Identification of Novel Human Immunodeficiency Virus-1 Integrase Inhibitors by Shape-Based Virtual Screening

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Abstract: Problem statement: Human Immunodeficiency Virus-1 (HIV-1) is causative agent of the immune system disease, Acquired Immune Deficiency Syndrome (AIDS). Majority of anti-HIV drugs target reverse transcriptase and protease enzymes. Toxicity and development of multidrug resistant HIV-1 virus strains are the reasons for studying new targets in HIV-1 replication process for identifying novel inhibitor with low toxicity and high activity. **Approach:** In this study, ROCS software was used to identify the novel HIV-1 Integrase (HIV-1 IN) inhibitor by shape-based virtual screening. The currently used drug raltegravir was used as query molecule. **Results:** Here five novel molecules were identified, among them Rank 5 molecule was shown to have higher structural and electrostatic similarity to query molecule and this molecule was considered as good inhibitor of HIV-1 IN enzyme. **Conclusion:** ROCS, EON and FRED effectively identified one active inhibitor of HIV-1 IN among five compounds, which was similar to the query molecule and this study showed that ROCS, EON and FRED can play a vital role in drug discovery projects.

Key words: HAART, human immunodeficiency, integrase inhibitors, virtual screening, ROCS

INTRODUCTION

The immune system disease, Acquired Immune Deficiency Syndrome (AIDS) is caused by Human Immunodeficiency Virus-1 (HIV-1), which belongs to Retrovirus family, which means RNA is genetic material for HIV-1. The size of HIV-1 is approximately 60 times smaller than a RBC and roughly spherical in shape and about 120 nm in diameter. Though it is a retrovirus, its shape is entirely different from other retroviruses. HIV-1 genome encodes three enzymes namely Reverse Transcriptase, Integrase and Protease which are essential for HIV-1 replication. The majority of anti-HIV drugs target Reverse Transcriptase and Protease enzymes. There are four different classes of drugs available to suppress the viral load, namely Nucleoside Reverse Transcriptase Inhibitors (NRTIs (e.g., AZT, DDI, DDC)), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's (e.g., Etravirine, Nevirapine)), Protease Inhibitors (PIs (e.g., Saquinavir, Lopinavir)) and fusion inhibitors (enfuvirtide). Highly Active Antiretroviral Therapy (HAART) combatively suppresses HIV-1 replication and progression of HIV-1 infection which uses combination of three or more drugs from at least two different classes approved by US Food and Drug Administration (FDA). Patients on long-term HAART medication were affected from development of multidrug resistant HIV-1 virus strains and severe side effects which are the reasons for studying new targets in HIV-1 replication process for discovering safe and potent drugs (Potter *et al.*, 2003; Yusa and Harada, 2004; Fontas *et al.*, 2004; De Bethune and Hertogs, 2006; Potter *et al.*, 2006; Machouf *et al.*, 2006; Tozzi *et al.*, 2006; Cozzi-Lepri, 2007).

HIV-1 Integrase (IN) is an effective target for antiretroviral therapy which inhibit the HIV-1 replication (Thomas and Brandy, 1997). The mechanism of integration of viral DNA into host DNA is carried out by HIV-1 IN enzyme (Asante-Appiah and Skalka, 1997; Hindmarsh and Leis, 1999). For this integration mechanism, the following two steps are essential. In the first step, two deoxynucleotides are removed from the 3'-ends of the viral DNA which is called 3'-end processing and the Second step, in which the processed 3'-ends of the viral DNA (target DNA) are covalently bind to the host chromosomal DNA is called strand transfer reaction. For both of the reactions (3'-end processing and Strand transfer) and for the formation of viral DNA-IN complex, divalent metal cations such as Mg^{2+} or Mn^{2+} are required (Ellison and Brown, 1994; Wolfe *et al.*, 1996). HIV-1 IN is a 32 kDa enzyme that consists of three functional domains (Andrake and Skalka, 1996). (i) N-terminal domain stabilized by Zn^{2+} which binds to HHCC motif ("Zinc finger"-like motif) (Lee and Han, 1996; Zheng *et al.*,

