ANTI-HIV AGENTS: A STEP TOWARDS FUTURE

Devendra Singh Thakur*, Chinmay Kumar Behera, Ajay Patidar, Pramod Kumar, Peeyush Kumar, Champa Lal
SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.) 495009.
***Email:** justmaildevendra@gmail.com

ABSTRACT

Novel targets for the management of HIV infection have become increasingly relevant in view of extensive drug resistance, side effects and high pill burden of some of the conventional anti-retroviral agents**.** These agents include chemokine receptor antagonists and the integrase inhibitors which were recently approved for HIV treatment, as well as numerous other agents directed to previously untested targets such as the maturation inhibitors, pharmacological CDK inhibitors, TateTAR interaction inhibitors, anti-CD4 monoclonal antibody, antisense oligonucleotides, Use of new agents with novel mechanism of action requires the development of new laboratory assays to detect viral tropism and new resistance mutations. This review discusses issues surrounding the development of these new agents as well as various traditional approaches for the treatment of AIDS.

Keywords: AIDS, Natural anti HIV agent, CCR5, Integrase inhibitors

INTRODUCTION

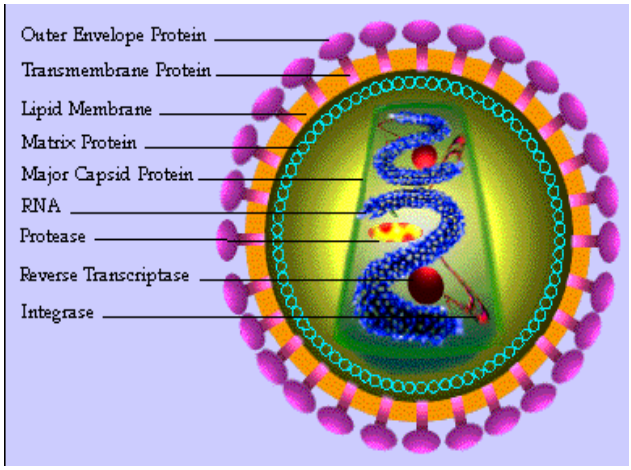
ACQUIRED IMMUNO DEFICIENCY SYNDROME (AIDS)

Acquired immunodeficiency syndrome (AIDS) is a collection of symptoms and infections resulting from the specific damage to the immune system caused by infection with the human immunodeficiency virus (HIV) which is a retrovirus of the lenitivirus family originally referred as HTLV-III. It results from the latter stages of advanced HIV infection in humans; thereby leaving compromised individuals prone to opportunistic infections and tumors. AIDS is the most severe manifestation of infection with HIV. HIV is a retrovirus that primarily infects vital components of the human immune system such as CD4+ T cells, macrophages and dendritic cells. It directly and indirectly destroys CD4+ T cells. CD4+ T cells are required for the proper functioning of the immune system. When HIV kills CD4+ T cells so that there are less than 200 CD4+ T cells per µl blood, cellular immunity is lost, leading to AIDS. People with AIDS often suffer infections of the lungs, intestinal tract, brain, eyes, and other organs, as well as debilitating weight loss, diarrhea, neurologic conditions, and cancers such as Kaposi's sarcoma and certain types of lymphomas.

ETIOLOGY OF AIDS

AIDS is caused by the HTLV-III (Human T-cell Leukemia Virus Type 3) and ARV (AIDS related virus) is now known as human immunodeficiency virus (HIV). There are two main types of HIV. HIV-1, which has 10 recognized subtypes (A-J), is responsible for the global epidemic and seems to have originated in western Africa in the early 1940s. The first documented case of infection with HIV occurred in 1959. HIV-2 is a less easily transmitted mutation of HIV-1 and remains primarily in Africa, although it has recently spread to India. Cases of HIV-2 infection have also been reported in the United States[1,2]. HIV uses cells of the immune system (macrophages and helper T cells) as sites for reproduction and multiple copies of the viral genetic material (RNA) are made and package into new viral particles ready for dispersal into new viral hosts. More cells of the immune system are killed or damaged with each round of infection, when millions of viral particles may be produced each day. Despite the production of antibodies and helper T cells that fight the disease, eventually the virus prevails and the infections and cancer associated with AIDS begin to appear.

STRUCTURE OF HIV (the viral envelop)



LIFE CYCLE OF HIV

STEPS IN HIV LIFE CYCLE

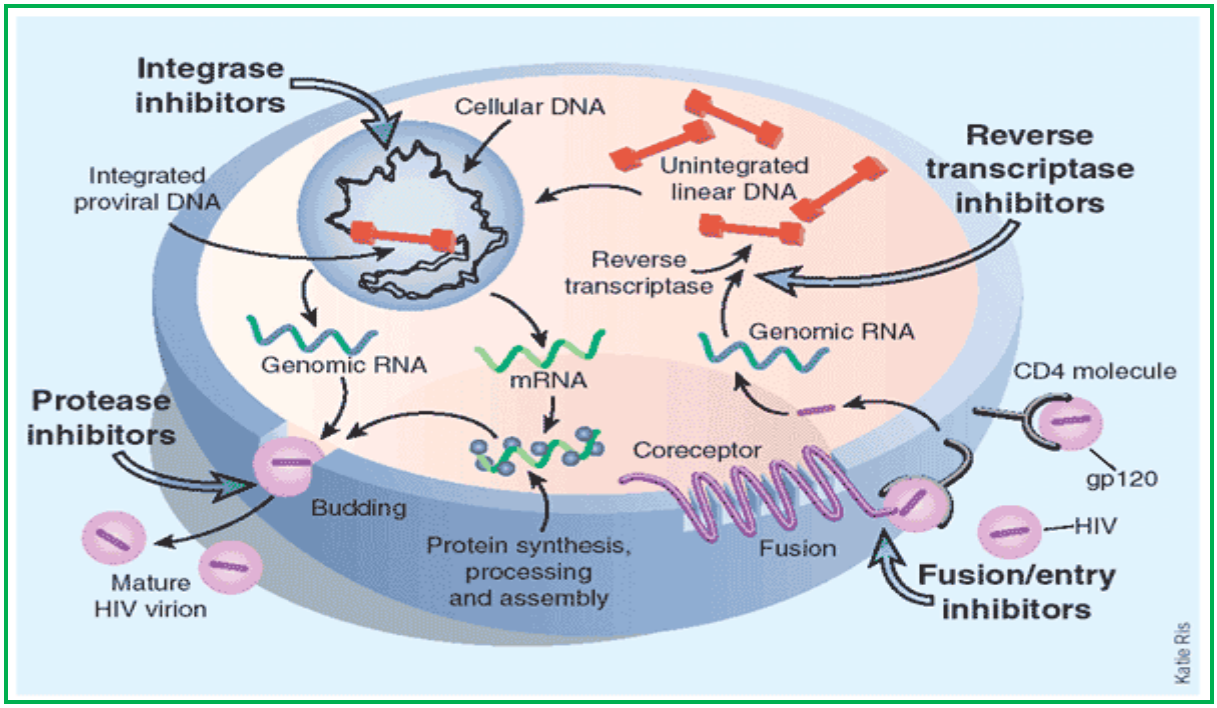
1. **Binding** - The HIV attaches to the immune cell when the gp120 protein of the HIV virus binds with the CD4 protein of the T-helper cell. The viral core enters the T-helper cell and the virion's protein membrane fuses with the cell membrane.
2. **Reverse transcription**- The viral enzyme, reverse transcriptase, copies the virus's RNA into DNA.
3. **Integration**- The newly created DNA is carried into the cell's nucleus by the enzyme, viral integrase, and it binds with cell's DNA. HIV DNA is called a provirus.

