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Comparative Studies of Various Nnrti's IN the Active Site of Different HIV-1RT Receptors †

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Abstract: HIV is one of the most deadly viruses known to humans. It causes a disease, known as Acquired Immuno Deficiency Syndrome (or, AIDS). HIV-AIDS, is one of those deadly diseases, which is a fatal disease. There are only a handful drugs which are totally effective against the virus. This is due to the enzyme, reverse transcriptase, present within the virus. Due to various mutations in the enzyme, the virus becomes unresponsive towards the drugs. In the present study, the docking studies of the standard non-nucleoside reverse transcriptase inhibitors were done, in the non-nucleoside inhibitory binding pocket of reverse transcriptase enzymes of wild type and the resistant strains of HIV-1RT virus with PDB ID's- 1RT2, 1KLM, 3BGR and 1JLB respectively by using Autodock version 4.5.6. Comparison of different compounds docked into the active site of various HIV-1RT strains was carried out. The obtained results indicate that most of the compounds docked into the active site of the different receptors, such as- 1RT2, 1KLM, 3BGR and 1JLB, with good docking scores, comparable to that of the internal standard (TNK 651) of the wild type strain of HIV-1 virus. A comparison was made based on the binding modes of the compounds in the active site of all the four receptors.

Keywords: HIV-AIDS; Reverse Transcriptase; Docking; active site; binding mode

1. Introduction

Human Immuno Deficiency virus contains a single strand of RNA belonging to retroviridae family. It can cause deadliest disease of the century called AIDS [1]. The enzyme Reverse Transcriptase helps in reverse transcription of cDNA (formation of double stranded DNA from single stranded RNA) and thus plays a crucial role in the life cycle of the virus. HIV can be categorized into two subtypes: HIV-1 (causes infections worldwide) and HIV-2 (confined to west part of Africa) [2]. The infections caused by HIV can be blocked by targeting various steps of the life cycle of the virus like attachment of the virus to human cell, entry of virus, uncoating of virus etc. Various enzymes like reverse transcriptase, protease, integrase plays a vital role in different processes of the viral life cycle and various classes of drugs helps inhibiting these enzymes like Non-Nucleoside reverse transcriptase inhibitors (NNRTIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Protease inhibitors, Nucleotide reverse transcriptase inhibitors (NtRTIs) etc [3,4].

Mode of action of NNRTIs is different from NRTIs because they directly bind with the active site of the reverse transcriptase enzyme and inhibits the movable proteins responsible for DNA synthesis and thus blocks the activity of the enzyme. This ultimately leads to termination of chain elongation process [5–7].

Although NNRTIs are chemically diversified in nature, their binding site is same in the enzyme. Their binding site is an allosteric site containing hydrophobic pocket, which is situated 10 Å (approximately) from the catalytic site of reverse transcriptase [8].

The butterfly like form is important for binding of NNRTIs. Chemically diversified drugs of this class assume a butterfly like form which is found to be similar in all cases. Two hydrophobic aromatic rings are found in NNRTIs. They mimic a butterfly's wings, whereas, the body of the butterfly structure is represented by a hydrophilic moiety [9]. One of the wings containing heteroaromatic ring, that can act as hydrogen bond acceptor and/or donator to the main chain of amino acids. Whereas the other wing capable of forming pi-pi interactions with a hydrophobic moiety.

The keystone of highly active antiretroviral therapy (HAART) is three drugs of the class NNRTIs have been approved by the FDA for HIV infection treatment, they are Nevirapine, Delavirdine and Efavirenz and they were approved in year 1996, 1997, 1998 respectively. Often, they are used along with Protease inhibitors and NRTIs for effective treatment of HIV infection [10].

Docking Strategies: In case of structure-based drug designing of drugs in pharmaceutical industries, molecular docking is a very commonly used method because it can predict the binding of various conformations of ligands to the target binding site. Binding activity characterization plays an important role in the rational drug designing and in the elucidation of essential biochemical processes [11,12].

The non-competitive binding of NNRTIs causes change in conformation of 3D structure of reverse transcriptase and leads to the formation of Non-nucleoside Inhibitory Binding Pocket (NNIBP). It is elastic in nature and various characteristics of NNRTIs like size, chemical composition, binding mode affects its conformation. Various amino acid residues are present in NNIBP for interactions with NNRTIs [13].