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1996; Lee *et al*., 1997) and this is also responsible for multimerization (Zheng *et al*., 1996). (ii) The catalytic domain consists of conserved D, D-35-E motif, which is represented in IN by the residues ASP 64, ASP 116 and Glu 152 and these residues are play a vital role for catalytic activity and are also coordinated by two metal ion cofactors (Ellison and Brown, 1994; Wolfe *et al*., 1996; Vink *et al*., 1994; Hazuda *et al*., 1997; Beese and Steitz, 1991) and (iii) C-terminal domain has DNA-binding activity which is non-specific (Woerner and Marcus-Sekura, 1993; Engelmen *et al*., 1994). Using Nuclear Magnetic Resonance (NMR) technique, the structure of N-terminal and C-terminal domain of HIV-1 IN was determined (Lodi *et al*., 1995; Cai *et al*., 1997; Eijkelenboom *et al*., 1995); on the other hand catalytic domain of HIV-1 IN was resolved by X-ray crystallography (Dyna *et al*., 1994; Bujacz *et al*., 1995).

Figure 1 shows some promising inhibitors of the HIV-1 IN (Cocohoba and Dong, 2008; Hazuda *et al*., 2000; Di Santo *et al*., 2005; Shiomi *et al*., 2005) and some natural peptides are also reported as inhibitor of HIV-1 IN (Marchand *et al*., 2006; Robinson *et al*., 1998; Krajewski *et al*., 2004). Raltegravir is the first HIV-1 IN inhibitor to be approved by the US FDA for antiretroviral treatment (Cocohoba and Dong, 2008) and it is active against multidrug resistant HIV-1 viruses (Morales-Ramirez *et al*., 2005; Summa *et al*., 2006; Laufer *et al*., 2006; Markowitz *et al*., 2006). Some computational studies on HIV-1 IN inhibitors have been published (Yuan and Parrill, 2002; Makhija and Kulkarni, 2001; 2002). The research reported in this article attempts to design novel HIV-1 IN inhibitors by shape-based virtual screening. In this method there is no need for any structural information of target protein and some researches on shape-based virtual screening were useful in this study (Rush *et al*., 2005; Gundersen *et al*., 2005; Muchmore *et al*., 2006).

Fig. 1: Some promising inhibitor of HIV integrase, (A) Raltegravir (Cocohoba and Dong, 2008), (B) Diketo Acids (DKA) (Hazuda *et al*., 2000), (C) Quinolin-4-one derivatives (Di Santo *et al*., 2005) and (D) natural products (Shiomi *et al*., 2005)

MATERIALS AND METHODS

Database preparation: Highly reliably commercial drug-like database was downloaded from ZINC website (John and Brain 2005). This database contains >3000 molecules. The drug-like molecules are present in the database as SMILES format. Then these molecules are expanded into a set of 3D conformers by OMEGA software with 0.8 rms and erange 5 kcal mol^{-1}. OMEGA generates different conformations of the ligands (OMEGA ver.2.1.0, 2006) and has been found to be successful in creating bioactive conformations (Bostrom *et al*., 2003). In OMEGA connection table method is used for generating initial set of 3D coordinates of the given molecule. Merck molecular force field is used to refine the molecules (Halgren, 1996). The Quacpac software is used to assign the AM1-BCC charges (QuacPac ver.1.3, 2006).