4. **Transcription** - The viral DNA in the nucleus separates and creates messenger RNA (mRNA), using the cell's own enzymes. The mRNA contains the instructions for making new viral proteins.

5. **Translation** - The mRNA is carried back out of the nucleus by the cell's enzymes. The virus then uses the cell's natural protein-making mechanisms to make long chains of viral proteins and enzymes.

6. **Assembly**- RNA and viral enzymes gather at the edge of the cell. An enzyme, called protease, cuts the polypeptides into viral proteins.

7. **Budding**- New HIV virus particles pinch out from the cell membrane and break away with a piece of the cell membrane surrounding them. This is how enveloped viruses leave the cell. In this way, the host cell is not destroyed. The newly replicated virions will infect other T-helper cells.



The Replication Cycle of HIV

CCR5 as a Target for HIV-1 Infection

Chemokine (C-C motif) receptor 5 (CCR5), is a chemokine receptor. The natural chemokines that bind to this receptor are RANTES, MIP-1α and MIP-1β. CCR5 is also the name of the gene that codes for the CCR5 receptor. It is located on chromosome 3 on the short (p) arm at position 21. CCR5 is predominantly expressed on T cells, macrophages, dendritic cells and microglia. It is likely that CCR5 plays a role in inflammatory responses to infection. Its exact role in normal immune function is unclear. CC chemokine receptors are integral membrane proteins that specifically bind and respond to cytokines of the CC chemokine family. HIV can infect a variety of cells such as CD4+ helper T-cells and macrophages that express the CD4 molecule on their surface. HIV-1 entry to macrophages and T helper cells is mediated not only through interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells but also with its chemokine coreceptors. Viruses that use only the CCR5 receptor are termed R5, those that only use CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection.

HIV uses CCR5 or another protein, CXCR4, as a co-receptor to enter its target cells. Several chemokine receptors can function as viral coreceptors, but CCR5 is likely the most physiologically important coreceptor during natural infection. The normal ligands for this receptor, RANTES, MIP-1β and MIP-1α, are able to suppress HIV-1 infection *in vitro*. In individuals infected with HIV, CCR5-using viruses are the predominant species isolated during the early stages of viral infection suggesting that these viruses may have a selective advantage during transmission of the acute phase of disease.

A number of new experimental HIV drugs, called entry inhibitors, have been designed to interfere with the interaction between CCR5 and HIV, including PRO140 (Progenics), Vicriviroc, Aplaviroc and Maraviroc A potential problem of this approach is that, while CCR5 is the major co-receptor by which HIV infects cells, it is not the only such co-receptor. It is possible that under selective pressure HIV will evolve to use another co-receptor. However, examination of molecular antagonist of CCR5, indicated that resistant viruses did not switch to another coreceptor (CXCR4) but persisted in using CCR5, either through binding to alternative domains of CCR5, or by binding to the receptor at a higher affinity. Two new proteins are present on immune cells, CCR5 and fusin (also known as CXCR4), play a key role in understanding how HIV infects cells. Though these discoveries may not have immediate impact on people with HIV, they may

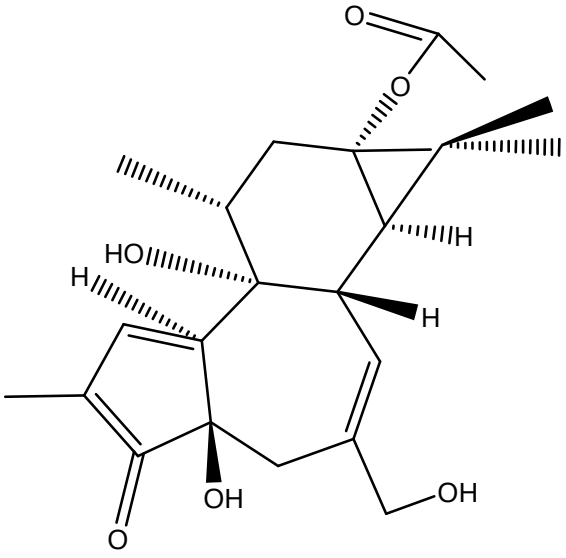
lead to important advances in HIV treatment, prevention and research in the future. One way HIV disables the immune system is by infecting and destroying CD4+ T-cells. These cells are critical in managing immune responses and when they are depleted, immune defenses are weakened. When HIV and other pathogens enter the body, CD4+ cells, operating through a network of chemical interactions, instruct other cells to disable the invading organisms. HIV actually attaches to the CD4+ protein on the surface of these and other cells to gain entry.

