In-Silico Studies and Lead Designing of Human Immunodeficiency Virus Inhibitors

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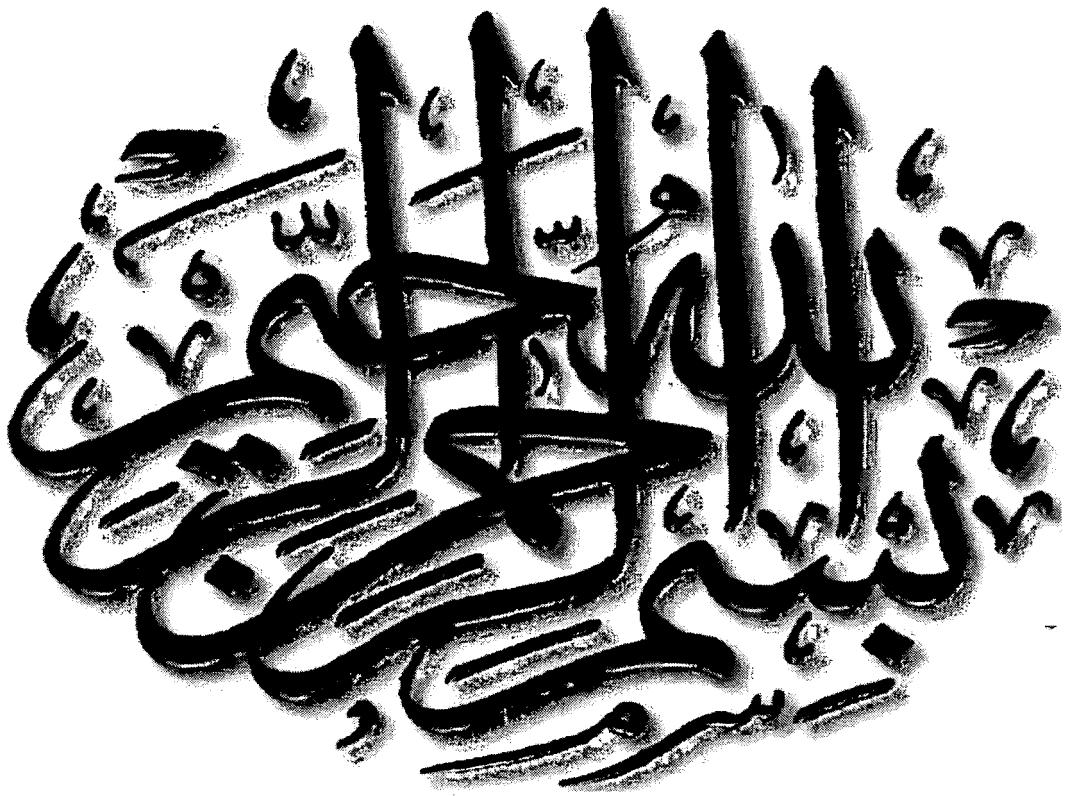

In the name of Allah Most Gracious and Most Beneficial

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where acknowledged otherwise, and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Pakeeza Akram

Certificate

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LIST OF ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
Å	Angstrom
AIDS	Acquired Immune Deficiency Syndrome
Ala	Alanine
Arg	Arginine
Asp	Aspartic Acid
CDC	U.S. Centers for Disease Control and Prevention
COMFA	Comparative Molecular Field Analysis
COMSIA	Comparative Molecular Similarity Indices Analysis
DNA	Deoxyribose Nucleic Acid
FDA	Food and Drug Administration
HAART	Highly Active Antiretroviral Therapy
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HIV	Human Immunodeficiency Virus
HIV PI	Human Immunodeficiency Virus Protease Inhibitor
HIV PR	Human Immunodeficiency Virus Protease
HOMO	Highest Occupied Molecular Orbital

HTS	High Throughput Screening
Gly	Glycine
IC_{50}	Half Maximal Inhibitory Concentration
Ile	Isoleucine
KP	Kaposi's Sarcoma
Log P	Partition Coefficient
LUMO	Lowest Unoccupied Molecular Orbital
Lue	Leucine
MOE	Molecular Orbital Environment
PCP	Pneumocystis Carinii Pneumonia
PDB	Protein Data Bank
Phe	Phenylalanine
PM	Pharmacophore Mapping
Pro	Proline
QSAR	Quantitative Structure Activity Relationship
Val	Valine
VS	Virtual Screening

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Starting from the foremost I thank to Almighty Allah (SWT); the creator who created me a human being and the Sovereign and the All Knowing, who showered such a knowledge upon me; the Guider who guided me well and broaden my understanding to differentiate between right and wrong; the Source of Peace an Provider due to Whom I was able to perform my task even in the time of severe depression due to loss of my loving Father; the loving and the Opener, Who opened the door leading to success generally throughout my life and specifically during my research. I am not even a particle without His soft and kind always upon my head.

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Last but not the least my deepest and cordial thanks to all those sacred souls especially my Papa watching me out there and not able to celebrate my success with me. My celebration is incomplete without them. May their souls rest in peace and May I follow the path taught by them throughout my life. Ameen

I pray to Allah (SWT) that may He bestow me with true success in all fields in both worlds and shower His blessed knowledge upon me for the betterment of all Muslims and whole Mankind.

Ameen

Pakeeza Akram

ABSTRACT

The advent of Computer-aided drug designing and discovery along with flavor of bioinformatics leads to the betterment of mankind by immediate result prediction and development specifically in human pathology. A pharmacophore is built from knowledge of the structure of the novel drugs. Ligand-based pharmacophore modeling is carried out on a set of 45 compounds alongside 4 different classes' of compounds were superimposed and all of them showed three common features: 2 hydrophobic units, 4 hydrogen bond acceptor and 2 hydrogen bond donor. *In-silico* approaches have been used to determine the pharmacophore triangle. Molecular docking study was conducted in order to identify the lead compound as the HIV protease inhibitor. AutoDock Vina was used for docking studies of data set and the target protein used was PDB ID: 1EBZ, the binding interactions of the active conformations of the ligands and the target protein have been identified by using VMD. Lead compound showed strong ligand-protein interaction which includes 3 hydrogen bonds and 6 hydrophobic interactions and IC_{50} value 0.000083 μM and Binding energy is 11.3Kcal/mol. Four analogues of the lead compound were made. They were also docked in order to predict their bioactivity. Quantitative structure-activity relationship was established to find dependency trend in HIV Protease Inhibitors and various molecular descriptors. Molecular descriptors were calculated and correlation was determined between IC_{50} and Heat of formation and E_{LUMO}. On the basis of above computational studies some new compounds were identified that act as HIV protease inhibitor and these new analogues have increased binding interactions.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Human Immunodeficiency Virus (HIV) is being the point of interest for the research hub since its identification. Numerous scientists placed their brick to develop a hospital for the cure of infection caused by HIV specifically Acquired Immune Deficiency Syndrome (AIDS). Still it seems that it requires much more exertion to complete this target and it should be done on priority basis. Statistical analysis suggest that on the globe number of people living with HIV in 2008 was total 33.4 million, people newly infected with HIV in 2008 total 2.7 million, AIDS-related deaths in 2008 is in total 2.0 million [1.7 million–2.4 million] (UNAIDS *et al.*, 2009).

The major reason behind choosing Protease Inhibitors as research issue is that it is the one of the best known drug type to cure HIV related Infections but an extended period in the treatment results in resistance where mutation enables natural substrate i.e. precursor poly protein again more prone to the HIV protease. Also viral replication of HIV itself is error-prone due to its inefficient proofreading process and most important all of the PI have low bioavailability. This is the major reason that although having 9 Food and Drug Administration (FDA) approved drugs as HIV Protease inhibitors; still the need of new potent compounds is demand of the world that is effective, selective and efficient.

Using *in-silico* drug designing techniques it is promised that novel drug for the treatment of HIV related infections such as AIDS will be developed in short time span.

Thus the hypothesis set forth states *"To identify lead compound that is potential candidate as HIV Protease Inhibitor using in-silico techniques by reducing the time required to develop new drug focusing on drug bioavailability by increasing binding interactions"*

- During this study a pharmacophore model is generated using the information derived from data set, as yet there is not a single confined pharmacophore model identified for the HIV protease inhibitors. Therefore it will contribute positively to the wild river of HIV pandemic.
- Secondly, a protocol is designed that will help in the *in-silico* drug development. This protocol incorporates pharmacophore modeling, molecular docking and quantitative structure-activity relationship (QSAR). This investigation resulted in identification of lead compound and its analogue formation having tendency to be next potential drug candidate.
- 2D QSAR analysis was also observed in which molecular descriptors were calculated and correlation was determined which resulted in finding relationship among certain biological activity and pharmacological descriptors.
- **Effectiveness of work states:** Lead compound was identified that enhance the therapeutic ability and will help to cure HIV infections such as AIDS by increasing the binding interactions and its bioavailability.

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CHAPTER 2

LITERATURE REVIEW

2.1 LITERATURE REVIEW

"Health has its science, as well as disease." --Elizabeth Blackwell

Health is negatively defined as the absence of illness; functionally as the ability to cope with everyday activities and positively as fitness and well-being (Bury M *et al.*, 2005). Health refers to homeostasis in input and output of energy necessary for growth and survival. Mortal nature of human leads him prone to illness and affect health since man creation but thanks to medicinal science that helps to combat with human health issues by identifying and curing certain diseases which elsewhere cause havoc to mankind. To find a cure for certain diseases is a strenuous task because sometimes the disease is not complex but symptoms related to disease occur too late to be treated e.g. hepatitis C and sometimes genes involved in causing certain disease are very complex and have structural variability e.g. HIV related infections.

The story of HIV is filled with conquest and defeat, success and loss, life and death. Perhaps the history began between 1980 and early 1981 when the first case of AIDS was observed. A group of five men showed symptoms of Pneumocystis Carinii Pneumonia (PCP) which is a rare opportunistic infection with a suppressed immune system. Along with another set of men who developed a rare skin cancer called Kaposi's sarcoma (KP) (CDC *et al.*, 1982; Barre *et al.*, 1983). U.S. Centers for Disease Control and Prevention

(CDC) was alarmed after several similar cases. A CDC task force monitored the outbreak by recognizing a pattern of symptoms present in patients and called this condition as acquired immune deficiency syndrome (AIDS) (Basavapathrunj *et al.,* 2007) compelling the scientists to identify the cause of this suppressed immune system condition. In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients (RC Gallo *et al.,* 1983; F Barre-Sinouaai *et al.,* 1983). Initially three different names were given to the virus isolated from AIDS patients but finally in 1986 eventually the AIDS-causing virus was given a comprehensive name, human immunodeficiency virus (HIV) (Coffin J *et al.,* 1986). HIV is a member of the genus Lentivirus, (NIH *et al.,* 1986) part of the family of Retroviridae (NIH *et al.,* 1986). Lentivirus infects many species and they are characterized by long-duration illnesses with a long incubation period and suppressed immune system (Levy J. A. *et al.,* 1993).

AIDS epidemic was like treating the untreatable but it influenced scientists who speeded up medicinal research for the development of compounds that inhibit the viral replication and halt the infections caused by them. In 1986, first compound, Suramin, inhibiting HIV replication both in vitro and in vivo was discovered (Smith Johanna A *et al.,* 1993). Discovery of this pioneer compound involved in the treatment of HIV opened a door of research for another field which set forth 25 anti-HIV compounds licensed and formally approved in nearly 29 years (Basavapathruni A *et al.,* 2007).

Every step of the HIV cycle can be considered as a potential drug target but anti-HIV chemotherapy decrease the number of potential targets as HIV is an intercellular parasite and may interrupt with host cell metabolic pathway causing the host cell death. It was the

reason scientist focus only on those processes which are essential for the survival of virus and inhibit them keeping in mind that it will not hurt the host cell (RC Gallo *et al.*, 1983; F Barre-Sinoussi *et al.*, 1983). Virus specific processes which may not infect host cell are: viral binding to host cell, virus cell fusion with host cell, virus uncoating, reverse transcription of genomic RNA, viral integration, and protease activity (Levy J. A *et al.*, 1984; Brun Vezinet *et al.*, 1984; Coffin, J *et al.*, 1986; NIH *et al.*, 2006)

Protease is a diverse class of enzyme that catalyzes the cleavage of peptides or proteins. HIV-1 protease is one of the best known aspartic proteases. The aspartic proteases are well-characterized group of enzymes that can be found in vertebrates, plants and fungi. Examples of aspartic proteases are Pepsin, Renin and Penicillopepsin. These all enzymes are two-domain with more than 300 residues in length and contain the Asp-Thr-Gly sequence in each domain that forms the active site and helping in cleavage reaction (Birch MR *et al.*, 2001; Dyer WB *et al.*, 1997).

HIV Protease (PR) cleaves newly synthesized poly-proteins to create the mature protein components of an infectious HIV virion (Greene *et al.*, 1993; Turner *et al.*, 1999). It is observed that mutation of active site of HIV PR or its activity inhibition interrupt ability of HIV to replicate and infect more host cells (McDougal, J. S *et al.*, 1986). It leads to HIV PR inhibition the subject of medicinal research for the treatment of HIV infections (Feng Y *et al.*, 1996). HIV gag–pol gene encodes translational products as shown in Figure 2.1. It also represents the sites at which the gene product is cleaved by the virus-encoded protease. Each gene performs its own specific such as p17 gene denotes capsid protein, p24 is a matrix protein, and p7 is a nucleocapsid protein; but p2, p1, and p6 are small proteins and their function is still unknown.

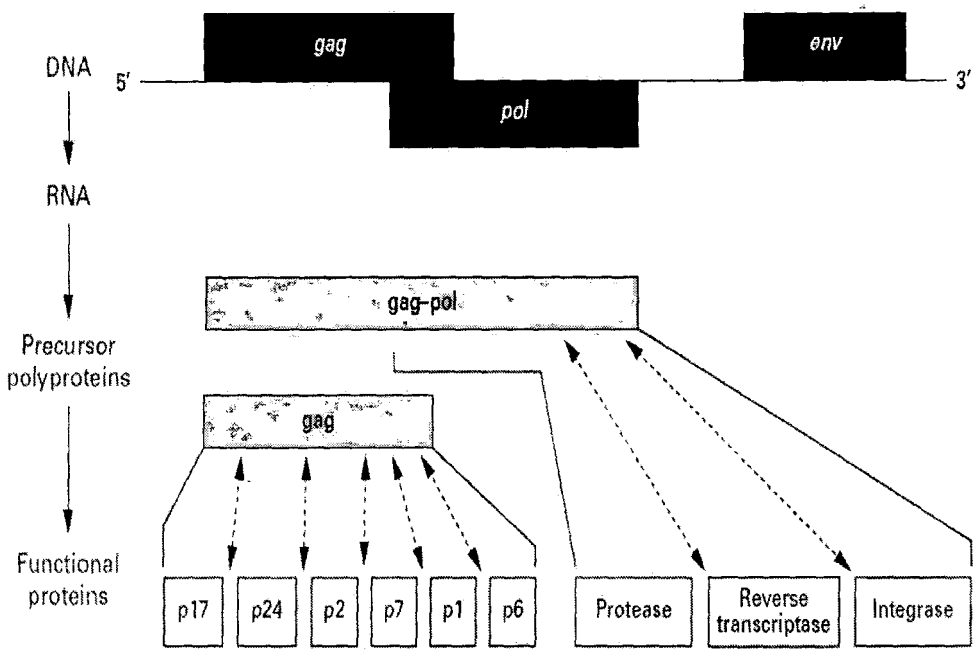


Figure 2.1: The translational products of the HIV gag–pol gene and the sites at which the gene product is cleaved by the virus-encoded protease.

The arrows denote cleavage events catalyzed by the HIV-specific protease. HIV protease protein structure has been investigated using X-Ray crystallography. It exists as a homo-dimer, with each subunit made up of 99 amino acids with a single active site (Doranz B.J *et al.,* 1996). There are three domains in HIV PR first the **terminal domain or dimerization domain**, this domain is quite crucial in dimer formation and stabilization of an active protease, second is the **core domains**, it contain active site and make the center of the protein. The conserved Asp25-Thr26-Gly27 forms catalytic triad of HIV Protease. The two Asp25 residues (one from each chain) act as the catalytic residues (Moore J. P *et al.,* 1997) and the third is the **flap domains**, they are flexible flaps which enclose the active site and provide important ligand binding interactions (A Wlodawer *et al.,* 1989). Under the normal condition, two identical monomers (folded) interact to form an active C2-symmetric PR dimer (Alfredo G. Tomasselli *et al.,* 1999). HIV I protease has in addition a second highly conserved sequence of Gly-Arg-Asn amino acids 86-88, which are located on the C-terminal domain and is not present in host cell domain and can be used as drug target (Pearl L.H *et al.,* 1987). The third highly conserved sequence is Asp29 which make it a specific structure for substrate binding (Blundell. T *et al.,* 1989). These highly conserved and unique sequences allows researcher to design specific inhibitors capable of recognizing the viral protease only and not the host protease. Complete structure of HIV protease along with its domain is given in Figure 2.2.

