IN SILICO ANALYSIS OF DRUG RESISTANCE IN HIV-REVERSE TRANSCRIPTASE INHIBITORS

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ABSTRACT

AIDS/HIV is the third cause of mortality by infectious diseases in the world. Drug resistance is the major clinical problem for the treatment of virus-infected individuals. The role of Reverse Transcriptase (RT) enzyme is vital in the life cycle of HIV virus. In this work, the role of single point mutations of reverse transcriptase on drug resistance has been studied based in silico methods. Through this study, the most important single point mutations of transcriptase enzyme have been simulated. Consequently, cross-docking simulations were used to compare binding energies of native and mutated enzymes with some known inhibitors. The purpose of the study was to find some structural features which are responsible for drug resistance against this target. Presence of phosphate groups showed to increase the chance of drug resistance while hydrazinecarboxamide substructure was beneficial to decrease less drug resistance.

Keywords*: HIV-I, Cross-docking simulations, reverse transcriptase, drug resistance.*

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Background and theory

Acquired immunodeficiency syndrome (AIDS) is a viral disease caused by human immunodeficiency virus type 1 (HIV-1). According to WHO More than 36.9 million people were infected with HIV in 2014[(1)]. HIV Infection can lead to progressive destruction of CD4+ T lymphocytes and inevitable defect of the immune system[(2)].

The essential components of antiretroviral therapy are non-nucleoside and nucleoside reverse transcriptase (RT) inhibitors (NNRTIs and NRTIs), HIV protease inhibitors, CCR5 antagonists, integrase strand transfer inhibitors (INSTIs) and fusion inhibitors[(3)].

The human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is a very critical target enzyme for drug development. HIV-1 RT catalyzes production of double-stranded proviral DNA (dsDNA) using single-stranded viral RNA (ssRNA) as template. A fundamental clinical problem for the treatment of virus-infected individuals is antiviral drug resistance[(7)].

The expansion of mutations in patients receiving different therapies has resulted in resistant viruses being more disseminated within the HIV-infected population[(8, 9)].

In one cross-docking study, an in-house pharmacophore docking method was used for 18 RT and ligand complexes[(10)].

Some other studies utilized MOE, DOCK4, and auto dock vina in docking simulation of RT enzyme with inhibitors[(11-15)].

In a more recent study docking of phytochemicals on the structure of RT was investigated[16]. A missing point not which is not largely discussed in most in silico studies is to present an applicable pattern for designing novel RT inhibitors. The current study focused on several single point mutations on the allosteric binding site residues of RT enzyme. Afterwards, cross-docking simulations of some known RT inhibitors were preceded by self-docking analysis to confirm the validity of docking simulations. The resulted matrix of binding energies was analyzed to find specific structural properties in ligands which introduce them as resistant agents against HIV-1 RT mutations.

Material and method

The 3D Crystallographic structures of HIV1RT enzyme (HXB2: Accession number: P03366) were downloaded from Protein Data Bank[17]. All PDB structures were selected with a maximum resolution value of 3 Å. Accordingly, 4 PDB codes (PDB ID) 1ikv, 2vg7, 4h4m and 4pqu were selected based on resolution and presence of a NNRTI cognate ligand in crystallographic data. Afterwards, the binding site of the endogenous ligand was investigated by means of chimera and PLIP for each PDB code[18, 19].

Ligand set up

A set of 71 small molecule ligands with known experimental activity against P03366 were retrieved from CHEMBL database as SMILES strings[20]. The structures were subsequently converted to 3D mol2 using open babel 2.3.2. Addition of Gasteiger partial charges and merging non-polar hydrogen atoms were done in case of all structures using MGLTOOLS 1.5.6 by means of a shell script to yield 71 pdbqt files.

Preparation of mutant PDB structures

Single point mutations on native structures were performed using Modeller 9.14 software according to data published in literature[21-23]. Modelface, an in-house application programming interface (API), was used to generate and run the needed modeller python scripts for each experiment[24]. 100 mutant structures were generated for each mutant structure and the best one based on DOPE score was used for further studies.

Self-Docking

All 82 native PDB structures were subjected to a bash script in linux operating system in order to extract the cognate ligands, add partial charges to both ligand and protein, generate configuration files for Autodock vina and finalize self-docking simulation[25]. Coordinates of the central atom of the cognate ligands were considered as the center of grid box for all PDB codes. According to the aforementioned criteria, self-docking procedure was repeated with modified parameters in such a way to result in RMSD values less than 2 Å.

The in-house bash scripts to perform cross-docking and extract the binding energies were previously presented[26]. The center of grid for each mutant enzyme was calculated after superposing the mutant PDB to the native structure. Superposition and saving of the translated PDBs were done by TCL scripting in VMD software[27]. Docking parameter include Exhaustiveness = 100, maximum number of modes = 9 and grid box size = 30*30*30 (A0). In cross-docking step, docking of all ligands on the enzyme structures was performed to yield 6106 binding energy scores as a data matrix.

The cut-off energy values for each ligand was defined based on the maximum possible binding energy values of that ligand in complex with all native structures. A heat map was therefore designed based on the cells with "0" and "1" values so that those cells with "0" score were colored green and those with "1" score were colored red (figure 1). The percentage of mutation for each ligand (column) and row (mutant enzyme) was calculated as bellow:

$$\text{Effective Mutation } (EM\%) = \frac{No.\,of\ cells\ with\ red\ color * 100}{Total\ No.\,of\ cells}$$

Finally, visualization of ligand-enzyme complex in case of ligands with high and low percentage of effective mutations was done by means of PLIP server to obtain the interaction map for each complex[19].