2. Materials and Methods

2.1. Software

Autodock v 4.5.6, was used for carrying out the computational studies [14], installed in a HP Precision workstation (Radeon Graphics) with an Intel Core 3 quad processor and 8 GB of RAM with Operating system as Windows 10.

2.2. Molecular Modelling Studies

2.2.1. Protein Preparation

The X-ray co-crystallized structures of all of the protein molecules (PDB ID: 1RT2, 1JLB, 1KLM, 3BGR) used in the study were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) [15]. From every protein molecule co-crystallized water molecule were deleted and polar hydrogens were added as well as Gasteiger charges were assigned and it was saved in PDBQT format using Autodock v 4.5.6 software.

2.2.2. Ligand Preparation

All of the ligands were prepared by minimizing their energies using PRODRG 2 server [16]. PDBQT format of all of the ligands were saved.

2.2.3. Receptor grid Generation

Autogrid was used to generate specific grid maps for each and every ligand.

The generation of the grid box was done by taking the dimensions of the three coordinates (X, Y and Z) at 24×24×24, with grid spacing of 0.100 Å. The values of X, Y and Z centres were taken according to the crystallographic positions of the native ligand of each and every receptor.

2.2.4. Docking Protocol Validation

For computational studies, Autodock v 4.5.6 was used. This software was used to predict the different binding mode of co-crystallized ligands as well as test molecules with all of the receptors taken to carry out the study.

To carry out the docking procedure the method was validated to check the robustness of the software. The extracted ligand (previously mentioned) was corrected and then it was redocked using the same protein. Other test molecules were docked using the same procedure and after that their conformations were compared with the co-crystallized one. The generated docking scores of the co-crystallized ligand was compared with the docking scores of other test molecules to choose the best molecule.

3. Results and Discussions

3.1. There are Many HIV-1 Protein Crystal Structures Available in the Literature

In this work we have considered four crystal structures (PDB Id- 1KLM, 3BGR, 1JLB & 1RT2) Co-crystallised with the ligands BHAP U-90152, Rilpivirine, Nevirapine and TNK 651 respectively. Docking studies were done using Autodock Tools (v-4.5.6), on four high resolution crystal structures of HIV protein. The NNRTI's were studied in the Non-nucleoside inhibitory binding pocket of the four receptors. The docking scores and the binding poses of the different NNRTI's were studied, the results are given in Table 1.

The software used for docking purpose was validated at first, to check its reliability for further docking procedures. The internal ligands were removed from the receptors and were redocked into the active site of the protein. Root mean square deviation (RMSD) values of 0.0 Å were obtained for the internal ligands- BHAP U-90152, Rilpivirine, Nevirapine and TNK 651 for the HIV-1 proteins with PDB Id- 1KLM, 3BGR, 1JLB & 1RT2 respectively. As the RMSD values were within the standard limits (i.e, 0.2 Å), the software was used for further docking procedures.

In the receptor (PDB Id- 1KLM), the docking score of the internal ligand was found to be -8.3, in the same active site, amongst the NNRTI's, doravirine had a comparable score with that of the internal ligand, (i.e, −8.6), whereas nevirapine, delaviridine, rilpivirine and dapivirine had docking scores higher than that of the internal ligand, (i.e, −10.2, −9.5, −9.9 and −10.7 respectively). From the binding mode analysis, it was found that amongst them, the best docking pose was obtained for dapivirine, in the NNIBP, 2-π(pi) bond interactions were obtained between the pyrimidine ring of the compound and the indole ring of the amino acid, with a bond length of 2.239 Å, i.e., dapivirine$_{\text{pyrimidine ring}}$----$_{\text{indole ring}}$ TRP229 = 2.239Å. (Figure 1)

In the receptor (PDB Id- 1JLB), the docking score of the internal ligand was found to be -9.7, in the same active site, amongst the NNRTI's, doravirine and efavirenz had a comparable score with that of the internal ligand, i.e., −9.7 and −9.4 respectively. From the binding mode analysis, it was found that amongst them, the best docking pose was obtained for doravirine, in the NNIBP, 1-Hydrogen bond interaction was obtained between the Hydrogen atom of the triazole ring of the compound and the oxygen atom of the amino acid, with a bond length of 2.196 Å, i.e., doravirine$_{\text{NH}}$---$_{\text{C=O}}$ LYS101 = 2.196Å. (Figure 2)

