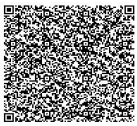

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Virtual Screening of Drugs against HIV-1 Protease

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Abstract

The life-threatening infections and pandemic spread of Human Immunodeficiency virus-1 (HIV-1), the etiologic agent of AIDS, has promoted an unending scientific effort to understand and control the disease. The resultant understanding of HIV-1 life cycle has defined many different targets for potential drug intervention. HIV protease enzyme responsible for cleaving large polyprotein precursors into biologically active protein products is an important target for the treatment of AIDS. However drug resistance is a persistent problem and new protease inhibitors are needed. Tipranavir, one of the protease inhibitors most recently approved for clinical use has been shown to be potent against viruses harbouring multidrug resistance mutations such as V82A and L90M, but even this drug is shown to lose potency due to certain mutations or mutation patterns. Thus 10 derivatives of the drug Tipranavir, chemically diverse from the initial hit were generated and screened to determine their ability to interact with protease. Further analysis revealed one unique compound with high binding ability from the initial hit and its possibility for new class of protease inhibitors is discussed in this report.

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Keywords: AIDS, HIV-1 Protease, Virtual Screening, Tipranavir, Protease inhibitors.

Introduction

AIDS is one of the most serious pandemic diseases of the modern era. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), at the end of 2010 there were approximately 34 million people infected by HIV-1 and about 30 million people have died of AIDS-related causes since the beginning of the epidemic [1]

The pharmaceutical industry is under ever increasing pressure to increase its success rate in bringing drugs to the market. It is estimated that on average it can take 14 years to bring a compound from hit identification through to an approved drug [11], and the costs associated with this process are enormous, with the bulk of the expense being incurred in the development phase of the value chain. Current efforts within the industry are directed at reducing the hit-to-drug timeline, increasing the number of quality candidate drugs that make the transition from discovery to clinical development, and decreasing the attrition rate (currently 90%) of candidate drugs in the clinical stages of the value chain. As a consequence, there has been an increased investment in discovery in technologies aimed at achieving these goals. This includes investments in functional genomics [3], for improved identification and validation of therapeutic targets; in HTS [5] and combinatorial chemistry [12] for hit identification; in both experimental [7] and predictive ADME methods [15]; and in structural biology (X-ray and NMR [1] for the identification of hits and for rational based evolution of hits to candidate drugs. HIV-1 protease is a C2 symmetric and an aspartic acid homodimeric enzyme, where aspartate 25 plays an important role in binding the substrate [16]**.** It is currently one of the most promising therapeutic targets for the treatment of AIDS due to its critical role in the virus maturation and replication. It is the target of 10 drugs approved by the U.S.

Food and Drug Administration (FDA) for the treatment of HIV/AIDS [16].

The enzyme's function is to cleave the Gag-Pol polyprotein precursor into shorter pieces to create the active protein components for viral packaging and maturation [6]. The protease active site is located in a tunnel (buried area), where two subunits meet each other. HIV-1 protease inhibitors are administered as a part of a drug combination in a treatment called Highly Active Antiretroviral Therapy (HAART), which is the most effective therapeutic strategy since the discovery of the virus. Nevertheless mutations in viral enzymes reduce drug affinity and breed resistance to HAART. Several mutations have been identified in the enzyme protease and resistance to all 10 FDA approved drugs have been reported [9], [16]. These mutations alter the viral enzymes in such a way that the drug no longer inhibits the enzyme functions and the virus restores its free replication power. Also the rate at which the virus reproduces and high number of errors made in the replication process creates a large amount of mutated viral strain [4]. Attempts to improve inhibitor quality continue with a common goal of increasing the specificity and affinity of enzyme drug interactions. Tipranavir (TPV) is a nonpeptidic PI, approved by the US FDA and the European Medicines Agency for the treatment of HIV infection. This molecule is a sulphonamide-substituted dihydropyrone. TPV like other PIs bind directly to HIV aspartyl protease. This disrupts the catalytic site of the enzyme and prevents the protease dependant cleavage of HIV gag and gag-pol polyproteins [14], [10]. TVP is active against PI multidrug resistant viruses and it has been very difficult to corner HIV to mutate through subsequent in vitro passages [13]. Tipranavir has been proved to be relatively safe in both adult and paediatric populations, giving a valuable alternative for these patients. This provides a promising line for therapy and the PI class effect in decreasing the formation of new mutations makes Tipranavir more desirable for HIV strains already highly resistant to other Antiretroviral Therapies (ARV) [2].

