Docking Studies on HIV Integrase Inhibitors Based On Potential Ligand Binding Sites

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Abstract
HIV integrase is a 32 kDa protein produced from the C-terminal portion of the Pol gene product, and is an attractive target for new anti-HIV drugs. Integrase is an enzyme produced by a retrovirus (such as HIV) that enables its genetic material to be integrated into the DNA of the infected cell. Raltegravir and Elvitegravir are two important drugs against integrase.

Keywords
Integrase, Chemsketch, Virtual screening.

1. Introduction
The human immunodeficiency virus type 1 (HIV-1) is the primary cause of the acquired immunodeficiency syndrome (AIDS), which is a slow, progressive and degenerative disease of the human immune system. HIV-1 is a lentivirus belonging to the retrovirus family. The virus is diploid and contains two plus-stranded RNA copies of its genome. The development of possible methods that can delay progression of the infection or block replication of HIV-1 in infected individuals has been the subject of dedicated research efforts over the past decades. One important issue is that HIV-1 makes use of the replication machinery of the host cell, which minimizes the number of potential viral targets. On the other hand, the close host-virus relationship limits the evolutionary freedom for the viral components that interact with the host molecules[1]. Integration of viral DNA into the host chromosome is a necessary process in the HIV replication cycle [2]. The key steps of DNA integration are carried out by the viral integrase protein, which, along with protease and reverse transcriptase, is one of three enzymes encoded by HIV. Combination antiviral therapy with protease and reverse transcriptase inhibitors has demonstrated the potential therapeutic efficacy of antiviral therapy for treatment for AIDS [3]. Since HIV integrase has no direct cellular counterpart it presents itself as an attractive target for therapeutic intervention [4]. Unlike the retroviral reverse transcriptase and protease enzymes, successful drug candidates based on the inhibition of integrase have yet to emerge despite the multitude of laboratories working on the problem [5]. The objective of this study is to discover new analogs with improved potency, physicochemical/metabolic properties and toxic effects, which can stand as potential inhibitors of AIDS.
2. Materials and Methods

For the present study bioinformatics tools, biological databases like PubMed, Drug Bank, PDB (Protein Data Bank) and software’s like Molegro Virtual Docker, ACD ChemSketch, Pharma Algorithms were used.

The structure of HIV-1 integrase catalytic domain was retrieved from Protein data bank (PDB) (PDB id: 1BL3) (http://www.pdb.org). It is a 160 residues long protein and contains three domains, an N-terminal HH-CC zinc finger domain believed to be partially responsible for multimerization, a central catalytic domain and a C-terminal domain. Both the Central catalytic domain and C-terminal domains have been shown to bind both viral and cellular DNA. Currently no crystal structure data exists with Integrase bound to its DNA substrates. Biochemical data and structural data suggest that integrase functions as a dimer or a tetramer [6].

The structures of the drugs Raltegravir and Elvitegravir were obtained from Drug Bank (www.drugbank.ca) and KEGG Drug (www.genome.jp/kegg/drug) respectively. Using ACDLABS/ChemSketch (www.acdlabs.com) the 2D structures of the analogues of these drugs were sketched. The analogs were screened for their physiochemical properties at FAF drugs. The analogs that do not follow Lipinski’s rules are discarded. The selected analogs are then screened for their bioactivity and toxicity under PASS software. The selected analogs were then searched against various chemical structure databases for similarity with a existing structure. The databases taken in this step are: PubChem (pubchem.ncbi.nlm.nih.gov), KEGG, Molsoft (MolCart) (www.molsoft.com/molcart.html), Hic-Up (xray.bmc.uu.se/hicup/), and ChemBank (chembank.broadinstitute.org/). No similarity has been detected. The analogs are then subjected to molecular dynamics analysis in ChemBio3D Ultra under optimum conditions. The screened analogs were then subjected to Pharma Algorithm predict various pharmacological effects like oral bioavailability, protein binding etc. The Molecular Docking is performed in Molegro Virtual Docker (MVD) (http://www.molegro.com/). Possible active sites and cavities were detected for Chain A of 1BL3 using Molegro Virtual Docker. The following Parameters were used for Cavity Detection:
3. Result

3.1 Result of Active Site Prediction and Cavity Detection

The structure of HIV-1 integrase catalytic domain (PDB id: 1BL3) is 160 residues long and contains three chains: A, B and C. Catalytic core domain is present between 50 – 212 residues [7]. The position of the active site are Thr (T) =66. Asp (D) =64,116. Val (V) =77. Glu (E) =15 Lys (K) =159 [8, 9]. Active sites and cavities were detected for Chain A of 1BL3 using Molegro Virtual Docker.
3.2 Result of Molecular Docking:

The Molecular Docking is performed in Molegro Virtual Docker (MVD). The following Parameters were used for Docking using Molegro Virtual Docker. Docker uses the MolDock docking engine to predict ligand - protein interactions. MolDock is based on a new hybrid search algorithm, called guided differential evolution [10].