In the receptor (PDB Id- 1RT2), the docking score of the internal ligand was found to be -11.9, in the same active site, amongst the NNRTI's, dapivirine had a comparable score with that of the internal ligand, (i.e, -11.7). From the binding mode analysis, it was found that amongst them, the best docking pose obtained for dapivirine, was in the NNIBP, 1- π(pi) bond interaction were obtained between the trimethyl substituted phenyl ring of the compound and the phenyl ring of the amino acid, with a bond length of 4.269 Å, i.e., dapivirine$_{\text{trisubstituted phenyl ring}}$----$_{\text{phenyl ring}}$ TYR188 = 4.269Å. (Figure 3a,b)

In the receptor (PDB Id-3BGR), the docking score of the internal ligand was found to be −8.9, in the same active site, amongst the NNRTI's, rilpivirine had a comparable score with that of the internal ligand, (i.e, −8.7),whereas, dapivirine, efavirenz and nevirapine had higher docking scores than that of the internal ligand, i.e., −9.3, −9.3 and −9.5 respectively. From the binding mode analysis, it was

found that amongst them, the best docking pose was obtained for dapivirine, in the NNIBP, 2- π(pi) bond interactions were obtained between the pyrimidine ring of the compound and the phenyl ring of the amino acid TYR188, with a bond length of 9.239 Å, and the other one was obtained for benzonitrile ring and the phenyl ring of the amino acid PHE227, with a bond length of 6.702 Å i.e., dapivirine$_{\text{pyrimidine ring----phenyl ring}}$ TYR188 = 9.239Å and dapivirine$_{\text{benzonitrile ring----phenyl ring}}$ PHE227 = 6.702Å. (Figure 4a,b)

3.2. Figures and Tables

Table 1. Docking scores of the NNRTI's in various HIV-1 RT receptors.

Compound	Docking Scores on			
	1KLM	**1JLB**	**1RT2**	**3BGR**
Nevirapine	−10.2	−9.7	−9.5	−9.5
Efavirenz	−7.6	−9.4	−9.4	−9.3
Delaviridine	−9.5	−6.9	−8.5	−6.8
Rilpivirine	−9.9	−8.4	−9.2	−8.7
Doravirine	−8.6	−9.7	−11.2	−7.1
Etravirine	−8.8	−6.9	−9.6	−6.9
Lersivirine	−6.5	−8.3	−6.5	−8.3
Dapivirine	−10.7	−8.9	−11.7	−9.3

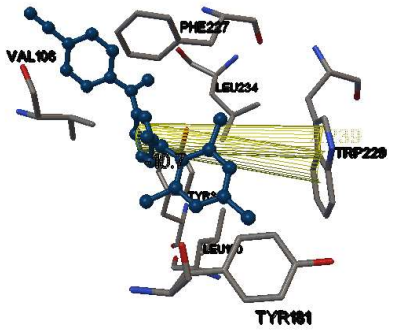


Figure 1. The binding pocket of HIV-1 reverse transcriptase (1KLM), showing the docking of dapivirine. The blue ball and stick model is the ligand (dapivirine) and the conventionally colored model are the amino acid residues interacting with the ligands. The lines are the π-(pi) bond interactions with amino acid residue of HIV-1 reverse transcriptase.