Virtual screening uses computer-based methods to discover new ligands on the basis of biological structures. It is an approach where computational screening of libraries of chemicals are done for

compounds that complement targets of known structures, and experimentally test those that are predicted to bind well. Such receptor-based virtual screening faces several fundamental challenges, including sampling of various conformations of flexible molecules and calculating absolute binding energies in an aqueous environment [8]. Virtual screening avoids problems like having to search a large chemical space. It restricts itself to only specific libraries with accessible compounds. In order to meet standards of 'drug likeness' or biological importance certain parameters or filters may be applied.

Materials and Methods

Hardware and software

The present work was carried out in Intel(R) core™ i3-3120M workstation with 2.50GHz processor. 2.00 GB RAM, with an Intel^{R} graphics card running in windows operating system. AutodockVina, MGLTools, Discovery Studio and online bioinformatics resources like Protein Data Bank (PDB), ZINC database, CHEMBL were employed to purpose the research findings.

Retrieval of HIV-1 protease and Tipranavir structures

The three dimensional structure of the HIV-1 protease-inhibitor complex (PDB code: 2BPX) was retrieved from Protein Data Bank at 2.80 A^{0} RMSD resolution (www.rcsb.org). Tipranavir and its derivatives were obtained from ZINC (Zinc Is Not Commercial) Database which consists of about 4.6 million commercially available compounds which are purchasable and ready for virtual screening. Initial refinement of both the receptor (protease) and the ligands (Tipranavir derivatives) were performed by means of MGLTools software.

Ligand and receptor preparation

Tipranavir and its derivatives, in MOL2 format obtained from the ZINC database, already contained 3-dimensional coordinates and were translated to PBD format. The drugs were chosen or modified based on Lipinsky's Rule of Five which shows 'drug like-ness'. Individual files were prepared and then processed using

MGLTools 1.5.6. These files were then converted into the PDBQT format that is the format which can be read by AutodockVina. To prepare the protease structure for docking, the ligand and water molecules were removed from the protein-inhibitor complex. Charges and polar hydrogen atoms were added to the protease using MGLTools and were also saved in the PDBQT format as well.

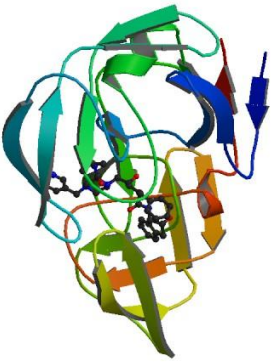
Protein-Ligand docking and virtual screening

The target protein was imported on the work space and the active site of the protein was defined by generating a grid box with grid points corresponding to X, Y and Z axis of size 26 A^0× 30 A^0× 32 A^0 and default grid spacing of 1.000 A^0 around the conserved triad forming residues Asp25-Thr26-Gly27 sites on the target protease enzyme. The TORSDOF (torsion degree of freedom) for the ligands/drug molecules were minimized to 6 and were introduced one by one into the docking panel available in the MGLTools program for performing docking calculations. Docking parameters were set to default. Prior to docking total Gasteiger charges were added to the protein and ligand. The binding affinity and the Root Mean Square Deviation (RMSD) for each docked conformation, calculated by the program autodockvina have been analysed.

