IMPACT: International Journal of Research in Applied Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 3, Issue 9, Sep 2015, 139-146

DESIGNING, DOCKING AND TOXICITY STUDIES OF NOVEL HIV-1 PROTEASE INHIBITORS

PAVANI ELIPILLA [1] & AMMANI KANDRU [2]

[1]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

[2]Co.ordinator, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

ABSTRACT

HIV virus causes Acquired immune deficiency syndrome (AIDS). HIV virus type-1 protease plays crucial role in the life cycle of the HIV viral particles. So this protein has been targeted as one of the antiretroviral treatment of AIDS, and HIV-1 Protease inhibitors as anti-HIV drugs. Due to the frequent development of drug resistance there is always a need to develop new drugs which are non toxic and efficient inhibitors. The present study aims to focus on designing of 10 non toxic, novel lead molecules which targets HIV 1 protease. And performing docking and toxicity studies to calculate their binding energies, and to predict their toxicity properties. Protein- ligand interactions were studied using HIV type 1 Protease protein, PDB ID - 1HXW extracted from PDB to evaluate the binding efficiency of various molecules towards the active site. And these values were compared with commercially available FDA approved HIV drugs Ritonavir, Saquinavir, Amprenavir, Indinavir, Lopinavir, Nelfinavir. The final docking and toxicity prediction studies proves that these novel drug molecules were satisfied all drug likeness rules, which are violated by FDA approved drugs, and have the binding energies and predicted inhibitory constant Ki nearly similar to commercially available drugs.

KEYWORDS: HIV, HIV-1 Protease, Anti Retroviral Drugs, Drug Designing, Docking, Predicted Toxicity Studies

INTRODUCTION

Most of the HIV epidemics are caused by HIV type 1, and type 2 variants (Alexander Wlodawer, 2002). HIV viral cells specifically choose T cells which are having CD4 receptors. Soon after binding to them, the viral genome enters the host cytoplasm and with the help of viral reverse transcriptase enzyme it converts its viral RNA into DNA then integrates into the host genome which is directed by viral integrase. During translation this genome will start synthesizing HIV viral proteins since it has integrated viral genome within it. Then HIV protease comes into action to process these proteins and activates them which eventually involves in the construction of viral mature proteins. These proteins and viral RNA packed together to form new virions and released to infect the new healthy host cells to spread the disease.

The most common checkpoints for the development of HIV drugs are, blocking the viral adhesion to host cells, blocking viral fusion and uncoating, inhibit the activity of reverse transcriptase, regulate the gene expression and block the HIV protease function. HIV 1 protease is a dimeric protein with 2 identical monomers. 25th aspartic acid of HIV protease imparts the catalytic activity of this protein (Ashraf Brik,Chi-Huey Wong, 2003). In addition to it, this protein's active site possessa signature amino acid sequence Asp-Thr-Gly which interacts strongly with the substrates or inhibitors. By designing ligands which could be able to bind and inhibit the activity of these catalytic amino acids, we can block the function of HIV protease. So ultimately it stops the processing and maturation of viral proteins thereby inhibits the

formation and release of new viral particles. There are 2 ways to block this protein, either the active site of this protein should be mutated or block it by using some inhibitors (Alexander Wlodawer, Jiri Vondrasek,1998). This present study focused on designing novel ligands which can efficiently block the HIV 1 protease by binding its activesite. These novel ligands binding energies were calculated by docking them into protein and these docking scores and Ki were compared with commercially available FDA approved HIV drugs Ritonavir, Saquinavir, Amprenavir, Indinavir, Lopinavir, Nelfinavir(Joseph J. Eron, Jr.,2000).