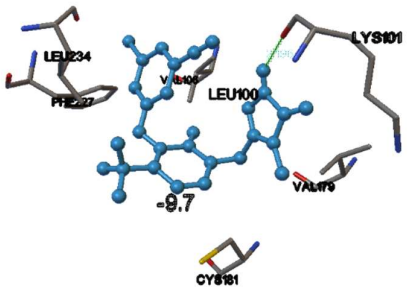
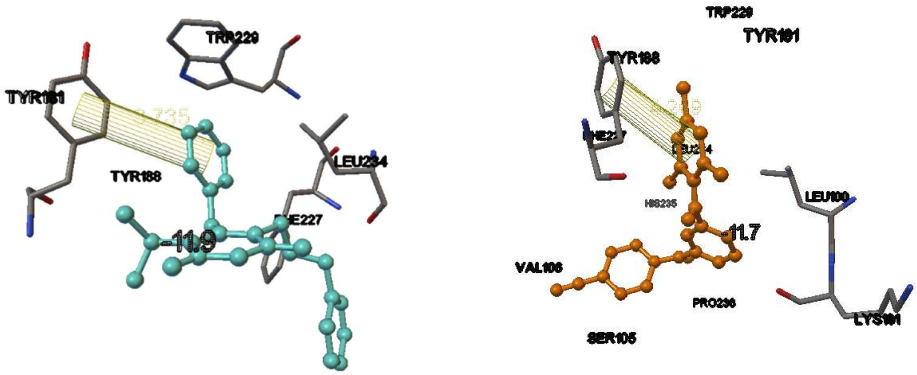
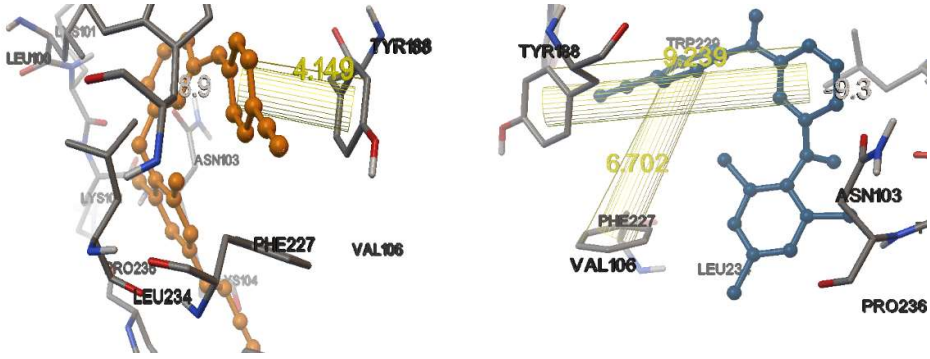


Figure 2. The binding pocket of HIV-1 reverse transcriptase (1JLB), showing the docking of doravirine. The blue ball and stick model is the ligand (doravirine) and the conventionally colored model are the amino acid residues interacting with the ligands. The green line is the hydrogen-bond interaction with amino acid residue of HIV-1 reverse transcriptase.



(**a**) (**b**)

Figure 3. (**a**): The binding pocket of HIV-1 reverse transcriptase (1RT2), showing the redocking of co-crystallized ligand TNK651. The blue ball and stick model is the ligand and the conventionally colored model is the amino acid residues interacting with the ligands. The lines are the π-(pi) bond interactions with amino acid residue of HIV-1 reverse transcriptase. (**b**): The binding pocket of HIV-1 reverse transcriptase (1RT2), showing the docking of dapivirine. The orange ball and stick model is the ligand (dapivirine) and the conventionally colored model is the amino acid residues interacting with the ligands. The lines are the π-(pi) bond interactions with amino acid residue of HIV-1 reverse transcriptase.



(**a**) (**b**)

Figure 4. (**a**): The binding pocket of HIV-1 reverse transcriptase (3BGR), showing the redocking of co-crystallized ligand T27. The orange colored ball and stick model is the ligand (T27) and the conventionally colored model is the amino acid residues interacting with the ligands. The lines are the π-(pi) bond interactions with amino acid residue of HIV-1 reverse transcriptase. (**b**): The binding pocket of HIV-1 reverse transcriptase (3BGR), showing the docking of dapivirine. The orange ball and stick model is the ligand (dapivirine) and the conventionally colored model is the amino acid residues interacting with the ligands. The lines are the π-(pi) bond interactions with amino acid residue of HIV-1 reverse transcriptase.

4. Conclusions

The NNRTI's were docked into the active site of four different HIV-1RT receptors. Amongst them, dapivirine, doravirine and efavirenz was found to have good docking scores and the binding mode analysis of these compounds revealed that they have similar interactions (pi- bond interactions) with the amino acid residues TYR188, TRP229 and LYS101 of the NNIBP. So, it can be predicted that these have the best activity and are the most effective among other NNRTI's.

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Conflicts of Interest: The authors declare no conflict of interest thereof.

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