disease. Vaccines against HIV are being developed and they are in various stages of clinical trials, but at present, none has proven effective [130].

In the early phase of HIV vaccine development, a preventive vaccine HIV envelope gp120 was found unsuccessful. Broadly neutralising antibodies and HIV-specific cytotoxic T lymphocytes (CTL) are two immune agents that an effective HIV vaccine should possess. Experiments in animal models have proved that sufficient levels of neutralising antibodies can clean up the virus and protect the animals from viral challenges. Therefore, the induction of neutralising antibody response remains a principal goal in HIV vaccine development. In the HIV infection, virus has evolved elegant strategies to evade host immune defence. These include envelope oligomerisation, rapid mutation, glycosylation, and conformational changes. Each level of the HIV's defence provides an additional dimension of complexity that has to be taken into account in order to come up with a vaccine conferring strong and long lasting immunity. Important progress has been made in recent years in understanding the structure of HIV envelopes and the mechanism of HIV evasion to the immune system. This in turn has greatly facilitated a rational design of immunogens capable of eliciting broadly neutralising antibodies against HIV.

There are certain classes of HIV vaccines based on process or factors involved in their development e.g. subunit vaccines, recombinant viral vectors based vaccine and Prime boost strategy based vaccine as they are currently under investigations. DNA based vaccines are also under development in several countries.

Subunit vaccines [131] are generally recombinant peptides including the envelope proteins of HIV-1, gp120 or gp160. This is also known as "component" vaccines, contain only individual proteins or peptides from HIV, rather than the whole virus. Instead of collecting protein or peptide components from the virus itself, they are made in the laboratory using genetic engineering techniques. Most HIV subunit vaccines are based on synthetic forms of the HIV envelope proteins that coat the outside of the virus. These envelope proteins can prompt the body to produce an anti-HIV immune response.

Another type of subunit vaccine is called a virus-like particle vaccine, also known as a VLP or pseudovirion vaccine. Virus-like particles are non-infectious HIV look alike that contain one or more, but not all, HIV proteins. They target antibody production and have been developed with the goal of prevention of primary infection. The VaxGen gp120 subtype B and E combination product is a prototype of this approach. Recently, the results of first phase III efficacy trials in humans were announced, showed no protective effect [132]. Newer formulations include oligomeric peptides, which may prove more immunogenic than monovalent formulations, and deglycosylated proteins, which may expose more neutralising antibody sites, holding better hopes.

Recombinant viral vectors comprising viral and bacterial vector system are being used to deliver HIV antigens to the immune system, targeting antigen-presenting cells in lymphatic tissues. They are based on viruses or bacteria that do not cause disease in humans or have been weakened so as not to cause disease. As mentioned above the viruses or bacteria are used as vectors, or carriers, to deliver harmless HIV genes into the cells of the body. In defence, body produces proteins from the HIV genes and these proteins stimulate an anti-HIV immune response. Some of the viral vectors being studied for HIV vaccines include ALVAC (a canarypox virus), MVA (modified vaccinia Ankara, a cow pox variant), and ADV5 (adenovirus 5). In this way most of the recombinant vector vaccines for HIV deliver several HIV genes. This approach targets cellular immune function, including CD8-mediated CTLs. Vectors investigated various studies in human have included ALVAC, ADV5 construct, and MVA. Other vectors under development include fowl pox, VEE (Venezuelan equine encephalitis virus), BCG, A modified version of the bacterium *Salmonella typhae* and Semliki Forest Virus (SFV). Most of these candidates include several HIV genes, gag, pol, nef, and sometimes env [133].

A prime-boost strategy is one of the approaches to HIV vaccination. In this approach, administration of one type of HIV vaccine (such as a recombinant vector vaccine) is followed by a second type of HIV vaccine (such as a subunit vaccine). The goal of this approach is to stimulate different kinds of immune responses and enhance the body's overall immune response to HIV. The first prime boost approaches tried to combine immune priming with a vectored vaccine followed by antigen boosting, usually with a subunit vaccine. The combination of an ALVAC recombinant prime followed by the VaxGen gp120 B/E boosting has entered in phase III

trials in Thailand only in this year. This would be the first efficacy trial of the prime-boost strategy.

Novel priming strategies with naked DNA products followed by MVA or other boosts have been proposed and are currently being pursued by several groups. Related research is being carried out with new adjuvants that may improve immunogenicity [134].

DNA vaccines [135] allow to introduce pieces of synthetic HIV DNA into the body. Unlike recombinant vector vaccines, DNA vaccines do not rely on a viral or bacterial vector. Instead, "naked" DNA containing HIV genes is injected directly into the body. Cells' genome incorporates this DNA and use it to produce HIV proteins. The proteins trigger the body to produce an anti-HIV immune response.

Recently, VRC of NIH is pursuing DNA vaccines (http://ocf.od.nih.gov/OCLUpdateJan_03.htm) with multiclade envelope components from clades A, B, and C of HIV-1.

CONCLUSION

Concerted efforts are being made worldwide by medicinal chemists, biologists and clinicians to eradicate most advanced HIV strains. The combination therapy to date involving synthetic drugs has been successful for partial cure of patients ailing from AIDS. The potential of some of the drugs isolated from plant to combat HIV strains resistant to combination therapy is of prime significance and is being studied by various research groups working in this area. The present review summarises recent advances made in anti-HIV/AIDS research.

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