MATERIALS AND METHODS

Ligands Preparation

10 novel non peptide ligands were designed by means of ligand based drug design method. Their structures were drawn using ACD labs chemsketch tool (Figure 1). 6 FDA approved HIV drugs were selected for comparative studies and their structures were also drawn using chemsketch. To remove the steric clashes these ligands were taken for energy minimization and optimization step, by using Prodrg online server (A. W. Schüttelkopf and D. M. F. van Aalten (2004).PRODRG: a tool for high throughput crystallography of protein ligand complexes, *Acta Crystallogr* D60, 1355–1363.). Then the corrected PDB files of all ligands were collected for further steps. IUPAC names of the 10 novel drugs were mentioned below.

- 2-amino-5-{[3-amino-4-(4-amino-3-hydroxybutan-2-yl)piperidin-1-yl]oxy}-N-tert-butyl-3-methyl benzamide
- [7-amino-6-methyl-2-(4-methylpentan-2-yl)-8-(trifluoromethyl)quinolin-4-yl](4-aminopiperidin-2-yl)methanol
- N-tert-butyl-4-{[3-(4,5-dimethyl-3H-pyrrol-2-yl)phenyl]methyl}-1-(2-hydroxypropyl)piperazine-2-carboxamide
- N-tert-butyl-1-(2-hydroxypropyl)-4-[3-(3H-pyrrol-2-yl)phenoxy]piperazine-2-carboxamide
- N-tert-butyl-2-{4-hydroxy-3-[(4-hydroxyphenyl)methyl]butyl}-decahydroisoquinoline-3-carboxamide
- 3-[6-(tert-butylamino)-8-(1-methoxypropan-2-yl)-3-methyl-7-oxo-decahydroisoquinolin-2-yl]butanamide
- N4-[5-(2-amino-4-tert-butylphenyl)pentan-2-yl]quinoline-3,4,6-triamine
- N-tert-butyl-6-(4-oxo-6-phenyl hexan-2-yl)-decahydro-2,6-naphthyridine-3-carboxamide
- {2-[(tert-butylamino) methyl] 8 (pyridin-2-yl) quinolin-4-yl}(piperidin-2-yl) methanol
- 1-[4-amino-1-(4-amino-2-cyclopentylbenzenesulfonyl)-5-methylpiperidin-3-yl]-2-(oxolan-3-yl) ethan-1-one
- The 6 FDA approved drug structures taken for comparative studies were Amprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir.