Detailed study of HIV Protease structure and the substrate which bind to it provide wide range of information that is constructive for the development of novel and specific protease inhibitors (PIs). Inhibitor incorporating high binding affinity and more space then the natural substrate has more potential to be the next candidate drug. HIV PIs bind

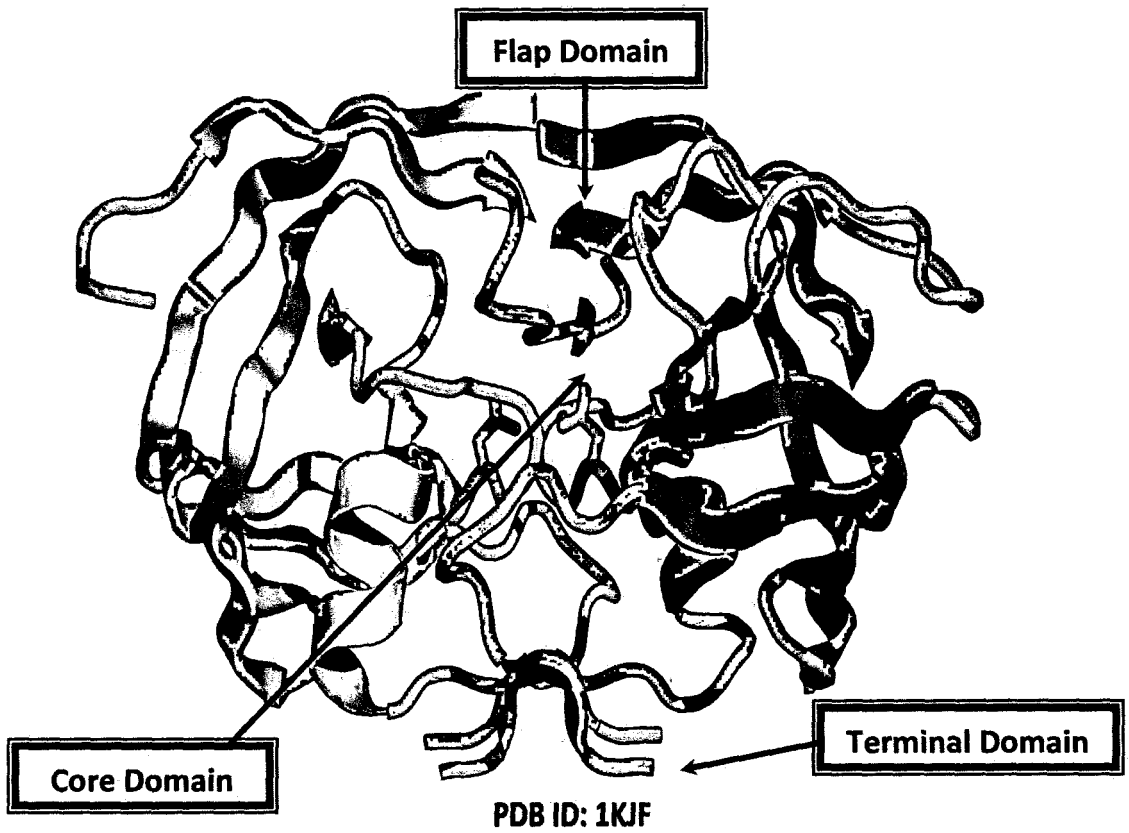


Figure 2.2: HIV Protease showing the three domains i.e. Flap Domain, Terminal Domain and Core Domain having active site

to the active site in competition with its natural substrate on the basis of structural and chemical properties along with thermodynamic stabilities thus they behave as a competitive inhibitor (Annemarie M. J *et al.,* 2009). They can be administered as single drug i.e. Monotherapy or as a combination of multiple drugs known as Highly Active Antiretroviral Therapy (HAART) (Annemarie M. J *et al.,* 2009).

HIV protease Inhibitor can be broadly categorized in to two major groups one is known as Peptide-based inhibitors and the other is Non-Peptide based inhibitors (D.J Kempf *et al.,* 1996). Except Tipranavir which contains a di-hydropyrone ring as a central part, all the other peptidomimetic drugs have core of hydroxyethylamine (Turner S.R *et al.,* 1998) as shown in Figure 2.3.

During the last 29 years of AIDS only 9 drugs were approved that are protease inhibitors and are in clinical use (Annemarie M.J *et al.,* 2009). These protease inhibitors along with their properties are listed in Table 2.1 (Craig J.C *et al.,* 1991; Kempf D.J *et al.,* 1995; Koh Y *et al.,* 2003; Partaledis J.A *et al.,* 1995; Patick A.K et al 1996, Robinson B.S *et al.,* 2000; Sham H.L *et al.,* 1998; Vacca J.P *et al.,* 1994).

These drugs have limited efficacy because of the natural selection phenomenon of protease variants which are catalytically competent with low affinity of drug than the wild type enzyme (Annemarie M.J *et al.,* 2009). Another issue is that high error rates of the HIV reverse transcriptase changes the DNA sequence which also affects the HIV Protease in the forth coming generations (Hendrik Weisser *et al.,* 2010). This continuous mutational change results in a different amino acid sequence of PR purified from a patient then from the wild type.

Table 2.1: FDA approved HIV Protease Inhibitors along with their properties

	Drug Name	Type	Year	Properties
1	**Saquinavir**	Peptidomimetic	1995	It is the first HIV protease inhibitor. Saquinavir is effective against both HIV-1 and HIV-2. Usually well tolerated but high serum concentration is not achieved.
2	**Ritonavir**	Peptidomimetic	1996	Designed to fit the C2-symmetry in the binding site of the protease. Gastrointestinal side effects and a large pill burden. Only used in a combination therapy with other protease inhibitors for pharmacokinetic boosting.
3	**Indinavir**	Peptidomimetic	1996	It increased potency.
4	**Nelfinavir**	Non-Peptide	1997	It was the first protease inhibitor that was not peptidomimetic.The first protease inhibitor to be indicated for pediatric AIDS.
5	**Amprenavir**	Non-Peptide	1999	It gives better oral bioavailability. Amprenavir was withdrawn from the market in 2004.
6	**Lopinavir**	Peptidomimetic	2000	It was originally designed to diminish the interactions of

G *et al.,* 1998), Chem Draw (A Zielesny *et al.,* 2005), Hyper Chem (Tsuji M *et al.,* 2010) and many more are used for finding molecular descriptors.

Chem Draw Ultra, using an add-on, Chem Prop/Draw, calculates predicted values for physical and thermodynamic properties of a selected structure of up to 100 atoms. (Loren D *et al.,* 2004) Chem draw software package is a chemical structure drawing tool which enables several features upon the drawing of structure which includes boiling point, melting point, and critical volume, heat of formation, Log P and molar refractivity (MR) Minimization of the energy of the compound is done by using Hyper Chem. Energy minimization alters molecular geometry to lower the energy of the system, and yields a more stable conformation. It generates a log file using computational chemistry techniques such as semi-empirical formula, molecular mechanics etc (hypercube *et al.,* 2002).

CHAPTER 3

METHODOLOGY

Protocol compiling the long story of indentifying lead compound for the cure of HIV-1 Protease is summarized in the figure 3.1.

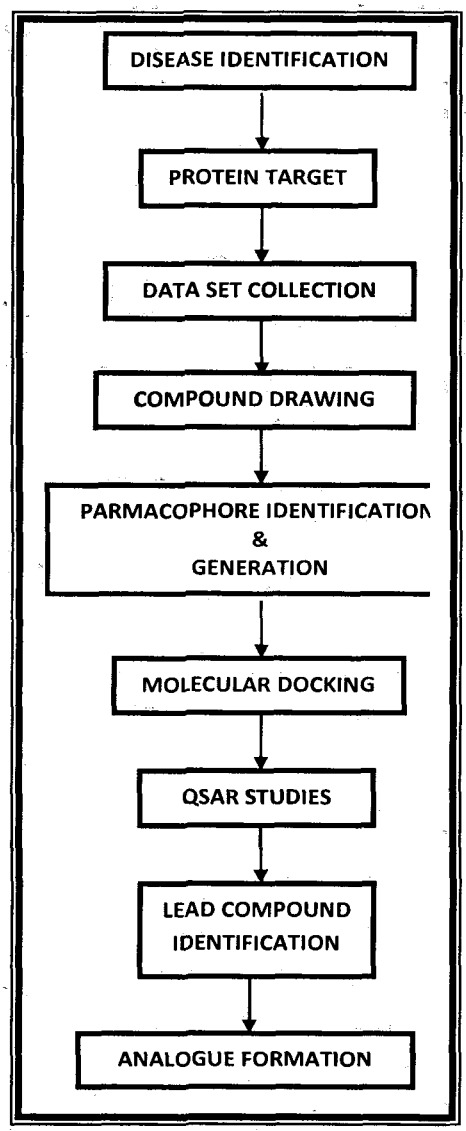


Figure 3.1: Protocol for the *Insilco* drug designing and development

As the pioneer step disease was identified leading to the potential protein target. Further data collection was incorporated; it was followed by drawing of drugs using 2D view. Later Pharmacophore identification and generation was done it led to the docking of compounds which resulted in the lead compound identification and analogues formations after this QSAR analysis was done. Each step of the protocol along with the challenges faced is discussed below.

3.1 DISEASE IDENTIFICATION

AIDS was selected as disease because it is considered as a pandemic. Susceptible individuals are increasing day by day and it is estimated that in 2007, a total of 2.0 million men, women, and children died of AIDS worldwide. The study states that death toll will remain high in the future because 33 million individuals were infected in 2009 and about 2.7 million new HIV infections occur each year (John Bongaarts *et al.*, 2009). In an attempt to combat this pandemic and make a novel drug this study is a subsequent contribution.

3.2 PROTEIN TARGET

There are multiple protein targets that can be considered to cure AIDS. Among several targets, HIV Protease is considered and for this study protein structure was taken from Protein Data Bank. The protein structure taken for this study was a wild type having PDB ID: **1EBZ** (Andersson HO *et al.*, 2003).

3.3 DATA SET FORMATION

Data set was made keeping in mind some considerations such as all the compounds had passed through bioassay and have reported IC_{50} value. The range of IC_{50} value up to 100 μM was only considered. Secondly it was considered that data set must be composed of

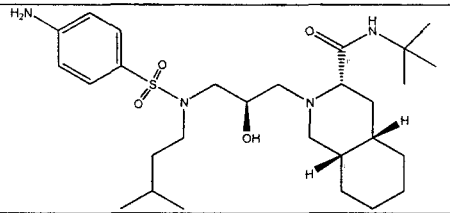
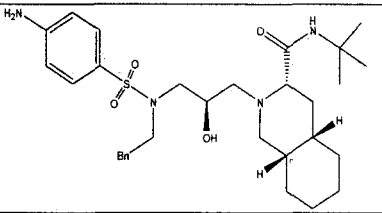
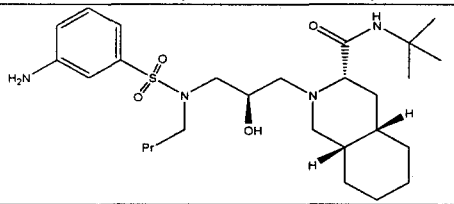
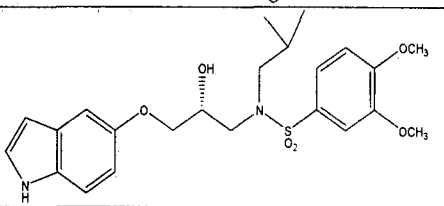
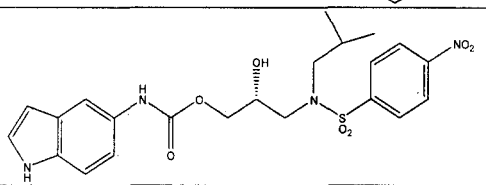
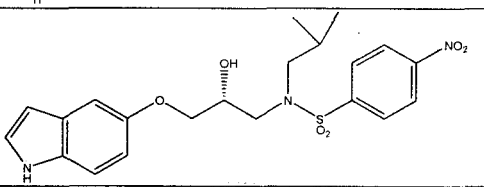
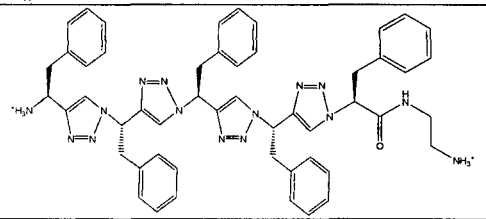
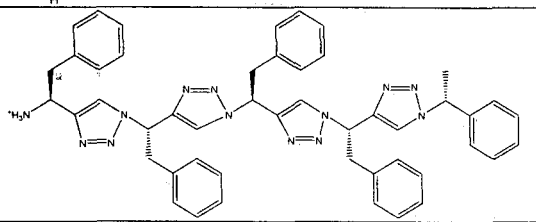
different classes of compounds having numerous functional groups so that highly active and potent lead is identified from a vast data set lastly all the selected compounds for this study must not be reported earlier than 2005 and all the drugs have HIV-1 Protease inhibition activity and can also take part in combinatorial drug therapy i.e. Highly Active Antiretroviral Therapy (HAART). Numerous anti-HIV protease drugs were studied and selected for this research, these drugs belongs to different classes having distinct functional groups. Along with these compounds some FDA approved drugs were also incorporated to be taken as standard drugs. The data consisted of 45 compounds in total (Lijun ZHOU *et al.,* 2008; Lucia Chiummiento *et al.,* 2009; Andrea L *et al.,* 2009; Abbas Raza *et al.,* 2008; Haibin Shi *et al.,* 2009; Michael Waibel *et al.,* 2009, Ying Wei *et al.,* 2009; Ying Wei *et al.,* 2009; Vladimir Frecer *et al.,* 2008; Ei'ichi Ami *et al.,* 2007).

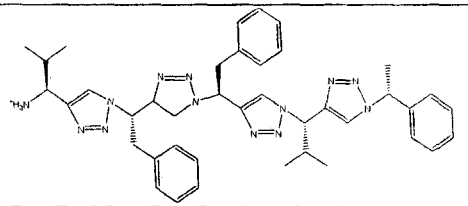
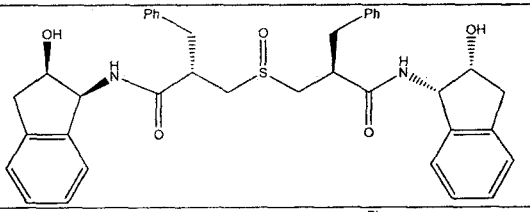
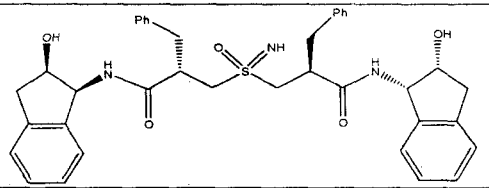
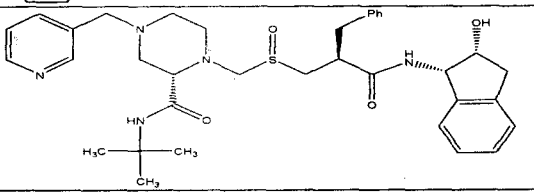
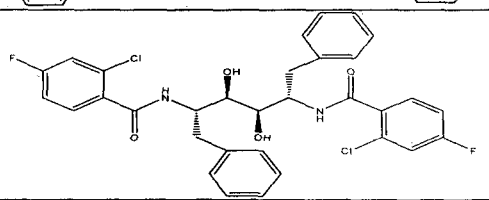
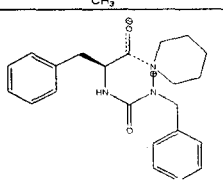
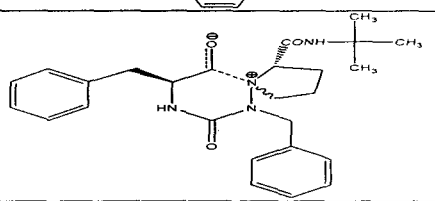
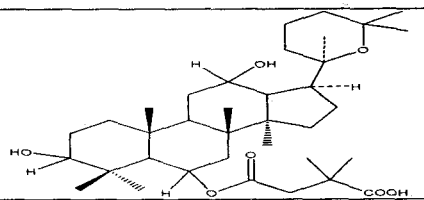
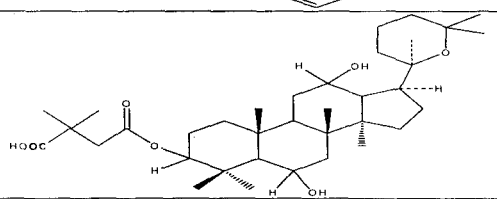
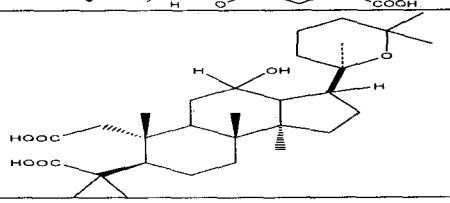
All the compounds were selected by keeping the IC_{50} value level under 100 μM. The standard drugs used are Amprenavir, Indinavir, Nelfinavir, Ritonavir and Saquinavir and they have IC_{50} values ranging from 0.01, 1.5, 1.05, 0.38, 0.42 (Lijun ZHOU *et al.,* 2008; Lucia Chiummiento *et al.,* 2009; Andrea L *et al.,* 2009; Abbas Raza *et al.,* 2008; Haibin Shi *et al.,* 2009; Michael Waibel *et al.,* 2009, Ying Wei *et al.,* 2009; Ying Wei *et al.,* 2009; Vladimir Frecer *et al.,* 2008; Ei'ichi Ami *et al.,* 2007; DALE J *et al.,* 1997). All the compounds are shown in Table 3.1 along with their IC_{50} Values.

