Chapter 2
2.0 AIM AND OBJECTIVES:
The research process for discovering, developing and testing new drugs is a complicated, time-consuming, and costly one whose end result is never known at the outset. The research runs from basic biomedical investigation of living cells and molecules to applied research that yields new products to improve healthcare. The complexity of the process can be attributed, in part, to the diversity of scientific disciplines involved in finding new drugs. Traditional organic chemists, physiologists and statisticians have been joined in recent years by new kinds of specialists. Biochemists study the chemistry of life processes. Protein crystallographers study the molecules that make up living matter. Toxicologists investigate the chemicals potential side effects. Pharmacologists look at how drugs work and computer scientists apply the power of their machines to analyze and assess new chemicals. Each discipline provides a different way of looking for that needle that has the properties of an inhibitor. The focus of the pharmaceutical industry has shifted from the trial and error process of drug discovery to a rational and structure based drug design. A successful and reliable drug design process could reduce the time and cost of developing useful pharmacological agents. Computational methods are used for the prediction of ‘drug-likeness’ which is nothing but the identification and elimination of candidate molecules that are unlikely to survive the later stages of discovery and development. There is a profound need for the identification and development of novel chemotherapeutic compounds active against HIV. For this, a new emerging field, Computer Aided Drug Design (CADD) occupied a special place in pharmaceutical industries. The tools of CADD and Bioinformatics offer significant benefit for the development of new drugs against HIV. Virtual screening, lead optimization, predictions of bioavailability and bioactivity of optimized lead molecules designed using Bioinformatics and Chemiinformatics tools can help to guide experimental research. When researchers show new lead molecules as inhibitors of drug targeted proteins, it is an intangible benefit that can help to design research programs especially in protein targeted drug design. The present thesis describes the attempts made to design the inhibitors with high potential as anti-HIV drugs. Availability of very few drugs in the market...
for HIV clearly indicates that there is greater necessity to propose modified drugs which are more potent and effective than the available ones.

2.1 Scope of the present study:
AIDS (Acquired Immuno Deficiency Syndrome) is a major epidemic caused by HIV (Human Immuno Deficiency Virus) and significant efforts are being made for years to develop drugs against the dreadful disease. HIV has just nine genes (compared to more than 500 genes in a bacterium, and around 20,000-25,000 in a human). Three of the HIV genes, called gag, pol and env, contain information needed to make structural proteins for new virus articles. The other six genes, known as tat, rev, nef, vif, vpr and vpu code for proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. Among the nine genes in HIV, Pol gene codes for three essential enzymes which are Integrase (IN), Reverse Transcriptase (RT) and Protease (PR), among other proteins.

**HIV-1 Integrase (HIV-1 IN)** is a 32 kDa protein produced from the C-terminal portion of the Pol gene product, and is an one of attractive target for new anti-HIV drugs. The role of HIV-1 IN is to insert the viral DNA into the host chromosomal DNA, a step that is essential for HIV replication. Integration is a point of no return for the cell, which becomes a permanent carrier of the viral genome (provirus). Integration is in part responsible for the persistence of retroviral infections. After integration, the viral gene expression and particle production may take place immediately or at some point in the future. The timing presumably depends on the activity of the chromosomal locus hosting the provirus.

Retroviral IN catalyzes two major reactions:
- 3'-processing, in which two or three nucleotides are removed from one or both 3' ends of the viral DNA to expose the invariant CA dinucleotides at both 3'-ends of the viral DNA.
- The strand transfer reaction, in which the processed 3' ends of the viral DNA are covalently ligated to the host chromosomal DNA.

HIV-1 Integrase inhibitors are a class of antiretroviral drug designed to block the action of integrase, a viral enzyme that inserts the viral genome into the DNA of
the host cell. Since integration is a vital step in retroviral replication, blocking it can halt further spread of the virus. Integrase inhibitors were initially developed for the treatment of HIV infection. Recently two major inhibitors identified for IN they are Elvitegravir and MK-2048.

**HIV-1 Reverse transcriptase (HIV-1 RT)** is a key enzyme in the HIV replication cycle and is one of the main targets in the development of drugs for treating HIV-infection and AIDS. Reverse transcriptase (RT), also known as RNA-dependent DNA polymerase, is a DNA polymerase enzyme that transcribes single-stranded RNA into double-stranded DNA. It also helps in the formation of a double helix DNA once the RNA has been reverse transcribed into a single strand cDNA and is one of attractive target for new anti-HIV drugs. The enzyme is encoded and used by reverse-transcribing viruses, which use the enzyme during the process of replication. Reverse-transcribing RNA viruses, such as retroviruses, use the enzyme to reverse-transcribe their RNA genomes into DNA, which is then integrated into the host genome and replicated along with it. Reverse-transcribing DNA viruses, such as the hepa dna viruses, can allow RNA to serve as a template in assembling, and making DNA strands. HIV infects humans with the use of this enzyme. Without reverse transcriptase, the viral genome would not be able to incorporate into the host cell, resulting in the failure of the ability to replicate. Unlike bacteria, retroviruses use preexisting host-encoded transfer RNAs as primers.

