Chapter-5
5.0 Summary and Conclusion:

Nearly 20 years have rolled by since AIDS has been recognized and the HIV has been identified as the causative agent and yet neither a cure nor a vaccine for AIDS. This epidemic is existing its incidence and enormity massively it is estimated 39.5 million people worldwide are present living with HIV, and 20 million people have already died, giving a total number of HIV infections of 56 million. Responding to HIV on a scale coextensive with the pandemic is a global threat. This process has already begun and the important advances have been made toward inhibition if the virus, bolstering the immune system, and extending and raise the lives of patients through combination antiretroviral therapy, which involves use of reverse transcriptase, protease inhibitors and Integrase inhibitor.

But challenges remain uncertain. Treatment does not inhibit HIV replication in all patients, and the emergence of drug resistant virus hinders subsequent treatment. This has entailed to search for new drugs and new therapeutic strategies to control deadly viral replication. Therefore, the present research work focused on design of more potent lead molecules against three important proteins Protease, Integrase, and Reverse Transcriptase of HIV which are essential for replication and survival of virus and also there are no known counterparts in the host cell.

Summary and workflow for present research work, 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.

HIV-1 Protease:

- To have a better understanding of the HIV-1 PR, its molecular organization and other allied parameters, it is necessary to build optimized computational 3-D model which requires the retrieval from RCSB.
- HIV-1 PR enzyme and mutants of HIV-1 PR analyzed using multiple sequence alignment clustalW, it reveals the conserve residues and also deleted residues in HIV PR. Phylogentic determined from HIV-1 PR sequence similarities and phylogenetic analyses that the members of HIV-1 PR may have evolved from different ancestral genes and through
convergent evolution, acquired the residues to recognize and bind substrate molecule

- The potential role of the HIV-1 PR is a retroviral aspartyl 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.

- The 3-D model of HIV-1 PR was Energy Minimized and MD simulation performed at 5000ps using Gromacs tool, the refined submitted to PROCHECK, What If and Prosa-Web view to evaluate reliability.

- MD simulation of HIV-1 PR at 5000ps shows the stability of the protein in RMSD plot at 1000ps and also high fluctuation at the active site amino acids in RMSF plot at 5000ps.

- PROCHECK analysis reports HIV-1 PR shown that 100% of amino acids are present in the allowed region and disallowed regions - 0% respectively. The analysis reports of PROCHECK, What-If and Prosa-Web view revealed that HIV-1 PR model is of good quality and is reliable.

- The secondary structure conformations of HIV-1 PR model revealed that the protein is composed of 2 beta sheets, 3 beta hairpins, 9 strands, 2 helices, 8 beta turns and 2 gamma turns.

- Active site has been characterized from literature survey, experimental data and from PDBSUM server. Active site was analyzed by visualization tools like Pymol and VMD and electrostatic potential reveals to find the positive and negative regions in HIV-1 PR.

- All the available HIV-1 PR drug saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, Lopinavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir docked with HIV-1 PR to find out more possible interaction. The results shown that Atazanavir has more potent towards the HIV-1 PR it showing highest docked energy of -09.25 Kcal/mol.

- The Tipranavir is considered a good scaffold to design lead molecules. All designed new lead molecules are energy minimized with PRODRG
Designed lead molecules are tested for Lipinski’s Rule-of-Five using MOLINSPIRATION server.

- The end results of Lipinski’s rule have enabled identification of 50 ranked lead molecules (TP-1 to TP50) through Molinspiration server some TP analogs do not satisfy drug-likeness properties so they are excluded from docking process with HIV-1 PR by AUTODOCK 4.0.

- Out of 50 lead molecules 10 molecules (TP-3, TP-5, TP-10, TP-16, TP-18, TP-21, TP-43, TP-49, TP-31, and TP-45) have given good docking and binding energies with receptor. The analysis reports have shown that the conducted study can be further utilized in designing of better Anti-HIV drugs.

**HIV-1 Integrase:**

- To have a better understanding of the HIV-1 IN, its molecular organization and other allied parameters, it is first necessary to build an optimized computational 3-D model which requires the retrieval from RCSB.

- HIV-1 IN enzyme and mutants of HIV-1 IN analyzed using multiple sequence alignment clustalW, it reveals the conserve residues and also deleted residues in HIV IN. Phylogentic determined from HIV-1 IN sequence similarities and phylogenetic analyses that the members of HIV-1 IN may have evolved from different ancestral genes and through convergent evolution, acquired the residues to recognize and bind substrate molecule

- The potential role of the HIV-1 IN The main function of 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).

- The 3-D model of HIV-1 IN is Energy Minimized and MD simulation performed at 5000ps using Gromacs tool, the refined submitted to PROCHECK, What If and Prosa-Web view to evaluate reliability.
MD simulation of HIV-1 PR at 5000ps shows the stability of the protein in RMSD plot at 500ps and also high fluctuation at the active site amino acids in RMSF plot at 5000ps.

PROCHECK analysis reports HIV-1 PR shows that 100% of amino acids are present in the allowed region and disallowed regions - 0% respectively. The analysis reports of PROCHECK, What-If and Prosa-Web view revealed that HIV-1 PR model is of good quality and is reliable.

The secondary structure conformations of HIV-1 IN model reveals that the protein is composed of 1 beta sheets, 1 beta-alpha-beta motif, 2 beta hairpins, 1 beta bulge, 5 strands, 6 helices, 13 beta turns, 5 helix-helix interaction and 4 gamma turns.