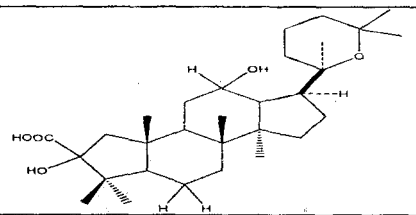
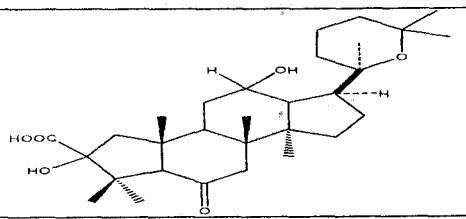
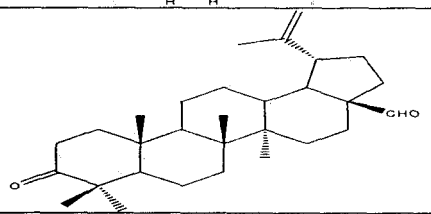
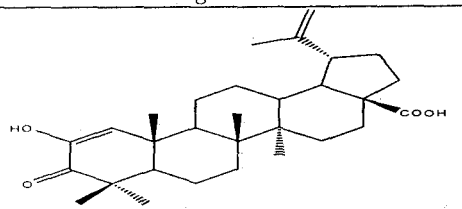
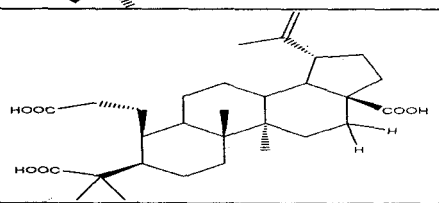
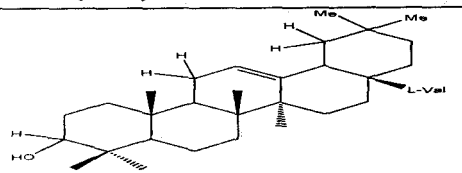
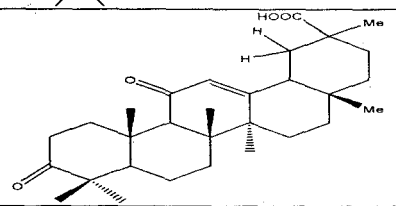
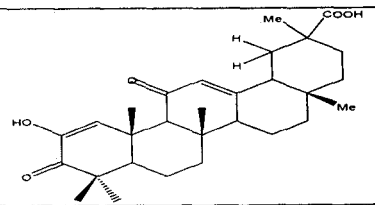
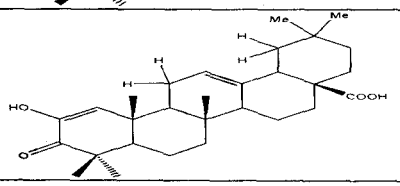
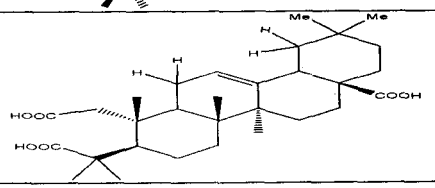
3.4 COMPOUNDS DRAWING:

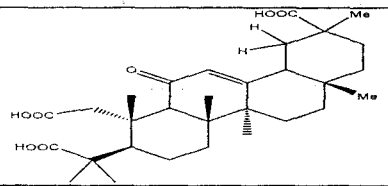
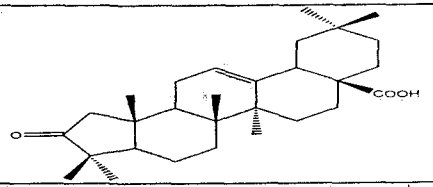
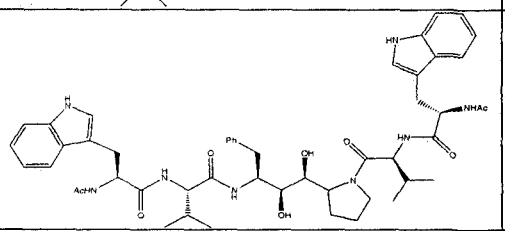
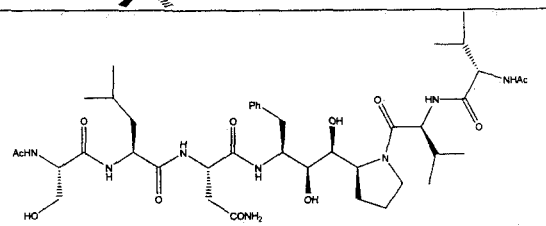
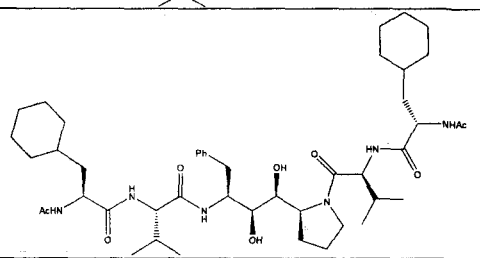
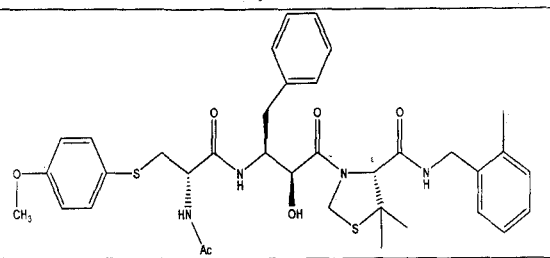
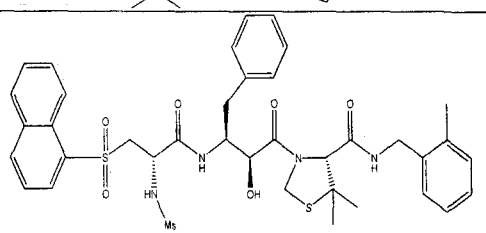
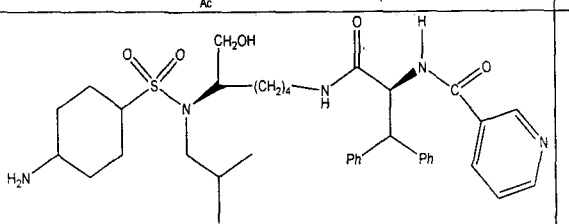
Many types of software are available for the drawing and representation of compounds. All the above mentioned compounds were drawn using Chem Draw Ultra Version 8.0 (Cambridgesoft.com) (Loren D *et al.,* 2004). Chem Draw provides user friendly drawing environment where one can draw structure by simple drag and drop method.

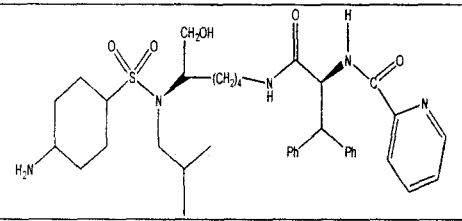
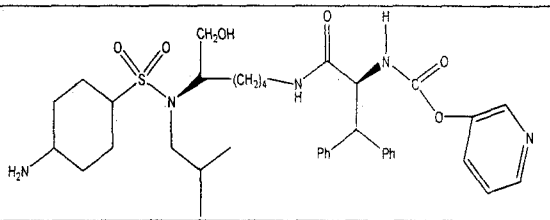
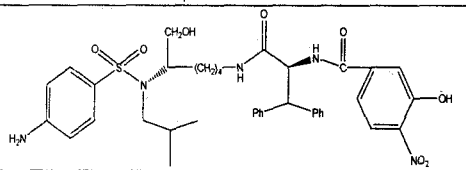
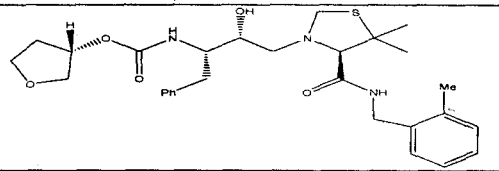
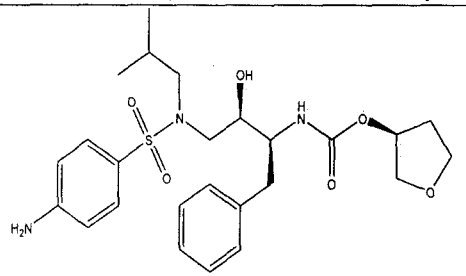
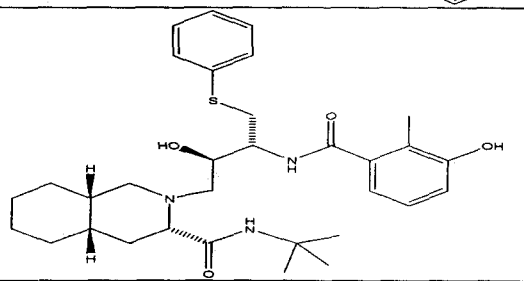
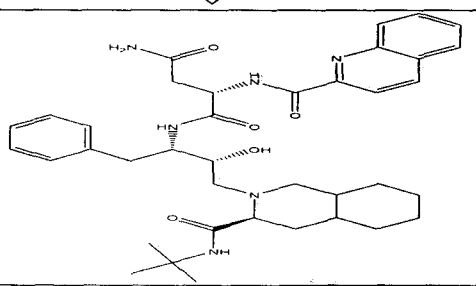
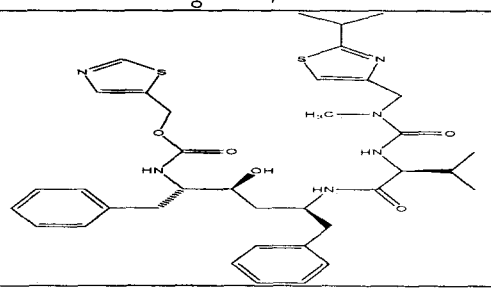
Table 3.1: Molecular Structures along with their IC_{50} Values of the dataset

Compound	STRUCTURE	IC_{50} (µM)	Compound	STRUCTURE	IC_{50} (µM)
PHIV 1		0.02	PHIV 2		0.05
PHIV 3		0.38	PHIV 4		1.04
PHIV 5		2.4	PHIV 6		10.3
PHIV 7		25	PHIV 8		29

Compound	STRUCTURE	IC_{50} (μM)	Compound	STRUCTURE	IC_{50} (μM)
PHIV 9		60	PHIV 10		0.021
PHIV 11		0.0025	PHIV 12		100
PHIV 13		0.3954	PHIV 14		37.2
PHIV 15		44.5	PHIV 16		2.7
PHIV 17		3.9	PHIV 18		11.9

Compound	STRUCTURE	IC_{50} (µM)	Compound	STRUCTURE	IC_{50} (µM)
PHIV 19		10.0	PHIV 20		29.9
PHIV 21		31.9	PHIV 22		18.8
PHIV 23		15.7	PHIV 24		10.6
PHIV 25		23.6	PHIV 26		15.8
PHIV 27		19.2	PHIV 28		3.9

Compound	STRUCTURE	IC_{50} (μM)	Compound	STRUCTURE	IC_{50} (μM)
PHIV 29		5.7	PHIV 30		28.6
PHIV 31		0.00006	PHIV 32		0.0006
PHIV 33		0.19	PHIV 34		0.00018
PHIV 35		0.00008 3	PHIV 36		0.24

Compound	STRUCTURE	IC_{50} (µM)	Compound	STRUCTURE	IC_{50} (µM)
PHIV 37		0.12	PHIV 38		0.48
PHIV 39		0.33	Inactive		nt
Amprinavir		0.01	Nelfinavir		1.05
Saquinavir		0.42	Ritonavir		0.38

3.5 2D AND 3D PHARMACOPHORE GENERATION:

The next step was to identify the pharmacophore of each compound. For the creation of pharmacophore Ligand Scout Software, Version 3.0, (Cambridge Med-Chem et al., 2009) was used in studies. It generates both ligand based and structure based pharmacophore models. As ligand based drug designing was main focus of this study therefore ligand based pharmacophore model was generated for the dataset. Input file for this study was in .mol format which was taken from Chem Draw software. Pharmacophore was generated by using simple commands. Pharmacophore of data set was compiled in the same manner. All the pharmacophore features i.e. aromatic rings; hydrophobic region, hydrogen bond acceptor and hydrogen bond donor were studied, predicted and compared. The common featured pharmacophore was identified and was then taken from Ligand Scout by the superimposition of the ligand. At the same time to more clearly verify the pharmacophore Visual Molecular Dynamics Software Version 1.8.7 (William Humphrey *et al.*, 1996) was used which along with visualization also help in calculating the distances. At the end unique pharmacophore and the pharmacophore triangle had been predicted for anti HIV protease.

3.6 MOLECULAR DOCKING:

Docking phase is meaningless without its two components target protein and ligand. To incorporate docking specific target protein should be identified thus PDB ID: **1EBZ** (Andersson HO *et al.*, 2003) met all the requirements to be a suitable protein and was taken from Protein Data Bank (RCSB) (pdb.org). Docking was done using software AutoDock 4.0 and its patch AutoDock Vina (Chang MW *et al.*, 2010).

3.6.1 STEPS FOR MOLECULAR DOCKING

Autodock 4.0 was used to modify the protein structure as well as the ligand structure. The pdb file of the macromolecule was taken as input of the software, modifications were done and macromolecule was saved in '.pdbqt' format. Similarly ligand file was modified by giving '.pdb' file of ligand as input. This allows the program to calculate its parameters such as non-polar hydrogen, aromatic carbons and rotatable bonds along with the ligand torsions. Simultaneously Gasteiger charges were added. Later torsions were modified by making amide bonds non-rotatable. Ligand file is saved with extension '.pdbqt' format. After that a text file which contains majorly four features: macromolecular file in '.pdbqt' format, ligand file in '.pdbqt' format, and 3D location of the grid from where docking algorithm search for docked site was made. Grid location depends upon the active site of the protein if it is the central pocket then grid must point at the center and so on and so forth. In case of HIV-1 Protease as mentioned earlier there is only one active site located at the center of the protein therefore grid pointer was set at the center where center points of x axis = 15.936, y axis= 26.459, z axis = 3.425 respectively and size of the affinity grid was set to 50 x 50 x 50 and then the docking was conducted.

3.6.2 LIGAND-PROTEIN INTERACTION

The ligand-protein interactions were predicted using Visual Molecular Dynamics (VMD), Version 1.8.7, (William Humphrey *et al.,* 1996). The active conformation of each ligand was obtained after docking studies was used to modify the .pdb file of the target protein and this was given as an input to the VMD. Then the interactions between the ligand and active site of the target were identified selecting the atoms within 5.0 Å. On the basis of

these detailed studies lead compound was identified. Binding interactions of all compounds were observed thoroughly and the compound showing the best interactions along with the energy value and IC_{50} among all was identified as Lead. Four structural analogues of the lead were made by introducing or eliminating various functional groups in it, focusing on increasing and decreasing hydrophobicity and hydrophilicity. Docking studies on the analogue were then performed using the same process using Auto Dock Vina.

3.7 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

A quantitative structure activity relationship was interpreted by calculating a number of electronic and steric parameters by the use of Chem Draw (Loren D *et al.*, 2004) and Hyper Chem (HyperChem *et al.*, 2002). Electronic parameter was studied by Hyper Chem which generates a log file. Semi-Emperical formula was used while Chem Draw was used for studying steric parameters which were calculated on several mouse-clicks.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 DATA SET FORMATION

Peptidomimetic and Non-Peptide groups of HIV-Protease both were taken into account for analysis. It incorporated 10 classes as the functional groups, making the total of 45 compounds in the data set. These compounds included 5 FDA Approved drugs which were taken as standard drugs along with 1 inactive compound and rest 39 as the potential hits for this study. These various compounds belong to following classes: Isopropanaol Amine, Indolic (S)-Glycidol, Triazolamers, Sulfoximine, Symmertric Asymmetric Diol, Hydrazino-Urea, Dammarane-type tritepene, Triterpenoids, Peptidomimetic dihydroxyethylenediamine, Allophenylnorstatine- incorporating D-Cysteine, and Sulfonamide. These entire compounds belong to the two major groups i.e. Peptidomimetic and Non-peptide, among 39 candidate hits 30 compounds belong to the Peptidomimetic and rest 9 belong to Non-Peptide groups of HIV Protease Inhibitors (Lijun ZHOU *et al.*, 2008; Lucia Chiummiento *et al.*, 2009; Andrea L *et al.*, 2009; Abbas Raza *et al.*, 2008; Haibin Shi *et al.*, 2009; Michael Waibel *et al.*, 2009, Ying Wei *et al.*, 2009; Ying Wei *et al.*, 2009; Vladimir Frecer *et al.*, 2008; Ei'ichi Ami *et al.*, 2007).

4.2 RULE OF FIVE

Although all the drugs have undergone the bioassay, to counter check there drug-likeness properties, In-Silico techniques i.e. rule of 5 or Lipinski rule was applied to incorporate the pharmacokinetics of the drug. The results are given in Table 4.1.

Table 4.1: Lipinski's rule (Rule of Five) applied to complete data set

Compound No.	HBA	HBD	Molecular Weight (amu)	Log P
PHIV1	4	4	490.41	1.60
PHIV 2	4	4	526.44	1.21
PHIV 3	4	4	478.40	0.83
PHIV 4	6	2	432.32	-1.75
PHIV 5	6	3	464.32	-2.68
PHIV 6	6	2	422.31	-2.79
PHIV 7	9	3	838.6	4.10
PHIV 8	4	5	758.61	4.08
PHIV 9	4	4	662.53	3.64
PHIV 10	5	3	596.49	4.04
PHIV 11	4	4	610.49	4.72
PHIV 12	5	4	586.48	2.02
PHIV 13	6	4	585.27	3.83
PHIV 14	2	2	338.26	4.24
PHIV 15	2	0	416.31	3.27
PHIV 16	7	3	548.42	3.82
PHIV 17	7	3	548.42	3.82
PHIV 18	6	1	458.34	3.91
PHIV 19	5	2	444.36	3.89
PHIV 20	6	3	458.34	3.97
PHIV 21	2	1	392.33	0.72
PHIV 22	4	0	424.33	2.89
PHIV 23	6	0	458.34	4.88
PHIV 24	2	3	515.44	3.67
PHIV 25	4	2	426.34	2.96
PHIV 26	5	0	430.33	3.61
PHIV 27	4	0	416.35	3.53

Compound No.	HBA	HBD	Molecular Weight	Log P
PHIV 28	6	0	460.36	4.86
PHIV 29	7	0	474.34	4.95
PHIV 30	3	0	396.32	2.24
PHIV 31	7	6	840.60	1.17
PHIV 32	10	10	780.50	3.88
PHIV 33	7	7	764.54	4.22
PHIV 34	6	5	648.54	1.03
PHIV 35	5	5	736.62	0.68
PHIV 36	6	7	626.50	1.28
PHIV 37	6	7	642.50	2.39
PHIV 38	7	7	642.50	1.25
PHIV 39	8	4	686.51	-0.53
Amprenavir	5	3	470.35	1.49
Indinavir	3	5	566.43	4.20
Nelfinavir	5	5	524.45	2.83
Ritonavir	6	4	672.56	3.07
Saquinavir	6	6	620.46	2.78
Inactive	5	4	546.751	3.72

The results of rule of five show that all the compounds follow the HBA and Log P constraints but some compounds deviate from the regular rule of five and this is shown in Table 4.2.

