June 2010 ISSN : 0974 - 7435 Volume 4 Issue 2

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 4(2), 2010 [89-94]

Comparitive modelling and docking studies of HIV 1 protease

B.Meenakshisundaram[1], R.Prasanna[2]*

[1]SRM Arts and Science College, Kattankulathur, Potheri, Chennai, (INDIA)

[2]Research and Development, BioGenex Life Sciences Pvt. Ltd., Secunderabad, (INDIA)

E-mail : prachu.rg@gmail.com

Received: 3rd March, 2010 ; Accepted: 13th March, 2010

ABSTRACT

Infection with Human Immunodeficiency Virus (HIV) followed by immune deficiency is a major threat to human. As HIV 1 protease is essential for the proteolytic cleavage of precursor viral protein, it remains as an effective and more reasonable target for drug design against HIV. In the present work, we resolved a three dimensional structure of HIV 1 protease using comparative modeling technique and identified the active sites. We designed a lead candidate using hydrogen bonding potential and amino acids active site affinities. Validation was done using *in silico* docking techniques. The result clearly demonstrates the binding affinity of the drug candidate with the HIV 1 protease. The current study offers a new drug candidate that has promising inhibitory activity on the HIV 1 protease.

KEYWORDS

HIV;
Protease;
Comparative modeling;
Active sites;
Hydrogen bonding potentials;
In silico docking.

INTRODUCTION

The first case of HIV infection was reported by CDC (USA) in 1981 from the blood samples of homosexual men suffering from severe immune deficiency. Later many strains such as HTLV III (Human T Lymphotropic Virus) and LAV (Lymphadenopathy associated Virus) were reported. Also the third virus ARV (AIDS-associated reterovirus) was reported in the same year[1,2]. HIV comprises of complex genome with regulatory and structural genes. Genes such as Gag, pol, env comprises of structural part whereas tat and rev are regulatory genes[3]. Pol genes codes for protease along with other products such as integrase and reverse transcriptase[4]. Current strategy is to suppress the replication of the virus through HAART (highly active anti-retroviral therapy)[5,6]. In this scenario protease inhibitors are more powerful in preventing viral replication. Currently there are eight protease inhibitors which are approved by the US.FDA which includes Ritonavir and Lopinavir. The most competitive research at present is finding the HIV 1 protease inhibitors because of its mutations[7]. The high rate of mutation in viral proteins occurs due to the mistranslation of 1 in every 10,000 codons. Thus the HIV 1 protease

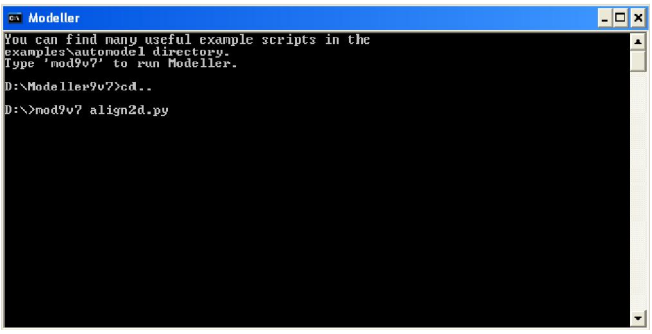

Figure 1 : Modeller command prompt

FULL PAPER

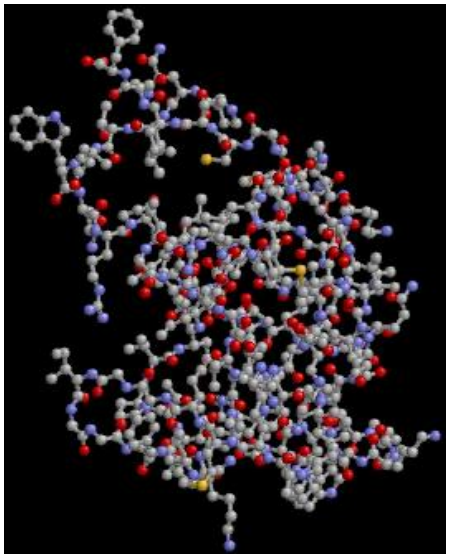

Figure 2 : Results for modeller 9v7. Three dimensional structure of HIV 1 protease was constructed using modeller 9v7. The template used here was crystal structure of HIV -1 CRF -01

