Targeting Strategies for Human Immunodeficiency Virus: A Combinatorial Approach

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Abstract: The battle between human and the Human immunodeficiency virus (HIV) is on, with both of them rapidly improving their attacking and defense strategies. Many therapeutic agents for HIV infection have been designed and developed, However there are various aspects, like novel targets against HIV, which are yet to be unfolded with a goal of designing and developing novel drug molecules against HIV. This article reviews the current status and innovative new options for antiretroviral therapy for HIV and also discusses the various mechanisms of action for each class of drugs, and the problems yet to be solved with respect to HIV as a target for improvised treatment against AIDS.

Keywords: Lentivirus, HIV, host cellular target, life cycle, treatment, nucleoside, reverse transcriptase, protease, integrase, carbohydrate binding proteins, Griffithsin, synthesis**,** and inhibitors.

INTRODUCTION

Lentivirus forms a distinctive genus of pathogens, which affects wide range of animals, including humans. The prefix 'lenti' (*lento* means slow) represents prolonged incubation without clinical symptoms, persistent viral replication, neurologic manifestations, and destruction of specific hematologic or immunologic cells leading to a chronic progression and death. Human immunodeficiency virus (HIV) belongs to a family of primate lentiviruses and is of two distinct subtypes namely, HIV-1 and HIV-2 [1]. HIV-1 is the most notorious and globally important lentivirus whereas HIV-2 has been isolated primarily in West African countries such as Guinea Bissau, Ivory Coast, and Senegal, With some cases also identified in America and Western Europe. Both agents are associated with the development of progressive immunologic deterioration which causes a dramatic decline in the number of $CD4^+$ T lymphocytes [2] and when the number of these cells becomes low enough, neurologic abnormalities, and opportunistic infections arise forming the pathological basis for acquired immunodeficiency syndrome (AIDS) in humans (Fig. (**1**)). However, epidemiologic studies suggest that the incubation period for HIV-2 for the development of disease is longer than for HIV-1 [3].

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HIV Infection

HIV is transmitted by direct sexual contact, either homosexual or heterosexual, by blood or blood products and from an infected mother to infant, either intrapartum, perinatally, or *via* breast milk [4]. Primary infection of humans with HIV-1 is associated with an acute mononucleosis-like clinical syndrome (Table **1**) which appears approximately 3–6 weeks following infection. However, HIV-2 is not as easily transmitted perinatally as HIV-1 [5]. Although HIV has been reported to infect a wide range of cells like B cells, natural killer cells, eosinophils, precursor $CD4^+$ bone marrow cells, $CD8^+$ T cells, renal epithelial cells, cervical cells, columnar epithelial cells, as well as cells and tissues from various organs like liver, lungs, salivary glands, eyes, prostate, and testes but $CD4^+$ T lymphocytes and macrophage-lineage cells are consistently found to be infected with HIV [6,7].

Overview of HIV Replication

HIV replication is a multi-step complex process, which involves attachment of the viral membrane protein to the cellular receptor (CD4) and co-receptors (CCR5, CXCR4) of the host cell membrane. Following this attachment, fusion peptide brings the host and viral membranes into close proximity creating a fusion pore and allowing entry of the HIV capsid into the host cell [8]. After entering the cytoplasm, reverse transcription begins and the preintegration complex is integrated into the host genome (Chromosomal DNA). New virus particles are produced by further transcription of new viral RNAs, which give rise to new envelope proteins. Viral core proteins are synthesised in

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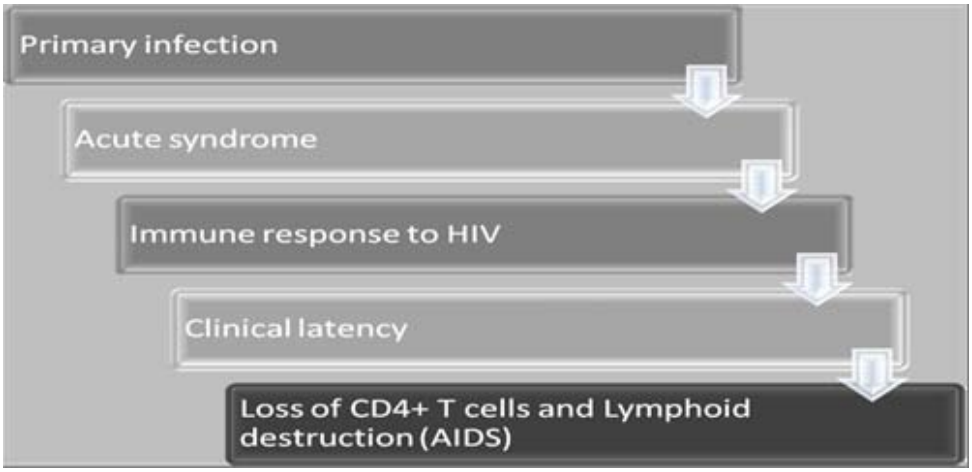


Fig. (1). A schematic diagram of the pathogenic events that occur from initial infection with HIV to the development of clinical disease.

Table 1. Pathologic Conditions Associated with HIV -Infection and AIDS [113, 114]

Primary infection	Mononucleosis-like syndrome: fever, malaise, pharyngitis, lymphadenopathy, headache, arthralgias, diarrhea, maculopapular rash, meningoencephalitis
Clinical latency	Often none, but can include: fatigue, mild weight loss, generalized lymphadenopathy, thrush, oral hairy leukoplakia, shingles
Clinical disease	200–500 $CD4^+$ T cells/ml: generalized lymphadenopathy, oral lesions (thrush, hairy leukoplakia, aphthous ulcers), reactivation of herpes zoster (shingles), thrombocytopenia, molluscum contagiosum, basal cell carcinomas of the skin, headache, condyloma acuminate, reactivation of latent Mycobacteria tuberculosis
Less than 200 $CD4^+$ T cells/ml:	Protozoal infections: Pneumocystis carinii, Toxoplasma gondii, Isospora belli, Cryptosporidia, Microsporidia
	Bacterial infections: Mycobacterium avium intracellulare, Treponema pallidum
	Fungal infections: Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum
	Viral infections and malignancies: cytomegalovirus (CMV), recurrent bouts of oral or genital herpes simplex virus
Lymphoma (mostly EBV, some HHV-8)	Kaposi's sarcoma (HHV-8), anogenital carcinoma (HPV), Neurological symptoms, aseptic meningitis, myelopathies such as vacuolar myelopathy
	pure sensory ataxia, paresthesia/dysesthesia, peripheral neuropathies such as acute demyelinating polyneuropathy, mononeuritis multiplex, and distal symmetric polyneuropathy, myopathy , AIDS dementia complex

the cytoplasm and simultaneously assembly of new virions occurs at the plasma membrane immediately before release of new virions. Then budding takes place and these viral proteins are processed into the individual components by the viral protease (Fig. (**2**)) [9].

Mechanisms of $CD4^+$ T Lymphocyte Dysfunction

One of the early hallmarks of HIV infection is the impairment of a variety of $CD4^+$ T-cell functions including T-cell colony formation, autologous mixed lymphocyte reactions, expression of interleukin-2 (IL-2) receptors, and IL-2 production [10]. Further, the T-cell proliferative response is lost to recall the antigens such as tetanus toxoid, influenza, *Candida albicans,* alloantigens and subsequently mitogens [11]. The cell surface expression of the CD4 receptor in infected $CD4^+$ T cells is down-regulated as the HIV gp120 molecules produced during viral replication bind to cytoplasmic CD4, forming intracellular gp120-CD4 complexes. Other viral products, Nef and Vpu, also contribute to the down regulation of CD4 [12-18] causing a decline in antigen-specific responses. Failure to respond to antigenic stimuli has also been seen in uninfected $CD4^+$ T cells in HIV-infected patients [19, 20]. HIV proteins such as purified Env glycoproteins (both HIV gp120 and gp41), Env peptides, and purified Tat protein have been reported to suppress, *in vitro*, proliferative responses to mitogens, antigens, and anti-CD3 antibody. Exposure of cells to HIV gp120 also inhibits both IL-2 production and IL-2 receptor expression. A number of possible mechanisms by which viral replication occur are: 1. The virus mutates rapidly and the resulting mutants can escape the neutralizing antibodies and cytotoxic T lymphocyte (CTL) responses. 2. The virus replicates in immunologically privileged sites where in the macrophage may limit the viral expression while effectively disseminating it [21]. 3. The presence of glycosylation sites on the Env proteins suggests that the virus becomes invisible to the immune system by using carbohydrates as a protective shield against the host's immune response [22-24]. Mutations in *gag* and *pol* allow the virus to escape from the cellular immune responses. CTLs are reactive against a specific viral sequence but are ineffective at controlling infection in an infected individual with virus expressing the same sequence.

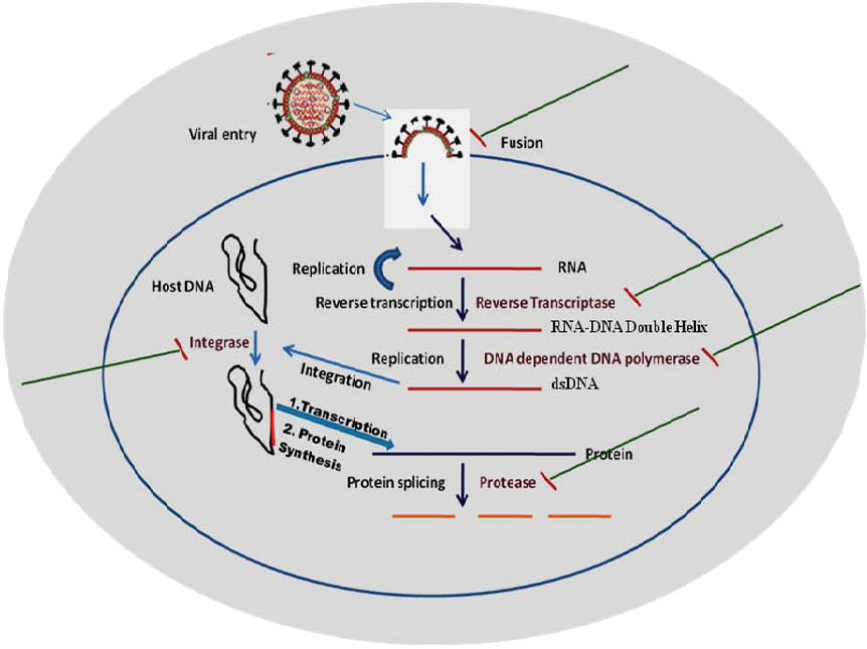


Fig. (2). Overview of HIV replication.