Active site has been characterized from literature survey, experimental data and from PDBSUM server. Active site is analyzed by visualization tools like Pymol and VMD and electrostatic potential reveals to find the positive and negative regions in HIV-1 IN.

The entire available HIV-1 IN drug Raltegravir (CID: 11598201) and Elvitegravir (CID: 444795) docked with HIV-1 IN to find out more possible interaction. The results show that Elvitegravir has more potent towards the HIV-1 IN shows highest docked energy of -05.78 Kcal/mol.

The Elvitegravir is considered good scaffold to design lead molecules. All designed new lead molecules are energy minimized with PRODRG server. Designed lead molecules are tested for Lipinski's Rule-of-Five using MOLINSPIRATION server.

The end results of Lipinski's rule have enabled identification of 50 ranked lead molecules (EL-1 to EL-50) through Molinspiration server some EL analogs do not satisfy drug-likeness properties so they are excluded from docking process with HIV-1 IN by AUTODOCK 4.0.

Out of 50 lead molecules 10 molecules (EL-1, EL-5, EL-8, EL-13, EL-18, EL-20, EL-26, EL-27 EL-39 and EL-46) have given good docking and binding energies with receptor. The analysis reports have shown that the
conducted study can be further utilized in designing of better Anti-HIV drugs.

**HIV-1 Reverse Transcriptase:**

- To have a better understanding of the HIV-1 RT, its molecular organization and other allied parameters, it is first necessary to build a optimized computational 3-D model which requires the retrieval from RCSB.
- HIV-1 RT enzyme and mutants of HIV-1 RT analyzed using multiple sequence alignment clustalW, it reveals the conserve residues and also deleted residues in HIV-1 RT. Phylogentic determined from HIV-1 RT sequence similarities and phylogenetic analyses that the members of HIV-1 RT may have evolved from different ancestral genes and through convergent evolution, acquired the residues to recognize and bind substrate molecule
- The potential role of the HIV-1 RT 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. The 3-D model of HIV-1 RT is Energy Minimized and MD simulation performed at 5000ps using Gromacs tool, the refined submitted to PROCHECK, What If and Prosa-Web view to evaluate reliability.
- MD simulation of HIV-1 RT at 5000ps shows the stability of the protein in RMSD plot at 100ps and also high fluctuation at the active site amino acids in RMSF plot at 5000ps.
- PROCHECK analysis reports HIV-1 RT shown that 100% of amino acids are present in the allowed region and disallowed regions - 0% respectively. The analysis reports of PROCHECK, What-If and Prosa-Web view revealed that HIV-1 RT model is of good quality and is reliable.
- The secondary structure conformations of HIV-1 RT model revealed that HIV-1 RT shows 7 beta sheets, 1 beta-alpha-beta motif, 8 beta hairpins, 5
beta bluges, 22 strands, 13 helix-helix interactions, 54 beta turns and 7 gamma..

- Active site has been characterized from literature survey, experimental data and from PDDBSUM server. Active site is analyzed by visualization tools like Pymol and VMD and electrostatic potential reveals to find the positive and negative regions in HIV-1 RT.

- The entire available HIV-1 RT inhibitors (Non-nucleoside reverse-transcriptase inhibitors, Nucleoside reverse-transcriptase inhibitor and Nucleotide reverse-transcriptase inhibitor) docked with HIV-1 RT to find out more possible interaction. The results shown that Nucleotide reverse-transcriptase inhibitor Adefovir had more potent towards the HIV-1 RT it showing highest docked energy of -10.50 Kcal/mol.

- The Adefovir is considered good scaffold to design lead molecules. All designed new lead molecules are energy minimized with PRODRG server. Designed lead molecules are tested for Lipinski’s Rule-of-Five using MOLINSPIRATION server.

- The end results of Lipinski’s rule have enabled identification of 50 ranked lead molecules (TP-1 to TP50) through Molinspiration server some TP analogs do not satisfy drug-likeness properties so they are excluded from docking process with HIV-1 RT by AUTODOCK 4.0.

- Out of 50 lead molecules 10 molecules (AV-2, AV-12, AV-16, AV-19, AV-22, AV-24, AV-35, AV-40, AV-47 and AV-50) have given good docking and binding energies with receptor. The analysis reports have shown that the conducted study can be further utilized in designing of better Anti-HIV drugs.

**Conclusion:**

Three vital enzymes are chosen for development of new drugs against HIV. These three enzymes play a key role in the survival of HIV, Even though all the three enzyme has inhibitors, but due to lack their action in some patients there is urgency of development of new potent inhibitors in HIV. In this work, we are developed new inhibitors based on available parental inhibitor which are binding
more potentially then than of available inhibitors. Based on the molecular interaction studies better drugs could be designed at a prime line level, lead molecule modification and designing, leads to identification of protein ligand interactions which do aid to rationalize effective Anti-HIV drugs. For a virtual screening of protein and ligand molecules, Chemiinformatics and Bioinformatics have provided tools for enhanced experimental research. This paves way for researchers to further analyze the data obtained through computational studies. The systematic stepwise analysis conducted in the present thesis on "*In silico Screening of Effective inhibitors for HIV-1 Protease, Integrase and Reverse Transcriptase using pharmacophore model*" yields useful information to design better drug formulations by pharmacists abiding to pharmaceutical norms.