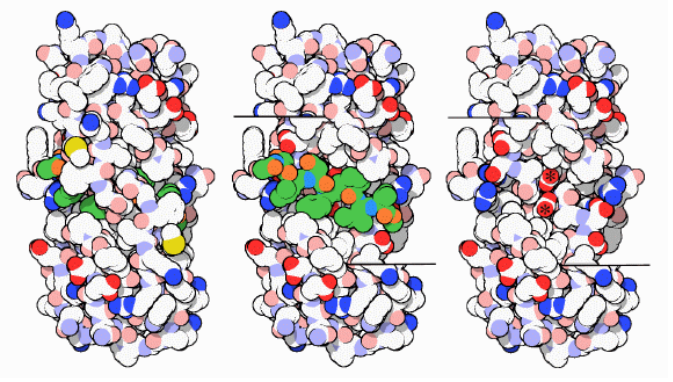
Results and Discussion

Analysis of structure and sequence of HIV-1 Protease

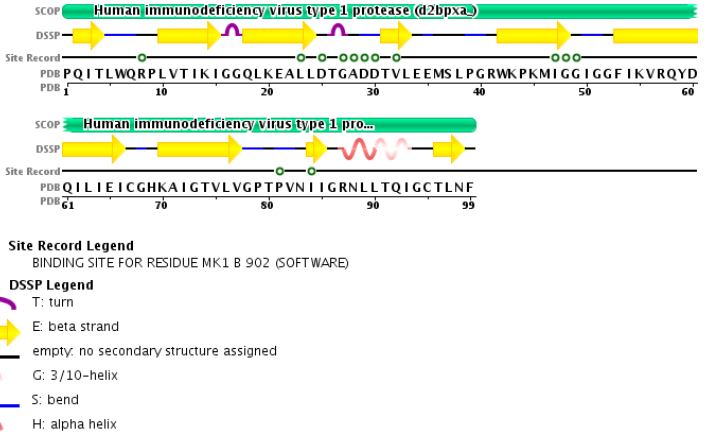
The 3 dimensional X-ray crystallographic protease-inhibitor complex (PDB code: 2BPX) and the protein sequence was retrieved from www.rcsb.org. The enzyme is a homodimer composed of two identical protein chains, 99 amino acids long with a resolution of 2.80A^0.The secondary structure of chain A consists of 7% helical (2 helices; 7residues) and 53% beta sheets (9 strands; 53 residues) and chain B has 4% helical (1 helices; 4 residues) and 54% beta sheets (9 strands; 54 residues). The active site is at the centre of the tunnel which is covered by two flexible protein flaps. **(Figure 1A, B and C)**



(A)



(B)


SCOP
Human immunodeficiency virus type 1 protease (d2bpxa_)
DSSP
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PDB 1 10 20 30 40 50 60
SCOP
Human immunodeficiency virus type 1 pro...
DSSP
Site Record
PDB QILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
PDB 61 70 80 90 99
Site Record Legend
BINDING SITE FOR RESIDUE MK1 B 902 (SOFTWARE)
DSSP Legend
T: turn
E: beta strand
empty: no secondary structure assigned
G: 3/10-helix
S: bend
H: alpha helix

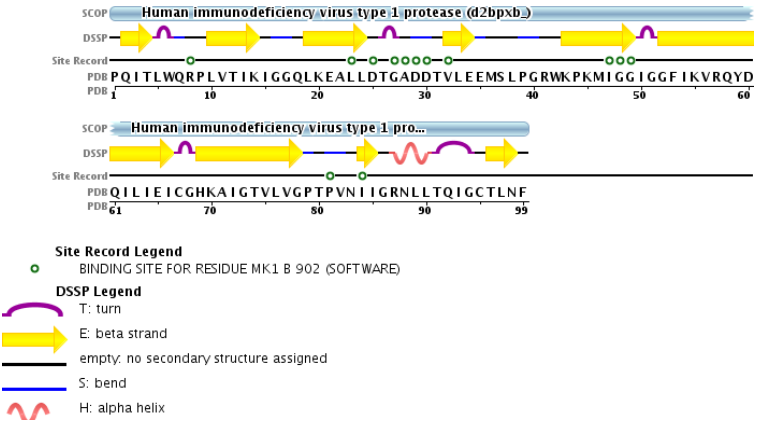
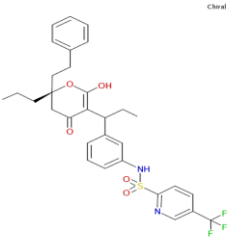