The reason for this is not yet clear. In addition, the complex replication cycle of the HIV leads to high-level viral production before being detected as foreign.

Viral Load and Progression to Disease

It is important to point out the difference between long-term non-progression of HIV disease and long-term survival. Long-term survivors are patients who although infected for 10 or more years are still alive, but whose HIV disease may or may not have progressed with regard to deterioration of their immune systems. Long-term non-progressors are those HIV-infected individuals who not only have survived 10–15 years of HIV infection, but are also free of symptoms and have $CD4^+$ T cell counts that have remained stable and reasonably normal. The genetic defects in the infecting virus may play a part in long-term non-progression. One patient was found to have a defect in *nef* gene [25]. Out of seven blood recipients from a donor of the infected virus that had sustained deletions in the *nef* gene, six remained free of AIDS and had stable and normal $CD4^+$ T-cell counts even after 10–14 years of infection. Virus obtained from these recipients had *nef* gene deletions [26]. This shows that the alterations of *nef* might be responsible for long-term non-progression of the disease. Adult rhesus monkeys were infected with cloned virus in which the *nef* gene was partially deleted. They showed very low levels of viremia analogous to the human long-term non-progressors and did not progress to disease [27, 28]. This suggests that *nef* gene is not necessary to establish or maintain infection, but it is required for high viral load. The mechanisms that underlie this effect are not yet understood [29, 30]. Nef protein seems to be required for efficient replication, increase cell division and expand the numbers of cells available for productive infection *in vivo*. The highly virulent SIVsmmPBj14 strain derives its pathogenicity from a point mutation in *nef* gene that may increase its interaction with cellular kinases [31, 32]. $CD8^+$ T-cell counts remain high in long-term survivors but decline during disease progression [33]. Non-progressors who maintain high levels of $CD8^+$ T-cell responses carry relatively non-cytopathic variants of HIV; such variants have a restricted cell tropism and grow to low titer in cells *in vitro* [34, 35]. The low titer may reflect selection for viral variants with greater resistance to the immune response. Variation in rate of disease progression may also reflect genetic variation in the host. Consistent with this idea, it has been reported that HIV-infected individuals who are heterozygous for a 32-base deletion in the CCR5 gene have slower rate of progression to AIDS [36, 37]. Genetic diversity in the variable regions of *env* is noticeably less in rapid progressors [38, 39]. This might mean that a less virulent virus variant is also less fit and therefore that there are more mutations that confer a positive selective benefit.

TARGETS FOR ANTIRETROVIRAL THERAPY

There are currently 6 different targets for antiretroviral therapy for the treatment of human immunodeficiency virus (HIV) infection. These include envelope (entry and fusion), integrase, reverse transcriptase and protease [40]. In addition to these essential viral enzymes that have been extensively explored as targets for antiretroviral drugs, a diverse array of potential targets for the development of new classes of HIV therapies have been reviewed that included Vif-APOBEC3G [41, 42], Integrase-LEDGF/p75 [43], TRIM5α [44, 45], virus assembly and maturation, and Vpu [46, 47].

Viral Entry: (Receptor Binding and Fusion with Cells)

The primary receptor for HIV is CD4, which is present on the surface of $CD4^+$ T lymphocytes, and certain other cells [48, 49]. In addition to its role as the principal receptor for HIV, CD4 serves as an adhesion molecule to stabilize the interaction. The entry of virus into host cells is a multi-step process including attachment to host cells and CD4 binding,

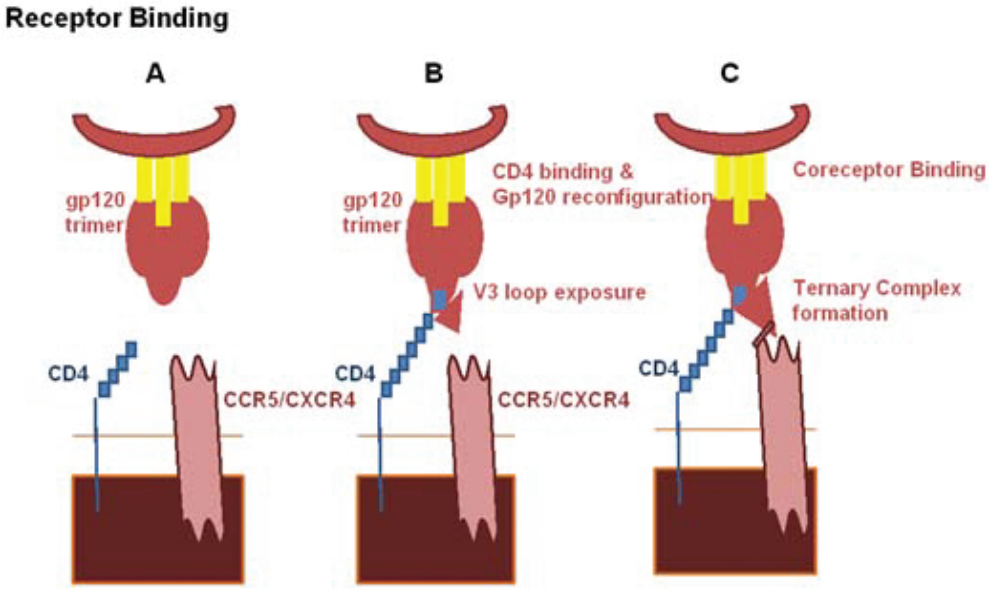


Fig. (3). Binding of HIV gp120 to the host cell CD4.

A. Virus entry into host cells mediated by glycoprotein. The functional unit is a trimer of covalently bonded gp41 (yellow bars) molecules and gp120 (majenta spheres) molecules.

B. Second step is the binding of gp120 to host cell CD4 (Blue squares) resulting in the reconfiguration of gp120 molecule.

C. CD4-bound gp120 interact with the coreceptor, either CCR5 or CXCR4 with the formation of ternary complex.

coreceptor binding, and membrane fusion. All of these steps are mediated by the noncovalently associated viral envelope (Env) proteins, gp120 and gp41. The gp120 subunit of Env mediates attachment, CD4 binding, and coreceptor binding, and consists of five conserved (C1–C5) and five variable (V1–V5) domains. These domains are critical for binding to host cells. The gp41 subunit of Env is responsible for the membrane fusion. Although HIV can bind to CD4 on cells, fusion between the HIV envelope and the cell membrane fails to occur, indicating the absence of a cell surface component essential for fusion [50]. The host-cell component (coreceptor) referred to as the "fusion receptor" permits HIV infection of mammalian cell that expresses CD4 [51]. The two most important coreceptors are CXCR4 (also called fusin or LESTR) and CCR5 (Fig. (**3**) & Fig. (**4**)) [52]. Coreceptors not only provide a crucial function for viral entry into cells, but they are also the principal determinants of tropism among $CD4^+$ cells. The chemokines that can bind to these receptors can block HIV-1 infection, and a defect in the gene encoding CCR5 causes natural resistance to HIV infection [53].

The entry sites for pH-independent viruses, such as HIV-1, have long been assumed to fuse directly with the plasma membrane, whereas enveloped viruses that rely on a low pH-dependent step for entry initiate infection by fusing with acidic endosomes. Fusion of HIV-1 with the plasma membrane did not progress beyond the lipid mixing step at the cell surface. The failure beyond this step indicates that the formation and/or dilation of a fusion pore depend on pH. The lack of complete fusion at the cell surface could be due to restrictions imposed by the cortical actin or other factors present in or around the plasma membrane. An alternative possibility is that viruses rely on yet unidentified endosomal factors to promote complete fusion. Miyauchi *et al.* recently reported that HIV-1 also opt for the endocytic machinery to enter into and fuse with target cells. A novel role for dynamins in HIV-1 content release from endosomes has been reported. The ability of dynamin to regulate actin

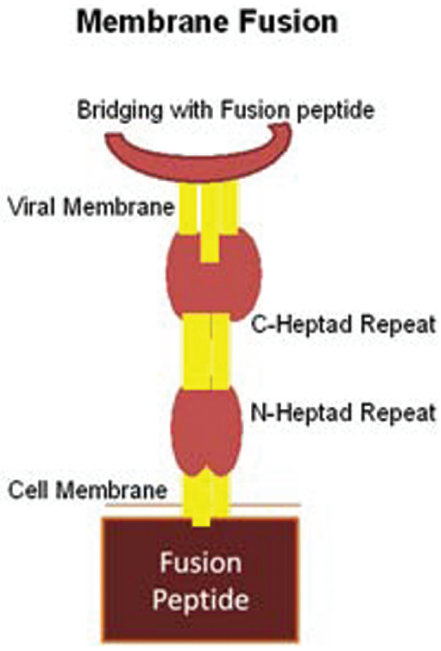


Fig. (4). Membrane fusion of HIV to release viral core in cytoplasm of host cell. In this step the insertion of gp41 in to the host cell membrane with the formation of a bridge between viral and cellular membrane occurs. Metastable prefusion takes place with the formation of a six-helix bundle and with the close approximation of the membranes a fusion pore is formed, which enlarges to release the viral core in to the cytoplasm.

remodelling and/or to associate with membrane-bending proteins could provide an additional driving force to expand pores and permit the release of the HIV-1 core. Endocytic entry offers several advantages, including the sheltering of HIV-1 from antibodies and inhibitors targeting intermediate conformations of Env during the unusually slow fusion reaction [54].