APPROACHES FOR TREATMENT OF AIDS

1. NATURAL ANTI HIV AGENTS

Homalanthus nutans (Euphorbiaceae) (Mamala)

Still in the search for new anti-AIDS compounds, Prostratin was isolated as the active constituent from an extract of the wood of the tree, Homalanthus nutans growing in Samoa. Prostratin is therefore, a potent activator of HIV expression in latently infected T-cell lines, and its potential value in HIV therapies more in its possible utility as a viral activator rather than as an anti-HIV agent.

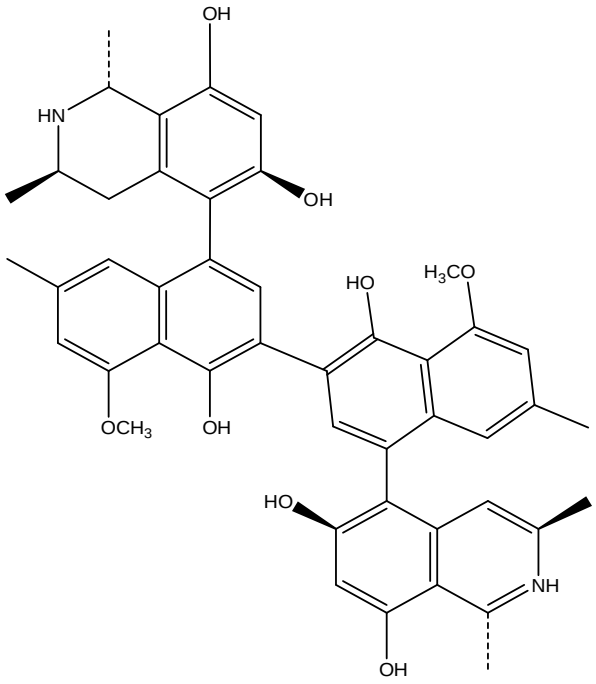


Prosratin

Catharanthus roseus (Apocynaceae) (Madagascan Periwinkle)

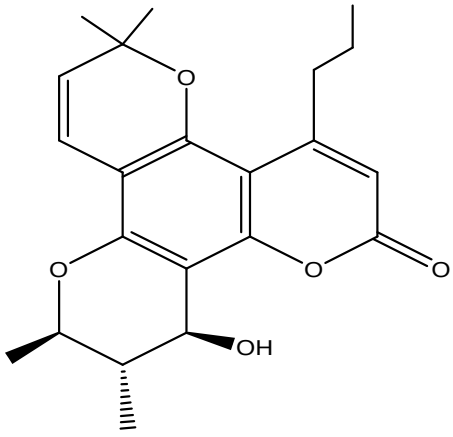
Vinorelbine, a semi-synthetic version of one anti-cancer alkaloid from the Madagascan Periwinkle (Catharanthus roseus, Apocynaceae) disrupts the spindle fibres that are responsible for separating chromosomes during cell division. It works at lower concentrations and with fewer side-effects than the alkaloids derived directly from the plants and it could be useful in combating Kaposis sarcoma, a rare skin cancer associated with AIDS.

The crude extract from Ancistrocladus species has yielded Michellamine B, a new alkaloid, which in the initial trials has been shown to work against the HIV virus.



Michellamine B

One of the most promising anti-AIDS compounds is produced by the Malaysian tree and latex of Calophyllum teysmanii yielded extracts with significant anti HIV activity. The active constituent was found to be (–)-calanolide B, which was isolated in yields of 20–30%. Eight compounds have been isolated from C. lagenirum with Calanolide A showing anti-HIV activity and C. teysmanni has yielded Calanolide B, equally found to be slightly less active than the (+)-Calanolide A, but it has the advantage of being readily available from the latex which is tapped in a sustainable manner by making small slash wounds in the bark of mature trees without causing any harm to the trees. Chemically, Calanolide A is a coumarin and is now beingtested in human trials. The drugs are being developed by Sarawak Medichem Pharmaceuticals, a joint venture company formed between the Sarawak State Government and Medichem Research, Inc. (+)-Calanolide A (which has been synthesized by Medichem chemists) is currently in Phase II clinical trials, while (–)-calanolide B is in pre-clinical development. These two Calanolides can also be isolated from other Calophyllum species, namely from the leaves or C. brasiliensis[3] and exhibiting more or less the same pattern of activity.



(+) –Calanolide A

2. CHEMOKINE (CCR5 and CXCR4) RECEPTOR ANTAGONIST

Maraviroc

It inhibits entry of HIV into CD4 cells by blocking binding of viral envelope protein gp120 to CCR5 co-receptor and can acts on R5 tropic and dual tropic viruses. It is pharmacologically a cyclohexane carboxamide with T ½ (time taken for the serum drug concentration to decline by 50%) of 14-18 h and it is metabolised by CYP3A and P-glycoprotein. It has an oral bioavailability of 33% which is unaffected by food. It has an I_C90 of 2 nM [4]. The drug was well tolerated but a few patients exhibited postural hypotension (dose limiting toxicity of maraviroc), headache, nausea, cystitis and myocardial ischaemia.

Vicriviroc (SCH-D, SCH 417690):

This medication is undergoing phase III trials in treatment-experienced patients, and phase II trials in ARV native patients[7]. Previous trials in treatment native patients were discontinued in October 2005 because of HIV viral load rebound and not because of any side effects[5]. This drug is piperazine based CCR5 antagonist.

Aplaviroc

Due to hepatotoxicity in 2005, GlaxoSmithKline halted the development of these compound.