Reverse transcriptase inhibitors are a class of antiretroviral drug used to treat HIV infection, tumors, and cancer. Reverse transcriptase inhibitors inhibit activity of reverse transcriptase, a viral DNA polymerase enzyme that retroviruses need to reproduce.

Reverse transcriptase inhibitors come in three forms:

- **Nucleoside analog reverse transcriptase inhibitors (NARTIs or NRTIs)**
  (Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine, lamivudine, Apricitabine)

- **Nucleotide analog reverse transcriptase inhibitors (NtARTIs or NtRTIs)**
  (Tenofovir, Adefovir)
• Non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Efavirenz, Nevirapine, Delavirdine, Etravirine)

The mode of action of NRTIs and NtRTIs is essentially the same; they are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. However, unlike the natural deoxynucleotides substrates, NRTIs and NtRTIs lack a 3'-hydroxyl group on the deoxyribose moiety. As a result, following incorporation of an NRTI or an NtRTI, the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, when an NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination. All NRTIs and NtRTIs are classified as competitive substrate inhibitors. In contrast, NNRTIs have a completely different mode of action. NNRTIs block reverse transcriptase by binding at a different site on the enzyme, compared to NRTIs and NtRTIs. NNRTIs are not incorporated into the viral DNA but instead inhibit the movement of protein domains of reverse transcriptase that are needed to carry out the process of DNA synthesis. NNRTIs are therefore classified as non-competitive inhibitors of reverse transcriptase.

**HIV-1 Protease** is an aspartic protease that is essential for the life-cycle of HIV, the retrovirus that causes AIDS. HIV-PR cleaves newly synthesized polyproteins at the appropriate places to create the mature protein components of an infectious HIV virion. Without effective HIV-PR, HIV virions remain uninfected. Thus, mutation of HIV-PR's active site or inhibition of its activity disrupts HIV's ability to replicate and infect additional cells, making HIV PR inhibition the subject of much pharmaceutical research. According to the mechanism for HIV PR protein cleaved and water acts as a nucleophile, which acts in simultaneous conjunction with a well-placed aspartic acid to hydrolyze the scissile peptide bond. Additionally, HIV PR has two molecular "flaps" which move a distance of up to 7 Å when the enzyme becomes associated with a substrate. Thus, new knowledge on inhibitors of these enzymes (HIV-RT, HIV-IN and HIV-PR) is of critical importance in the anti-HIV drug discovery area. Interestingly HIV-IN, HIV-
RT and HIV-PR have no sequence homologue in the human host and hence, it is considered as a potential drug target.

Protease inhibitors are a class of drugs used to treat or prevent infection by HIV, antiretroviral protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, Lopinavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir). Protease inhibitors were the second class of antiretroviral drugs developed. In all cases, patents remain in force until 2010 or beyond.

Given the specificity of the target of these drugs there is the risk, as in antibiotics, of the development of drug-resistant mutated viruses. To reduce this risk it is common to use several different drugs together that are each aimed at different targets and also pharmacophores understanding the interactions between protein targets and ligands are important in screening of drugs.

2.2 Objectives of present study:

Therefore, the present research work was focused on design and development of more potent lead molecules against HIV-1-IN, HIV-1-RT and HIV-1-PR through computer aided drug design technique. The main objectives of the present study are

- Sequence analysis and Phylogenic analysis of HIV-1 RT, HIV-1 IN and HIV-1 PR mutant families to find out the evolutionary relationships.
- Energy minimization and MD simulation of HIV-1 RT, HIV-1 IN and HIV-1 PR to check the stability of the protein models in solvent system in define temperature and time intervals.
- Validation of refined models of HIV-1 RT, HIV-1 IN and HIV-1 PR using advanced sterochemical properties analyzers PROCHECK, What If and Prosa Web.
- Active site analysis and Electrostatic potential analysis of HIV-1 RT, HIV-1 IN and HIV-1 PR
- Screening of All available drug against HIV-1 RT, HIV-1 IN and HIV-1 PR and docking Analysis of All Available drugs against HIV-1 RT, HIV-1 IN and HIV-1 PR using molecular docking application Autodock 4.0.
- Identification of Suitable inhibitor against HIV-RT, HIV-IN and HIV-PR
• Designing of HIV-1 PR inhibitor Tipranavir analogs and selection of best of ten Tipranavir analogs based on various filters (molecular weight, log P, number of Hydrogen acceptors and donors.
• Lead optimization and Autodock analysis of Tipranavir analogs against HIV-PR
• Designing of HIV-1 RT inhibitor Adefovir analog and selection of best of ten Adefovir analogs based on various filters (molecular weight, log P, number of Hydrogen acceptors and donors.
• Lead optimization and Autodock analysis of Adefovir analog against HIV-1 RT
• Designing of HIV-1 IN inhibitor Elvitegravir analogs and election of best of ten Elvitegravir analogs based on various filters (molecular weight, log P, number of Hydrogen acceptors and donors.
• Lead optimization and Autodock analysis of Elvitegravir analogs against HIV-1 IN