Integrase-LEDGF/p75 Axis

As per the co-crystal structure (PDB Id 2BJ4) of the interacting domains of integrase and LEDGF/p75, LEDGF/p75 is a protein with 533 amino acids which while interacting with HIV-1 integrase, a highly conserved integrase binding domain (IBD) is present in the C-terminus (amino acids 347–429) of HIV-1 integrase whose two monomers interacts with a dimer of the catalytic core domain (CCD) of integrase at an interhelical loop of "LEDGF/p75". The most critical interacting residues of the IBD are I365, D366 and F406 [55, 56]. Further, to establish the role of LEDGF/p75 in the replication of HIV-1, many cellular and biochemical experiments were performed like reduction of HIV-1 integration in homozygous *LEDGF* disrupted murine cells. Mutagenesis studies like substitution of integrase amino acids with alanine diminished the interaction with LEDGF/p75 and showed that LEDGF/p75 is an important integrase cofactor which plays an active part in HIV-1 replication at the step of integration and proved this LEDGF/p75-integrase interaction as a novel target for antiviral therapy. Thus, small molecules that can enter the binding pocket in integrase, may prevent the interaction with the cofactor and will provide a vast momentum to this field [57]. However, during replication the majority of HIV-1 DNA remains unintegrated (uDNA) and is generally considered a replication dead-end. But recently, Gelderblom, H. C. *et al.* has reported that virus alternatively replicates by coinfection with integrating and non-integrating HIV-1 resulting in a several fold increase in the number of cells displaying uDNA early gene expression and efficiently driving uDNA into late gene expression. Further it was reported that uDNA-derived genomes undergo recombination with the integrated provirus-derived genomes during second round infection and generates virions by viral complementation. This novel mode of retroviral replication allows survival of viruses which would increase the replicating HIV-1 gene pool [58-60].

Reverse Transcriptase

HIV are enveloped viruses that have RNA as their genetic material and utilize the enzyme reverse transcriptase (RNA-directed DNA polymerase) for the transcription of RNA genome into its complimentary DNA strand to form a double-stranded DNA:RNA hybrid which further gets transcribed into a double-stranded DNA copy of the HIV genome and gets incorporated into the human genome through the actions of HIV integrase enzyme.

Protease

Newly formed HIV particles are not infectious until they undergo ''maturation phase.'' This phase involves the cleavage of viral protein precursors by HIV protease enzymes which are encoded by HIV and offer an attractive target to design and develop drugs against maturation phase of HIV. These protease enzymes are symmetrical in nature. The structure shows a dimer with a central core that binds the peptides which are to be tailored by the enzyme [61].

Vif-APOBEC3G Axis

APOBEC (*apo*lipoprotein *B* mRNA-*e*diting *c*atalytic polypeptide) proteins are a group of cytidine deaminases, which include APOBEC1 (A1), AID, APOBEC2 (A2), APOBEC3 (A3), and APOBEC4 (A4), proteins in humans. All of these proteins contain one or two Zn^{2+} binding motifs which catalyze cytidine deamination. These proteins target cytosines and convert them to uracils (C to U) on a variety of vertebrate-specific RNA/DNA targets. Further, A3 gene is a tandem arrayed cluster of genes encoding seven cytidine deaminase, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3DE, APOBEC3F, APOBEC3G, and APOBEC3H. The function of the A3 genes remained unknown until it was discovered that human APOBEC3G (A3G) inhibits human immunodeficiency virus type 1 (HIV-1) replication [62-64]. In the later phase of the viral life cycle, a 23-kDa phosphoprotein, "Vif" (viral infectivity factor) is expressed which directly binds to an anti-HIV factor, APOBEC3G (A3G) a single-strand DNA deoxycytidine deaminase [65, 66], leading to its polyubiquitination and subsequent degradation by the 26S proteasome [67-69]. There are two important Vif domains, i.e, the central hydrophilic domain with amino acids 88 to 93 and the proline-rich domain with amino acids 161 to 164. These domains are essential to maintain the level of Vif function and to interact with tyrosine kinase [70, 71]. As a result of these activities, the concentration of A3G is effectively reduced from the infected cells leading to full infectivity of viral progeny. Therefore, when viruses lack this Vif functional gene they do not replicate in $CD4^+$ T-cells and macrophage. When cells are infected by viruses without Vif, A3G is effectively incorporated into budding HIV-1 virions. Incorporation of A3G molecules into HIV-1 virions inhibits its replication during the next round of infection therefore Vif-A3G axis can be regarded as a new drug target for designing small-molecule selective inhibitors of HIV-1 Vif to prevent Vif-mediated ubiquitination of A3G and its degradation by proteasome. However, there are certain potential drawbacks also to this approach. In the complete absence of Vif expression, APOBEC3 is incorporated into HIV-1 virions and the viral genome undergoes hypermutation (extensive G-to-A mutation), rendering it non-viable in a single replicative cycle. Although a highly effective Vif inhibitor may result in mutational meltdown of the viral quasispecies, a partially effective Vif inhibitor may accelerate the evolution of drug resistance and immune escape due to the codon structure and recombinogenic nature of HIV-1 (72-74). Therefore, further investigation is required to facilitate the rational design of such inhibitors.

TRIM 5αrh Factor

TRIM5αrh is a cellular factor that recognises the incoming viral capsid core structure and blocks HIV-1 infection at a post-entry, pre-integration stage in the viral life cycle (Fig. (**2**)) and was first identified in a Rhesus monkey [75]. It is a member of TRIpartite Motif (TRIM) family of

proteins and is comprised of a RING, a B-box, coiled-coil motifs (RBCC) and a B30.2 (PRYSPRY) domain which determine the potency and specificity for post-entry restriction and works against the late phase of HIV-1 replication for AIDS gene therapy [76]. For example, transgenic macrophages with high resistance to HIV-1 infection have been generated by introducing TRIM5α into hematopoietic stem cells proving this therapy an important application to treat HIV-1 and AIDS [77]. TRIM5α mediated antiviral therapy can be utilised for cost-effective HIV-1 therapy by synthesis of efficient peptidomimics, which can mimic TRIM5αrh protein. For example, It is reported that the removal of arginine 332 allows human TRIM5α to bind human immunodeficiency virus capsids and to restrict the infection [78, 79]. Mutation or change in the conformation of TRIM5αrh, which can mimic original protein or by increasing the *in vivo* expression of endogenous TRIM5αrh by interferon-like small molecules could also be alternative approaches [80, 81] but unfortunately interferon technique is not so active and could lead to toxicity [82, 83]. Since studies have shown that TRIM family proteins have anti-HIV-1 activity therefore designed TRIM family protein analogs may be used for future HIV-1 therapy.

Vpu Protein

Vpu is a small transmembrane protein of HIV-1 with a short amino terminal domain, transmembrane domain and cytoplasmic domain, which is translated from the same mRNA that encodes the envelope glycoprotein of HIV-1 [84]. It contains two kinase sites in the hinge region, which functions for the degradation of CD4 [85]. It interacts with the CD4 receptor in the rough endoplasmic reticulum (RER) and shunts it to proteasome for degradation [86]. The Vpu protein has a property to form an ion channel, "viroporins" and thus it also function to release virions from the cells through the transmembrane (TM) domain and ion channel. M2 protein of influenza is a viroporin, which has a similar structure to Vpu, therefore, the TM domain of Vpu could possibly be used as a target for novel antiviral drugs [87].

INHIBITORS

Entry Inhibitors

Infection of target cells by HIV is a complex, multi-stage process involving attachment to host cells and CD4 binding, coreceptor binding, and membrane fusion. Drugs that block HIV entry are collectively known as entry inhibitors, but comprise a complex group of drugs with multiple mechanisms of action depending on the stage of the entry process at which they act. All of these stages are mediated by the viral envelope (Env) proteins, gp120 and gp41, which are the only viral proteins that project from the membrane of the virion. Generally, the group of entry inhibitors can be subdivided into classes of agents that act at different stages of entry: attachment and CD4 binding, coreceptor binding, and fusion. Currently, only antagonists that block CCR5 binding (maraviroc) and fusion (enfuvirtide) have been approved by the FDA for treatment of HIV-infected patients,

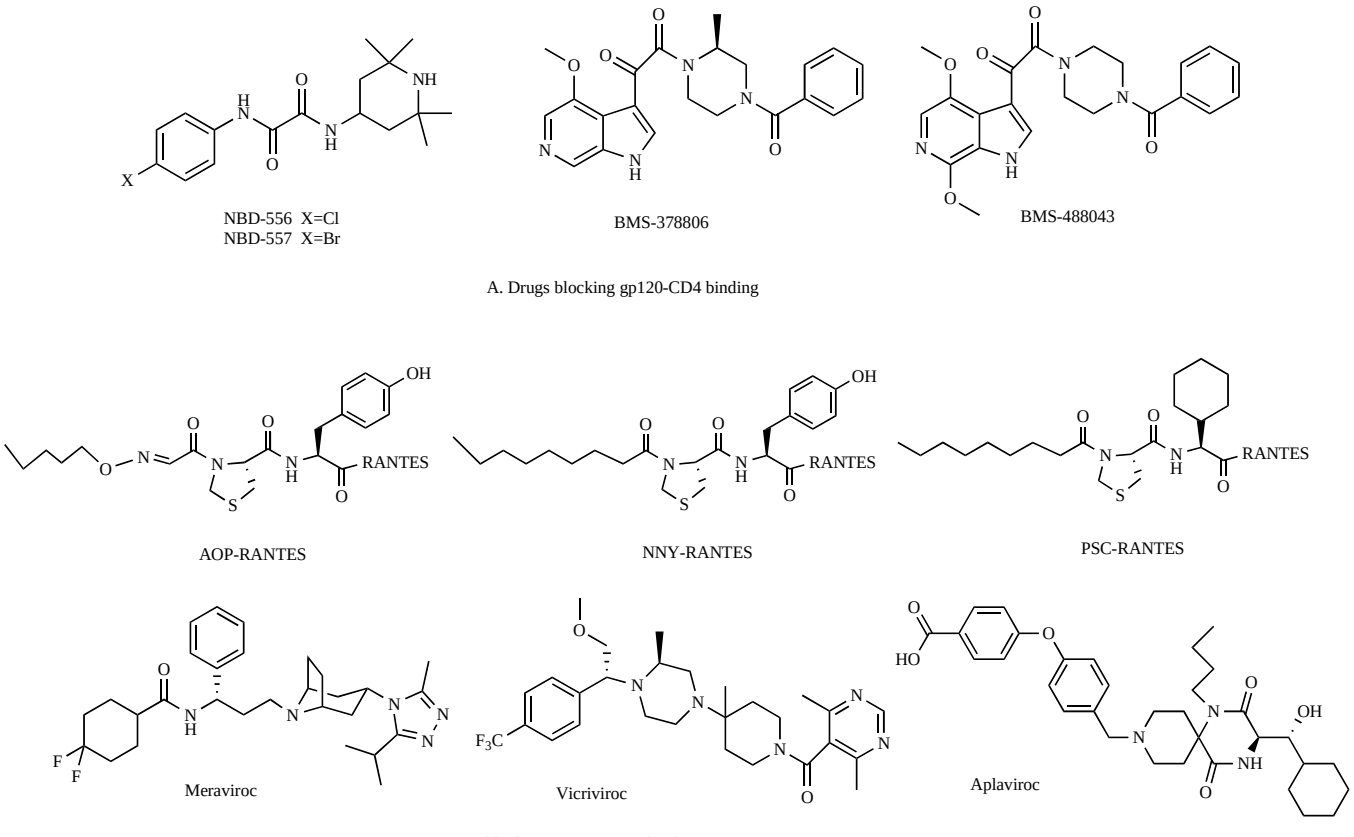


Fig. (5). Entry Inhibitors.

although strategies to inhibit other aspects of HIV entry are under development.