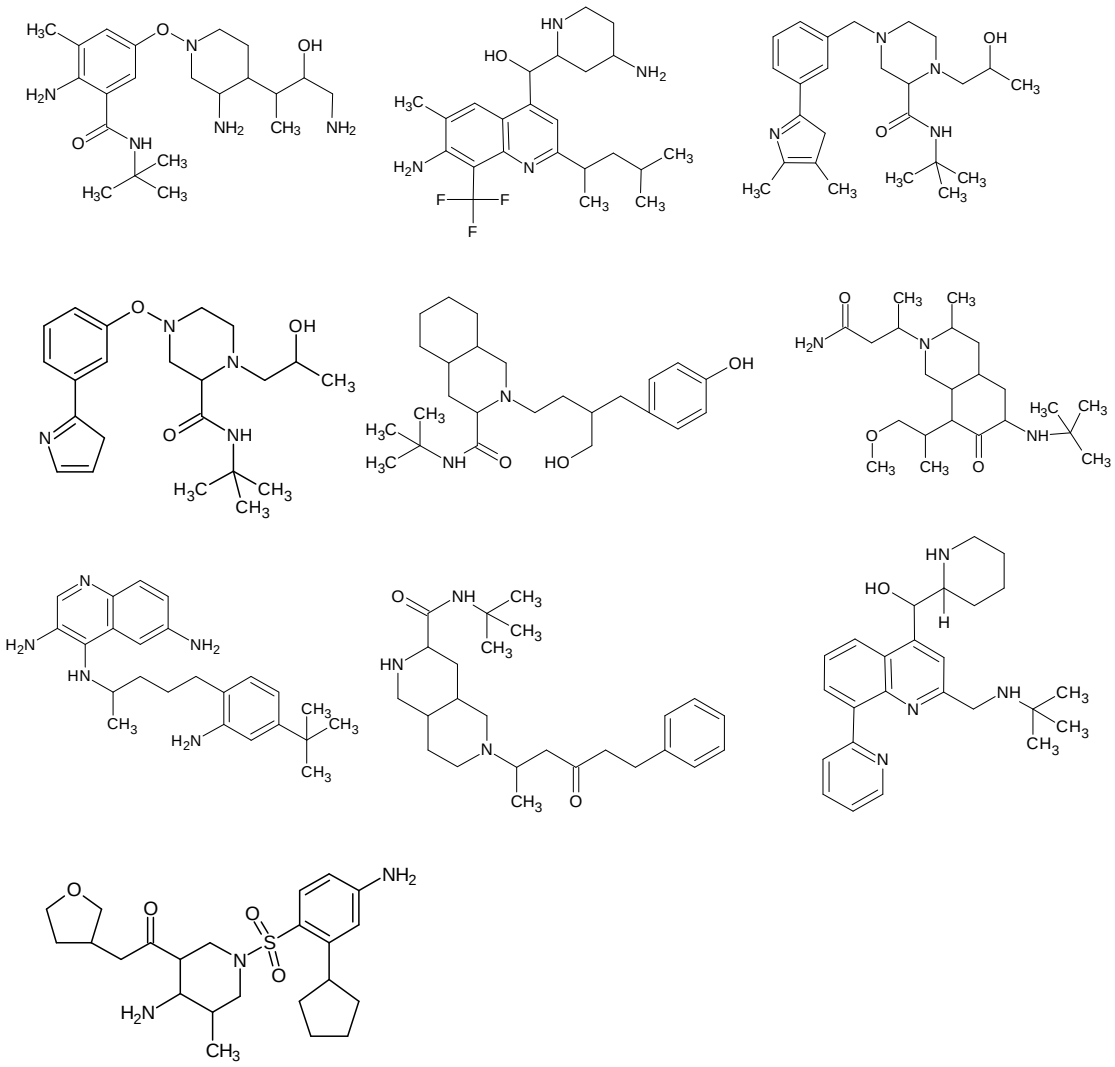

Figure 1: 10-Non Peptide Novel Ligands were Designed. Structures were Drawn in ACD Labs - Chem Sketch Tool (1- 10, from Left to Right)

Protein Preparation

In this present study a crystal structure of HIV-1 Protease (PDB: 1HXW) was extracted from RCSB Protein Data Bank. All HETATM were deleted. To repair distorted geometries and to fill the missing atoms in the crystallographic structure, the protein was subjected to protein optimization and energy minimization step by using SPDV tool, where it was processed using 200 steps of steepest descent method. Then this optimized structure was taken to docking step.

Molecular Docking

Molecular docking was carried out using Autodock4.0 tools. Above optimized protein (1 HXW) was taken and water molecules were removed then polar hydrogens and kollman charges were assigned. Ligand Torsions were allowed to rotate Auto grid generated grid parameter files (.gpf). The grid dimensions on X, Y, Z were set to 60×60×60 Å. It covers all the active site area of the protein which accommodates the ligands. Through literature it was known that the catalytic amino acid of HIV 1 protease is, 25th asparticacid. Autodock generated dock parameter files (.dpf). Then Lamarckian Genetic Algorithm (LGA) was run by using cygwin which has generated logarithmic files.glg,.dlg. Then autodock was run

to dock the ligand into the protein active site and generates 10 best conformational poses, which can be visualized using UCSF-chimera and Ligplot to get 2d images of docked protein. We can visualize the hydrogen bonds, hydrophobic interactions and vanderwaal interactions between ligand and active site amino acids (Figures 2a -2g).