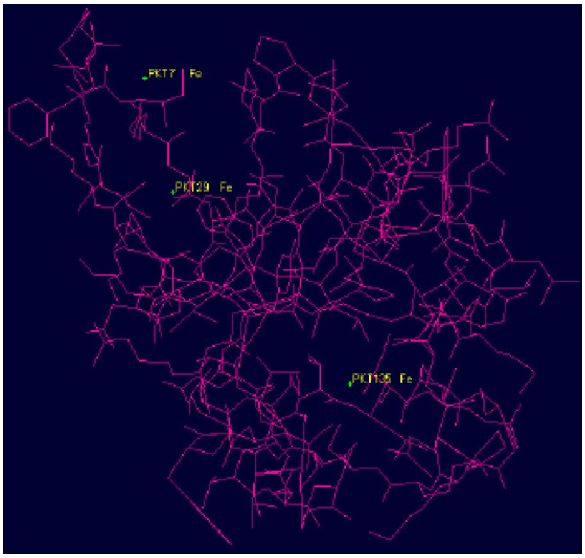

Figure 3 : Results for the pockets present in the HIV 1 protease. Ligsite was used to determine the active sites present the HIV-1 Protease

becomes drug-resistance to the protease inhibitors[8]. All the approved protease inhibitor drugs have large hydrophobic moieties that interacts with the hydrophobic P2-P2' pockets in the active sites[9]. Therefore to find an effective HIV 1 protease inhibitor the good structure of protein is necessary.

The three dimensional structure of the protein provides a valuable information, which is usually performed using X-ray crystallography and NMR studies. A rapid alternate for comparative modeling is to use a theoretical model. The current study uses a restraint based method to build such model using Modeller software. By comparing the spatial restraints of the homologous template the structure of the target can be defined. Experimentally defined structure serves as a template. The current study uses Auto-Dock Vina for docking analysis.

METHODOLOGY

Homology modeling

The three dimensional structure of HIV 1 protease was designed using Modeller. Modeller uses restraint based technique for the prediction of three dimensional structures. For the better resolution of the structure the template was selected based on many criteria such as e Value, number of positives and number of identities. Blast search was performed to find the best template. The alignment score for target and crystal structure of HIV 1 crf01 (3D3T) are as follows.

Expect value = 1- 49
Positives = 100%
Identities = 93%
Gaps = 0 %

Using the spatial restraints of the template, three dimensional structure of the HIV1 Protease was constructed[10].

The protein can form pockets where the ligand binds. Thus the identification of pockets becomes more important in protein-ligand docking studies. Ligsite, an online web server extends the pockets by scanning along the four cubic diagonals along with x, y, z axes[11]. This identifies the pockets in the HIV 1 Protease which were used for docking the ligand with the protein.

Pocket identification

Three pockets were identified.

PKT-135
PKT-29
PKT-7

Ferrous (d_2sp^3) atoms were present in the active site.

Following this, the amino acids nearer to the active site were identified. The distance between the center of the pockets and the nearest amino acid were calculated using SWISS PDB viewer. After analyzing the nature of the active site amino acid such as hydrophobic or hydrophilic the drug was searched.