Drugs Blocking the gp120–CD4 Interaction

Small-molecule inhibitors of the CD4-gp120 binding interaction are NBD-556, NBD-557, BMS-378806 and BMS-488043 compounds (Fig. (**5**)). The precise mechanism of action of these molecules is unclear yet but some studies have suggested that these compounds compete with CD4 for binding to gp120 while others indicate that they prevent conformational changes in gp120 upon CD4 engagement. BMS-806 has been discontinued in phase II clinical trial since amino acids, Trp 112, Thr 257, Ser 375, Phe 382, Met 426, and 'phenylalanine 43 cavity' on gp120 confer resistance to BMS-806.

Drugs Blocking the gp120–Coreceptor Interaction

CCR5 and CXCR4 are the critical coreceptors for HIV entry. A number of naturally occurring ligands block HIV infection by blocking Env binding to CCR5 but they have potentially undesirable agonist activity on CCR5. Therefore, several RANTES derivatives, including AOP-RANTES, NNY-RANTES, and PSC-RANTES, (Fig. (**5**)) have been developed in an effort to maintain anti-HIV activity while reducing or eliminating the agonistic effects on CCR5. These agents compete with gp120 for binding to coreceptor and therefore are competitive antagonists of HIV infection. Another strategy developed was to design small molecules that can potentially bind to a hydrophobic pocket in the transmembrane helices of CCR5 and alter the conformation of the extracellular loops of HIV during coreceptor binding. The designed molecules aplaviroc (GW873140) and ancriviroc (SCH-C) (Fig. (**5**)) went up to clinical trials but were discontinued due to idiosyncratic hepatotoxicity & elongated QT cardiac interval respectively. Vicriviroc (SCH-417690), a second-generation molecule of ancriviroc (SCH-C), is currently in phase III clinical trials. Maraviroc (UK-427857) got FDA approval in 2007 for the treatment of HIV-infected patients and is effective against CCR5-using (R5-tropic) HIV strains in the low nanomolar range. It is administered twice daily by oral route but dose depends on the presence or absence of CYP3A inducers or inhibitors in the antiviral regimen [88].

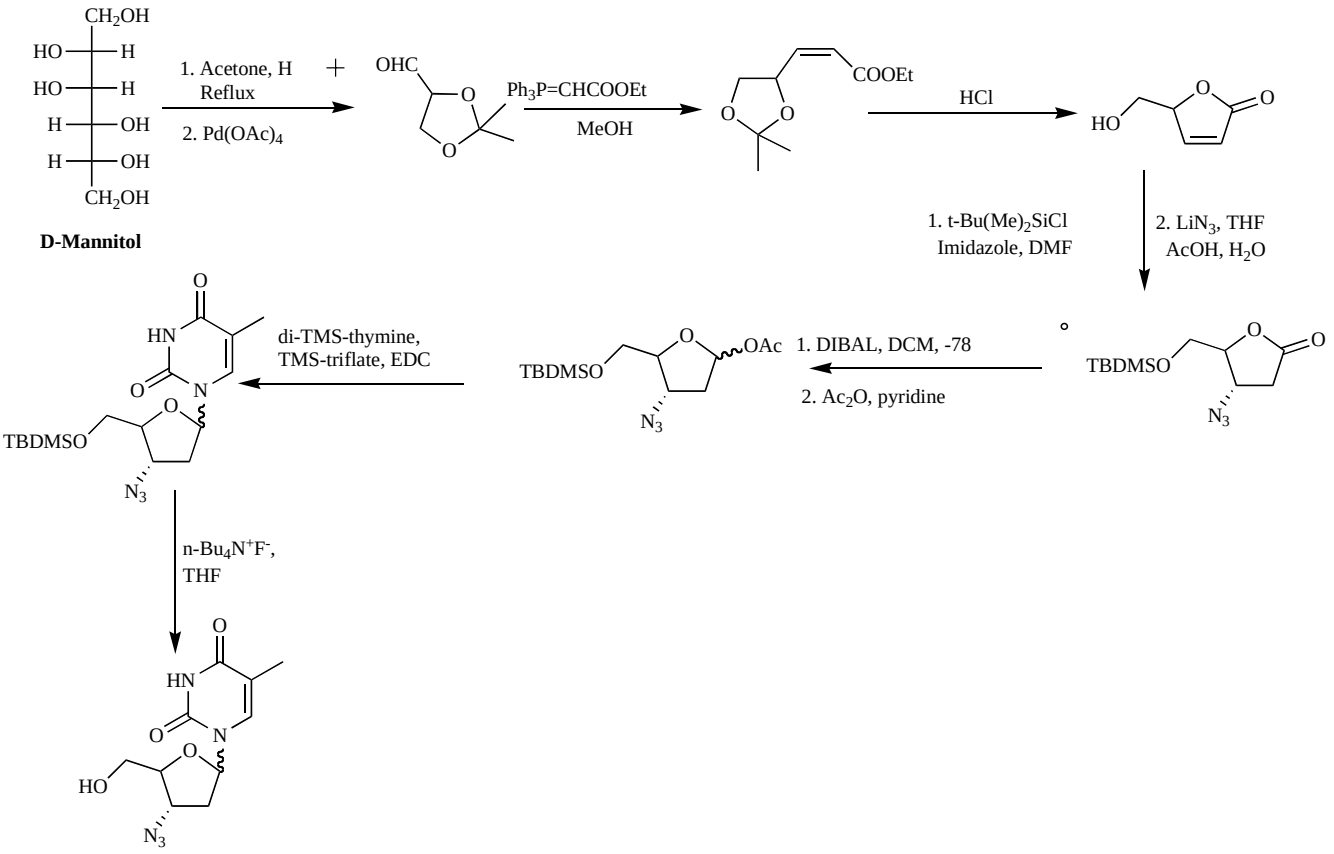
Fusion Inhibitors

Pharmacological agents that disrupt gp41-mediated membrane fusion, collectively called fusion inhibitors, were the first entry inhibitors to be approved for the treatment of HIV infection. Synthetic peptides corresponding to the HR1 and HR2 domains of gp41 were found to have potent antiviral effects. The fusion inhibitor enfuvirtide (T-20) was approved by the FDA for the treatment of HIV-infected patients in 2003. Enfuvirtide is a linear, 36 amino acid synthetic peptide with a sequence identical to part of the HR2 region of gp41 and competes for binding to HR1. A number of other next-generation peptidic fusion inhibitors are under investigation, several of which have improved pharmacodynamics and efficacy compared with enfuvirtide. Additionally, certain agents are active against some enfuvirtide-resistant strains of HIV, and fusion inhibitors that bind to different functional domains of gp41 can have synergistic effects. However, since peptidic fusion inhibitors are not orally bioavailable and must be administered *via* injection, the development of small-molecule inhibitors of gp41 mediated fusion remains a goal in drug development [89].

Nucleoside Reverse Transcriptase Inhibitors

The first ever reported anti-retroviral drug candidate zidovudine (AZT, Retrovir) is a thymidine analog, which was first synthesised in 1964 [90] but the synthetic procedure for zidovudine was further improved so as to use the D-mannitol as the starting material in place of the expensive thymidine (Scheme **1**) [91]. Zidovudine was successfully evaluated as an antiviral agent in 1980 but its activity as a potential inhibitor of a viral enzyme, RNA dependent DNA polymerase (reverse transcriptase), was established in 1985 [92]. Zidovudine first gets phosphorylated into 5'-monophosphate and finally to the 5'-triphosphate by the cellular kinase enzymes to form zidovudine-5'-triphosphate (AZT-TP) which gets incorporated on to the growing complementary DNA chain of the virion. This incorporation blocks the DNA polymerisation reaction and disrupts the proviral DNA synthesis leading to the termination of the viral life cycle (Fig. (**6**)) [93, 94]. The loss in the activity is generally corresponded to the number of mutations taken place in the active site. Fig. (**7**) shows the chimera view of the active site of RT of a crystal structure (PDB id 3KLE) interacting with zidovudine terminating the DNA synthesis. The active site shows the interaction of AZT with the complementary base on the DNA strand. It also inhibits cellular α-DNA polymerase but at a hundred fold higher concentration as compared to the concentration required to inhibit reverse transcriptase. Abacavir (ABC), Didanonise (ddI), Emtricitabine (FTC), Stavudine (d4T), Lamivudine (3TC), Tenofovir disoproxil fumarate (TDF), Zalcitabine (ddC) are other drugs in this class (Table **2**) which work on the similar mechanism to inhibit the viral replication. Out of these drugs, three anti-HIV drugs, i.e. lamivudine, zalcitabine, and emtricitabine, are based on cytidine derivatives. The synthesis of lamivudine (Scheme **2**) was first reported in 1992 in a racemic form where in levorotatory (-) enantiomer was found to be less toxic as compared to the dextrorotatory (+) enantiomer [95]. The 5-fluoro derivative of lamivudine (emtricitabine) was synthesised in 1992 [96]. Further, Didanosine, Abacavir and Tenofovir were reported as guanosine-based inhibitors with significant anti-HIV activity [97].