As we can see that HBD and molecular weight features are not following the Lipinski rule in certain compounds but when compared to the standard drugs all the five standard drugs except Amprenavir have molecular weight greater than 500 and similarly Saquinavir (standard drug) have 6 HBD i.e. more than 5. So the result is verified and compatible with the standard drugs concluding that all the potential hits have druggable properties.

Table 4.2: Detailed Analysis of Rule of Five in percentage form

RULE OF FIVE CONSTRAINT	PERCENTAGE
Hydrogen Bond Acceptor	100%
Hydrogen Bond Donor	84%
Molecular Weight	48%
Log P	100%

4.3 PHARMACOPHORE MODELING

The chemical features which enhance the binding affinity to the target protein are always of keen importance. Thus the dataset selected from previous experiments and literature described in this study before (Lijun ZHOU *et al.,* 2008; Lucia Chiummiento *et al.,* 2009; Andrea L *et al.,* 2009; Abbas Raza *et al.,* 2008; Haibin Shi *et al.,* 2009; Michael Waibel *et al.,* 2009, Ying Wei *et al.,* 2009; Ying Wei *et al.,* 2009; Vladimir Frecer *et al.,* 2008; Ei'ichi Ami *et al.,* 2007) were taken for the search of spatial arrangements of chemical features that confers drug activity towards active target. The pharmacophore model of anti-HIV drugs has not been reported yet therefore it is an attempt to generate the general pharmacophore model.

Technique followed for identifying pharmacophore is reported in (Nighat N *et al.,* 2010). Ligand-Scout was used to generate the pharmacophore model of all the compounds of the dataset essentially focusing on the common features such as H-bonding and Hydrophobic volumes. The Figure 4.1 shows the pharmacophore features of standard drug Ritonavir.

Figure 4.2-4.6 represents the 3D and 2D view of compounds PHIV16, PHIV20, PHIV31, PHIV35 and PHIV39. These compounds are taken as the candidate compound of classes i.e. triterpene, Peptidomimetic dihydroxyethylenediamine, Allophenylnorstatine-incorporating D-cysteine and sulfonamide. Figure 4.2-4.6 shows that the compounds consist of hydrophobic unit, hydrogen bonds (acceptor and donor) and aromatic rings. Ligand Scout suggests that every single compound contains hydrophobic patch (yellow spheres), hydrogen bond donors (Green spheres) and hydrogen bond acceptors (red spheres) and rare aromatic rings (Blue Circles).

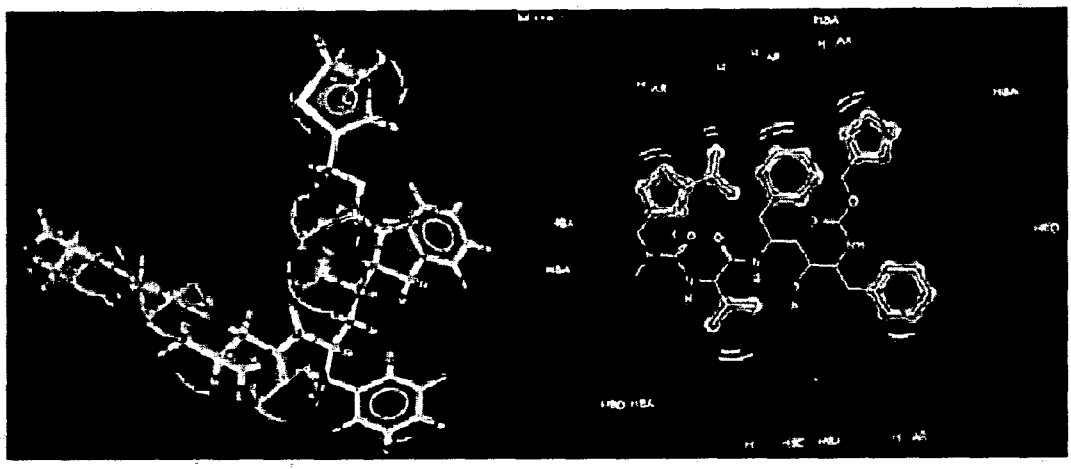


Figure 4.1: 3D and 2D Pharmacophore Model of Standard drug Ritonavir from LigandScout 3.0

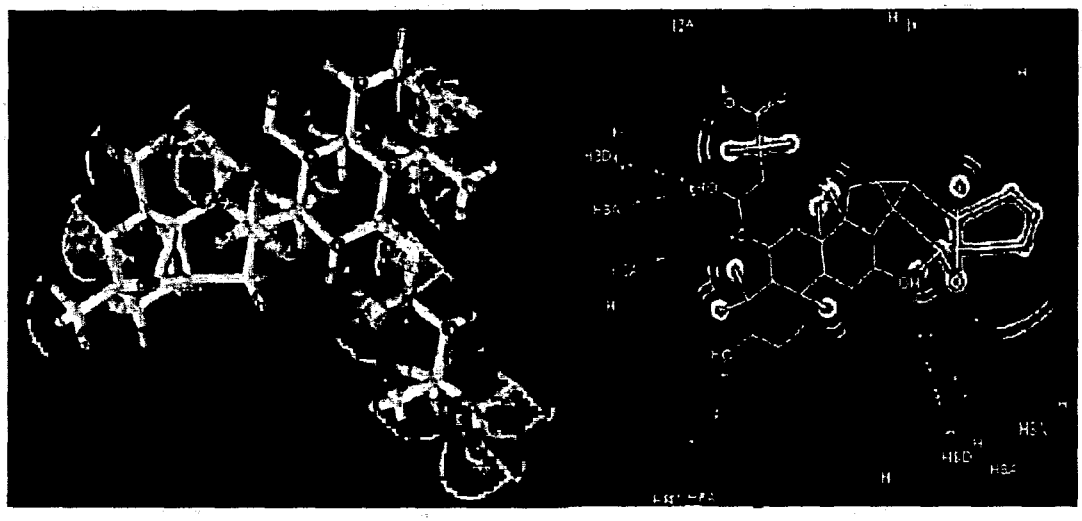


Figure 4.2: 3D and 2D Pharmacophore Model of PHIV16 from LigandScout 3.0

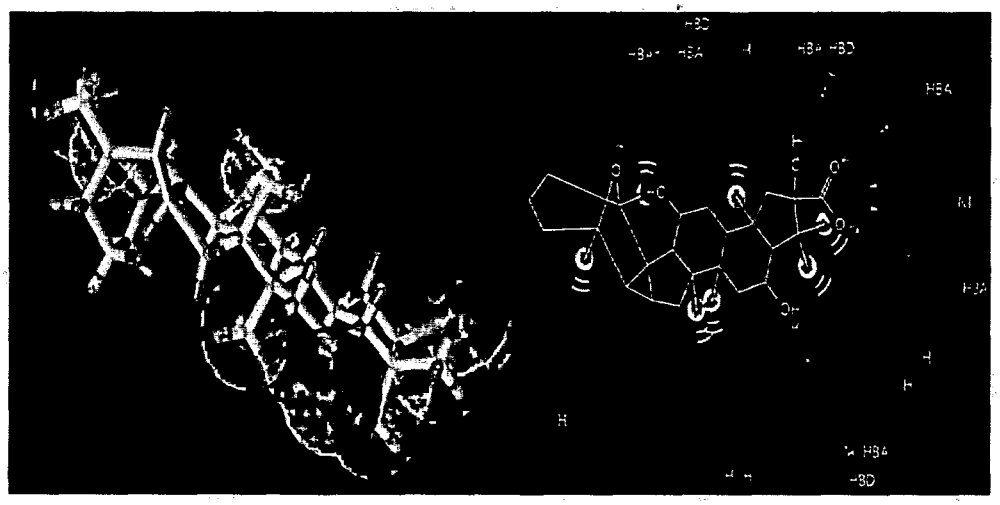


Figure 4.3: 3D and 2D Pharmacophore Model of PHIV20 from LigandScout 3.0

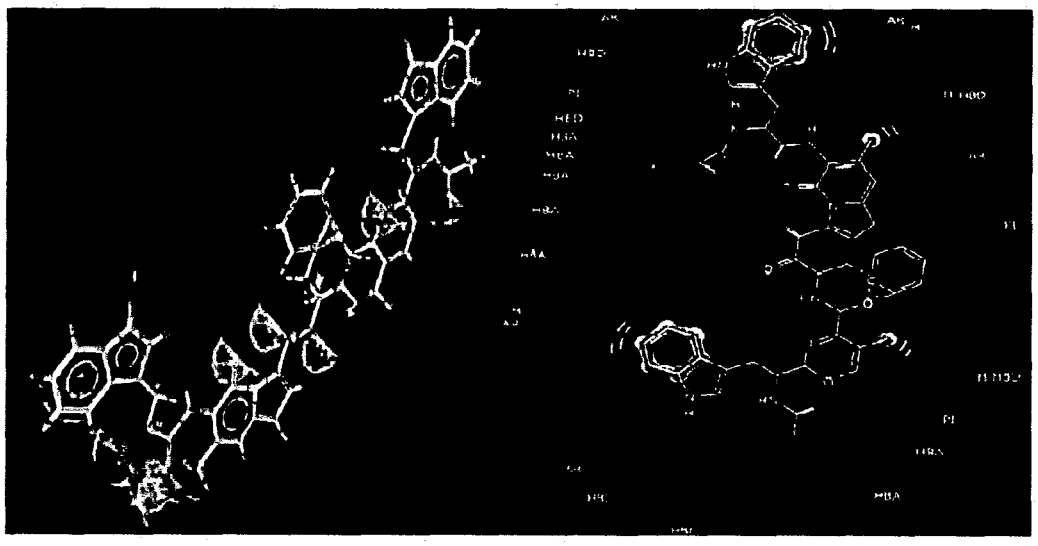


Figure 4.4: 3D and 2D Pharmacophore Model of PHIV31 from LigandScout 3.0

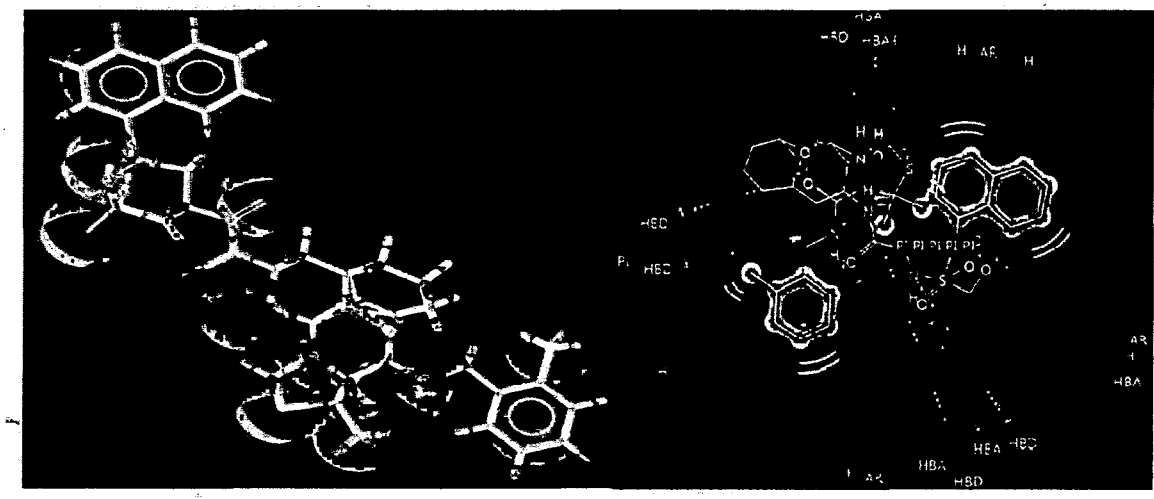


Figure 4.5: 3D and 2D Pharmacophore Model of PHIV35 from LigandScout 3.0

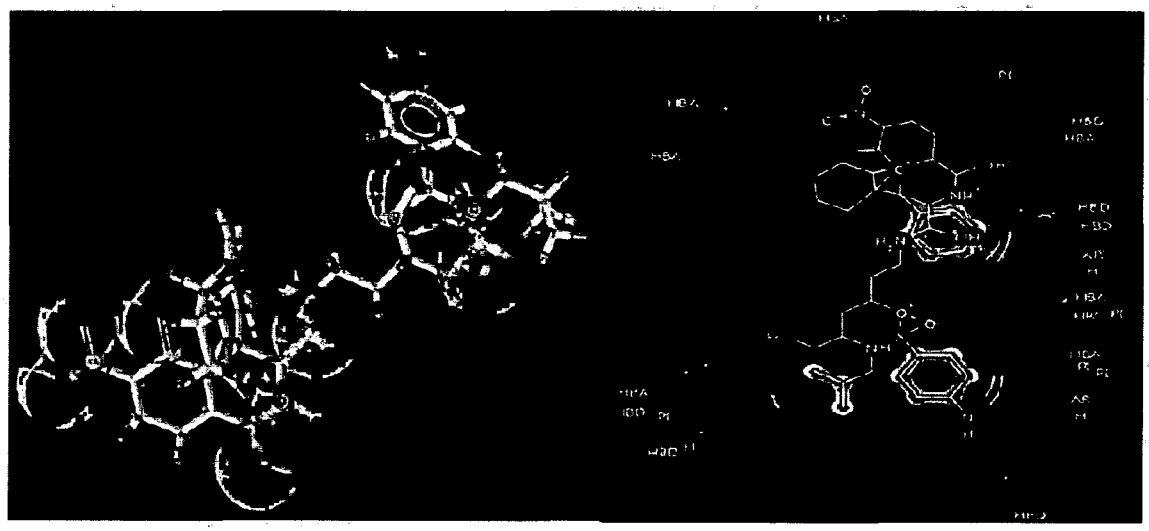


Figure 4.6: 3D and 2D Pharmacophore Model of PHIV39 from LigandScout 3.0

To generate a pharmacophore model based on several ligands, ligands i.e. PHIV16, PHIV20, PHIV31, PHIV35 and PHIV39 were superimposed along with a standard drug Ritonavir and the shared pharmacophore was produced as shown in Figure 4.7. This shared pharmacophore represent that every candidate compound active as HIV protease inhibitor must have 2 hydrophobic volumes, 4 hydrogen bond acceptors (HBA) and 2 hydrogen bond donors (HBD).

On the basis of above information pharmacophore distance triangle was made which is basically four features triangle i.e. it incorporate 1 hydrophobic feature, 1 HBD, and 2 HBA. Distance range is also give for the pharmacophore triangle. These distances were calculated with the help of VMD software. The pharmacophore triangle is given in Figure 4.8.