FULL PAPER

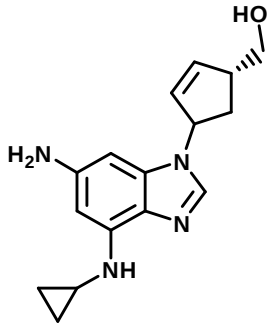

Figure 4 : Structure of abacavir

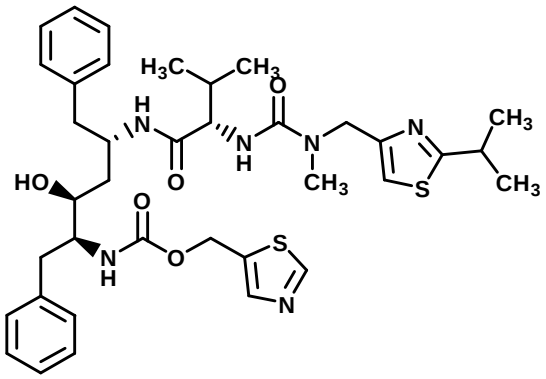

Figure 5 : Structure of ritonavir

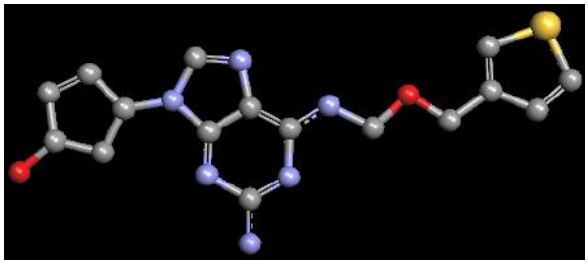

Figure 6 : Structure of new drug candidate-Abarito

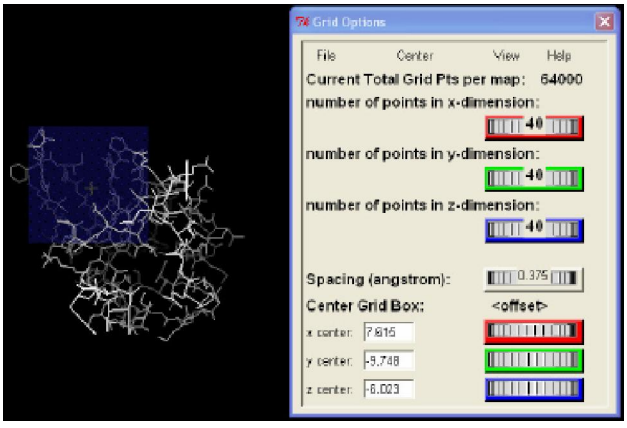

Figure 7 : Autodock-vina showing grid box

Ritonavir, Abacavir are the two drugs, currently used in the treatment of HIV 1 infection. Abacavir and Ritonavir has Lopinavir as basic compound. Lopinavir is a potent inhibitor of the cytochrome P_{450} $3A_4$

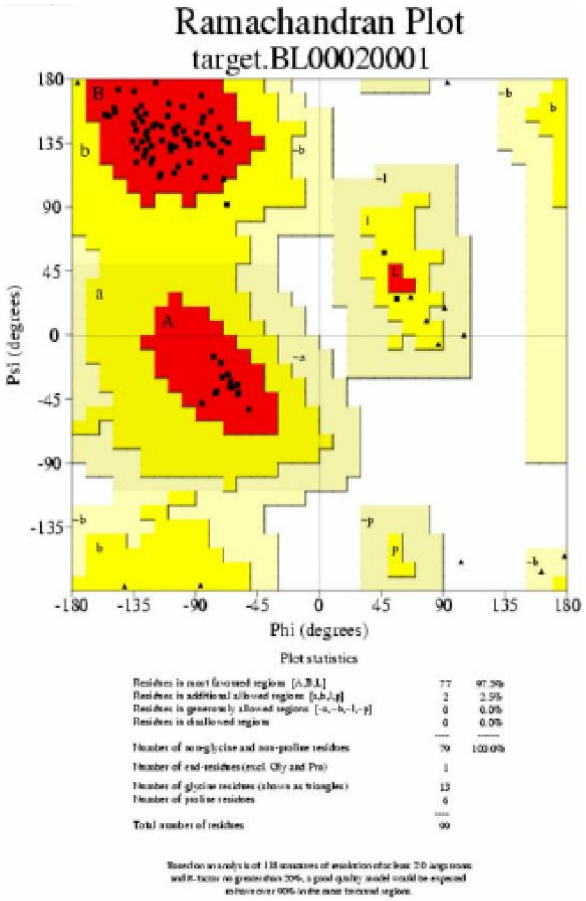

Figure 8 : Ramachandran plot showing the result for the HIV 1 protease after comparative modelling