Although these drugs were well evaluated with respect to their pharmacokinetics (ADMET) but due to the emergence of viral resistance and toxicity in monotherapy of HIV, combination chemotherapy was evaluated using zidovudine and zalcitabin which proved to be well tolerated and showed significant potential to reduce the viral load after alternative 7-days regimen. NRTIs were invariable tested for combination therapy and the results showed that the combination of lamivudine with zidovudine was found to be significantly efficacious as compared to the monotherapy of zidovudine [98].



Scheme 1. Synthesis of Zidovudine.

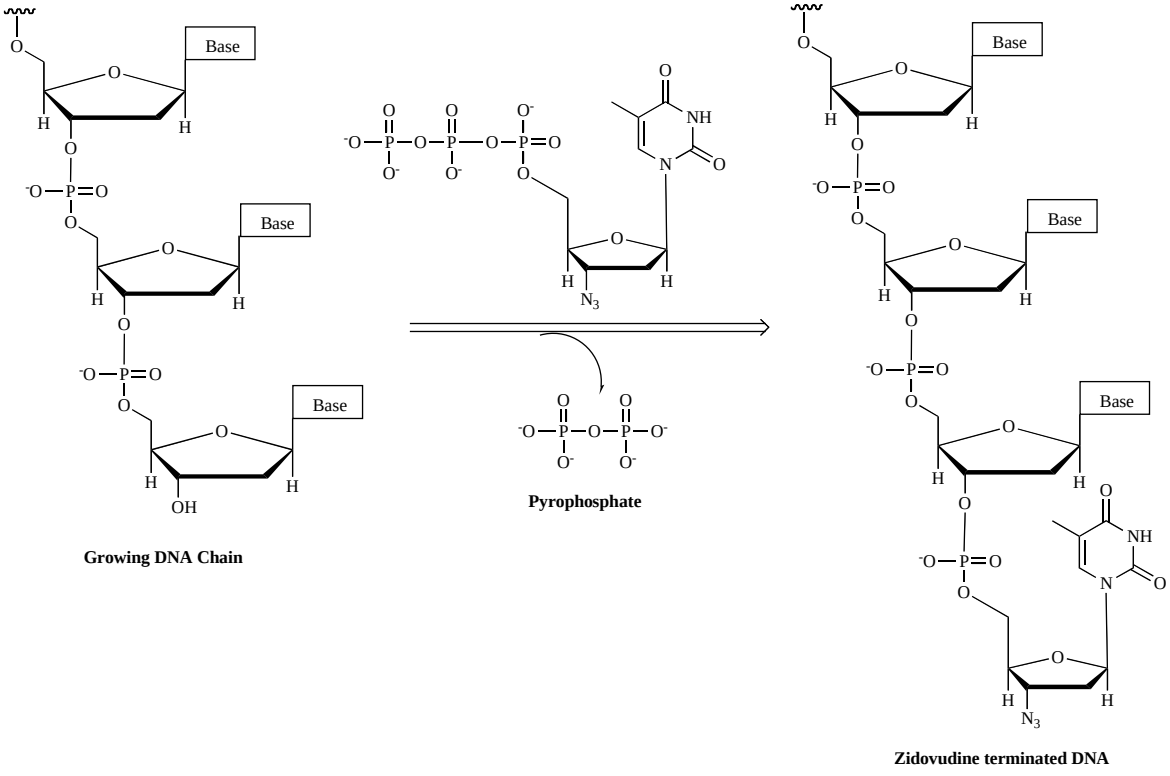


Fig. (6). Mode of action of Zidovudine.

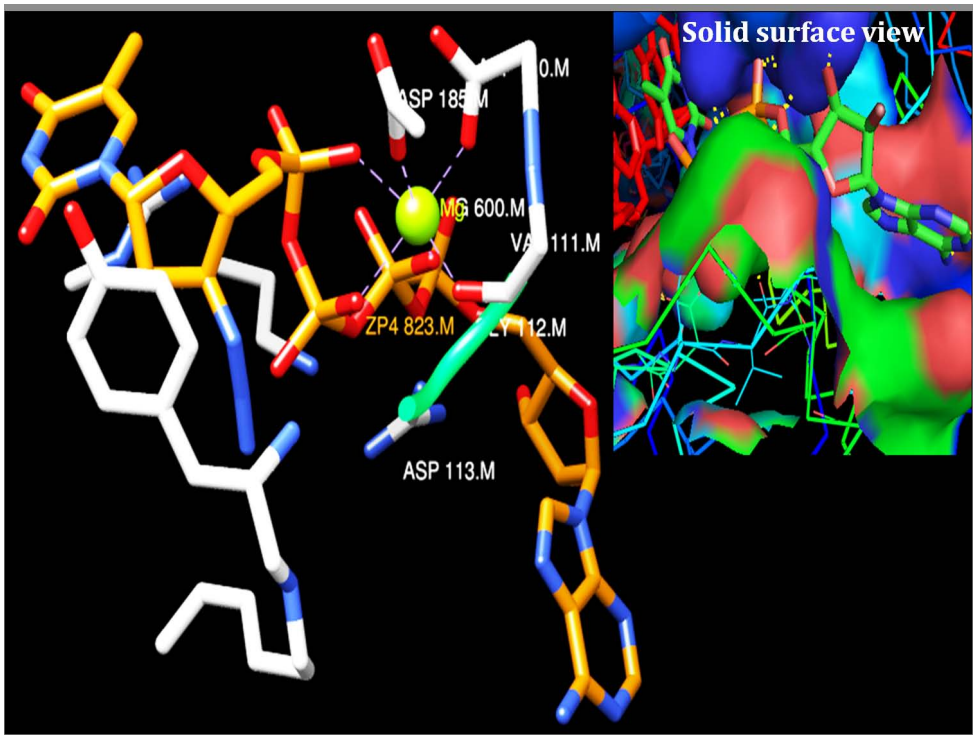
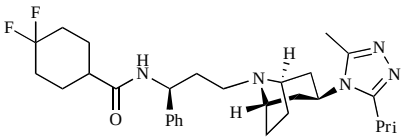
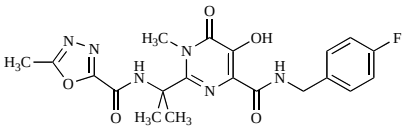
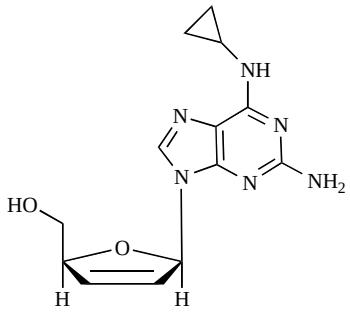
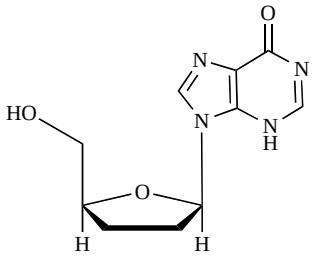
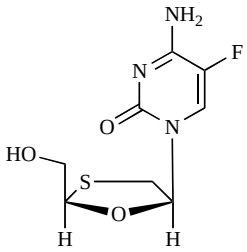
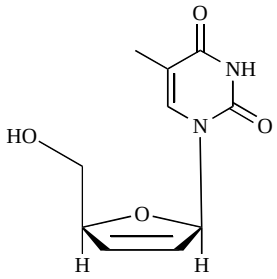
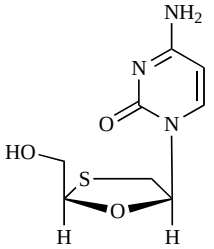
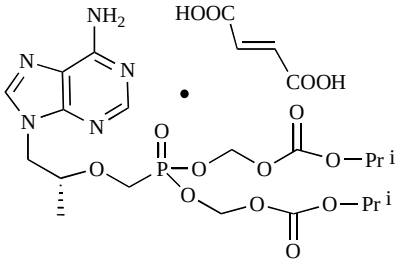
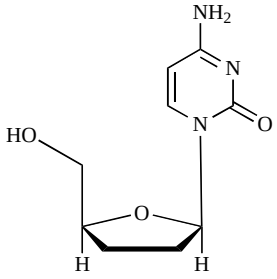


Fig. (7). Chimera view of the interaction of AZT with the complementary base of the DNA strand (orange colour) and active site magnesium atom (PDB Id 3KLE) of HIV-1 reverse transcriptase. The active site magnesium atom is shown as the large green sphere. Pymol generated solid surface view of AZT interaction in HIV-1 reverse transcriptase is shown in green colour smaller picture.

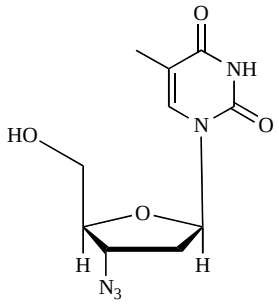
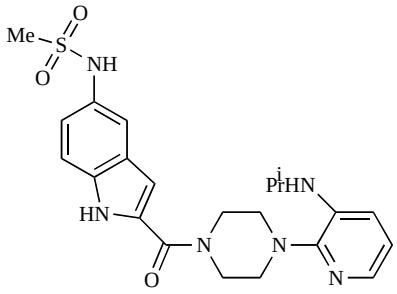
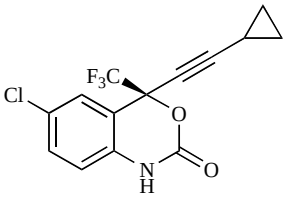
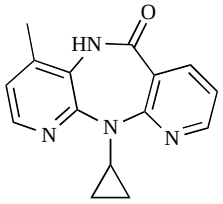
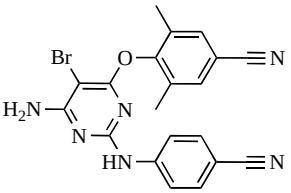
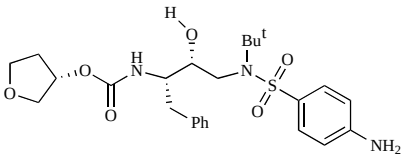
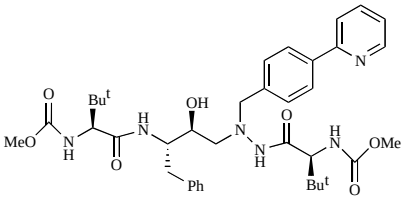
Table 2. Drugs for the Treatment of HIV Infection [115]