Toxicity Prediction

All of the10 novel ligands and FDA approved drugs were checked for the violation of Lipinsky filter, Viber filter, Ghose filter, lead likeness rules (Table 1 and 2). Other properties like LogP and LogS, Protease inhibitory property, Solvent Accessible Surface Area, Polar surface area, Estimated Binding Energy, Inhibition Constant Ki were predicted using online servers like chemicalize, Molinspiration, ALOGPS.

RESULTS AND DISCUSSIONS

A series of 10 non peptide novel ligands were designed (Figure 1) and they were along with 6 FDA approved commercially available HIV drugs subjected to docking with HIV-1 protease. All of the docking results and toxicity prediction studies values were incorporated in (Table 1 and 2). The docking studies reveals that all the novel ligands were having strong binding affinity towards the active site of HIV 1 protease. The predicted binding energy of these 10 ligands were in between -8.26 to -11.51 Kcal/mol and predicted inhibitory constant Ki values were in between 3.65 nM to 882.45 nM. (Table 1). Protein-Ligand interactions were visualized using LigPlot software which generated 2d images of the formation of hydrogen bonding between active site amino acids of HIV protease and the ligand molecule (Figures 2a -2g).

Table 1: Calculation of Binding Energy, Inhibitory Constant and Other Properties of Novel Ligands and FDA Approved HIV Drugs

Ligands	Estimated Binding Energy Kcal/mol	Estimated Inhibition Constant Ki.	H Bonds Formed	Molecular weight	LOGP	LOGS	Polar Surface Area:
Ligand-1.	-11.32	5.02 nM	4	407.55	0.59	-3.22	139.86
Ligand-2.	-11.51	3.65 nM	5	438.52	3.44	-4.51	97.19
Ligand-3.	-10.91	10.07 nM	5	426.5	2.00	-3.70	68.17
Ligand-4.	-8.26	882.45nM	1	400.51	1.23	-3.43	77.40
Ligand-5.	-10.77	12.65 nM	7	416.5	3.58	-4.41	72.80
Ligand-6.	-8.28	848.78 nM	4	395.5	1.75	-3.72	84.66
Ligand-7.	-8.53	558.20 nM	8	391.55	3.98	-5.43	102.98
Ligand-8.	-9.77	68.57 nM	4	413.5	3.09	-4.53	61.44
Ligand-9.	-11.14	6.86 nM	4	404.54	3.48	-5.10	70.07
Ligand-10	-10.88	10.62 nM	6	449.6	1.74	-4.11	115.72
Amprenavir	-8.26	877.36 nM	6	505.62	2.43	-4.01	131.19
Indinavir	-12.33	912.42 pM	5	613.7	2.81	-4.11	118.03
Lopinavir	-9.57	96.10 nM	5	628.3	4.69	-5.51	120.00
Nelfinavir	-10.34	26.51 nM	4	567.31	4.72	-5.47	101.90
Ritonavir	-8.00	1.36 uM	6	720.31	5.22	-5.76	145.78
Saquinavir	-12.05	1.46 nM	8	670.38	3.16	-5.43	166.75

And these 10 novel ligands and FDA approved drugs were checked for the violation of Lipinski filter, Viber filter, Ghose filter, like rules (Table 2). Other properties like LogP and LogS, Protease inhibitory property, Solvent Accessible Surface Area, Polar surface area values were predicted using online servers like chemicalize, Molinspiration, ALOGPS. A ligand should satisfy all the ADME properties to be a promising drug molecule. These novel ligands were accepted by all the drug likeness filters like veber filter, lipinski rule, ghose filter, muegge filter. A drug molecule will have a poor

permeability if its polar surface area exceeds 140 Å², The absorption of a drug will be more when it's LogP (lipophilicity) is < 5, and LogS (aqueous solubility) is ≤ 0 (Bob Gotwals, NCSSM Chemistry 2009), and Molecular weight should be < 500 dalton. These 10 novel ligands were having polar surface area ranging from 61.44 Å² to 139.86 Å², LogP values were ranging from 0.59 to 3.98, LogS values falls within the range of -3.22 to -5.43 and molecular weight ranged between 391.55 to 449.6 daltons, which were proven to be having good pharmacological properties.