AMD070

It inhibits entry of HIV into CD4 cells by blocking the binding of viral envelope protein gp120 to CXCR4 coreceptor. It acts on X4 tropic and dual tropic virus. Pharmacologically it is a bicyclam derived from prototype AMD3100. It has a half-life of 14 h and metabolised by CYP3A4 and P-glycoprotein and inhibits CYP3D6. Clinically it is likely to need ritonavir booster. The drug is orally bioavailable with absorption unaffected by food. The I_C50 is 12.5 nM [6]

INTEGRASE INHIBITORS

Integrase is a 32 kDa 288 amino acid HIV enzyme, which enables the integration of the viral genetic material into host cell DNA. The structure is confirmed by NMR spectroscopy which elaborate that structure has 3 domain. The C- terminal domain responsible for DNA binding as well as catalytic activity. Integration of cDNA derived from the retroviral RNA to the host cell DNA occurs through the PIC (preintegration complex) in three steps: 3' end processing, strand transfer and gap repairs. The first two steps are mediated through the viral integrase whereas the third step is mediated by cellular proteins[7]. Integrase inhibitors such as raltegravir (previously known as MK-0518; Merck & Co., Inc.) inhibit the strand transfer process. Raltegravir is a hydroxypyrimidinone carboxamide.

Elvitegravir

Elvitegravir is chemically a dihydroquinoline carboxylic acid and structurally similar to quinolone antibiotics. It binds to magnesium cations and inhibits the strand transfer reaction. The common side effects being headache, muscle spasm, increase in serum triglyceride and amylase [8]. E29Q and T66I are the two mutation pathways associated with elvitegravir resistance [9].

3. ANTI-SENSE OLIGONUCLEOTIDES

Anti-sense oligonucleotides are short RNAs, which can be designed to target various functionally important sites of HIV-1 RNA. The oligonucleotides act on targets such as the 5'-UTR region of HIV-1, TAR (transactivator response region) of HIV-1 genome, Psi domain responsible for dimerization of HIV-1 RNA, or HIV-1 envelope gene. These agents act by neutralisating HIV-1 expression. The LNA (locked nucleic acid) modified antisense oligonucleotide was found to be superior to DNAzymes in inhibiting HIV expression[10,11,12,13,14].

4. ANTI-CD4 MONOCLONAL ANTIBODY TNX - 355

This is a humanized anti-CD4 IgG4 monoclonal antibody, which acts on domain 2 of the CD4 receptor. The agent is not immunosuppressive and does on interfere with the immunological functions of CD4 cells like antigen presentation. It is administered by i.v. infusion once every other week. The common adverse effects including depression, vasovagal attacks, seizures and acute renal failure[15,16].

One of the most important agent used in AIDS are inhibitor of the interaction between between transactivator of transcription (Tat protein) and the transactivator response region (TAR) of HIV RNA. This interaction is primarily between the ARM (arginine rich motif) of Tat and the three base bulge region of TAR RNA. The compounds within this category can be classified between those that act on the 3 base bulge region and those that act on the TAT protein. They include agents such as WM5 (6-aminoquinolone derivative), which is a substituted purine having side chains with amino or guanidyl group, and trehalose derivatives with guanidine groups [17,18,19].

5. NANOTECHNOLOGY FOR HIV/AIDS TREATMENT

Nanotechnology involves the understanding, design, engineering and fabrication of materials at the atomic and molecular level**.** Applications of nanotechnology for prevention and treatment of HIV/AIDS have also gained attention in recent years. There are emerging novel approaches in which nanotechnology can enhance current treatment as well as advance new therapeutic strategies, such as gene therapy and immunotherapy. Nanoscale delivery systems also enhance and modulate the distribution of hydrophobic and hydrophilic drugs into and within different tissues due to their small size. This particular feature of nanoscale delivery systems appears to hold the most promise for their use in clinical treatment and prevention of HIV. Specifcally, targeted delivery of antiretroviral drugs to $CD4^+$ T cells and macrophages as well as delivery to the brain and other organ systems could ensure that drugs reach latent reservoirs [20,21,22,23,24,25]. The use of nanotechnology systems for delivery of antiretroviral drugs has been extensively reviewed by Nowacek et al. and Amiji et al [20,22,25]. In a recent study based on polymeric systems, nanosuspensions (200 nm) of the drug rilpivirine (TMC278) stabilized by polyethylene-

polypro-pylene glycol (poloxamer 338) and PEGylated tocopheryl succinate ester (TPGS 1000) were studied in dogs and mice [26].

A part of these Dou et al. showed that nanosuspension of the drug indinavir can be stabilized by a surfactant system comprised of Lipoid E80 for effective delivery to various tissues[29,30,31]. The indinavir nanosuspensions were loaded into macrophages and their uptake was investigated. Macrophages loaded with indinavir nanosuspensions were then injected intravenously into mice, resulting in a high distribution in the lungs, liver and spleen.

Macrophages, which are the major HIV reservoir cells, have various receptors on their surface such as formyl peptide, mannose, galactose and Fc receptors, which could be utilized for receptor-mediated internalization. The drug stavudine was encapsulated using various liposomes (120–200 nm) conjugated with mannose and galactose, resulting in increased cellular uptake compared with free drug or plain liposomes, and generating significant level of the drug in liver, spleen and lungs [30,31,32,33].

In separate work, a mannose-targeted poly (propyleneimine) dendrimer nanocarrier was used to deliver the drug efavirenz to human monocytes /macrophages in vitro[34].

In a more recent study, the tetra-peptide tuftsin (Thr-Lys-Pro-Arg) was conjugated to the same dendrimer to target the drug efavirenz to macrophages in vitro [35]. The targeted dendrimer system resulted in sixfold prolonged release, 34-fold increased cellular uptake and sevenfold increase in anti-HIV activity compared with free drug. In a new approach to target macrophage HIV reservoirs, a peptide nanocarrier was proposed as a model where a drug is conjugated to the backbone of peptide-PEG and N-formyl-methionyl-leucyl-phenylalanine (fMLF), a bacterial peptide sequence for which macrophages express a receptor, is attached to the PEG for targeting. The study found that fMLF-targeted peptide-PEG nanocarriers show increased cellular uptake and increased accumulation in macrophages of liver, kidney and spleen compared with those which are nontargeted [36].