Brand Name/ Manufacturer	Generic Name (Drug)	Structure	Class	Dosing regimen mg/ Frequency
Selzentry/ Pfizer	Maraviroc (MVC)		CCR5/ Entry Inhibitor	-
Fuzeon/ HoffmanLaRoche and Trimeris	Enfuvirtide (T-20)	CH_3CO—Tyr—Thr—Ser—Leu—Ile—His—Ser—Leu—Ile—Glu—Glu—Ser—Gln-Asn-Gln-Gln-Glu—Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys—Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2	Fusion Inhibitor	-
Isentress/ Merck	Raltegravir (RTV)		Integrase	-
Ziagen/ GlaxoSmithKline	Abacavir (ABC)		NRTI	600/qd 300/bid

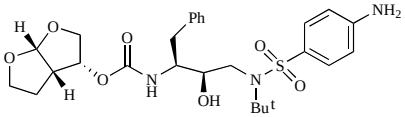
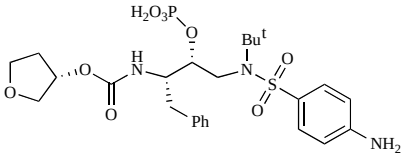
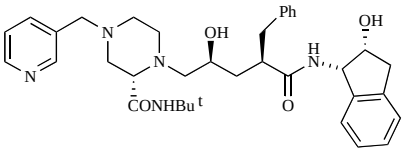
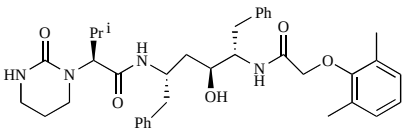
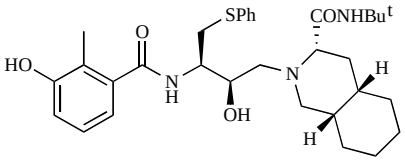
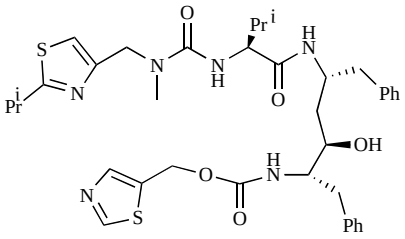
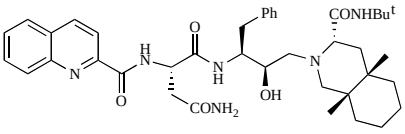
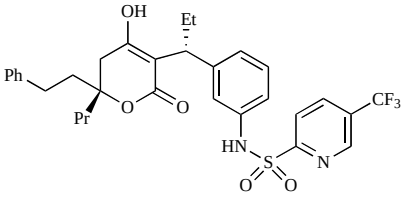
(Table 2). Contd…..

Brand Name/ Manufacturer	Generic Name (Drug)	Structure	Class	Dosing regimen mg/ Frequency
Videx/ Bristol-Myers Squibb	Didanonise (ddI)		NRTI	250-400/qd 125-200/bid
Emtriva/ Gilead Sciences	Emtricitabine (FTC)		NRTI	200/qd
Zerit/ Bristol-Myers Squibb	Stavudine (d4T)		NRTI	30-40/bid
Epivir/ GlaxoSmithKline	Lamivudine (3TC)		NRTI	300/qd 150/bid
Viread/ Gilead Sciences	Tenofovir disoproxil fumarate (TDF)		NRTI	300/qd
Hivid/ Hoffmann-LaRoche	Zalcitabine (ddC)		NRTI	0.75/tid

(Table 2). Contd…..

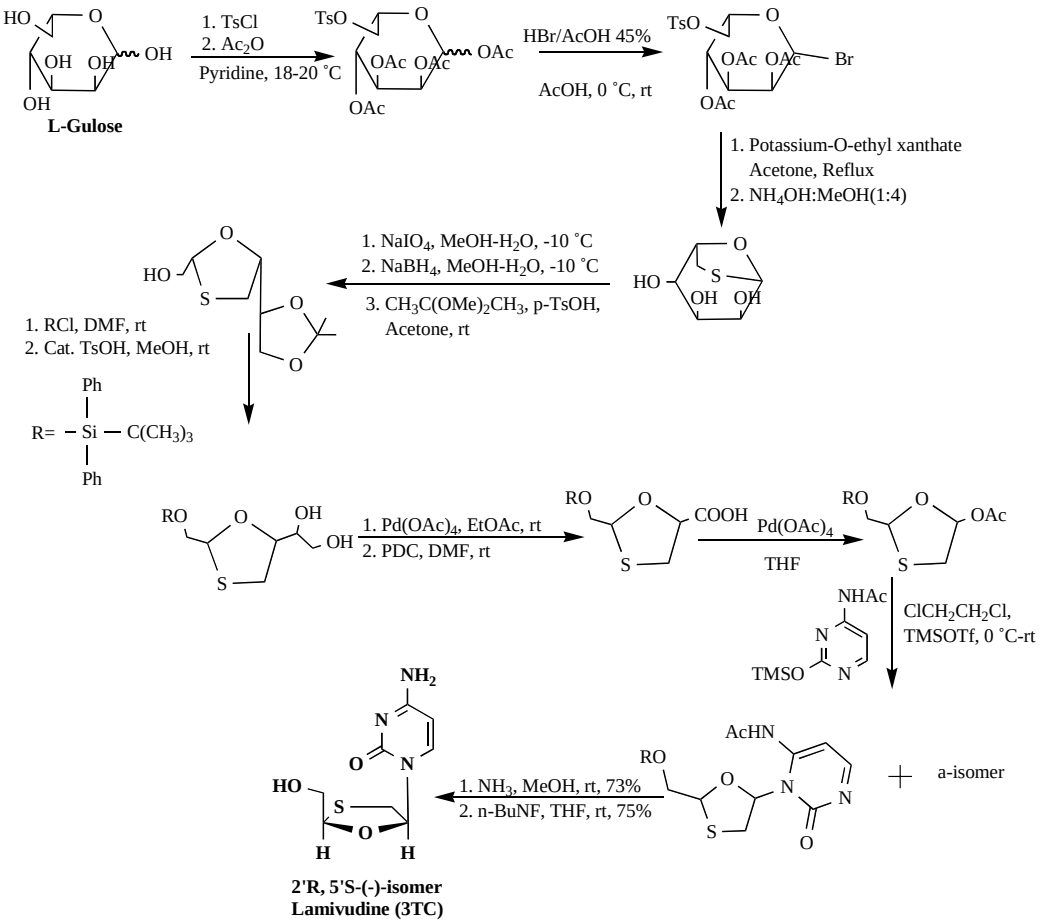
Brand Name/ Manufacturer	Generic Name (Drug)	Structure	Class	Dosing regimen mg/ Frequency
Retrovir/ GlaxoSmithKline	Zidovudine (AZT)		NRTI	300/bid 200/tid
Rescriptor/ Pfizer	Delavirdine (DLV)		NNRTI	400/tid
Sustiva/ Bristol-Myers Squibb	Efavirenz (EVF)		NNRTI	600/qd
Viramune/ Boehringer Ingelheim	Nevirapine (NVP)		NNRTI	200/qd 14days 200/bid after
Intelence/ Tibotec Therapeutics	Etravirine (ETV)		NNRTI	-
Agenerase/ GlaxoSmithKline	Amprenavir (APV)		PI	1400/bid
Reyataz/ Bristol-Myers Squibb	Atazanavir (ATV)		PI	400/qd

(Table 2). Contd…..

Brand Name/ Manufacturer	Generic Name (Drug)	Structure	Class	Dosing regimen mg/ Frequency
Prezista/ Tibotec, Inc.	Darunavir (TMC-114)		PI	-
Lexiva/ GlaxoSmithKline	Fosamprenavir (APV)		PI	1400/bid
Crixivan/ Merck	Indinavir (IDV)		PI	800/tid
-	Lopinavir (LPV)		PI	-
Viracept/ Agouron	Nelfinavir (NFV)		PI	1250/bid 750/tid
Norvir/ Abbott Laboratories	Ritonavir (RTV)		PI	600/bid
Invirase/ Hoffmann-LaRoche	Saquinavir (SQV)		PI	1200/tid
Aptivus/ Boehringer Ingelheim	Tipranavir (TPV)		PI	-

Nonnucleoside Reverse Transcriptase Inhibitors

This class of inhibitor was first discovered in 1990 with the identification of the first tricyclic benzodiazepine molecule R14458 (Fig. (**8a**)) from anti-HIV screening of 600 compounds otherwise inactive against various other biochemical targets. Then a series of similar compounds with improved anti-HIV activity were synthesized on the basis of structure activity relationship (SAR) studies leading to a molecule R82913 (Fig. (**8a**)) with more potent activity [99]. Further, another group reported a series of diaryldiazepinones out of which, BI-RG-587 was identified as a potent NNRT inhibitor in cell culture and after many preclinical and clinical trials it managed to get the first ever NNRTI regulatory approval with a generic name nevirapine



Scheme 2. Synthesis of Lamivudine (3TC).