Distances of all the compounds merged for identifying pharmacophore model are given in Table 4.3. It states that distance from HBA to HBA should lie in range 3.7-4.3Å and the distance from HBA-HBD is between 3.8-4.3 Å and the distance between HBD and hydrophobic is between 2.1-2.3 Å, lastly the distance between hydrophobic and HBA is 3.1-3.4 Å. Individual distances of dataset are mentioned in Table 4.3. All compounds were also undergone the process of distance calculation for the formation of distance triangle and these are mentioned in Table 4.4

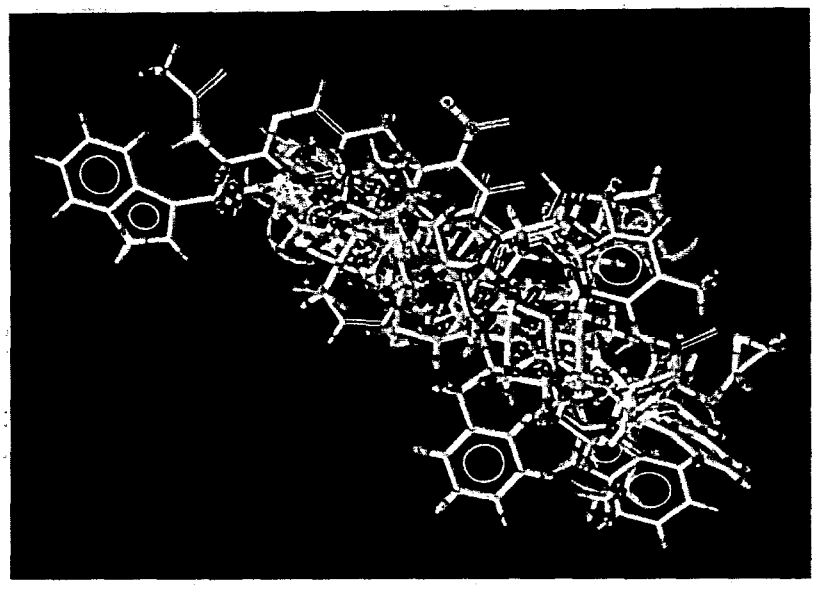


Figure 4.7a: Merged Pharmacophore of compounds PHIV16, PHIV20, PHIV31, PHIV35, PHIV39 generated by LigandScout

Figure 4.7b: Shared Pharmacophore showing 4 Hydrogen Bond Acceptors, 2 hydrogen Bond Donors and Two Hydrophobic volumes

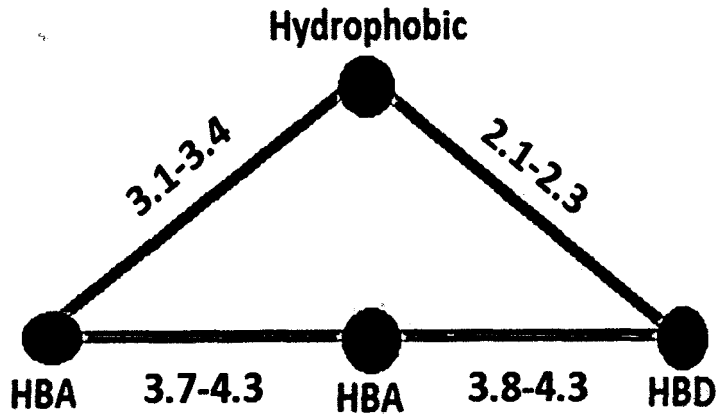


Figure 4.8: Four featured pharmacophore triangle of HIV Protease Inhibitors

Table 4.3: Distance of the compounds incorporated to identify the general pharmacophore model

Compound	H-HBA	HBA-HBA	HBA-HBD	HBD-H
Ritonavir	3.3	4.5	3.5	2.2
PHIV16	3.1	4.3	4.2	2.3
PHIV20	3.3	3.7	4.2	2.3
PHIV31	3.2	4.2	3.8	2.2
PHIV35	3.4	4.3	3.8	2.2
PHIV39	3.2	3.7	4.3	2.1

Table 4.4: 2D Pharmacophore Model of HIV Protease Inhibitors

Compound Number	H-HBA	HBA-HBA	HBA-HBD	HBD-H
Amprenavir	3.2	3.9	4.0	1.7
Indinavir	3.9	4.0	4.2	1.3
Nelfinavir	3.3	4.0	3.0	1.7
Ritonavir	3.3	4.5	3.5	2.2
Saquinavir	3.0	3.7	4.2	2.0
PHIV1	3.0	3.5	4.2	1.7
PHIV 2	3.0	3.5	4.2	1.7
PHIV 3	3.0	3.5	4.2	1.7
PHIV 4	3.5	4.1	3.8	1.6
PHIV 5	3.6	3.7	3.5	1.9
PHIV 6	3.8	4.0	3.5	1.7
PHIV 7	3.2	4.4	3.7	1.6
PHIV 8	3.2	2.6	2.9	1.8
PHIV 9	3.1	4.4	3.7	1.3
PHIV 10	No Hydrophobic			
PHIV 11	No Hydrophobic			
PHIV 12	3.4	4.4	4.1	2.0
PHIV 13	3.8	3.8	3.9	2.3
PHIV 14	No Hydrophobic			
PHIV 15	3.3	3.4	3.0	2.1
PHIV 16	3.1	4.3	4.2	2.3
PHIV 17	3.8	4.4	4.0	2.3
PHIV 18	3.2	3.9	4.2	1.5
PHIV 19	3.7	4.2	4.2	1.4
PHIV 20	3.3	3.7	4.2	2.3
PHIV 21	No HBD			
PHIV 22	2.4	1.25	1.25	2.3
PHIV 23	No HBD			

Compound Number	H-HBA	HBA-HBA	HBA-HBD	HBD-H
PHIV 24	3.1			
PHIV 25	No HBD			
PHIV 26	3.0	3.6	3.5	2.3
PHIV 27	3.3	2.4	5.4	1.9
PHIV 28	No HBD			
PHIV 29	No HBD			
PHIV 30	No HBD			
PHIV 31	3.2	4.2	3.8	2.2
PHIV 32	3.4	3.9	4.2	2.2
PHIV 33	3.4	4.4	3.9	1.9
PHIV 34	3.4	4.3	4.2	2.2
PHIV 35	3.4	4.3	3.8	2.2
PHIV 36	3.2	3.7	4.3	2.1
PHIV 37	3.2	3.7	4.3	2.1
PHIV 38	3.2	3.7	4.3	2.1
PHIV 39	3.2	3.7	4.3	2.1

4.4 MOLECULAR DOCKING

AutoDock Vina was used to predict that how ligand will bind to protein target and to select the best conformation in ligand protein binding with increasing affinity.

4.4.1 ACTIVE SITE OF HIV-1 PROTEASE

HIV Protease has only one active site located at the center of the both homo-dimer. Complete data set was docked and found to bind at the same active site position; the active site amino acids were identified by looking the 5 Å vicinity. Table 4.5 show the list of all the amino acid within 5 Å of the ligand docked with HIV-Protease. The residues found were Arg8, Asp25, Gly27, Asp29, Asp30, Ile47, Gly49, Ile50, Phe53, Val82, Ile84, Arg108, Lue123, Asp125, Gly127, Ala128, Asp129, Asp130, Ile147, Gly149, Ile150, Pro181, Val182, and Ile184. The study revealed that Arg8, Asp25, Asp29, Asp30, Ile47, Ile50, Val82, Ile84, Arg108, Asp125, Ala128, Ile147, Ile150, Pro181, Val182, and Ile184 amino acids are significant for binding interactions. Figure 4.9 shows amino acids in 5 Å of Ritonavir.

Table 4.5: Amino acids Present within the 5 Å Vicinity of the Ligand where + and – signs indicate the presence and absence of amino acid

COMPOUND	ARG 8	ASP 25	GLY 27	ALA 28	ASP 29	ASP 30	ILE 47	GLY 49	ILE 50	PHE 53	VAL 82	ILE 84
Amprenavir	-	-	-	-	+	+	-	-	-	-	-	-
Indinavir	-	+	-	+	+	+	+	-	+	+	+	+
Nelfinavir	+	+	-	+	+	+	+	-	-	-	-	+
Ritonavir	+	+	+	+	+	+	+	+	-	+	+	+
Saquinavir	-	-	-	-	+	+	-	-	-	-	-	-
PHIV1	-	+	+	+	+	+	+	+	+	-	+	+
PHIV2	-	-	-	-	-	-	-	-	-	-	-	-
PHIV3	+	+	+	+	+	+	+	+	+	-	+	+
PHIV4	+	+	+	+	+	+	+	+	+	-	-	-
PHIV5	+	+	+	+	-	+	+	+	+	-	+	+
PHIV6	+	+	+	+	-	+	+	-	+	-	+	+
PHIV7	-	-	-	-	-	-	-	-	-	-	-	-
PHIV8	-	-	-	-	-	-	-	-	-	-	-	-
PHIV9	-	-	-	-	+	+	-	-	-	-	-	-
PHIV10	+	+	-	+	+	+	+	+	+	-	+	+
PHIV11	+		-	-	-	-	-	-	-	-	-	-
PHIV12	-	+	+	-	-	+	+	+	+	-	+	+
PHIV13	-	-	-	-	-	-	-	+	+	+	-	-
PHIV14	-	+	+	+	+	+	+	+	+	-	+	+
PHIV15	-	+	+	-	+	+	+	-	+	+	-	+
PHIV16	-	-	-	-	-	+	-	-	-	-	-	-
PHIV17	-	-	-	-	+	-	-	-	-	-	-	-
PHIV18	+	-	-	-	-	-	-	-	-	-	-	-
PHIV19	-	-	-	-	-	-	-	-	-	+	-	-
PHIV20	-	-	-	-	-	-	-	-	-	+	-	-
PHIV21	-	+	+	+	+	-	-	+	+	-	+	+
PHIV22	+	-	-	-	-	-	-	-	-	-	-	-

COMPOUND	ARG 108	LEU 123	ASP 125	GLY 127	ALA 128	ASP 129	ASP 130	ILE 147	GLY 149	ILE 150	PRO 181
Amprenavir	-	-	-	-	-	-	-	-	-	-	-
Indinavir	+	+	+	-	+	-	-	-	-	-	+
Nelfinavir	+	+	+	+	+	+	+	+	+	+	+
Ritonavir	+	-	+	+	+	+	-	+	+	+	-
Saquinavir	-	-	-	-	-	-	-	-	-	-	-
PHIV1	+	+	+	+	+	-	-	+	+	+	+
PHIV2	-	-	-	-	-	-	-	-	-	-	-
PHIV3	-	+	+	+	+	-	-	-	+	+	+
PHIV4	-	-	+	+	+	+	+	+	+	+	-
PHIV5	-	-	+	+	-	+	+	+	+	+	-
PHIV6	-	-	+	+	-	+	+	+	+	+	-
PHIV7	-	-	-	-	-	-	-	-	-	-	-
PHIV8	-	-	-	-	-	-	-	-	-	-	-
PHIV9	-	-	-	-	-	-	-	-	-	-	-
PHIV10	-	+	+	+	+	+	+	+	+	+	+
PHIV11	-	-	-	-	-	-	-	-	-	-	-
PHIV12	+	-	+	+	+	+	+	+	-	+	+
PHIV13	-	-	-	-	-	-	-	-	-	-	+
PHIV14	+	+	+	+	+	-	-	-	-	+	+
PHIV15	+	+	+	+	+	-	+	+	+	+	+
PHIV16	-	-	-	-	-	-	-	-	-	-	-
PHIV17	-	-	-	-	-	-	-	-	-	-	-
PHIV18	-	-	-	-	-	-	-	-	-	-	-
PHIV19	-	-	-	-	-	-	-	-	+	-	-
PHIV20	-	-	-	-	-	-	-	-	+	+	-
PHIV21	+	+	+	+	+	-	-	-	+	+	+
PHIV22	-	-	-	-	-	-	-	-	-	-	-
PHIV23	-	-	-	-	-	-	-	-	-	-	-

COMPOUND	ARG 8	ASP 25	GLY 27	ALA 28	ASP 29	ASP 30	ILE 47	GLY 49	ILE 50	PHE 53	VAL 82	ILE 84
PHIV23	-	-	-	-	+	+	-	-	-	-	-	-
PHIV24	+	-	-	-	-	-	-	-	-	-	-	-
PHIV25	-	-	-	-	-	-	-	-	-	-	-	-
PHIV26	-	-	-	-	-	-	-	-	-	-	-	-
PHIV27	+	-	-	-	-	-	-	-	-	-	-	-
PHIV28	-	-	-	-	+	-	-	-	-	-	-	-
PHIV29	-	-	-	-	-	+	-	-	-	-	-	-
PHIV30	-	-	-	-	-	-	-	-	-	-	-	-
PHIV31	+	+	+	+	+	+	+	+	+	-	+	+
PHIV32	+	-	-	-	-	-	-	-	-	-	-	-
PHIV33	-	+	+	+	+	+	-	-	+	+	-	+
PHIV34	+	+	+	+	-	-	-	-	+	-	+	+
PHIV35	+	+	+	+	-	-	-	+	+	-	+	+
PHIV36	-	-	-	-	+	+	-	-	-	-	-	-
PHIV37	-	-	-	-	+	+	-	-	-	-	-	-
PHIV38	+	+	+	+	+	+	+	+	+	-	+	+
PHIV39	-	-	-	-	+	+	-	-	-	-	-	-
Inactive	-	-	-	-	-	-	-	+	-	-	-	-

COMPOUND	ARG 108	LEU 123	ASP 125	GLY 127	ALA 128	ASP 129	ASP130	ILE 147	GLY149	ILE 150	PRO 181
PHIV24	-	-	-	-	-	-	-	-	-	-	-
PHIV25	-	-	-	-	-	-	-	-	-	-	-
PHIV26	-	-	-	-	-	-	-	-	-	-	-
PHIV27	-	-	-	-	-	-	-	-	-	-	-
PHIV28	+	-	-	-	-	-	-	-	-	-	-
PHIV29	-	-	-	-	-	-	-	-	-	-	-
PHIV30	-	-	-	-	-	-	-	-	-	-	-
PHIV31	-	-	-	-	+	+	+	+	+	+	-
PHIV32	-	-	-	-	-	-	-	-	-	-	-
PHIV33	-	-	-	-	-	-	-	-	-	-	+
PHIV34	-	-	-	-	+	-	+	-	+	+	-
PHIV35	-	-	-	-	-	-	-	-	+	+	-
PHIV36	+	-	-	-	-	-	-	-	-	-	-
PHIV37	-	-	-	-	-	-	-	-	-	-	-
PHIV38	-	-	-	-	+	+	+	-	+	+	-
PHIV39	+	-	-	-	-	-	-	-	-	-	-
Inactive	-	-	-	-	-	-	-	-	-	-	-

4.4.2 MOLECULAR DOCKING OF STANDARD DRUG:

Standard drugs in the data set were selected for the docking with HIV-Protease using the same molecular docking software and parameters. A detailed 3D analysis of the docked site of these drugs indicated that they bind to same active site. Figure 4.10-4.12 shows the hydrogen, hydrophobic and ionic interactions of the standard drugs Nelfinavir, Ritonavir and Saquinavir.

In case of **Amprenavir,** it showed three active binding interactions i.e. binding interaction has distance less than 4 Å two are hydrogen bonding with Gln58 and ligands O and N of Asn88 having distance 2.98 Å and 3.31 Å respectively and a hydrophobic interaction of C of Thr74 and ligand C having distance 3.75 Å

In case of **Indinavir,** there were three types of interactions that is Ionic, hydrogen and Hydrophobic: Ionic interaction was between O of Asp125 and N of ligand having distance of 3.98 Å. Hydrogen bonding was between N of Arg108 and O of ligand having distance 3.41 Å. Hydrophobic interactions were 3 in total between C of ligand and C of Ala28, Ile47 and Pro181 having distances 3.06 Å, 3.23 Å and 3.75 Å, respectively. In case of **Nelfinavir,** there were two hydrogen bonding and 8 hydrophobic interactions. Hydrogen bonding was between OH of ligand and O of Asp25 and Asp125 having distance 2.99 Å and 3.05 Å respectively while hydrophobic interactions were between C of ligand and C of Ala128, Val132, Ile184, Val182, Leu123, Val32, Ile84, Ile150 and Ile147 having distances 3.6 Å, 3.32 Å, 3.42 Å, 3.43 Å, 3.43 Å, 3.74 Å, 3.84 Å and 3.75 Å respectively. In case of **Ritonavir,** there were 2 hydrogen bonding and 6 hydrophobic interactions. Hydrogen bonding was between O of ligand and OH and N of Asp25 and Asp125 having distances 3.17 Å and 3.78 Å respectively. Hydrophobic interactions were

between C of ligand and C of Val31, Ile47, Leu23, Phe53, Ala28 and Val82 having distances 3.41 Å, 3.84 Å, 3.66 Å, 3.64 Å, 3.87 Å and 3.54 Å respectively. Figure 4.9 reflects amino acid residues of active site of the FDA approved standard drug Ritonavir. In case of **Saquinavir,** there was only hydrogen bonding it is between O of Asp60 and N of Ligand having distance 3.85 Å, N of Gln58 and O of ligand having distance of 3.68 Å, N of Lys45 and O of ligand having distance of 3.15 Å and O of Asp30 and N of ligand having distance of 2.94 Å. Keeping in view these interactions it was observed that Nelfinavir and Ritonavir have better efficacy than other standard drugs.

4.4.3 DOCKING OF INACTIVE COMPOUND

Docking of inactive compound was done but no active interaction was deduced and the binding site was also slightly different from the rest data set showing that the amino acids in the near vicinity are Pro179, Ile154, Thr180 and Gly49 as shown in figure 4.13.. Pro179 and Thr180 were not appeared for any other compound or standard drugs.

4.4.4 DOCKING OF DATA SET COMPOUNDS

In order to explain the activity of the data set compounds on the basis of docking studies, they were docked into the active site of HIV-Protease. Best conformation and its binding energy value were recorded and are shown in Table 4.6a. The docked files were visualized in VMD software to get the interactions i.e. hydrogen bonding, hydrophobic, ionic and van der Waal interactions. These interactions are enlisted in Table 4.6b. On the basis of these binding interactions, binding energy value and IC_{50} value lead compound was identified. It is evident that compound PHIV16 had the lowest binding energy i.e. -12.4 Kcal/mol and hence it must be more potent than other compound but it was biased to take this decision only considering binding affinity.