Nanomaterials as therapeutic agents [36,37]

- Various nanomaterials such as fullerenes, dendrimers, silver and gold nanoparticles have shown anti-HIV effects in vitro.
- Silver nanoparticles showed size-dependent interaction with HIV, inhibiting the virus from binding to CD4+ T cells while gold nanoparticles conjugated to the molecule SDC-1721 (a segment of the CCR5 inhibitor TAK-779) showed strong anti-HIV activity compared with free SDC-1721.

Gene therapy for HIV/AIDS treatment [38,39,40,41,42]

- Gene therapy based on siRNA has shown promise for HIV/AIDS treatment. Nanotechnology platforms for delivery of siRNA for HIV/AIDS treatment are in their early stages but recent work has been met with optimism.
- Single-walled nanotubes, dendrimers, fusion proteins, peptide–antibody conjugates have all been used for delivery of siRNA to HIV-specifc cells.

Immunotherapy for HIV/AIDS [43,44]

- Immunotherapy for HIV/AIDS based on viral agents and administration of ex vivo-generated autologous dendritic cells involves risks due to the viral agents and the complicated procedures used in ex vivo dendritic cell generation and manipulation.
- Nanotechnology-based immunotherapy that uses DNA plasmid delivered through mannose targeted polyethyleimine has reached Phase II clinical trials.

Nanotechnology-based preventive HIV/AIDS vaccine [45,46]

- Generation of an effective HIV/AIDS vaccine has been notoriously difficult and new approaches are always sought.
- Nanoparticles have various advantages in improving delivery of antigens to enhance the immune response. They can be used both for encapsulating antigens in their core from which antigen presenting cells can process and present and cross-present antigen to CD4+ and CD8+ T cells respectively, or absorbing the antigens on their surfaces, allowing B cells to generate humoral responses. Nanoparticle vaccines can also be optimized for various routes of administration.
- Various polymeric and lipid-based nanoparticles have been used to deliver DNA-, protein- or peptide-based antigens in vivo, eliciting strong cellular, humoral and mucosal immune responses.

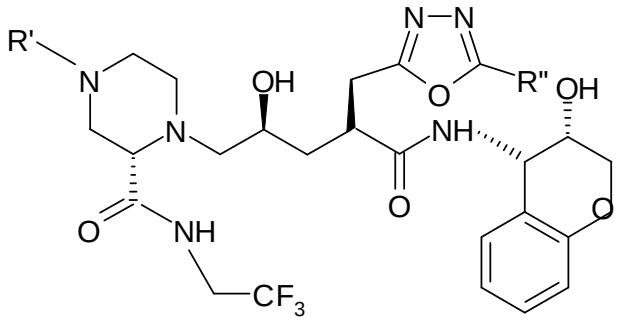
Nanotechnology-based intravaginal microbicides

- Intravaginal microbicides are preventive agents that are topically applied to the vagina to prevent the transmission of HIV/AIDS or other sexually transmitted diseases.
- Nanotechnology-based approaches are being developed to use dendrimers, siRNA and nanoparticles for microbicidal functions.
- VivaGel is a dendrimer-based microbicide gel that has been shown to be safe in humans in Phase I clinical trials.
- Polymeric nanoparticles have been used to deliver the CCR5 inhibitor PSC-RANTES and HIV-specifc siRNA as microbicides.

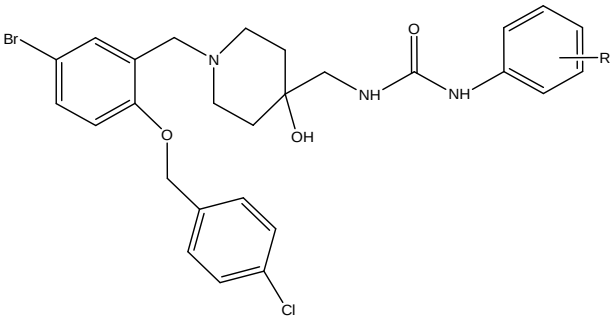
6. MEDICINAL CHEMISTRY FOR THE TREATMENT OF AIDS

- A part of these **Kim RM et al** in 2004 studied HIV-1 protease inhibitors (PI's) bearing 1,3,4-oxadiazoles at the P1' position were synthesized by a novel method involving the diastereoselective installtion of a carboxylic

acid and conversion to the P1' heterocycle exhibited excellent activities [47].

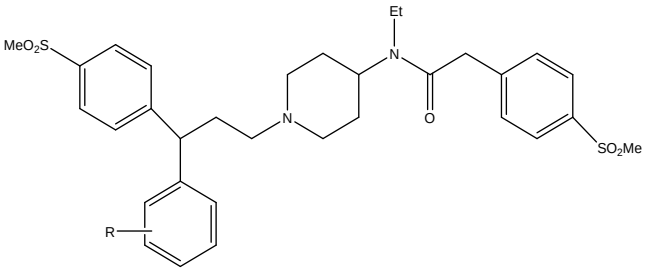


- **Shou-Fu Lu *et. al,*** described novel 4-hydroxypiperidine derivatives as CCR5 receptor antagonist [49].



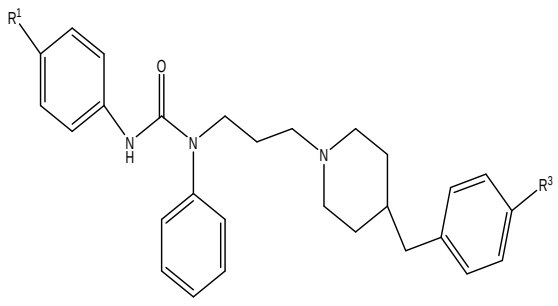
4-Hydroxypiperidin urea

- **John G cumming *et. al,*** described substituted 1-[3-(4-methanesulfonylpheny)-3-phenylpropyl]-piperidinyl phenylacetamides as a modulator of human CCR5 receptor [50].