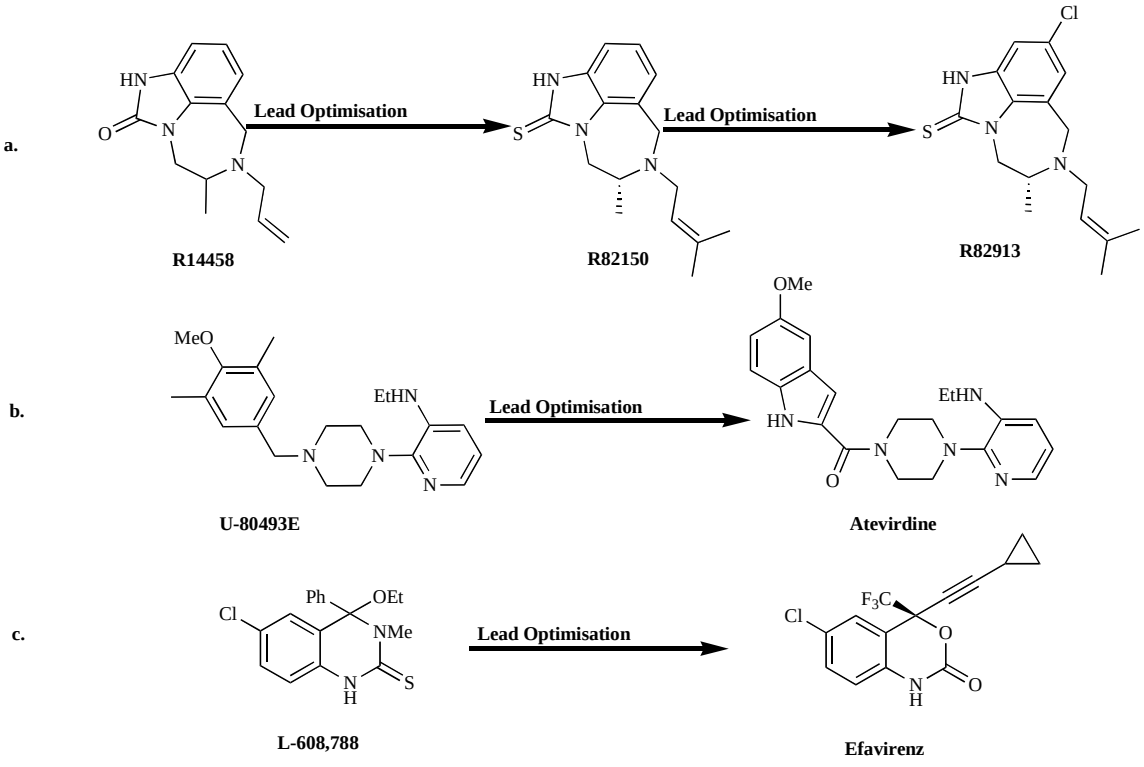
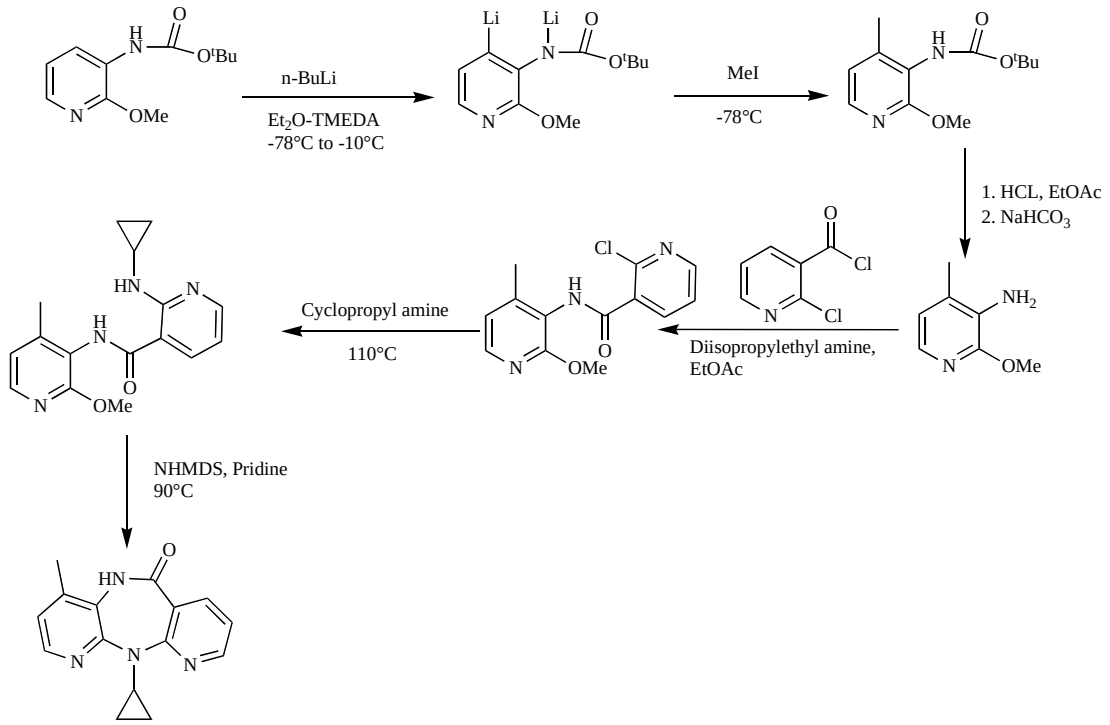
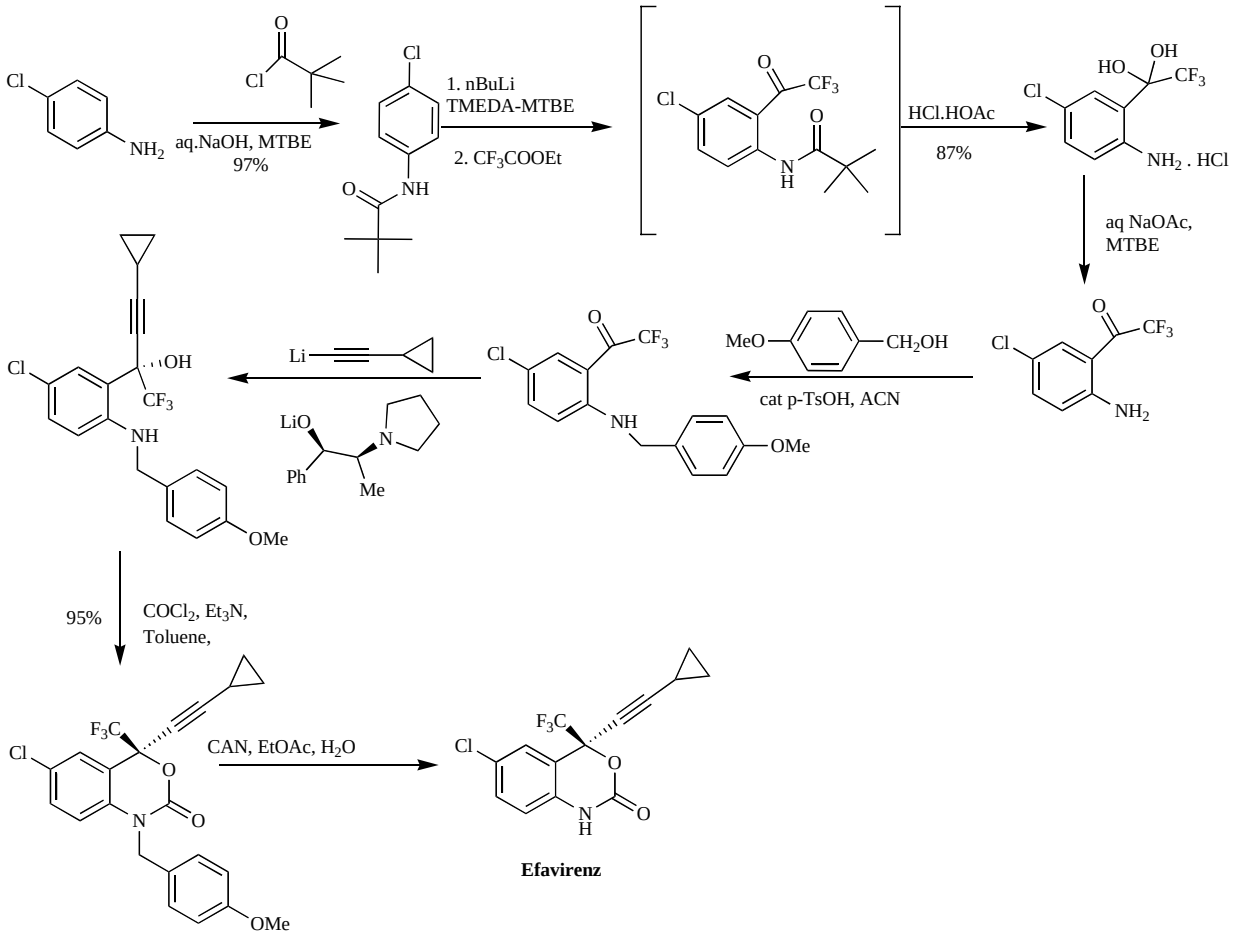


Fig. (8). Lead optimization of NNRTIs.



Scheme 3. Synthesis of Nevirapine.



Scheme 4. Synthesis of Efavirenz.

[100]. The synthetic route using 4-methyl-3-[(tert-butoxycarbonyl)amino]-2-methoxypyridin as an intermediate compound can be utilised for the synthesis of nevirapine (Scheme **3**) [101] and efavirenz was reported to be enantioselectively synthesized by 4-chloroaniline as the starting material (Scheme **4**) [102].

Nevirapine binds directly to reverse transcriptase and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing the disruption of the catalytic site. Fig. (**9**) shows the pymol view of the x-ray crystal structure of Nevirapine bound to HIV-1 RT. It does not compete with nucleoside triphosphate. In cell culture, nevirapine demonstrated the synergistic effect against HIV in combination with NRTIs like AZT, ddI, d4T, 3TC and protease inhibitor saquinavir.

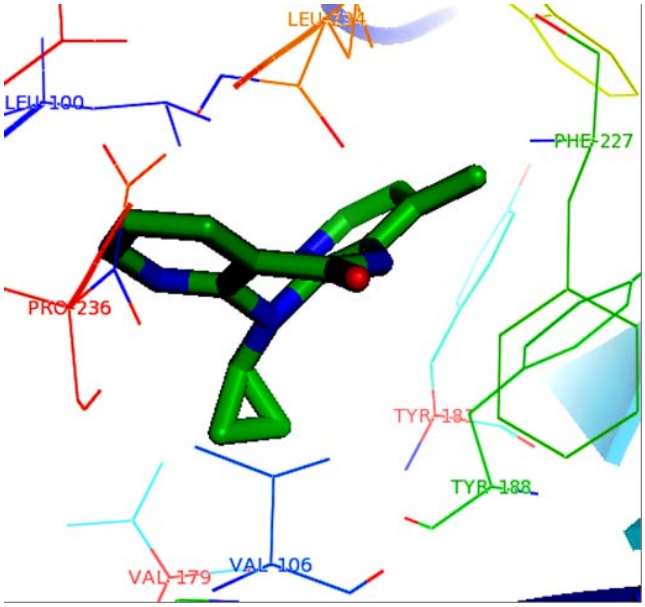


Fig. (9). Pymol view of the hydrophobic interactions at the active site of the crystal structure (PDB id 1S1X) of V108I mutant HIV-1 reverse transcriptase in complex with Nevirapine.