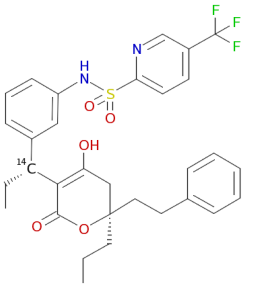
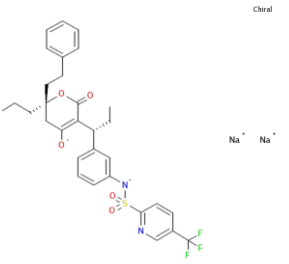
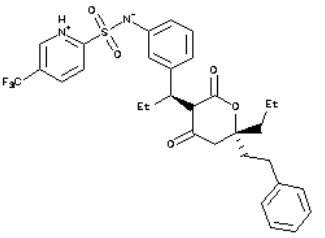
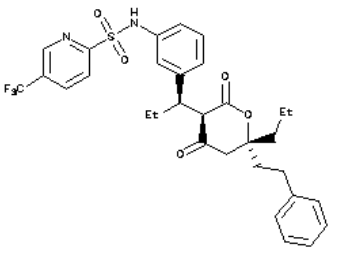
Figure 1: (A) Helix-sheet diagram of HIV-1 Protease with Inhibitor. (B)Protease enzyme viewed from above with an inhibitor in the active site (shown in green) and two aspartate amino acids (shown with asterisks) which attack the protein. (C) Graphical representation of HIV-1 Protease sequence; Chain A and Chain B.

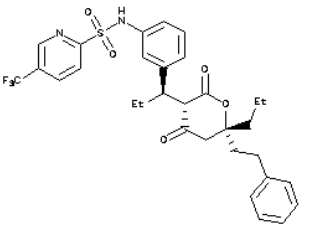
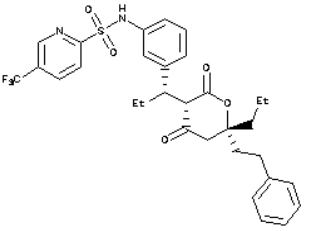
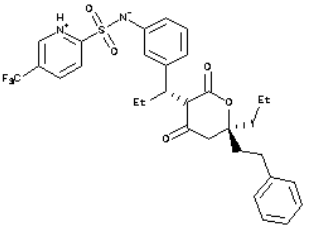
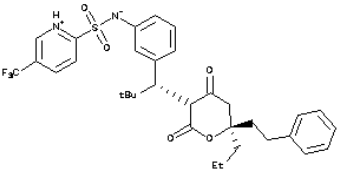
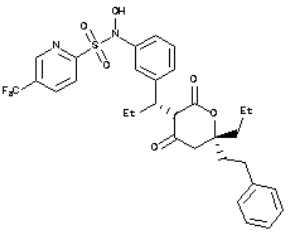
Novel HIV-1 Protease Inhibitors

The 10 derivatives of Tipranavir chosen for screening was retrieved from www.zinc.docking.org in the MOL2 format. It was then translated to PDB format using Discovery Studios software. Each ligand was imported to the autodock tools Graphical User Interface (GUI) one by one for docking and screening purposes. A summary of the derivates chosen for screening is given in the table below.

Table 1: Tipranavir derivatives chosen for virtual screening.

Code	Zinc ID	Mol Weight (g/mol)	xlogP	Structure
TPV1	**332684**	**602.7**	**7.38**	

Code	Zinc ID	Mol Weight (g/mol)	xlogP	Structure
TPV2	**445699**	**602.7**	**6.95**	
TPV3	**2103936**	**602.7**	**6.95**	
TPV4	**3815630**	**602.675**	**7.52**	
TPV5	**8536340**	**601.667**	**7.52**	

Code	Zinc ID	Mol Weight (g/mol)	xlogP	Structure
TPV6	**8536341**	**601.667**	**7.52**	
TPV7	**8536343**	**601.667**	**7.52**	
TPV8	**11616947**	**601.667**	**7.52**	
TPV9	**14943261**	**629.721**	**8.29**	
TPV10	**72131424**	**618.674**	**7.44**	

HIV-1 protease-inhibitor complex was prepared for docking by removing the water molecules and the inhibitor using Discovery Studio software. It was then saved in the PDB format.

The protease and each ligand were initialised in autodock tools. Polar hydrogen atoms and Gasteiger charges were added to the protease and non polar hydrogen atoms were merged. The torsion for each ligand was minimized to six and they were saved it the PDBQT format ready for docking.

Grid parameters for the protease enzyme was set around the active site with grid spacing of 1.000 A^0 and grid values for the x, y and z coordinates were set to 26, 30 and 32 respectively. The docking parameters were kept to default with 2, 70, 000 evaluations for each docking experiment. The PDBQT files of the protease and the ligands prepared for screening were imported to a New folder where AutodockVina software was used to find the binding affinities in gram/mol and the RMSD values.