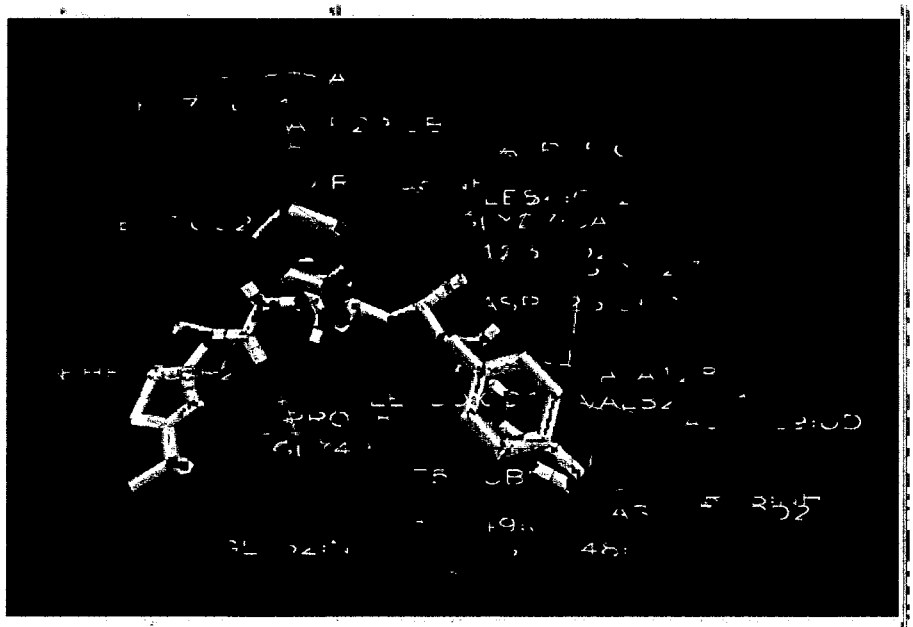


Figure 4.9: Amino acid residues of active site of HIV-1 Protease bind with FDA approved standard drug Ritonavir

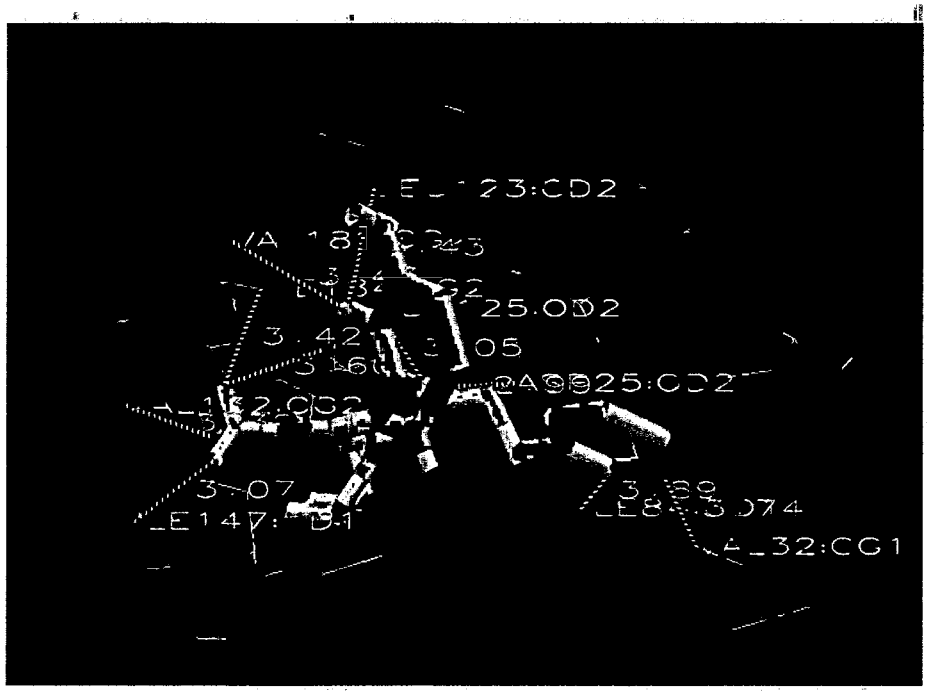


Figure 4.10: Binding interactions of Nelfinavir with HIV-1 Protease Target protein

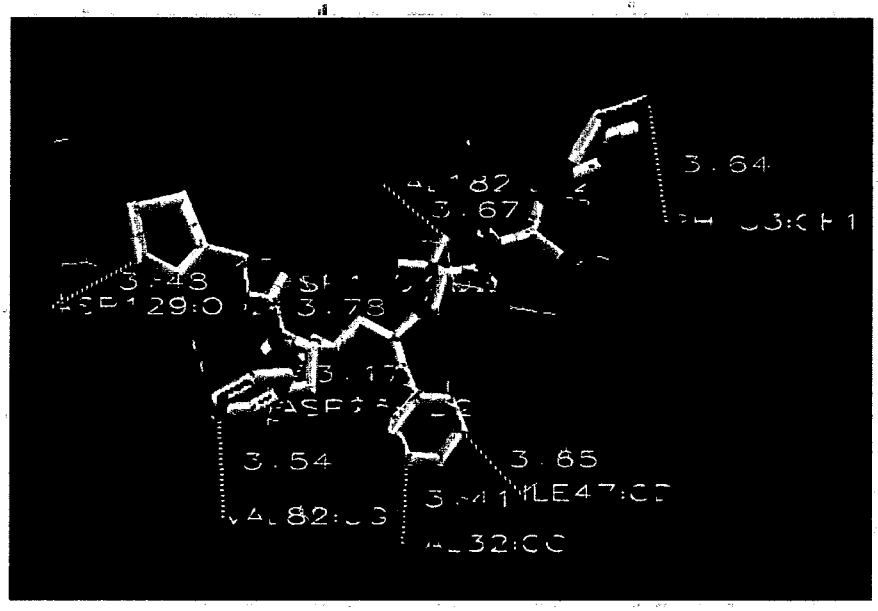


Figure 4.11: Binding interactions of Ritonavir with HIV-1 Protease Target protein

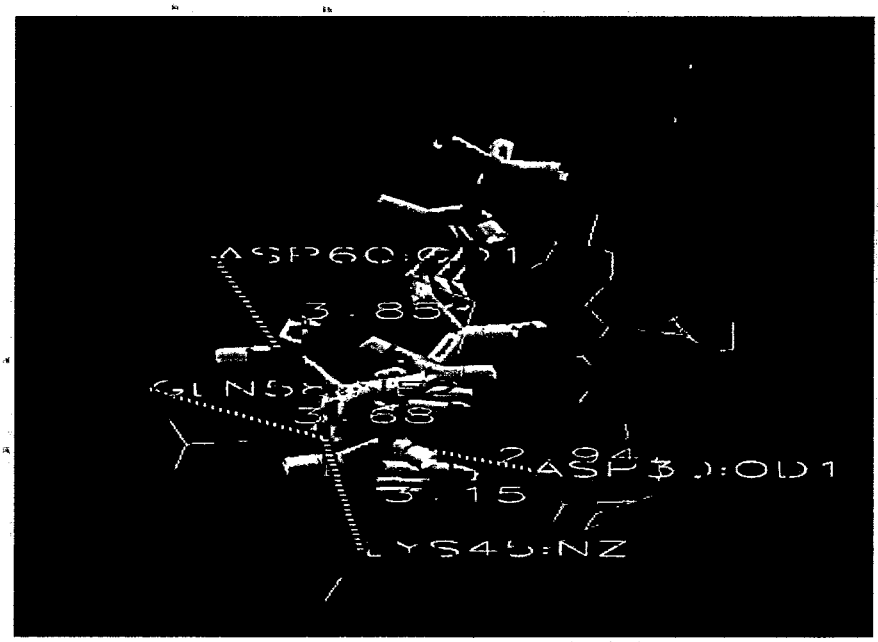


Figure 4.12: Binding interactions of Saquinavir with HIV-1 Protease Target protein

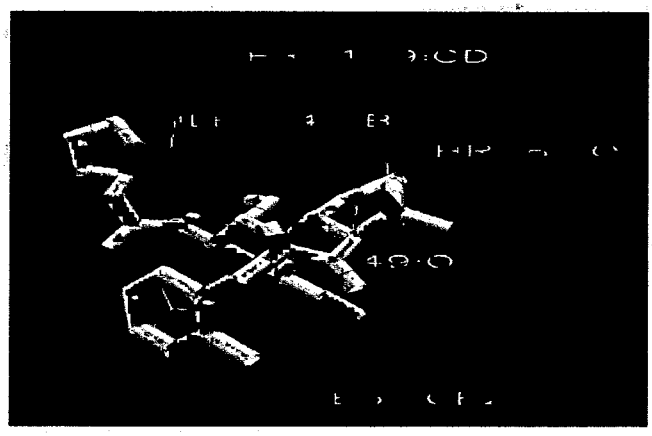


Figure 4.13: Binding interactions of Inactive compound with HIV-1 Protease Target protein showing not a single significant interaction within 4.0 Å

Table 4.6a: Inhibition Concentration and Energy Value of the Data Set

Compound Number	IC_{50} (μM)	Energy Value (Kcal/mol)
PHIV1	0.02	-8.9
PHIV 2	0.05	-5.0
PHIV 3	0.38	-8.2
PHIV 4	1.04	-8.1
PHIV 5	2.4	-9.4
PHIV 6	10.3	-9.0
PHIV 7	25	-9.6
PHIV 8	29	-10.1
PHIV 9	60	-8.0
PHIV 10	0.021	-10.4
PHIV 11	0.0025	-5.8
PHIV 12	100	-10.8
PHIV 13	0.3954	-7.4
PHIV 14	37.2	-8.2
PHIV 15	44.5	-12.4
PHIV 16	2.7	-7.1
PHIV 17	3.9	-6.5

Compound Number	IC_{50} (μM)	Energy Value (Kcal/mol)
PHIV 18	11.9	-5.8
PHIV 19	10.0	-6.2
PHIV 20	29.9	-6.3
PHIV 21	31.9	-6.6
PHIV 22	18.8	-6.3
PHIV 23	15.7	-5.3
PHIV 24	10.6	-7.1
PHIV 25	23.6	-6.6
PHIV 26	15.8	-7.1
PHIV 27	19.2	-6.3
PHIV 28	3.9	-5.2
PHIV 29	5.7	-5.7
PHIV 30	28.6	-7.1
PHIV 31	0.00006	-10.8
PHIV 32	0.0006	-6.5
PHIV 33	0.19	-7.6
PHIV 34	0.00018	-9.4
PHIV 35	0.000083	-11.3
PHIV 36	0.24	-4.8
PHIV 37	0.12	-5.3
PHIV 38	0.48	-7.4
PHIV 39	0.33	5.7
Inactive	Nt	-5.7
Amprenavir	0.33	-5.7
Indinavir	1.5	-10.2
Nelfinavir	1.05	-7.0
Ritonavir	0.38	-9.3
Saquinavir	0.42	-5.8

To predict compound activeness IC_{50} value and binding interactions were also incorporated. It is evident from Table 4.6b that compounds PHIV9, PHIV10, PHIV12, PHIV24, PHIV35 and PHIV38 had good hydrogen and hydrophobic interactions. Van der Waal interactions were rare and were only observed in compounds PHIV13, PHIV19, PHIV20 and PHIV28 and these interactions were not present in any standard drug. Likewise ionic interaction, which was only observed in Indinavir standard drug, is present in compounds PHIV9, PHIV15 and PHIV28. All the distances and interactions along with the residues involved in interactions are mentioned in Table 4.6b. It must be noted that all the interactions were taken with the side chain functional groups of the target protein amino acids as rest are bonded with peptide bond therefore none of the interaction is taken with those functional groups.

A detailed 3D study of docked files revealed that all the compounds of dataset have the same amino acids with the 5 Å of the ligand and all the interactions were calculated after selecting the best conformation based on energy values. It was observed that amino acids such as Asp25, Asp125, Asp30, Lys45, Gln92, Val132, Val182, Ile84, Ile184, Ile150 and Ile50 were important in binding interactions.

Table 4.6b: Binding Interactions and distances of Data Set showing all the three kinds of interactions including Hydrogen Bonding, Hydrophobic Interactions and Van der Waal Interactions

Compounds	Ionic Bond Distances	Hydrogen Bond Distances	Hydrophobic Bond Distances	Van der Waal Forces Distances
Amprenavir		GLN58 (N)-UNK(O)-2.98 ASN88 (N)-UNK(O)-3.31	THR74 (C)-UNK(C)-3.75	
Indinavir	ASP125(O)-UNK(N)-3.98	ARG108(N)-UNK(O)-3.41	PRO181(C)-UNK(C)-3.75 ILE47(C)-UNK(C)-3.23 ALA28(C)-UNK(C)-3.06	
Nelfinavir		ASP25(O)-UNK(OH)-2.99 ASP125(O)-UNK(OH)-3.05	ALA128(C)-UNK(C)-3.6 VAL132(C)-UNK(C)-3.32 ILE184(C)-UNK(C)-3.42 VAL182(C)-UNK(C)-3.43 LEU123(C)-UNK(C)-3.43 VAL32(C)-UNK(C)-3.74 ILE84(C)-UNK(C)-3.84 ILE147(C)-UNK(C)-3.75	
Ritonavir		ASP25(O)-UNK(OH)-3.17 ASP125(O)- UNK(N)-3.78	VAL32(C)-UNK(C)-3.41 ILE47(C)-UNK(C)-3.84 LEU23(C)-UNK(C)-3.66 PHE53(C)-UNK(C)-3.64 ALA28(C)-UNK(C)-3.87 VAL82(C)- UNK(C)-3.54	
Saquinavir		ASP60(O)- UNK(N)-3.85 GLN58(N)-UNK(O)-3.68 LYS45(N)- UNK(O)-3.15 ASP30(O)-UNK(N)-2.94		
PHIV 1			VAL82(C)- UNK(C)-3.62 VAL32(C)- UNK(C)-2.96 ILE47(C)- UNK(C)-2.93 ILE184(C)- UNK(C)-3.82 PRO81(C)-UNK(C)-3.83	

Compounds	Ionic Bond Distances	Hydrogen Bond Distances	Hydrophobic Bond Distances	Van der Waal Forces Distances
PHIV 2			PRO1(C)- UNK(C)-3.59	
PHIV 3		ASP25(O)- UNK(OH)-2.86 ASP125(O)-UNK(OH)-3.09	VAL82(C)- UNK(C)-3.72 LEU23(C)- UNK(C)-3.75 VAL32(C)-UNK(C)-3.75 ILE47(C)- UNK(C)-3.87	
PHIV 4		ASP25(O)-UNK(OH)-2.66 ASP30(O)-UNK(N)-3.31		
PHIV 5			ILE50(C)- UNK(C)-3.81 ILE84(C)- UNK(C)-3.52 ALA28(C)- UNK(C)-3.33	
PHIV 6		ASP25(O)-UNK(OH)-2.91 ASP125(O)-UNK(OH)-2.87 ARG8(N)-UNK(O)-3.02	ALA28(C)- UNK(C)-3.33 ILE84(C)- UNK(C)-3.52	
PHIV 7		GLN92(N)- UNK(O)-2.80		
PHIV 8		GLN102(O)-UNK(N)-3.56 ASN198(O)-UNK(N)-3.65 ASN98(N)-UNK(N)-3.63	PRO101(C)- UNK(C)-3.72	
PHIV 9	ASP30(O)-UNK(N)-2.96	ASN88(N)-UNK(N)-3.53 GLN107(N)-UNK(N)-3.44 GLN107(O)-UNK(N)-3.44	THR74(C)- UNK(C)-3.71	
PHIV 10			ILE184(C)-UNK(C)-3.34 ALA128(C)-UNK(C)-3.81 VAL132(C)-UNK(C)-3.53 VAL182(C)-UNK(C)-3.18 ILE184(C)-UNK(C)-3.34 PRO181(C)-UNK(C)-3.31 VAL132(C)-UNK(C)-3.53	

Compounds	Ionic Bond	Hydrogen Bond	Hydrophobic Bond	Van der Waal Forces
PHIV 11		CYS67(S)-UNK(N)-3.55 THR12(O)-UNK(O)-3.20		
PHIV 12		ASP30(O)-UNK(N)-3.58	ALA128(C)-UNK(C)-3.72 ILE84(C)-UNK(C)-3.78 VAL32(C)-UNK(C)-3.48 ILE47(C)-UNK(C)-2.92 PRO181(C)-UNK(C)-3.72 VAL182(C)-UNK(C)-3.60	
PHIV 13			ILE154(C)-UNK(C)-3.58	PRO181(C)-UNK(C)-3.72
PHIV 14		ASP25(O)-UNK(N)-3.97 ASP125(O)-UNK(N)-3.52	ILE84(C)- UNK(C)-3.37 VAL32(C)- UNK(C)-3.46 ALA28(C)- UNK(C)-3.94 ILE150(C)- UNK(C)-3.93 ILE47(C)-UNK(C)-3.50	
PHIV 15	ASP25(O)-UNK(N)-3.56	ASP125(O)-UNK(OH)-3.04	VAL32(C)-UNK(C)-3.31 ILE147(C) -UNK(C)-3.71 ILE47(C) -UNK(C)-3.71 PHE53(C) -UNK(C)-3.60 PRO181(C) -UNK(C)-3.62	
PHIV 16		LYS45(O) -UNK(O)-3.09 ASP30(O)-UNK(OH)-2.96 ARG87(N)-UNK(O)-3.70 GLN58(N)-UNK(O)-3.28	ILE72(C)-UNK(C)-3.99	
PHIV 17		ARG87(N)-UNK(O)-2.96 THR91(O)-UNK(O)-3.78 GLN92(N)-UNK(O)-2.91		
PHIV 18			ILE3(C) -UNK(C)-3.85	
PHIV 19			ILE54(C)-UNK(C)-3.77	PHE153(C) -UNK(C)-3.8
PHIV 20			ILE54(C) -UNK(C)-3.74	PHE153(C) -UNK(C)-3.82

Compounds	Ionic Bond	Hydrogen Bond	Hydrophobic Bond	Van der Waal Forces
PHIV 21		ARG108(N)-UNK(O)-3.03 ASP29(O)-UNK(O)-3.43	ILE150(C) -UNK(C)-3.55 VAL182(C) -UNK(C)-3.81 LEU123(C)- UNK(C)-3.73 LEU23(C)-UNK(C)-3.65 ILE84(C)-UNK(C)-3.18 VAL82(C)-UNK(C)-3.43	
PHIV 22		CYS67(S)-UNK(O)-3.48 GLN7(O)-UNK(O)-2.82		
PHIV 23		ASP30(O) -UNK(OH)-3.06 ASP60(O) -UNK(OH)-3.08 GLN58(N) -UNK(O)-3.00 ASN88(N) -UNK(O)-3.24 THR74(O)-UNK(O)-2.91	LYS43(C)-UNK(C)-4.32	
PHIV 24			PRO1(C) -UNK(C)-3.53 VAL11(C) -UNK(C)-3.69	
PHIV 25		GLN2(N)-UNK(O)-2.98 THR196(O)-UNK(O)-3.78 ASN198(N)-UNK(O)-3.36		
PHIV 26		THR12(O)-UNK(O)3.52	THR12(C) -UNK(C)-3.65 LEU19(C)-UNK(C)-3.48	
PHIV 27		CYS67(S)-UNK(O)-3.61	PRO1(C) -UNK(C)-3.84 VAL11(C) -UNK(C)-3.60	
PHIV 28	ARG87(N)-UNK(O)-2.92	ASP29(O)-UNK(OH)-3.48		TRP106(C) -UNK(C)-3.84
PHIV 29		ASP30(O)-UNK(OH)-2.91 GLN58(N)-UNK(O)-3.36 THR91(O)-UNK9O)-3.78		
PHIV 30		ARG157(N)-UNK(O)-3.24	TRP142(C)-UNK(C)-3.60	
PHIV 31		ARG8(N)-UNK(O)-3.79	VAL32(C)-UNK(C)-3.65	
PHIV 32		GLU21(O)-UNK(O)-2.96 THR12(O)-UNK(O)-3.58	LEU10(C)- -UNK(C)-3.80 THR12(C)-UNK(C)-3.74 LEU12(C)-UNK(C)-3.74	