Similarly, a large number of compounds were screened in a cell culture method for their anti-HIV-1RT activity from which aryl piperazine derivative; U-80493E (Fig. (**8b**)) was selected as a lead molecule which was optimised to give a clinical candidate atevirdine. Unfortunately, atevirdine (Fig. (**8b**)) was not very active even at a dose of 600mg *tid* so further optimization was done on the indole and pyridine portion of the molecule to finally give delavirdine (Table **2**) [103, 104]. Likewise, L-608,788 (Fig. (**8c**)) became the lead molecule in another class of dihydroquinazolinethione derivatives and optimisation on this molecule led to a very potent once daily dosing molecule Efavirenz [105]. Fig. **10** shows the pymol view of the x-ray crystal structure of efavirenz bound to HIV-1 RT.

Integrase Inhibitor

October 2007, FDA approved the first drug Raltegravir (MK-0518) in the integrase-inhibitor class for the effective treatment of HIV as a part of combination antiretroviral therapy. Raltegravir is a structural analogue of di-keto acid class of compounds and constitutes a β-hydroxy-ketone structural motif. This structural motif possesses metal-chelating functions which interact with divalent metals within the active site of HIV-1 integrase. The insertion of HIV-1 viral genomic DNA into the host chromosome is a process often referred to as strand transfer. Raltegravir and its related molecules inhibit this latter step, and as a result are often referred to as "strand-transfer inhibitors." Raltegravir has been shown to have a 50% inhibitory concentration (IC50) of approximately 10 nM [106]. Further, various quinolone acid derivatives have been developed as an integrase inhibitors [107].

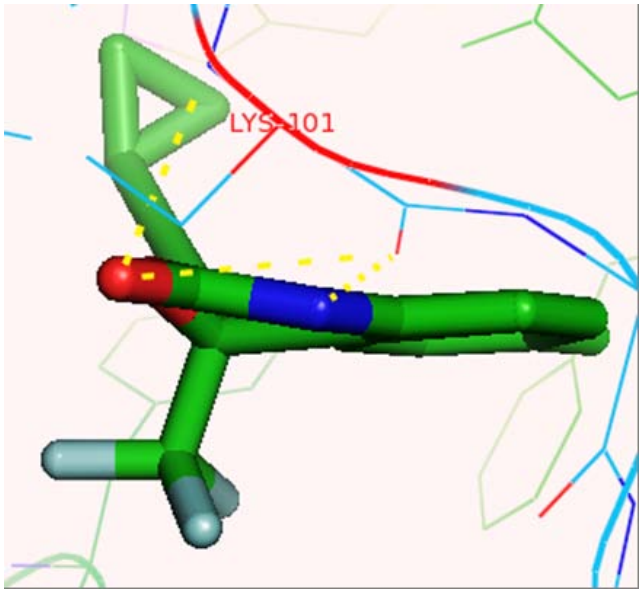


Fig. (10). Pymol view of the active site of crystal structure (PDB id 1IKW) wild type HIV-1 reverse transcriptase in complex with Efavirenz at Lys101.

Protease Inhibitors

Protease is one of the most important targets of HIV-1, which have been identified and utilised for the design and development of anti-HIV drugs. Various molecules have been co-crystallised with the enzyme protease and submitted to protein data bank. Fig. **11** shows the pymol view of the co-crystal structure and drug-amino acid interactions of indinavir at the active site of HIV protease enzyme. Mutagenic efficiency of HIV is very high which results in the generation of new highly resistant viral strains of HIV with multiple mutations in their proteases. Therefore, novel inhibitors with new mode of action at the enzyme are required. Recently, Shityakov *et al.* developed a library of diverse chemical compounds on the bases of Indinavir and screened them for plausible anti-protease activity. One unique compound with different protease binding ability was reported for which further studies may provide useful information towards the rational design of HIV-1 protease inhibitors [108].

New Therapeutics (Carbohydrate-Binding Proteins)

Carbohydrate-binding proteins (CBP) show specific recognition for mannose (Man) and *N*-acetylglucosamine

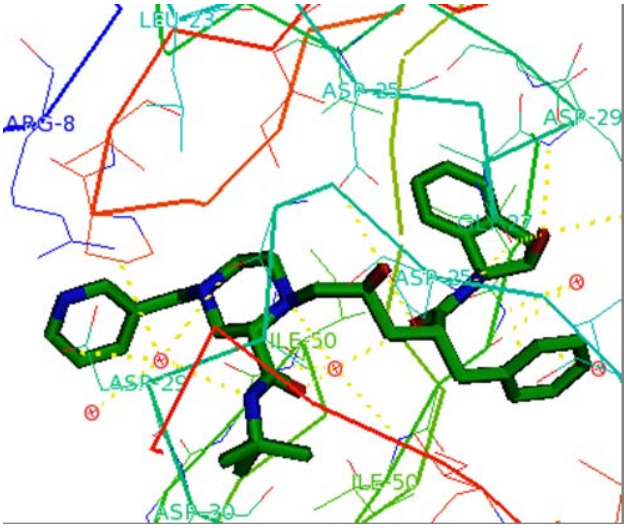


Fig. (11). Pymol view at the active site of the crystal structure (PDB id 2BPX) of Indinavir bound to HIV-1 protease at ARG8, LEU23, ASP25, ASP30, GLY27, bound water molecules (red spheres with cross) ASP29, ILE50.

(GlcNAc) and are endowed with a remarkable anti-HIV activity in cell culture. CBP occur as monomer, dimer, trimer or tetramer peptides with a molecular weight of approx. 8.5 kDa upto 50 kDa. CBP may contain 2 to 12 carbohydrate-binding sites per molecule, depending on the nature and its oligomerization state. CBP shows potential anti-HIV activity because they inhibit viral infection in the lower nano- or even subnanomolar range. The CBP's also prevent virus transmission from virus-infected cells to uninfected T-lymphocytes. They act by inhibiting the virus entry (i.e. fusion) into its target cell by direct binding to the glycans that are abundantly present on the HIV-1 gp120 envelope. They cross-link several glycans during virus/cell interaction and/or freeze the conformation of gp120 consequently preventing further interaction with the coreceptor. CBP's have a unique mechanism of antiviral action, drug resistance profile and an intrinsic capacity to trigger a specific immune response against HIV strains after glycan deletions. CBP from various sources have been reported [109]. Griffithsin (GRFT) is one such CBP with a novel anti-HIV protein consisting of 121-amino acid sequence isolated from an aqueous extract of the red alga *Griffithsia* sp. The pymol view of the crystal structure of griffithsin (Fig. (**12**)) shows that it is a dimer with a unique structure. GRFT forms a complex with mannose at three almost identical carbohydrate binding sites on each monomer displaying a potent antiviral activity against HIV-1 with EC50 values ranging from 0.043 to 0.63 nM. At high concentration of 783 nM, GRFT was not lethal to the host cells. GRFT blocks gp120 binding to receptor-expressing cells and itself gets bind to the viral coat glycoproteins gp120, gp41, and gp160 in a glycosylation-dependent manner. Antiviral potency of griffithsin is likely due to the presence of multiple, similar sugar binding sites that provide redundant attachment points for complex carbohydrate molecules present on viral envelopes [110].

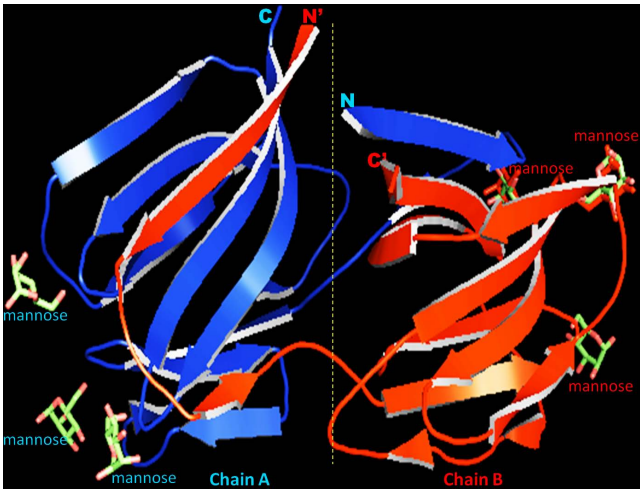


Fig. (12). Pymol based representation of the two fold symmetry of crystal structure (PDB id 2GUD) of Griffithsin shown as a yellow broken line. Chain A is represented in blue and Chain B in red. Each monomer is bound to three molecules of mannose.

FUTURE STRATEGIES & CONCLUSION

The following section concludes some of the challenges to be encountered during the design and development of new classes of antiretroviral inhibitors acting on some of the discussed targets. Recent, Shityakov and group has opened a new avenue in the HIV research claiming that protein 'transportin-SR2' is the actual nuclear import protein for HIV-1 integrase rather than transportin-SR1 [111]. Since very limited information is available about the novel targets therefore further improvement in the existing inhibitors against established targets using QSAR techniques is comparatively less challenging than the de novo design of antiretroviral drugs against novel therapeutic targets (112). Although various drug design and discovery technologies have been introduced over the past few years, the result remains unpredictable in finding effective inhibitors for these novel targets. Nevertheless new therapeutic approaches like carbohydrate binding proteins from various sources including marine has shown the promising results. Therefore the focus of the antiretroviral research should be on such therapeutics. Further, these proteins should be taken to the clinical trials and should also be tried in combinations with other antiretroviral drugs as a multi drug therapy for the treatment of HIV/AIDS. The goal of this review was to provide the essential steps in the HIV replication cycle, to cover all potential HIV targets and their available inhibitors in view of a medicinal chemist. After an exhaustive exploration of therapeutic targets and inhibitors against HIV we need to further expand the existing weapon store of antiretroviral drugs by focusing on novel targets, novel therapeutics from various sources including marine and interdisciplinary collaborative effort.

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