Autodock seeks the best interaction energy between a flexible ligand and the protein surface. Computed energies are typically favourable since the docking procedure searches widely. The compounds were screened for the ability to inhibit HIV-1 Protease which has undergone mutation. In each docking run, 9 conformations for each drug molecule were reported. The best binding affinity in grams/mol with RMSD value of 0.000 was considered in each docking run. Nearly all the derivates of Tipranavir exhibits equal or weak binding energies when compared to the parental molecule. (Known inhibitor Tipranavir). The results for the 10 derivates are typical of AutodockVina docking experiments (Table 2).

Table 2: Summary of docking results

Sl. No.	Ligand name	Binding affinity energy value score
1	**chEMBL332684 (TPV1)**	-10.2
2	**chEMBL445699 (TPV2)**	-9.0
3	**chEMBL2103936 (TPV3)**	-10.3
4	**Zinc_3815630 (TPV4)**	-10.5
5	**Zinc_8536340 (TPV5)**	-9.9
6	**Zinc_8536341 (TPV6)**	-10.0
7	**Zinc_8536343 (TPV7)**	-10.0
8	**Zinc_11616947 (TPV8)**	-10.1
9	**Zinc_14943261 (TPV9)**	-9.5
10	**Zinc_72131424 (TPV10)**	-10.2

The best ranked lead (Score -10.5) had shown an overall good interaction and no ugly contacts. The docking complex revealed that Asp30 Gly27 Asp29 Asp30 Gly48 and Ile50 were involved in good van der Walls interaction and 2 hydrogen bonds were formed with Ile50 and Gly48 (Figure 2)

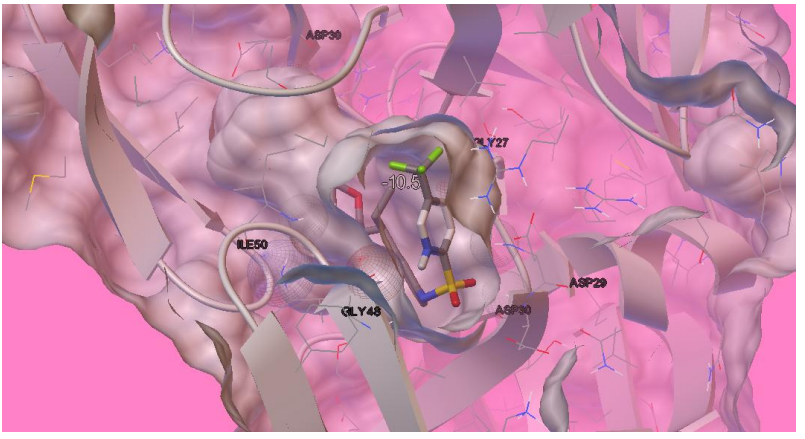


Figure 2: Docking interactions of best ranked inhibitor and HIV-1 protease. The protein is shown in ribbons and the ligand in sticks. Hydrogen bonds are shown as spheres.

Conclusion

Virtual screening (VS) has proven to be useful in the discovery of new antiviral drugs. The advantages of a rationalized and faster drug discovery process are obvious, especially in anti-viral research, where the sudden emergence of new diseases of drug resistances is common. During the past years, VS has been mainly used for lead-discovery and to a smaller extent for compound development and optimization. Most virtual screening efforts employ docking as their principal screening technique. Less common are 2D or 3D similarity searches with validated ligands or pharmacophore based screening. The major advantage of docking for VS is that it can be utilized to screen for compounds acting on a certain protein as soon as one crystal structure exists. This is especially useful in early lead discovery, as at this point only scarce information is accessible.

To employ similarity search methods for screening, at least one known ligand must exist. To build a pharmacophore, several known ligands or a crystal structure with co-crystallized ligand are necessary, which makes these methods difficult to employ when little information is available. By contrast, in later drug development stages with more experimental data available, especially 3D pharmacophores can be efficiently used for VS leading to hit compounds with a high structural variability.Based on the results obtained in this project, it was observed that TPV4 showed lesser Binding energy of -10.5 and RMSD od 0.000 than that as seen in Tipranavir where the binding energy was -10.4 at RMSD of 0.000.Therefore, **(TPV4)** can be used as a potential drug when compared to Tipranavir.

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