Results and discussion

All docking results led to reasonable RMSD values of less than 2, meaning that this docking protocol can be used for other studies. The best convergence between docking result and crystallographic data was seen in 4h4m (RMSD=0.35). Afterwards, cross-docking simulations were carried

out using the parameters of self-docking step to result in the heat map depicted in figure1.

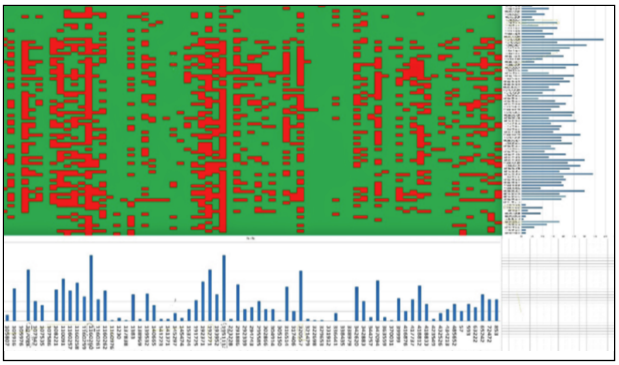


Figure 1: Heat map projection of cross-docking results (the rows represent mutant targets and the columns represent ligands).

A primary purpose of this study was to find out which single point mutations of RT are responsible for maximum drug resistance. As seen in Figure1. The maximum effective mutations were seen in mutations of 4pqu; Arg72-His72 and Arg72-Glu72 (48% and 38%, respectively). This finding can be explained by mutation of a positively charged aliphatic residue to a bulkier positively charged aromatic residue (His) or to a negatively charged residue (Glu). On the other hand, the minimum effect of mutation was seen in 4h4m; Tyr188-Phe188 (0%), explaining the role of π-π interaction for Tyr188 which can be well retained by Phe188. In addition mutations of LYS101 in 1ikv to different residues including ALA (2%), ARG (2%) and MET (1%) led to insignificant effective mutations. For this purpose, column-wise comparison of the heat map which is provided in table 1.

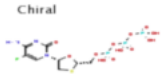
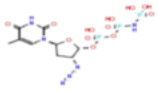
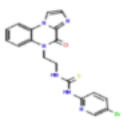
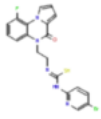
CHEMBL ID	Structure	CHEMBL ID	
195111		192771	
1160260		320557	
107099			

Table 1: The structure of the ligands with maximum distructive mutaions (>35%).

As seen in table 1, the compounds chembl195111, chembl192771, chembl1160260 bear triphosphate moiety and are susceptible to mutations of RT. In addition, other sensitive compounds, chembl107099 and chembl320557 are with different tautomers of urea substructure attached to a fused tricyclic system composed of phenyl, diazine and imidazole. The compounds described above are showing more than 35% effective mutations against different mutations of RT. On the other hand, based on data displayed in table 2, chembl331912 chembl338435 and chembl338979 are with indole substructure attached to piperidine via a carbonyl group. This pattern showed reasonable resistance against different mutations of RT. The compound chembl15979 was also well tolerated against different mutations of RT. An important structural modification which led to a significant increase of resistance against different mutations was seen in compound chembl107586 which was designed by replacement of carbon with nitrogen in the linker connecting urea to the aforementioned tricyclic system. Visualization of the enzyme inhibitor complexes were done using PLIP to compare the interactions in the native and mutant structures[19].

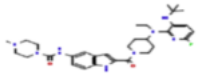
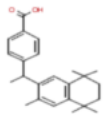
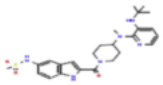
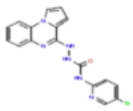
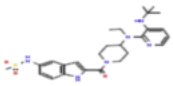
CHEMBL ID	Structure	CHEMBL ID	Structure
331912		105979	
338435		107586	
338979			

Table 2: The structure of the ligands with minimum effective mutaions (< 2%).

For instance, in native structure (4pqu) with CHEMBL107586 with indole moiety was accommodated in the binding cavity of the enzyme to have interaction with Asp185, Ala114, Gln151 and Gly112. Mutation of 4pqu; Val111-Cys111with a high effective mutation percentage (34%), was not effective for this structure (DM=0%). The flexibility of this ligand to adopt different conformations as seen in mutated structure (mut_4pqu_111_val_cys) with CHEMBL107586 can explain its ability to retain its similar binding mode as well as binding energy with different mutant and native RT enzymes. On the other hand, compounds such as native structure (4pqu) with CHEMBL1160260.

Bearing phosphate moieties are able to interact with Arg72 in native structure. Mutation of this residue to a negatively charged residue such as Glu72 could largely devastate the ionic interaction between the inhibitor and the enzyme.

In mutated structure (mut_4pqu_72_arg_glu) with CHEMBL1160260, the ionic bond in native structure was replaced by a weak hydrogen bond. As a result, the presence of an indole moiety as well as hydrazinecarboxamide structure could be beneficial to design inhibitors less susceptible to drug resistance. On the contrary phosphate groups are not suggested to be present in RT inhibitors. Although, cross-docking simulation of some co-crystal structures with RT have been reported in literature, few points about analysis of the binding energies and the role of different substructures were discussed[28]. In this study a heat map was suggested for docking binding energies which made it possible to simply analyze the mutations row-wise and column-wise. This novel strategy can be implemented in future docking softwares to develop more powerful toolboxes in drug discovery.

Conclusion

A novel strategy was based on a heat map resulted from cross-docking simulations of native and mutant HIV-1 reverse transcriptase with different bioactive inhibitors of the enzyme was developed. The result of this study revealed the reasonable success of this strategy in detection of structural features leading to drug resistance for this target. It is suggested to use this analysis in docking programs for other drug resistance related enzyme targets.

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