Table 2: Calculation of Predicted Pharmacological Properties of Novel and FDA Approved Drugs

Ligands	Solvent Accessible Surface Area:	Protease Inhibitory Property	Lipinski's Rule of five	Bio Availability Filter	GHOSE Filter	Lead Likeness Filter	MUEGGE Filter	VEBER Filter
Ligand-1.	675.50	0.63	Yes	yes	yes	yes	yes	yes
Ligand-2.	661.93	0.51	yes	yes	yes	yes	yes	yes
Ligand-3.	716.16	0.25	yes	yes	yes	yes	yes	yes
Ligand-4.	633.09	0.52	yes	yes	yes	yes	yes	yes
Ligand-5.	720.69	0.61	yes	yes	yes	yes	yes	yes
Ligand-6.	711.18	0.60	yes	yes	yes	yes	yes	yes
Ligand-7.	641.49	0.12	yes	yes	yes	yes	yes	yes
Ligand-8.	719.52	0.54	yes	yes	yes	yes	yes	yes
Ligand-9.	653.09	0.45	yes	yes	yes	yes	yes	yes
Ligand-10	694.22	0.49	yes	yes	yes	yes	yes	yes
Amprenavir	767.65	1.01	no	no	no	no	yes	no
Indinavir	964.60	0.66	no	no	no	no	no	no
Lopinavir	994.10	0.42	no	no	no	no	no	no
Nelfinavir	892.55	0.58	no	yes	no	no	yes	yes
Ritonavir	1066.46	0.35	no	no	no	no	no	no
Saquinavir	1031.69	0.40	no	no	no	no	no	no

CONCLUSIONS

It was concluded that, these newly designed 10 ligand molecules can be considered as a better HIV drugs, than the commercially available HIV drugs since they have shown nearly same binding efficiency and better pharmacological properties compared to FDA approved drugs. All the FDA approved 6 HIV protease inhibitors violated the Lipinski rule, veber rule and all other standard drug approval rules, whereas these novel ligands satisfied all the drug likeness rules which reduces the side effects and safe to clinical trials. If these designed novel analogues were synthesized and tested in animal models theywould gives us promising results for the discovery of better drugs.

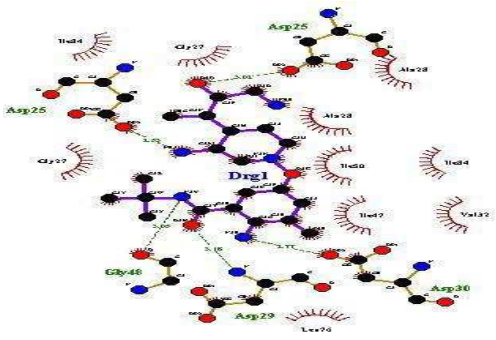

Figure 2a: 1HXW and Ligand 1

Figure 2a: Ligplot 2d Analysis of the Active Site of HIV-1 Protease Complexed with the Novel Ligand-1 and the

Green Dots Represents the Hydrogen Bond Formation between Liand and Active Site Amino Acids of HIV Protease

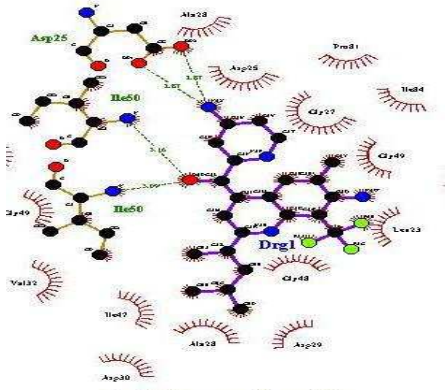

Figure 2b: 1hxw and Ligand 2

Figure 2b: Ligplot 2d Analysis of the Active Site of HIV-1 Protease Complexed with the Novel Ligand-2 and Ligand-2 and the Green Dots Represents the Hydrogen Bond Formation between Ligand 2 AND Active Site Amino Acids of HIV Protease.