Virtual screening: Rapid Overlay of Chemical Structures (ROCS) software is used for to screening the database which contains >30000 conformers. Raltegravir was used as query molecule to screen this database. The query file contains different conformers of the Raltegravir and this approach is called multiconformer query and Fig. 2 shows different conformers of Raltegravir generated by OMEGA. Shape-based superimposition method is used in ROCS. The shape-based virtual screening allows the screening of novel HIV-1 IN inhibitors that are similar in shape with that of the query molecule. In this method there is no need for any structural information of the targets (enzymes and proteins). To maximize the volume overlap between the molecules (query and database molecules), solid-body optimization process is used when molecules are aligned and to compute the geometric overlap atom-centered Gaussian model is used. Shape Tanimoto values were used in this study. Based on ST values, similarities between the two molecules (query and database molecule) were studied and this value ranges from 0-1; 1 for similarity and 0 for dissimilarity. Finally we got three sets of hits and each hit contains best 100 molecules ranked by ST values. Figure 3 shows the query molecule overlapped with database molecules.

Hits from ROCS were used as input to EON which calculates the electrostatic similarity between the query and the database (here ROCS hits are considered as a database) (ROCS ver.2.2 and EON ver.1.1, 2006). Poisson-Boltzmann function is used to calculate the electrostatic similarity and it is measured by Electrostatic Tanimoto (ET) values. This method uses an outer dielectric of 80. Spin 90° command was set to maximize electrostatic similarity by spin terminal rotors.
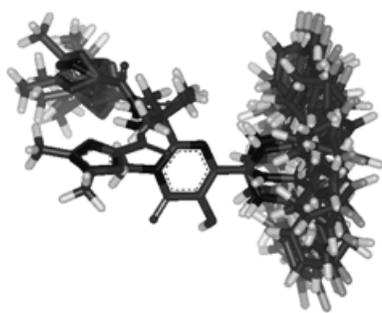
Fig. 2: Different conformers of Raltegravir generated by OMEGA

Fig. 3: A rank 5 molecule in Set 1 compounds overlapped with query molecule

Molecular docking: In order to study the binding mode of screened molecules to HIV-1 IN enzyme (PDB id: 1QS4), docking studies were performed. FRED was used to perform docking studies (FRED ver.2.2, 2006). Conformational orientations that clash with the protein or those which are distant from the active sites were rejected (Schulz-Gasch and Stahl, 2003). The default step sizes were 1 and 1.5 Å for translational and rotational modes respectively, which roughly give a Root Mean Square Deviation (RMSD) of 1 for the change. To calculate the docking energies, Chemscore, Shapegauss and PLP scoring functions in FRED were selected.

RESULTS

A shape-based virtual screening was carried out using ROCS software. Raltegravir was used as query to screen the database for identifying the novel leads which inhibit the HIV-1 IN enzyme. There are two types of methods available for screening, namely single conformer query method and multiconformer query method. In case of multiconformer method, we get the hits for each and every conformer. For example, if multiconformer query file contains 10 conformers of the same molecule, then we get 10 hits and every hit contains different screened molecule. In this study, we have generated 106 conformers for Raltegravir
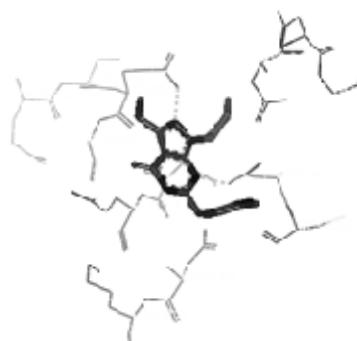

Fig. 4: A rank 5 molecule in set 1 compounds docked with active site of the HIV-1 integrase

Table 1: Hits obtained from rank 1 conformer used as a query

Rank	ROCS hits	Shape tanimoto	Electrostatic tanimoto
1	ZINC00017019	0.736	0.004
2	ZINC00018810	0.726	0.007
3	ZINC0006117	0.720	0.004
4	ZINC00023049	0.718	0.163
5	ZINC0006708	0.711	0.510

Table 2: Hits obtained from rank 2 conformer used as a query

Rank	ROCS hits	Shape tanimoto	Electrostatic tanimoto
1	ZINC00023931	0.730	-0.011
2	ZINC00021206	0.724	-0.049
3	ZINC00023049	0.720	-0.059
4	ZINC0002303	0.718	0.006
5	ZINC00021208	0.714	-0.020