Compounds	Ionic Bond	Hydrogen Bond	Hydrophobic Bond	Van der Waal Forces
PHIV 33			ARG108(C)-UNK(C)-3.81 PRO181(C) –UNK(C)-3.95 VAL182(C)-UNK(C)-3.80 LEU123(C)-UNK(C)-3.97	
PHIV 34		ARG8(N)-UNK(O)-3.28 ASP129(O)-UNK(OH)-3.54		
PHIV 35		ARG8(N)-UNK(OH)-2.75 ARG8(N)-UNK(S)-3.82 ASP129(O)-UNK(OH)-3.57	ALA28(C)-UNK(C)-4.02 ILE84(C)-UNK(C)-3.44 VAL82(C)-UNK(C)-3.62 ILE50(C)-UNK(C)-3.44 ILE184(C)-UNK(C)-3.78 ALA128(C)-UNK(C)-3.59	
PHIV 36		ASP60(O)-UNK(OH)-3.24 GLU58(N)-UNK(O)-2.81 THR74(O)-UNK(N)-3.29 ASP30(O)-UNK(OH)-2.7 ARG87(N)-UNK(O0-2.94		
PHIV 37	ASP30(O)-UNK(N)-3.89	LYS43(N)-UNK(O)-3.15 GLN58(N)-UNK(O)-2.81 ASP60(O)-UNK(OH)-3.15 GLN92(O)-UNK(O)-3.94 GLN92(N)-UNK(O)-2.94		
PHIV 38		ASP29(O)-UNK(OH)-3.12 ASP125(O)-UNK(OH)-2.93 ASP25(O)-UNK(OH)-3.18 ASP129(O)-UNK(N)-2.96	VAL82(C)-UNK(C)-3.93 VAL182(C)-UNK(C)-3.63 ILE47(C)-UNK(C)-3.16 PRO181(C)-UNK(C)-3.11	
PHIV39	ASP29(O)-UNK(N)-3.26	ASP30(O)-UNK(OH)-3.79 ARG87(N)-UNK(O)-2.78 THR74(O)-UNK(OH)-3.00 Trp106(O)-UNK(N)-3.53		
Inactive	-	-	-	-

4.5 LEAD COMPOUND IDENTIFICATION

After the identification of binding interactions and binding affinities of data set, possible active compounds were chosen i.e. hits were identified in order to get a lead compound. According to binding interactions PHIV9, PHIV 12, PHIV 15, PHIV35 and PHIV38 were showing strong binding affinity. The interactions of these compounds were as follow: **PHIV9** had 1 ionic interaction with distance 2.96 Å, 3 hydrogen bonding having distances 3.53 Å, 3.44 Å and 3.44 Å and 1 hydrophobic interaction of distance 3.71 Å. **PHIV12** had 1 hydrogen bonding between O of Asp30 and N of target protein having distance 3.58 Å and 6 hydrophobic interactions having C to C distance from Ala128, Ile84, Val32, Ile47, Pro181 and Val182 to protein target as 3.72 Å, 3.78 Å, 3.48 Å, 2.92 Å, 3.72 Å and 3.60 Å respectively. **PHIV18** had 1 ionic, 1 hydrogen and 5 hydrophobic interactions having distances as 3.56 Å for ionic, 3.04 Å for hydrogen and 3.31 Å, 3.71 Å, 3.71 Å, 3.60 Å and 3.62 Å respectively. **PHIV15** had 1 ionic having distance 3.56 Å, 1 hydrogen having distance 3.04 Å and 5 hydrophobic interactions having distances 3.31 Å, 3.71 Å, 3.71 Å, 3.60 Å and 3.62 Å respectively. **PHIV38** had 4 hydrogen bonds and 4 hydrophobic interactions, hydrogen bonds were at the distance of 3.12 Å, 2.93 Å, 3.18 Å and 2.96 Å respectively and hydrophobic interactions were at distance of 3.93 Å, 3.63 Å, 3.16 Å and 3.11 Å respectively.

PHIV35 had 3 hydrogen bonds and 6 hydrophobic interaction, H-bond was between N of Arg8 and OH of ligand having distance 2.75 Å, and between N of Arg8 and S of ligand and the distance is was 3.82 Å and the last is between O of Asp129 and OH of ligand and the distance was 3.57 Å. The hydrophobic interaction were between C of ligand is with C of Ala28 having distance 4.02 Å, C of Ile84 having distance 3.44 Å, C of Val82 having

distance of 3.62 Å, C of Ile50 having distance of 3.44 Å, C of Ile184 having distance of 3.78 Å and C of Ala128 having distance of 3.59 Å.

But the binding affinity of the following compounds was least in the data set PHIV10, PHIV15, PHIV31, PHIV35 i.e. -10.8, -12.4, -10.8 and -11.3 Kcal/mol respectively reducing the hits to two i.e. PHIV15 and PHIV35. Although IC_{50} value have 30% role in identifying the lead compound but when the IC_{50} value of PHIV15 and pHIV35 were compared there was a remarkable difference because IC_{50} value of PHIV15 was 44.5 μM and that of PHIV35 is 0.000083 μM.

All these calculations led to the result that PHIV35 was the lead compound having binding affinity -11.3Kcal/mol (2^{nd} lowest binding affinity), IC_{50} value 0.000083 μM (lowest IC_{50} value) and 3 hydrogen and 1 hydrophobic interaction as shown in Table 4.6a and Table 4.6b.

4.6 ANALOGUES OF LEAD COMPOUND

On the basis of binding interactions, binding affinity and IC_{50} value compound PHIV35 had been considered as the lead compound in this study. Analogues were made of this compound in order to get most active compound to use as HIV Protease inhibitors. Table 4.7 shows the analogues of the lead compound with their IUPAC names obtained from ChemDraw software. Analogues were made by changing the functional groups which either increase the hydrophobicity or hydrophilicity of the analogue made thus reworking the efficacy of the compound.

All the analogues were docked within the active site of HIV Protease with the earlier mentioned procedure. The best conformation was selected and visualized in the VMD software in order to calculate binding interactions. These binding interactions are shown

in Table 4.8. In first analogue alkyl oxidation of the lead compound was done it increased the hydrophilicity of the compound and this resulted in the 5 hydrogen and 5 hydrophobic interactions. The H-bond was between OH of the ligand and O of the Asp129 having distance 3.56 Å, and O of ligand and N of Arg8 having distance 2.79 Å, O of ligand and N of Arg8 having distance 2.91 Å and OH of ligand and O of Asp25 having distance 2.99 Å while hydrophobic interactions were between C of ligand and C of Ile84 having distance 3.62 Å, C of Val82 having distance 3.85 Å, C of Ile50 having distance 3.43 Å, C of Ile184 having distance of 3.41 Å and C of Ala128 having distance 3.73 Å. This analogue increased the activity and interactions than the lead compound by increasing its H-Bonds. Binding energy of this analogue was -11.5kcal/mol interestingly this value is greater than the binding energy value of the lead compound

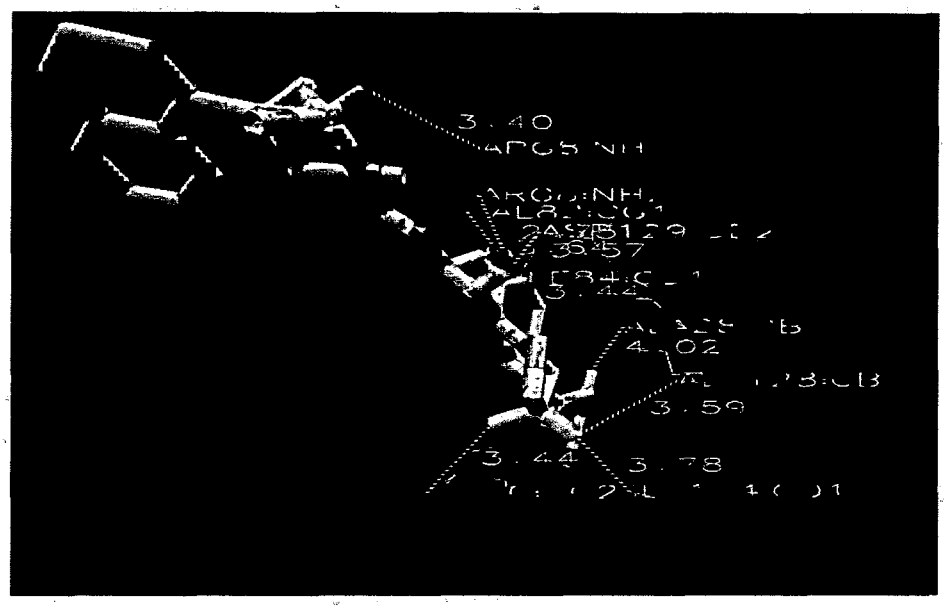


Figure 4.14: Binding interactions of PHIV35 (lead compound) showing the 3 hydrogen and 6 hydrophobic interactions

Table 4.7: Analogues formed from lead compound along with their IUPAC names

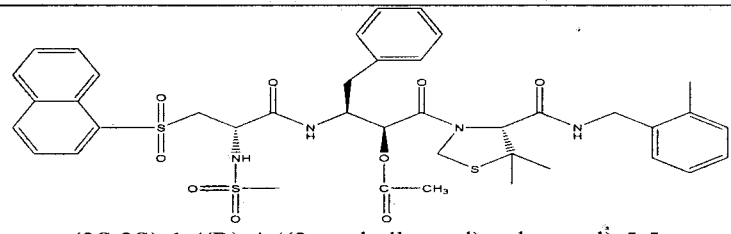
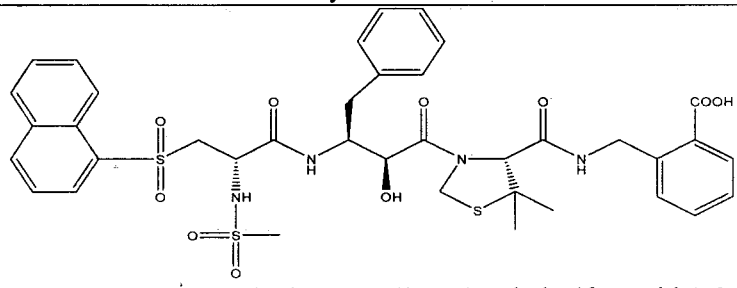
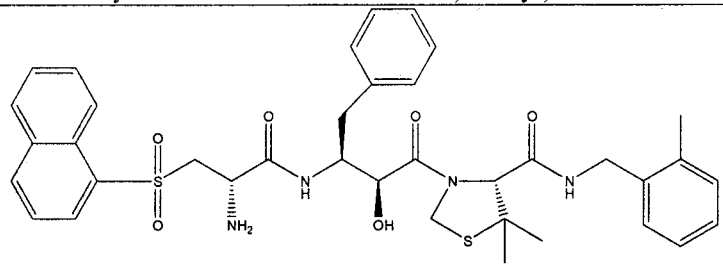
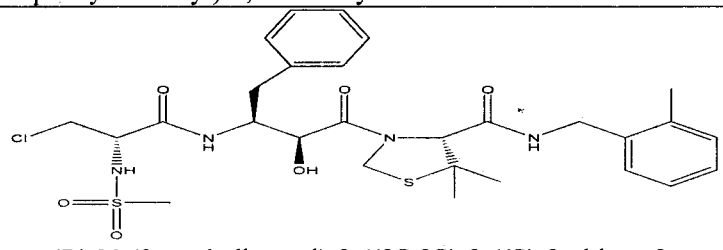
Compound	Structure	Energy Value
Alcohol Protection	 (2S,3S)-1-((R)-4-((2-methylbenzyl)carbamoyl)-5,5-dimethylthiazolidin-3-yl)-3-((S)-2-(methylsulfonamido)-3-(naphthalen-1-ylsulfonyl)propanamido)-1-oxo-4-phenylbutan-2-yl acetate	-7.7
Alkyl Oxidation	 2-(((R)-3-((2S,3S)-2-hydroxy-3-((S)-2-(methylsulfonamido)-3-(naphthalen-1-ylsulfonyl)propanamido)-4-phenylbutanoyl)-5,5-dimethylthiazolidine-4-carboxamido)methyl)benzoic acid	-11.5
Amine Deprotection	 (R)-N-(2-methylbenzyl)-3-((2S,3S)-3-((S)-2-amino-3-(naphthalen-1-ylsulfonyl)propanamido)-2-hydroxy-4-phenylbutanoyl)-5,5-dimethylthiazolidine-4-carboxamide	-10.5
Retro of Lead Compound	 (R)-N-(2-methylbenzyl)-3-((2S,3S)-3-((S)-3-chloro-2-(methylsulfonamido)propanamido)-2-hydroxy-4-phenylbutanoyl)-5,5-dimethylthiazolidine-4-carboxamide	-10.9

Table 4.8: Binding interactions of the analogues which include hydrophobic, hydrogen bonding and Van der Waal interactions along with distances in Angstrom

Compound	Ionic Bonding	Hydrogen Bonding	Hydrophobic	Van der Waal Forces
Alcohol Protection		LYS45(N)-UNK(O)-3.08 ASP60(O)-UNK(N)-4.00 GLN58(N)-UNK(O)-3.62 THR74(O)-UNK(N)-3.19		TRP106(C)-UNK(C)-3.81
Alkyl Oxidation		ASP129(O)-UNK(OH)-3.56 ARG8(N)-UNK(O)-2.79 ARG8(N)-UNK(O)-3.39 ASP125(O)-UNK(OH)-2.91 ASP25(O)-UNK(OH)-2.91	ILE84(C)-UNK(C)-3.62 VAL82(C)-UNK(C)-3.85 ILE50(C)-UNK(C)-3.43 ILE184(C)-UNK(C)-3.41 ALA128(C)-UNK(C)-3.73	
Amine Deprotection	ASP25(O)-UNK(N)-3.89	ARG108(N)UNK(OH)-2.98	VAL182(C)-UNK(C)-3.06 ILE84(C)UNK(C)-3.57 ALA28(C)UNK(C)-3.24 ALA128(C)-UNK(C)-3.92 ILE184(C)-UNK(C)-3.50 LEU123(C)-UNK(C)-3.77 PRO181(C)-UNK(C)-3.89	PHE53(C)-UNK(C)-3.51
Retro of Lead Structure		ARG8(N)-UNK(O)-3.39 ASP129)O)-UNK(OH)-3.56	ILE84(C)-UNK(C)-3.35 LEU23(C)-UNK(C)-4.05 VAL82(C)-UNK(C)-3.55 ALA128(C)-UNK(C)-3.73	

It can be observed from Figure 4.15- 4.16 that docking of the 1st analogue was at the same position where the rest dataset compounds were docked i.e. same active site position it include amino acid Arg8, Asp129, Asp125, Asp25, Val82, Ile84, Ile184, Ala128 and Ile50

In second analogue De-protection of amine group was done and its effect of the binding interactions and binding affinity were observed. Binding energy of 2nd analogue was -10.5 Kcal/mol. Amine de-protection is done to increase hydrophobicity of the compound. In case of second analogue all four types of interactions exist. There is 1 ionic, 1 hydrogen, 1 Van der Waal and 7 hydrophobic interactions.

It can be observed from Figure 4.17-4.18 that docking of the 2nd analogue is at the same position of active site it include amino acids Arg108, Asp25, Phe53, Val182, Ile84, Ala28, Ala128, Ile184, Leu123 and Pro181.

In third analogue, retro of lead compound was done which decreased the hydrophilicity of the compound. Binding energy of this analogue is -10.9Kcal/mol. It produces 2 hydrogen and 4 hydrophobic interactions.

In the last analogue, protection of the alcohol in the lead compound was done and increased hydrophobicity was expected but result was opposite and binding interactions observed in this analogue were 4 hydrogen bonds and 1 Van der Waal interactions.