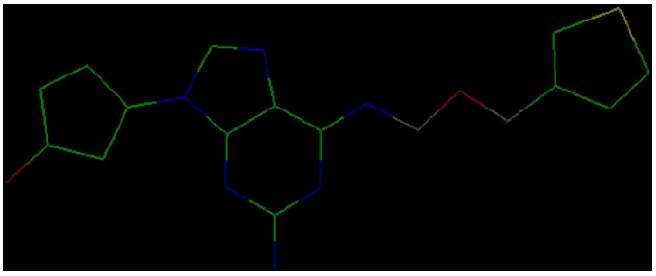

Figure 9 : Structure of abarito before docking

(CYP_3A_4). Lopinavir/Ritonavir causes increase in inhibitory quotient (IQ), this high IQ acts as a barrier for the formation of viral resistance to the drug[12]. From the drug bank report it has been identified that Abacavir is a powerful nucleoside analog of reverse transcriptase receptor.

It has an average molecular weight of 286.3323. Abacavir was converted into active metabolites and incorporates with the viral DNA. This induces the negative regulation of HIV 1 reverse transcriptase and act as a chain terminator. Same way the information and the structure of the ritonavir was also collected and ana-

FULL PAPER

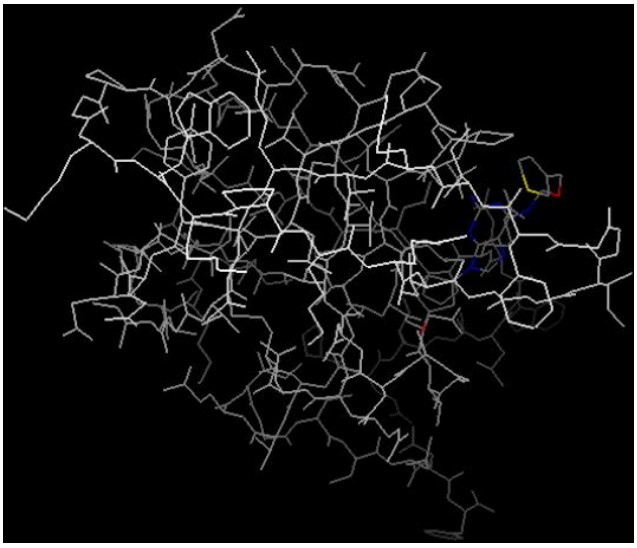

Figure 10 : Newly formulated drug candidate abarito showing interaction with the active site

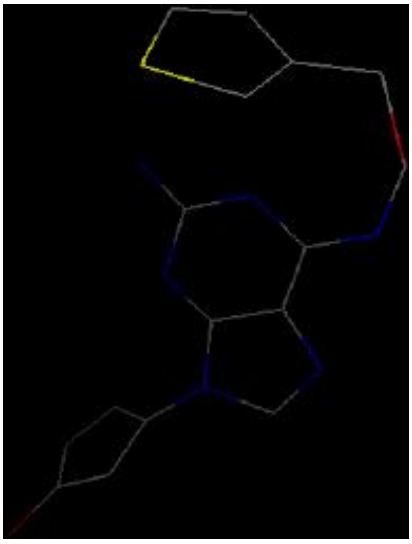

Figure 11 : Structure of the newly formulated HIV 1 protease inhibitor after docking

lyzed from drug bank. The 3'OH of the drug prevents the formation of 5'to3' phosphodiester linkage of viral DNA[13]. The co-formulated drug named Kaletra contains the combination of lopinavir/ritonavir[14].