Table 3: Hits obtained from rank 3 conformer used as a query

Rank	ROCS hits	Shape tanimoto	Electrostatic tanimoto
1	ZINC00022237	0.742	-0.049
2	ZINC00018810	0.742	0.001
3	ZINC00014016	0.727	-0.014
4	ZINC0006117	0.725	0.002
5	ZINC00014017	0.725	-0.015

Table 4: Docking scores of set 1 hits

Rank	ROCS hits	Chemscore	Shapegauss	PLP
1	ZINC00017019	-7.612	-267.2	-29.25
2	ZINC00018810	-8.365	-329.4	-36.22
3	ZINC0006117	-9.464	-273.7	-18.92
4	ZINC00023049	-3.401	-238.4	-31.85
5	ZINC0006708	-4.591	-257.7	-16.59
Query molecule		3.840	-257.4	-24.32

molecule. We have randomly selected only Rank 1, 8 and 20 conformers for screening purpose, namely Set 1-3 conformers respectively and hits obtained using these conformers are named as Set 1-3 molecules, respectively. Totally 300 hits were obtained and each 100 hits for each set was ranked by ST value.

These ROCS hits were used as an input to EON in order to calculate electrostatic similarity between the query and ROCS hits. Spin 90° command was used to maximize the electrostatic similarity. Finally it is

ranked by Electrostatic Similarity (ET) value. Top five ranked molecules in each set were selected for further study (Table 1-3). The selection of hits based on ST and ET values elaborated in discussion. On the basis of higher structural and electrostatic similarity to query, five hits from Set 1 data set were docked into the active site of HIV-1 IN enzyme. Docking energy of five new molecules and Raltegravir (query) with HIV-1 IN are given in Table 4. Three scoring functions were used to compare the results. Figure 4 shows rank 5 molecule docked with HIV-IN active site.

DISCUSSION

This research has shown that it is possible to detect compounds with similar shape and electrostatics to a query molecule using ROCS and EON software. ROCS rapidly identify very similar compounds to Raltegravir such as Set 1 molecules. However, it fails when the electrostatics similarity in terms of ET value was compared, because of large similar shaped compounds does not show same activity. To solve this problem, other conformers of Raltegravir such as Set 2 and 3 were chosen for screening. But, in the case of Set 2 molecules, ST values were almost similar to Set 1 and ET values were very low than that of Set 1 compounds. Surprisingly ST values of Set 3 molecules were slightly higher than that of compounds in Sets 1 and 2. But ET values were very low than that of the other two sets, because of EON is conformer specific, that means different ET values we can obtain for different conformers of the same molecule.

To maximize the electrostatic similarity, spin 90° command was used in EON for all the three sets. But there was no significant improvement in ET values of compounds in Sets 2 and 3. Among Set 1 compounds, fourth and fifth ranked compounds showed higher ET values. Between these two, Rank 5 compound was shown to have 50% similarity to query molecule.

Docking energies of Set 1 compounds were shown to have good binding affinity than query. Fifth ranked compound was selected as good inhibitor of HIV-1 IN enzyme, because it was showed higher binding affinity than query and 71% of structural similarity and 51% of electrostatic similarity to query. Hence the fifth ranked molecule ZINC0006708 has satisfied both shape and electrostatic similarity criteria.

CONCLUSION

ROCS, EON and FRED effectively identified one active inhibitor of HIV-1 IN among five compounds, which was similar to the query molecule and this study shows that ROCS, EON and FRED can play a vital role in drug discovery projects. ROCS is conformer specific, for example if we increase the number of conformers of query, then we get more number of hits. Increasing the number of molecules in the database is another way to get more number of hits. The selection of most active compounds from hits is very essential in virtual screening. In this study, EON and FRED played a vital role in compound selection. Synthesis and *in vitro* studies of hit molecules will support our study. Further study in this area will be required to design novel HIV-1 IN inhibitors.

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