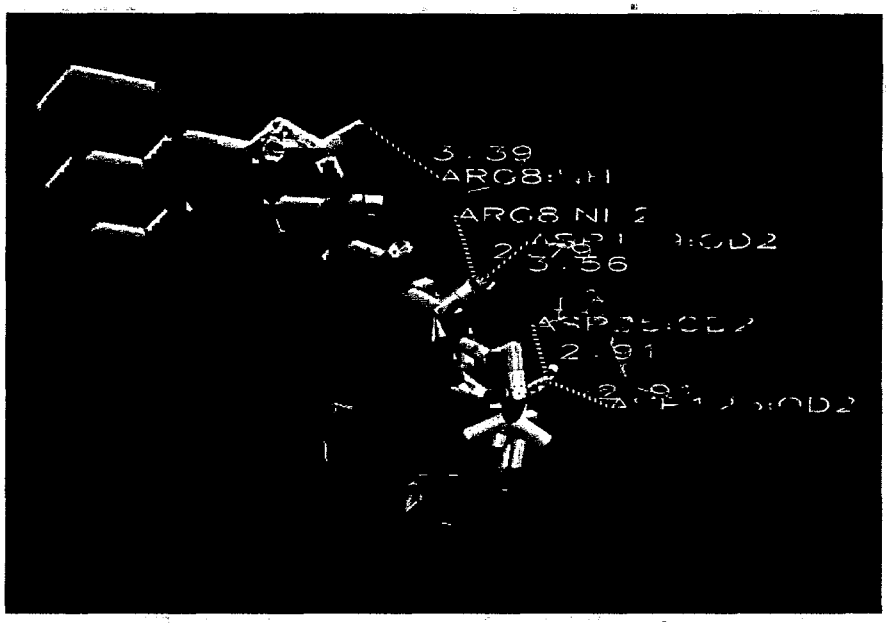


Figure 4.15: Hydrogen Bonds of the Analogue 1 (alcohol protection) and the target protein HIV-1 Protease

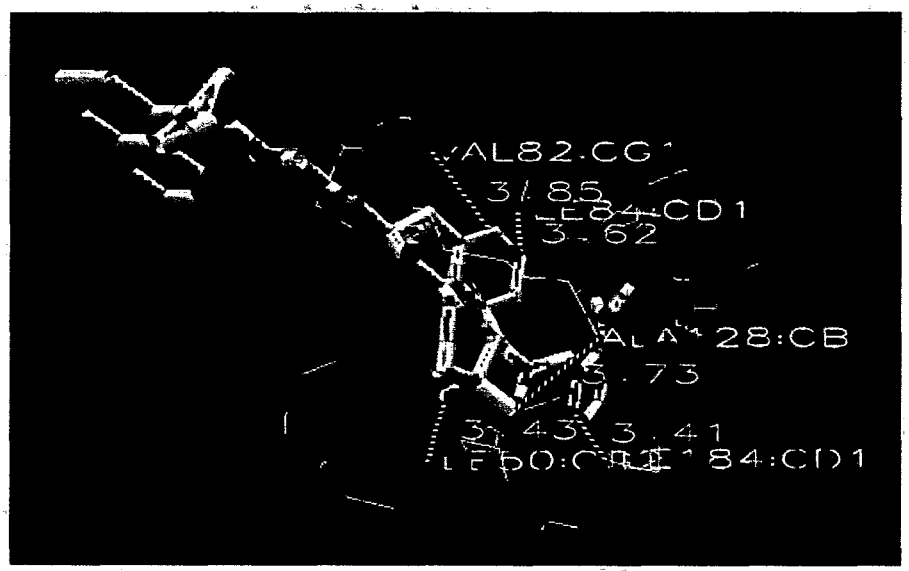


Figure 4.16: Hydrophobic Interactions of the Analogue 1 (alcohol protection) and the target protein HIV-1 Protease

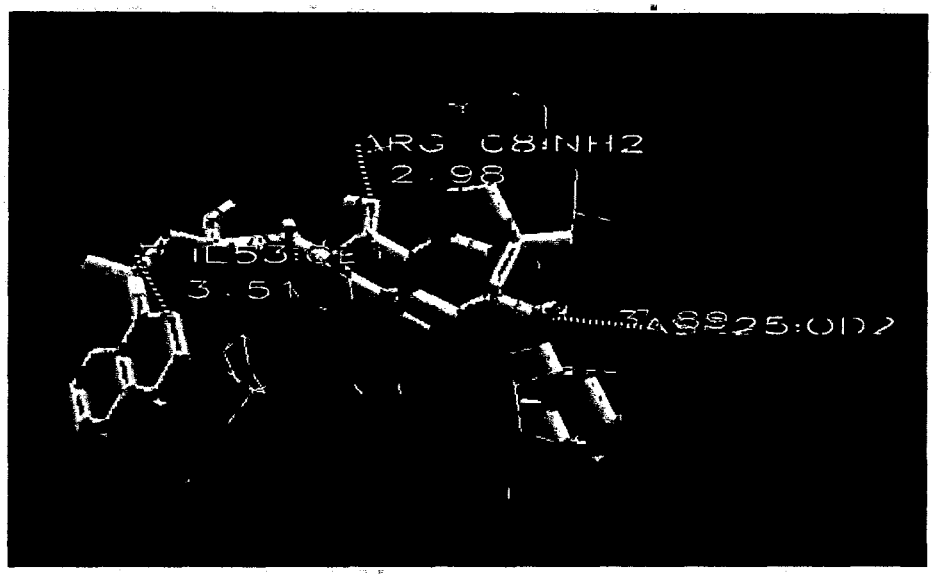


Figure 4.17: Hydrogen Bonds of the Analogue 2 (alkyl oxidation) and the target protein HIV-1 Protease

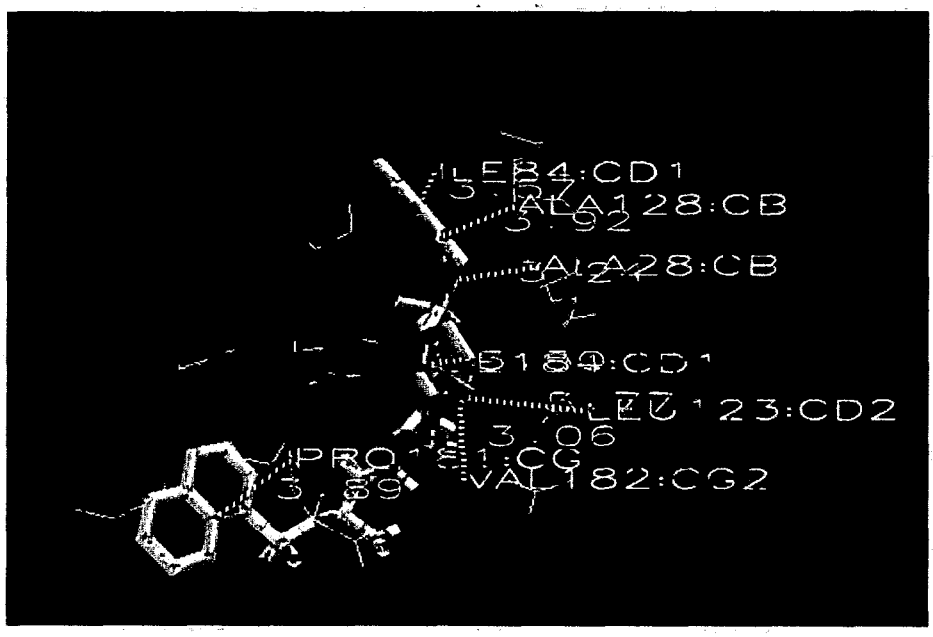


Figure 4.18: Hydrophobic interactions of the Analogue 2 (alkyl oxidation) and the target protein HIV-1 Protease

4.7 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

QSAR model was built for describing how some descriptors were directly or indirectly related to the biological activity i.e. pharmacokinetics of a compound. A set of descriptor was chosen and this set was then applied to data set. These descriptors were assumed to influence whether a given compound will succeed or fail in binding to the target protein (Barrat, M.D *et al.,* 1995). Sets of 14 compounds from sulfonamide derivatives class of protease inhibitors were selected as data sets shown in Table 4.9 (Brent R. *et al.,* 2006). A QSAR has been interpreted by calculating a number of steric and electronic parameters by the use of software Hyper Chem and Chem Draw. The descriptors included partition coefficient i.e. Log P and molar refractivity as steric parameter, total binding energy, heat of formation, E_{HOMO}, E_{LUMO} as electronic parameters. The calculated descriptor values are mentioned in Table 4.10. In order to have direct correlation between the descriptor and the compound activity the regression coefficient was supposed be greater than 0.6 and as the regression coefficient decreased it indicated that there was no correlation among the both variables. Descriptors i.e. electronic and steric parameters were taken as dependent while IC_{50} value as independent variables. The regression values were calculated these regression values were recorded as 0.09 for Log P, 0.00 for molar refractivity, 0.04 for total energy, 0.6 for both heat of formation and E_{LUMO} and 0.01 for E_{HOMO} and the plots of E_{LUMO} and heat of formation are shown in Figure 4.19-4.20. This analysis suggested that there was no correlation between IC_{50} value and Log P, molar refractivity, E_{HOMO} and total energy but IC_{50} value was found to be directly related to heat of formation and E_{LUMO} as the regression value of these parameters was greater or equivalent to 0.6

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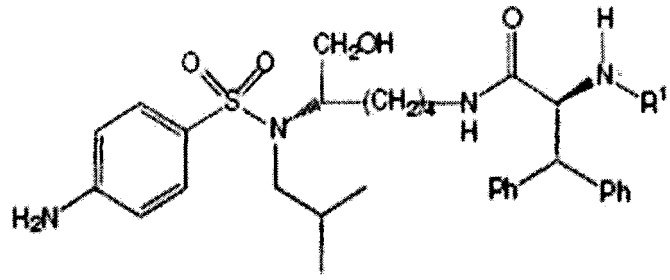
4.7 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

QSAR model was built for describing how some descriptors were directly or indirectly related to the biological activity i.e. pharmacokinetics of a compound. A set of descriptor was chosen and this set was then applied to data set. These descriptors were assumed to influence whether a given compound will succeed or fail in binding to the target protein (Barrat, M.D *et al.,* 1995). Sets of 14 compounds from sulfonamide derivatives class of protease inhibitors were selected as data sets shown in Table 4.9 (Brent R. *et al.,* 2006). A QSAR has been interpreted by calculating a number of steric and electronic parameters by the use of software Hyper Chem and Chem Draw. The descriptors included partition coefficient i.e. Log P and molar refractivity as steric parameter, total binding energy, heat of formation, E_{HOMO}, E_{LUMO} as electronic parameters. The calculated descriptor values are mentioned in Table 4.10. In order to have direct correlation between the descriptor and the compound activity the regression coefficient was supposed be greater than 0.6 and as the regression coefficient decreased it indicated that there was no correlation among the both variables. Descriptors i.e. electronic and steric parameters were taken as dependent while IC_{50} value as independent variables. The regression values were calculated these regression values were recorded as 0.09 for Log P, 0.00 for molar refractivity, 0.04 for total energy, 0.6 for both heat of formation and E_{LUMO} and 0.01 for E_{HOMO} and the plots of E_{LUMO} and heat of formation are shown in Figure 4.19-4.20. This analysis suggested that there was no correlation between IC_{50} value and Log P, molar refractivity, E_{HOMO} and total energy but IC_{50} value was found to be directly related to heat of formation and E_{LUMO} as the regression value of these parameters was greater or equivalent to 0.6

Table 4.9: Data set of sulfonamide class chosen for the QSAR studies along with the IC_{50} values. These compounds are named as QHIV from QHIV1 - QHIV14



Compound	R^1	IC_{50} (µmol)
QHIV1	CH_3O-CO	0.0005
QHIV2	Cyclopropyl-CO	0.00043
QHIV3	Pyrazine-CO	0.00052
QHIV4	2-Pyridyl-CO	0.00046
QHIV5	3-Pyridyl-CO	0.00068
QHIV6	4-Pyridyl-CO	0.00065
QHIV7	6-CH_3-3-Pyridyl-CO	0.0004
QHIV8	6-HO-3-Pyridyl-CO	0.0005
QHIV9	3-Picolyl-CO	0.00024
QHIV10	4-Picolyl-O-CO	0.00039
QHIV11	2-HO-phenyl-CO	0.00063
QHIV12	3-HO-phenyl-CO	0.0004
QHIV13	4-HO-phenyl-CO	0.00053
QHIV14	2-HO-3-CH_3O-phenyl-CO	0.0005

Table 4.10: Steric and Electronic descriptors along with IC_{50} value of the data set chosen for QSAR studies

Compound	IC_{50} (Kcal/mol)	Log P	Molar Refractivity (cm^3/mol)	E_{HOMO} (Kcal/mol)	E_{LUMO} (Kcal/mol)	Heat of Formation (Kcal/mol)	Total Energy (Kcal/mol)
QHIV1	0.0005	4.4	172.83	-41.39	10.93	121718.399	-8920.78
QHIV2	0.00043	4.54	178.25	-0.29	16.59	108511.085	-28042.15
QHIV3	0.00052	2.36	187.6	-4.11	9.91	137403.42	-1668.3
QHIV4	0.00046	3.7	189.13	-50.45	7.54	132948.08	17143.88
QHIV5	0.00068	3.28	190.09	-42.18	20.97	189715.13	33910.933
QHIV6	0.00065	3.28	190.09	-48.4	10.61	173210.65	17406.46
QHIV7	0.0004	4.86	195.37	-7.88	2.17	141910.9	23149.66
QHIV8	0.0005	3.98	191.28	-55.12	6.38	138897.56	15744.23
QHIV9	0.00024	4.37	189.47	-55.4	1.61	123188.82	17384.62
QHIV10	0.00039[a]	4.73	190.38	-55.53	2.8	126414.93	13261.6
QHIV11	0.00063	5.32	192.81	-43.18	16.59	173023.22	14335.71
QHIV12	0.0004	5.32	192.81	-3.59	4.44	138484.35	5710.89
QHIV13	0.00053	5.32	192.81	-7.57	7.46	139470.17	5696.717
QHIV14	0.0005	5.19	200.06	-41.72	12.72	128428.9	5349.31

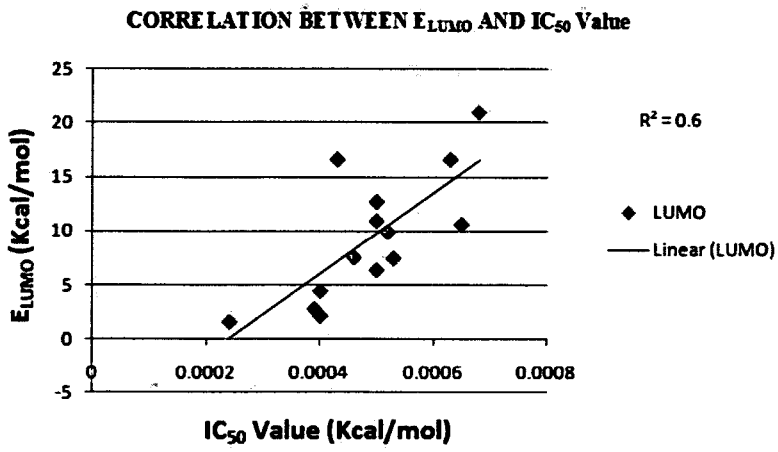


Fig 4.19: Graphical representation showing correlation between E_{LUMO} and IC_{50} value

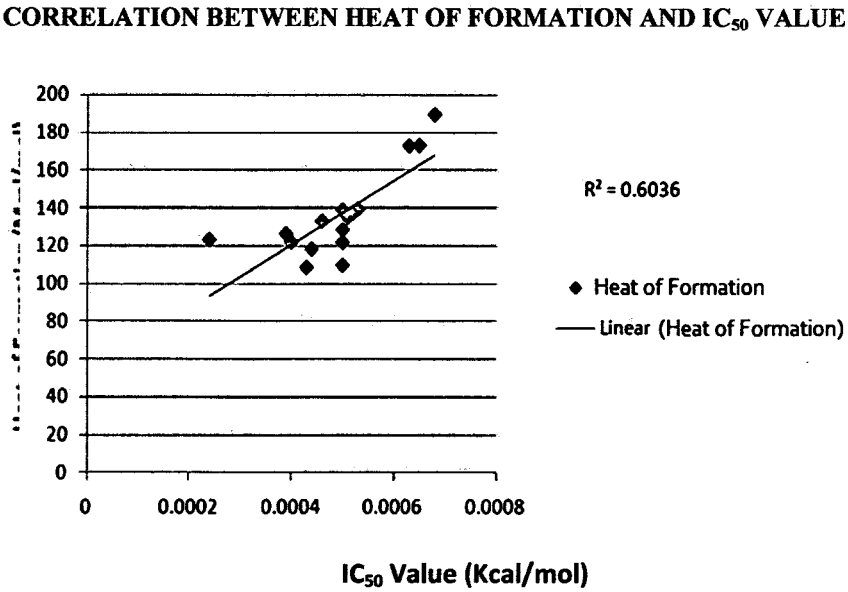


Fig 4.20: Graphical representation showing correlation between heat of formation and IC_{50} value

CONCLUSION

FUTURE ENHANCEMENTS

The present study was aimed at finding novel drug for the treatment of HIV related infections i.e. AIDS that had best pharmacophore features and reversible binding interaction.

Ligand based pharmacophore modeling was carried out on 45 compounds of different classes i.e. isopropanaol amine, indolic (S)-glycidol, triazolamers, sulfoximine, symmertric asymmetric diol, hydrazino-urea, dammarane-type tritepene, triterpenoids, peptidomimetic dihydroxyethylenediamine, allophenylnorstatine- incorporating D-cysteine, and sulfonamide.

Identified pharmacophore features consisted of four hydrogen bond acceptor, twp hydrogen bond donor and two hydrophobic volumes. It is the first pharmacophore model identified specific for the HIV protease inhibitors. Pharmacophore model was proposed using four different classes of compounds so that it can be further tested on the other classes therefore a more generalized pharmacophore model be presented.

Lead compound was identified on the basis of best energy score and interactions by using Auto Dock Vina. On the basis of this lead compound four analogues were designed among them two analogues are identified having potential to be the next HIV protease inhibitors as they have more binding interactions than the lead compound which concludes that they will have more bioavailability than the lead compound. The two analogues that show best interactions are proposed for clinical trials and can be

synthesized in laboratory in order to bring forth a better drug having high bioavailability than the prior drugs which may treat HIV related infections.

QSAR studies were done using one class of compound that is sulfonamide which concluded that biological activity of the compound was directly related to the two descriptors i.e. heat of formation (HF) and E_{LUMO} both of them having regression coefficient equal to 0.6. The lead compound was from the major HIV class division i.e. peptidomimetic to which the boosting compounds do belong hence the analogues proposed here should be analyzed as the next boosting elements. The two descriptors which were found to be critical for HIV protease inhibitor in this study may be evaluated for other classes of compounds to get a general view.

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