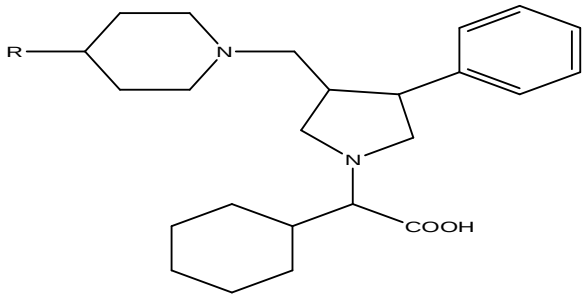
Diphenylpropylpiperidine compounds

- **Shinichi Imamura *et. al,*** described synthesis and biological evaluation of N-[3-(4-benzylpiperidin-1-yl) propyl]-N,N-diphenylureas [51].



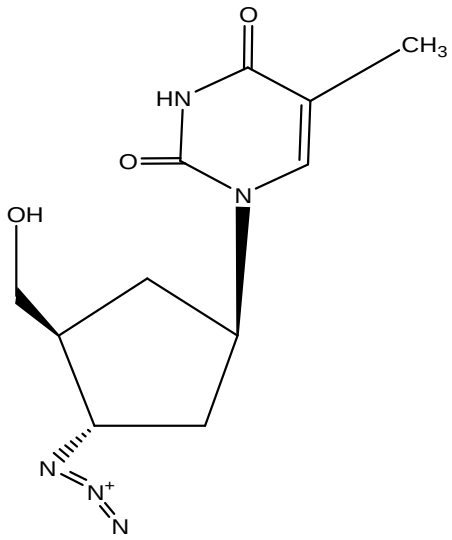
N, N-diphenylurea derivatives

- **K. Shankaran *et. al,*** described 4-(heteroarylpeperidin-1-yl-methyl)-pyrrolidin-1-yl-acetic acid as antagonists of the human CCR5 chemokine receptor [52].

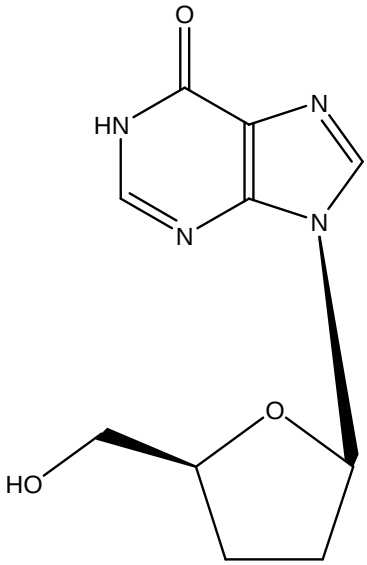


Miscellaneous sulfones

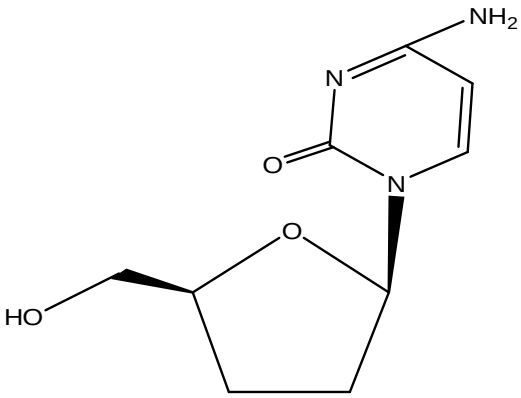
Structure of classical antiretroviral agents



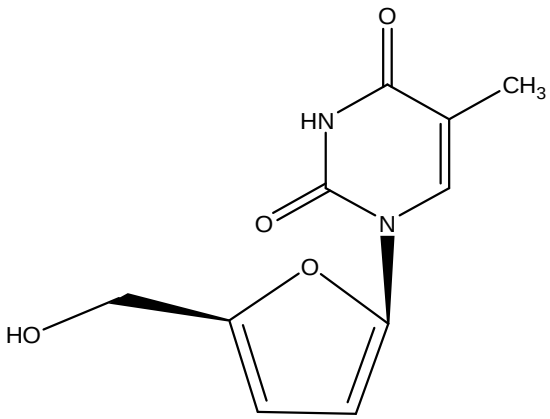
Zidovudine



Didanosine



Zalcitabine



Stavudine

Approved antiretroviral drugs[48]

Generic Name	Brand Name	Manufacturer
Zalcitabine	Hivid	Hoffmann-La Roche
Stavudine	Zerit	Bristol-Myers Squibb
Lamivudine	Epivir	GlaxoSmithKline
Saquinavir	Invirase (hard gel capsule)	Hoffmann-La Roche
Ritonavir	Norvir	Abbott Laboratories
Indinavir	Crixivan	Merck
Nevirapine	Viramune	Boehringer Ingelheim
Nelfinavir	Viracept	Agouron Pharmaceuticals
Delavirdine	Rescriptor	Pfizer
Efavirenz	Sustiva (USA)	Bristol-Myers Squibb
Zidovudine	Retrovir	GlaxoSmithKline
Didanosine	Videx (tablet)	Bristol-Myers Squibb
Enfuvirtide	Fuzeon	Hoffmann-La Roche & Trimeris
Atazanavir	Reyataz	Bristol-Myers Squibb
Emtricitabine	Emtriva	Gilead Sciences
Fosamprenavir	Lexiva (USA)	GlaxoSmithKline
Tipranavir	Aptivus	Boehringer Ingelheim
Darunavir	Prezista	Tibotec, Inc.
Maraviroc	Celsentri (Europe)	Pfizer
Raltegravir	Isentress	Merck & Co., Inc.
Etravirine	Intelence	Tibotec Therapeutics

CONCLUSION

Undoubtedly, the identification of novel targets and the development of new agents for the treatment of HIV is unmitigated good news for patients, clinicians, scientific community and the pharmaceutical industry**.** There is no doubt that most of the new agents would generate new resistance mutations, and development of resistance assays to identify novel mutations, resistance phenotypes and breakpoints would be required. In addition use of agents such as the chemokine receptor antagonists would need simultaneous development and availability of Tropism assays before they can be used effectively. Development of new anti-retroviral agents (ARVs) is expensive and time consuming and with few exceptions most of the R&D activities in this area are occurring in the industrialised world.