The imported ligands were manually checked before docking and corrected in those cases where it had failed. Water molecules with the protein structures were excluded from the docking
experiments, The docking is then allowed to run for some time for Raltegravir and Elvitegravir analogs (remained after virtual screening) respectively with the target protein. The poses having the good MolDock and Docking score are selected for the further analysis.

Figure 4. The result of Docking shown with contacts.

Docking results tabulated between HIV integrase and the conventional drug Raltegravir (Table 1) and Elvitegravir (Table 2) analogs are shown below.

| Mol_id | MolDock Score | Docking score | Mol_id | MolDock Score | Docking score |
|--------|---------------|---------------|--------|---------------|---------------|
| RL 40  | -150.584      | -160.781      | EL 9   | -114.166      | -199.066      |
| RL 13  | -144.854      | -144.94       | EL 15  | -113.443      | -118.713      |
| RL 4   | -143.524      | -154.891      | EL 10  | -112.471      | -112.235      |
| RL 3   | -142.581      | -148.704      | EL 7   | -111.277      | 113.903       |

The interactions between the residues of the target protein and analogs showing good docking score which are derived from the two lead molecules are given below-
Table 3. Interaction of Raltegravir analogs

| Mol_id | Residue | Interaction | Distance (in Armstrong) | Mol_id | Residue | Interaction | Distance (in Armstrong) |
|--------|---------|-------------|-------------------------|--------|---------|-------------|-------------------------|
| RL 9   | Glu (152) | H - H       | 1.57                    | RL 28  | Glu (152) | H - H       | 1.82                    |
|        | Glu (152) | H – H       | 1.36                    |        | Glu (152) | H - H       | 2.01                    |
|        | Gly (140) | H – O       | 2.22                    |        | Glu (152) | H - H       | 1.91                    |
|        | Asp(116)  | H - O       | 2.28                    |        | Asp(64)   | H - H       | 2.32                    |
| RL 10  | Asp(116)  | O – H       | 2.19                    | RL 40  | His(114)  | H - H       | 1.71                    |
|        | Asp(64)   | H – H       | 0.90                    |        | Gln(62)   | N – H       | 1.80                    |
|        | Gly(140)  | H – O       | 2.00                    |        | Asp(64)   | H - H       | 1.92                    |
|        | Gly(140)  | H – H       | 1.66                    |        | His(114)  | H - H       | 1.78                    |

Table 4. Interaction of Elvitegravir analogs

| Mol_id | Residue | Interaction | Distance (in Armstrong) | Mol_id | Residue | Interaction | Distance (in Armstrong) |
|--------|---------|-------------|-------------------------|--------|---------|-------------|-------------------------|
| EL 7   | Glu (152) | H - H       | 2.09                    | EL 9   | Glu (152) | H - H       | 2.02                    |
|        | Glu (152) | H - H       | 2.36                    |        | His(114)  | H - Cl      | 2.38                    |
|        | Glu (152) | H - H       | 2.02                    |        | Asp(116)  | H - H       | 1.57                    |
|        | Thr (115) | H - H       | 2.08                    |        | Asp(64)   | H - H       | 1.87                    |
|        | Asp(64)   | H - O       | 2.32                    |        | Asp(64)   | H - H       | 2.36                    |
|        | Asp(64)   | H - O       | 2.37                    |        | His(114)  | H - O       | 2.15                    |

3.4 Result of Pharma algorithm

Pharma algorithm is a tool for in silico physicochemical, ADME, Metabolism, and Toxicology screening and prediction. The results for Raltegravir (Table 5) and Elvitegravir (Table 6) analogs are shown below.

Table 5. Pharma algorithm results for Raltegravir

| ADME BOX | Raltegravir | RL 40 | RL 28 | RL 10 | RL 9 |
|----------|-------------|-------|-------|-------|------|
| Oral bioavailability between 30% and 70% | | | | | |
| %F(Oral) > 30%: | 0.721 | 0.827 | 0.721 | 0.099 | 0.391 |
| %F(Oral) >70%: | 0.351 | 0.282 | 0.282 | 0.080 | 0.091 |
| Absorption | | | | | |
Maximum passive absorption
Contribution from:

| Route                  | Pe, Jejunum | Pe, Caco-2 |
|------------------------|-------------|------------|
| Trancellular route     | 100%        | 2.37x10^-4 cm/s | 61.04x10^-6 cm/s |
|                        | 100%        | 2.62x10^-4 cm/s | 121.86x10^-6 cm/s |
|                        | 100%        | 3.60x10^-4 cm/s | 159.45x10^-6 cm/s |
|                        | 100%        | 2.35x10^-4 cm/s | 59.88x10^-6 cm/s  |
|                        | 100%        | 1.71x10^-4 cm/s | 27.88x10^-6 cm/s  |
| Paracellular route     | 0%          | 0%          | 0%            |
| Permeability:          |             |             |               |
| Human Jejunum scale    |             |             |               |
| (pH=6.5) Pe, Jejunum   |             |             |               |
|                        |             |             |               |
| Caco-2 scale (pH=7.4, 500 rpm): Pe, Caco-2 | | |
| Absorption rate Ka := | 0.080 min-1 | 0.085 min-1 | 0.095 min-1 | 0.080 min-1 | 0.062 min-1 |

Table 6. Pharma algorithm results for Elvitegravir
The structures of the two drugs Raltegravir and Elvitegravir and the changes or modifications in the two analogs (RL-10, EL -7) showing good protein binding are shown below.

Figure 5. The structure of Raltegravir  
Figure 6. Structural analog of Raltegravir RL10

Figure 7. The structure of Elvitegravir  
Figure 8. Structural analog of Elvitegravir EL7

**Conclusion**

From the molecular docking result carrying out using Molegro virtual docker, the best four analog form each inhibitor ( Raltegravir- RL-40,RL-13, RL -10, RL-9 ; Elvitegravir- EL-27,EL-47,EL-28, EL-21 ) based on the mol dock score and the docking score were selected. The close contacts shows that there is a high possibility of interaction of these analogs with the amino acids of the active site of the protein. These analogs also show better bioavailability (RL 40 ; EL47) , protein binding (RL 10, EL 7) ,solubility (EL7, RL 10) and low toxicity than existing two inhibitors (Raltegravir , Elvitegravir). These inhibitor analogs will provide a platform for structure-based design of an additional class of inhibitors for antiviral therapy.

**References**

[1] M. H. Nielsen, F. S. Pedersen, and J. Kjems, "Molecular strategies to inhibit HIV-1 replication," *Retrovirology*, vol. 2, p. 10, 2005.

[2] P. O. Brown, "Integration," in *Retroviruses*, J. M. Coffin, S. H. Hughes, and H. E. Varmus, Eds., ed Cold Spring Harbor (NY), 1997.

[3] A. M. Vandamme, K. Van Vaerenbergh, and E. De Clercq. "Anti-human immunodeficiency virus drug combination strategies," *Antivir Chem Chemother*, vol. 9, pp. 187-203, May 1998.

[4] N. J. Anthony, "HIV-1 integrase: a target for new AIDS chemotherapeutics," *Curr Top Med Chem*, vol. 4, pp. 979-90, 2004.
[5] S. D. Young, "Inhibition of HIV-1 integrase by small molecules: the potential for a new class of AIDS chemotherapeutics," *Curr Opin Drug Discov Devel*, vol. 4, pp. 402-10, Jul 2001.

[6] S. Maignan, J. P. Guilloteau, Q. Zhou-Liu, C. Clement-Mella, and V. Mikol, "Crystal structures of the catalytic domain of HIV-1 integrase free and complexed with its metal cofactor: high level of similarity of the active site with other viral integrases," *J Mol Biol*, vol. 282, pp. 359-68, Sep 18 1998.

[7] T. M. Jenkins, A. B. Hickman, F. Dyda, R. Ghirlando, D. R. Davies, and R. Craigie, "Catalytic domain of human immunodeficiency virus type 1 integrase: identification of a soluble mutant by systematic replacement of hydrophobic residues," *Proc Natl Acad Sci U S A*, vol. 92, pp. 6057-61, Jun 20 1995.

[8] J. L. Gerton and P. O. Brown, "The core domain of HIV-1 integrase recognizes key features of its DNA substrates," *J Biol Chem*, vol. 272, pp. 25809-15, Oct 10 1997.

[9] A. A. Adesokan, V. A. Roberts, K. W. Lee, R. D. Lins, and J. M. Briggs, "Prediction of HIV-1 integrase/viral DNA interactions in the catalytic domain by fast molecular docking," *J Med Chem*, vol. 47, pp. 821-8, Feb 12 2004.

[10] R. Thomsen and M. H. Christensen, "MolDock: a new technique for high-accuracy molecular docking," *J Med Chem*, vol. 49, pp. 3315-21, Jun 1 2006.