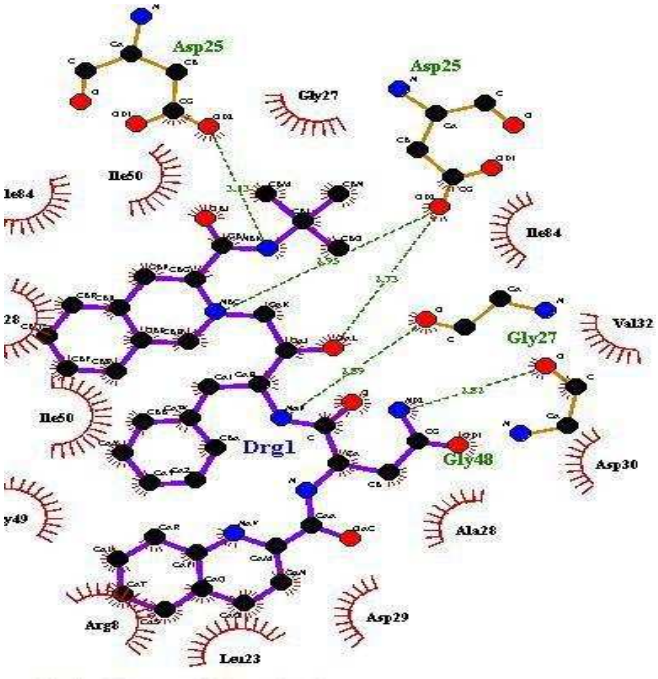

Figure 2c: 1HXW and Saquinavir

Figure 2c: Ligplot 2D analysis of the Active Site of HIV-1 Protease Complexed with anti Retroviral Drug Saquinavir and the Green dots represents the Hydrogen bond Formation between Ligand and active site Amino Acids of HIV Protease.

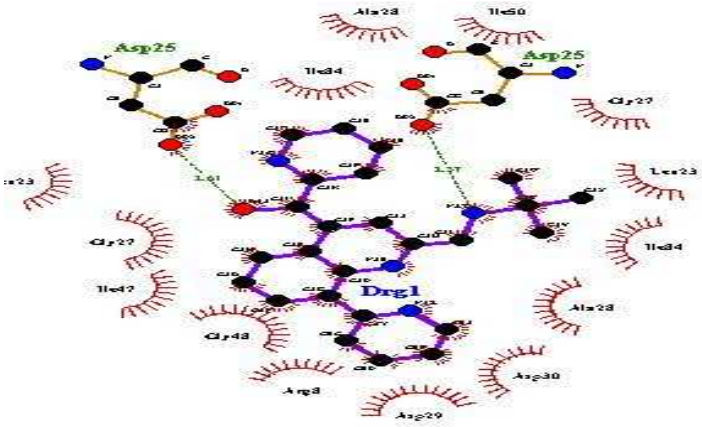

Figure 2d: 1HXW and Ligand 9

Figure 2d: Ligplot 2D Analysis of the Active site of HIV-1 Protease Complexed with Anti Retroviral Drug Ligand 9 and the Green Dots Represents the Hydrogen Bond Formation between Ligand and Active Site Amino acids of HIV protease.