REFERENCES

1. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg C M, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, and Hahn BH, Origin of HIV-1 in the chimpanzee pantroglodytes troglodytes, Nature, 397, 1999,436-441.
2. Keele BF, van Heuverswyn F., Li YY, Bailes E., Takehisa J, Santiago M L, Bibollet-Ruche F, Chen Y, Wain LV, Liegois F, Loul S, Mpoudi Ngole E, Bienvenue, H., et al., Chimpanzee Reservoirs of Pandemic and Nonpandemic HIV-1, 3, 2006,5-25.
3. Huerta-Reyes M, Basualdo Mdel C, Abe F, Jimenez-Estrada M, Soler C, Reyes-Chilpa, R., HIV-1 inhibitory compounds from Calophyllum brasiliensis leaves. Biol. Pharm. Bull. 27 (9), 2004, 1471–1475.

4. Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, et al. Time trends in primary HIV-1 drug resistance among recently infected persons, JAMA 288,2002,181-188.
5. Schering,Plough, Expands vicriviroc phase II study in treatment-naïve patients with HIV, http:/www.scheringplough.com/news article.aspx? regidZ1307827 [accessed 12.09.09].
6. Tagat JR, McCombie SW, Nazareno D, Labroli MA, Xiao Y, Steensma RW, et al. Piperazine-Based CCR5 antagonists as HIV-1 inhibitors. IV. Discovery of 1-[(4,6-Dimethyl-5-pyrimidi-nyl)carbonyl]- 4-[4-{2-methoxy-1(R)- 4-(trifluoromethyl)phe nyl}ethyl-3(S)-methyl-1-piperazinyl]- 4-methylpiperidine(Sch-417690/Sch-D), a potent, highly Selective, and orally Bioavailable CCR5 antagonist, J Med Chem, 47,2004,2405-2408.
7. Cao YJ, Flexner CW, Dunaway S, Park JG, Klingman K, Wiggins I, et al. Effect of low-dose ritonavir on the pharmacokinetics of the CXCR4 antagonist AMD070 in healthy volunteers. Antimicrob Agents Chemother, 52, 2008, 1630-1634.
8. Esposito D, Craigie R. HIV integrase structure and function. Adv Virus Res, 52, 1999, 319-333.
9. DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, et al. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. J Acquir Immune Defic Syndr; 19, 2006, 1-5.
10. Shimura K, Kodama E, Sakagami Y, Matsuzaki Y, Watanabe W, Yamataka K, et al. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137), J Virol, 82,2008, 764-774.
11. Gu S, Ji J, Kim JD, Yee JK, Rossi JJ, Inhibition of infectious human immunodeficiency virus type 1 virions via lentiviral vector encoded short antisense RNAs. Oligonucleotides 16 , 2006, 287-295.
12. Jakobsen MR, Haasnoot J, Wengel J, Berkhout B, Kjems J, Efficient inhibition of HIV-1 expression by LNA modified antisense oligonucleotides and DNAzymes targeted to functionally selected binding sites. Retrovirology , 9,2007, 264.
13. Lebars I, Richard T, Di Primo C, Toulme ´ JJ. LNA derivatives of a kissing aptamer targeted to the trans-activating responsive RNA element of HIV-1. Blood Cells Mol Dis 38, 2007, 204-209.
14. Lu X, Yu Q, Binder GK, Chen Z, Slepushkina T, Rossi J, Dropulic B. Antisense-mediated inhibition of human immunodeficiency virus (HIV) replication by use of an HIV type 1-based vector results in severely attenuated mutants incapable of developing resistance. J Virol, 78, 2004, 7079-7088.
15. Kuritzkes DR, Jacobson J, Powderly WG, Godofsky E, DeJesus E, Haas F, et al. Antiretroviral activity of the antiCD4 monoclonal antibody TNX-355 in patients infected with HIV type 1. J Infect Dis, 189, 2004, 286-291.
16. Zhang XQ, Sorensen M, Fung M, Schooley RT, Synergistic in vitro antiretroviral activity of a humanized monoclonal anti-CD4 antibody (TNX-355) and enfuvirtide (T-20). Antimicrob Agents Chemother, 50, 2006, 2231-2233.
17. Richter S, Parolin C, Gatto B, Del Vecchio C, Brocca-Cofano E, Fravolini A, et al. Inhibition of human immunodeficiency virus type 1 tat-trans-activation-responsive region interaction by an antiviral quinolone derivative. Antimicrob Agents Chemother , 48, 2004, 1895-1899.
18. Yang M, Discoveries of TateTAR interaction inhibitors for HIV-1. Curr Drug Targets Infect Disord, 5, 2005, 433-444.
19. Yuan D, He M, Pang R, Lin SS, Li Z, Yang M. The design, synthesis, and biological evaluation of novel substituted purines as HIV-1 TateTAR inhibitors. Bioorg Med Chem 15, 2007, 265-272.
20. Vyas TK, Shah L, Amiji MM, Nanoparticulate drug carriers for delivery of HIV/AIDS therapy to viral reservoir sites. Expert Opin. Drug Deliv. 3(5), 2006, 613–628.
21. Wan L, Pooyan S, Hu P, Leibowitz MJ, Stein S, Sinko PJ, Peritoneal macrophage uptake, pharmacokinetics and biodistribution of macrophage-targeted peg-fmlf (n-formyl-methionyl-leucyl-phenylalanine) nanocarriers for improving HIV drug delivery. Pharm. Res. 24(11), 2007, 2110–2119
22. Nowacek A, Gendelman HE, Nanoart, neuro AIDS and CNS drug delivery. Nanomed. 4(5), 2009, 557–574.
23. Farokhzad OC, Langer R, Impact of nanotechnology on drug delivery. ACS Nano 3(1), 2009, 16–20.
24. Davis ME, Chen ZG, Shin DM, Nanoparticle therapeutics: An emerging treatment modality for cancer. Nat. Rev. Drug Discov. 7(9), 2008, 771–782.
25. Amiji MM, Vyas TK, Shah LK, Role of nanotechnology in HIV/AIDS treatment: Potential to overcome the viral reservoir challenge. Discov. Med. 6(34), 2006, 157-162.
26. Baert L, van't Klooster G, Dries W et al.: Development of a long-acting injectable formulation with nanoparticles of rilpivirine (tmc278) for HIV treatment. Eur. J. Pharm. Biopharm. 72(3), 2009, 502–508.
27. Dou H, Destache CJ, Morehead JR et al.: Development of a macrophage-based nanoparticle platform for antiretroviral drug delivery. Blood 108(8), 2006, 2827–2835.
28. Dou H, Morehead J, Destache CJ et al.: Laboratory investigations for the morphologic, pharmacokinetic, and anti-retroviral properties of indinavir

nanoparticles in human monocyte-derived macrophages. Virology 358(1), 2007, 148–158.