Ritonavir is a powerful inhibitor of HIV1 protease. HIV 1 protease is required for cleavage of certain viral polyprotein precursors in to functional proteins. This Ritonavir binds to the active site of the HIV 1 protease and inhibit its activity[17,18].

In this study we have designed a potent HIV 1 inhibitor which has the properties of both Abacavir and Ritonavir.

Formulation of the drug abarito

The lead molecule was designed using chemsketch1.1. We made changes in side chains of the molecules except the 3'OH group of Abacavir. This drug also shares the Ritonavir structure. The sulphur group in the benzene ring of the ritonavir was coformulated with the new HIV 1 protease inhibitor. Since it shares the properties of both abacavir and ritonavir it was named "Abarito".

After the drug formulation, the energy optimization and geometrical optimization was done using Argus lab. The formula molecular weight ranges 372.401.

Energy minimization and geometrical optimization

Conformational analysis of the lead molecule is based on the molecular mechanics. Using this method the molecular structure, energies and other properties can be calculated. Energy minimization is the integral part of the molecular mechanics. In the present work, Argus lab was used for performing energy minimization and geometrical optimization[16].

Energy calculations using arguslab

Method – AM1
SCF type – RHF
Total energy = -93387.29 Kcal/mol

Calculations for geometrical optimization

Method – UFF
Linear search – BFGS
Total energy = 108.83 Kcal/mol

Docking

Docking was performed using Autodock vina 4.2. As Autodock 4.0 has better resolution than autodock 3.05, we used quite more advanced version than autodock 4.0. Autodock vina 4.2 runs on windows platform and unlike other autodock versions it does not need Cygwin platform to run on windows machine[15].

RESULTS AND DISCUSSION

After the HIV-1 protease has been modeled using Modeller9v7, it was validated using Ramachadran plot from savs server. The above result indicates that there are no molecules in the disallowed regions. Also there are 97.5% amino acids in the most favored regions which imply the stability of the model.

TABLE 1

Drug pose	Binding affinity(kcal/mol)	RMSD value
1	-6.1	0.000
2	-6.1	6.355
3	-6.0	21.248

TABLE 2

Drug pose	Binding affinity(kcal/mol)	RMSD value
1	-6.5	0.000
2	-6.4	20.509
3	-6.3	19.876

After the drug candidate has passed all the entire prerequisite steps such as energy minimization and geometrical optimization, the drug was docked with all the three active sites present in the HIV 1 protease.

The inhibitor after formulation contains 6 torsions angles namely. They are C10, N9, C1, N17, C18, and C21. Thus the inhibitor can rotate in these torsions angles in order to get the good dock pose.

The result (Figure 8) confirms the docking ability of newly formulated drug abarito which consist of essential 3'OH of abacavir and sulphur group of ritonavir. The inhibitor is docked with all active site –PKT 135, PKT, 29, PKT 7. Among the three active sites the inhibitor had more affinity with PKT 29.

TABLE 1 gives the binding affinity and the RMSD value of the newly formulated inhibitor on docking with PKT 135. The first pose in the auto dock result gives the reasonable binding affinity and RMSD value.

The inhibitor was also docked with the PKT 29; TABLE 2 gives the results of interaction of inhibitor with the active site 29. Comparing the two results, drug interaction on PKT 29 was more than the PKT 135. PKT 7 does not contain any amino acid in close to active site and subsequently it is impossible for an inhibitor to bind with those amino acids. Hence the inhibitor was not docked with PKT 7.

The newly formulated inhibitor abarito had more affinity toward PKT 29 (active site). The binding affinity -6.5kcal/mol was more reasonable than -6.1 along with its RMSD value (0.000) which was a good sign for a perfect docking.

When comparing the structure of the inhibitor before (Figure 8) and after docking (Figure 10), it is evident that the designed inhibitor has ability to bind with the active site of HIV 1 protease. At this dock pose the binding affinity of the inhibitor was high as -6.5 kcal/mol.