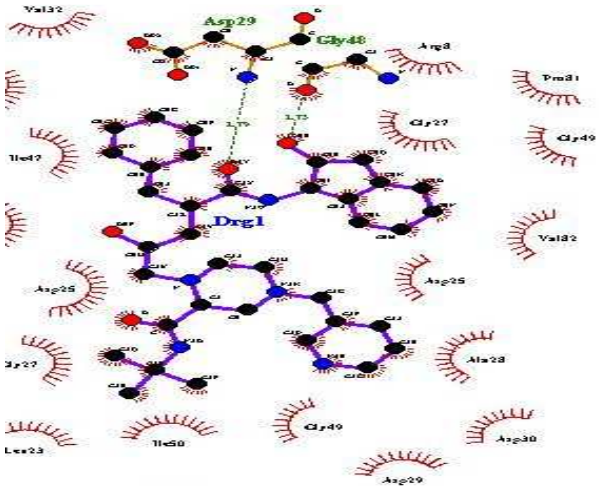

Figure 2e: 1HXW and Indinavir

Figure 2e: Ligplot 2D Analysis of the Active Site of HIV-1 Protease Complexed with Anti Retroviral Drug Indinavir and the Green Dots Represents the Hydrogen bond Formation between Ligand and active site amino acids of HIV Protease.

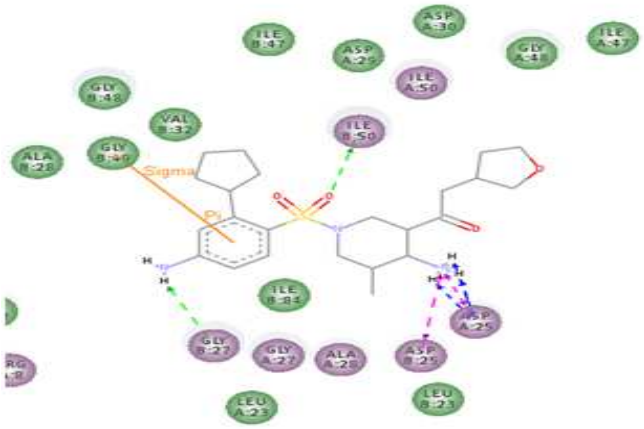

Figure 2f: 1HXW and Ligand 10

Figure 2f: Schematic 2D Representation of the Interation between HIV Protease Active Site Amino Acids with Novel Ligand-10, Image was Generated Using Accelrys Discovery Studio.

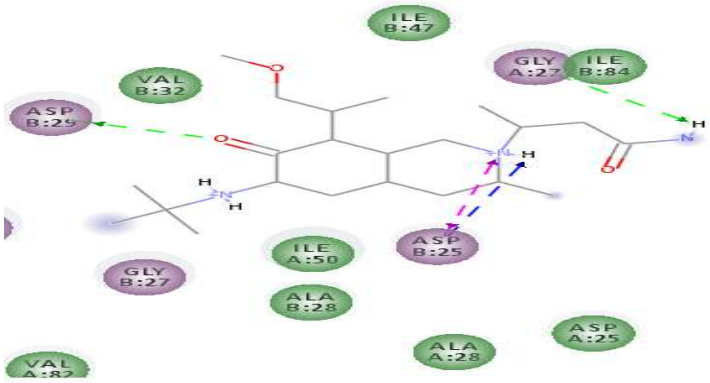

Figure 2g: 1HXW and Ligand 6

Figure 2g: Schematic 2D Representation of the Interation between HIV Protease Active Site Amino Acids with Novel Ligand-10, Image was Generated Using Accelrys Discovery Studio

REFERENCES

1. Ashraf Brik and Chi-Huey Wong, HIV-1 protease: mechanism and drug discovery, Org, Bio mol Chem.,2003,1, 5-14, DOI: 10.1039/ b208248a
2. Alexander Wlodawer and Jiri Vondrasek, Inhibitors of HIV-1 protease: A Major Success of Structure-Assisted Drug Design, Annu. Rev. Biophys.Biomol. Struct. 1998. 27:249–84
3. Alexander Wlodawer, Rational approach to aids drug design through structural biology, Annu. Rev. Med. 2002. 53:595–614
4. Bob gotwals, Case Study: Drug Solubility, NCSSM Chemistry, Feb,12, 2009
5. Joseph J. Eron, Jr., HIV-1 Protease Inhibitors, Clinical Infectious Diseases 2000; 30 (Suppl 2):S160–70