29. Dou H, Grotepas CB, McMillan JM et al.:Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuroAIDS. J. Immunol. 183(1), 2009, 661–669.
30. Garg M, Asthana A, Agashe HB, Agrawal GP, Jain NK: Stavudine-loaded mannosylated liposomes: In-vitro anti-HIV-activity, tissue distribution and pharmacokinetics. J. Pharm. Pharmacol. 58(5), 2006, 605–616.
31. Garg M, Dutta T, Jain NK, Reduced hepatic toxicity, enhanced cellular uptake and altered pharmacokinetics of stavudine loaded galactosylated liposomes. Eur. J. Pharm. Biopharm 67(1), 2007, 76–85.
32. Garg M, Garg BR, Jain S et al.: Radiolabeling, pharmacoscintigraphic evaluation and antiretroviral effcacy of stavudine loaded 99mtc labeled galactosylated liposomes. Eur. J. Pharm. Sci, 33(3), 2008, 271–281.
33. Dutta T, Agashe HB, Garg M, Balakrishnan P, Kabra M, Jain NK, Poly (propyleneimine) dendrimer based nanocontainers for targeting of efavirenz tohuman monocytes/macrophages in vitro. J. Drug Target 15(1), 2007, 89–98.
34. Dutta T, Garg M, Jain NK, Targeting of efavirenz loaded tuftsin conjugated poly(propyleneimine) dendrimers to HIVinfected macrophages in vitro. Eur. J. Pharm. Sci. 34(2–3), 2008, 181–189.
35. Wan L, Zhang X, Pooyan S et al.: Optimizing size and copy number for PEG-FMLF (n-formyl-methionyl-leucyl-phenylalanine) nanocarrier uptake by macrophages. Bioconjug. Chem, 19(1), 2008, 28–38.
36. Elechiguerra JL, Burt JL, Morones JR et al.: Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnology 3, 2005, 6.
37. Sun RW, Chen R, Chung NP, Ho CM, Lin CL, Che CM: Silver nanoparticles fabricated in hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. Chem. Commun. (40), 2005, 5059–5061.
38. Eguchi A, Meade BR, Chang YC et al. Efficient siRNA delivery into primary cells by a peptide transduction domain-dsRNA binding domain fusion protein. Nat. Biotechnol. 27(6), 2009, 567–571.
39. Song E, Zhu P, Lee SK et al.: Antibody mediated in vivo delivery of small interfering RNAS via cell-surface receptors. Nat. Biotechnol. 23(6), 2005, 709–717.
40. Liu Z, Winters M, Holodniy M, Dai H: Sirna delivery into human t cells and primary cells with carbon-nanotube transporters. Angew. Chem. Int. Ed. 46(12), 2007, 2023–2027.
41. Weber N, Ortega P, Clemente MI et al. Characterization of carbosilane dendrimers as effective carriers of siRNA to HIV-infected lymphocytes. J. Control Release 132(1), 2008, 55–64.
42. Kumar P, Ban HS, Kim SS et al. T cell-specifc siRNA delivery suppresses HIV-1 infection in humanized mice. Cell 134(4), 2008, 577–586.
43. Aline F, Brand D, Pierre J et al. Dendritic cells loaded with HIV-1 p24 proteins adsorbe on surfactant-free anionic pla nanoparticles induce enhanced cellular immune responses against HIV-1 after vaccination. Vaccine 27(38), 2009, 5284–5291.
44. Lori F, Calarota SA, Lisziewicz J, Nanochemistry-based immunotherapy for HIV-1. Curr. Med. Chem. 14(18), 2007, 1911–1919.
45. Fairman J, Moore J, Lemieux M et al. Enhanced in vivo immunogenicity of SIV vaccine candidates with cationic liposome-DNA complexes in a rhesus macaque pilot study. Hum. Vaccin. 5(2), 2008, 3.
46. Burke B, Gomez-Roman VR, Lian Y et al. Neutralizing antibody responses to subtype b and c adjuvanted HIV envelope protein vaccination in rabbits. Virology 387(1), 2009 , 147–156
47. Kim RM, Rouse EA, Chapman KT, Schleif WA, Olsen DB, Stahlhut M, Rutkowski CA, Emini EA and Tata JR. P1' oxadiazole protease inhibitors with excellent activity against native and protease inhibitor-resistant HIV-1. Bioorg. Med. Chem. 14, 2004, 4651–4654.
48. Erik De Clercq, Anti-HIV drugs: 25 compounds approved within 25 years after the discovery (Review), International Journal of Antimicrobial Agents 33, 2009, 307–320.
49. S. Fu Lu, B. Chen, D. Davey, L. Dunning, S. Jaroch, K. May, J. Onuffer, G. Phillips, B. Subramanyam, J. Tseng, R. G. Wei, M. Wei and B. Ye, Bioorg. Med. Chem. Lett. 17 2007, 1883–1887.
50. Cumming JG, Brown SJ, Cooper AE, Faull AW, Flynn AP, Grime K, Oldfield J, Shaw JS, et al. Bioorg. Med. Chem. Lett. 16, 2006, 3533–3536.
51. Imamura S, Kurasawa O, Nara Y, Ichikawa T, Nishikawa Y, Iida T, Hashiguchi S, Kanzaki N, et al. Bioorg. Med. Chem, 12 , 2004, 2295–2306.
52. Shankaran K, Donnelly KL, et al., Bioorg. Med. Chem. Lett, 14, 2004, 3419–3424.

****