CONCLUSION

The present study gives a new drug candidate that could be used as and inhibitor to HIV 1 protease. The 3'OH group present can bind with the HIV 1 protease and inhibit the enzyme activity. Since the drug candidate has low molecular weight, the absorption and excretion rate becomes high. The number of acceptors and donors was also less than 5 following Lipinski rule of drug. Since abarito possess the property of both Abacavir and Ritonavir it is postulated to be more effective in the treatment of HIV. Thus the newly formulated abarito gives promising hopes of inhibitory activity against HIV 1 protease.

REFERENCES

[1] Afshan Naz, Khalida Bano, Farhat Bano, Najaf Abbas Ghafoor, Naheed Akhtar; J.Pharm.Sci., **22(1)**, 78-82 **(2009)**.

[2] Ashish Chandwani, Jonathan Shuter; Lopinavir/ Ritonavir in the Treatment of HIV-1 Infection: Review.

[3] F.Barre-Sinoussi, J.C.Chermann, F.Rey, M.T.Nugeyre, S.Chamaret, J.Gruest, C.Dauguet, C.Axler-Blin, F.Vezinet-Brun, C.Rouzioux; Science, **220**, 868-871 **(1983)**.

[4] Bingding Huang, Michael Schroeder; BMC Structural Biology, **6**, 19 **(2006)**.

[5] Caroline Solas, Isabelle Poizot-Martin, Marie-Pierre Drogoul, Isabelle Ravaux, Catherine Dhiver, Alain Lafeuillade, Thierry Allegre, Malika Mokhtari, Jacques Moreau, Gérard Lepeu, Nathalie Petit, Alain Durand, Bruno Lacarelle; 'Therapeutic Drug Monitoring of Lopinavir/Ritonavir Given Alone or With a Non-Nucleoside Reverse Transcriptase Inhibitor British Journal of Pharmacology.

[6] P.Carrieri, B.Spire, S.Duran, C.Katlama, D.Peyramond, C.Francois, G.Chene, J.M.Lang, J.P.Moatti, C.Leport; J.Acquired Immune Defic.Syndr., **32**, 38-47.

[7] A.Das, D.R.Rao, M.Hosur; Protein Pept.Lett., **14(6)**, 565-8 **(2007)**.

[8] E.De Clercq; Med.Res.Rev., **22(6)**, 531-65 **(2002)**.

[9] R.C.Gallo, L.Montagnier; Science, **298**, 1730-1731 **(2002)**.

[10] M.Joshua; Virology Journal, **4**, 100 **(2007)**.

FULL PAPER

[11] J.A.Levy; Microbiol.Rev., **57**, 183-289 **(1993)**.
[12] N.L.Madhavi Nalam, A.Celia Schiffer; Curr.Opin. HIVAIDS, **3(6)**, 642-646 **(2008)**.
[13] Mukesh Kumar, Vishal Prashar, Smita Mahale, Madhusoodan V.Hosur; Biochem.J., **389**, 365-371 **(2005)**.
[14] Nicholas Furnham, I.W.Paul de Bakker, Swanand Gore1, F.David Burke, L.Tom Blundell1; BMC Structural Biology, **8**, 7 **(2008)**.
[15] O.Trott, A.J.Olson, Auto Dock Vina; Journal of Computational Chemistry, **31**, 455-461**(2010)**.
[16] K.Porter, A.Babiker, K.Bhaskaran, J.Darbyshire, P.Pezzotti, K.Porter, A.S.Walker; Lancet, **362**, 1267-1274 **(2003)**.
[17] K.Wittayanarakul, S.Hannongbua, M.Feig; J.Comput.Chem., **29(5)**, 673-85 **(2008)**.
[18] A.Wlodawer, J.W.Erickson; Annu.Rev.Biochem., **62**, 543-585 **(1993)**.