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The mystery of chemistry behind the mechanism of action of anti-HIV drugs: A docking approach at an atomic level

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RESEARCH ARTICLE

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ABSTRACT

The effect of HIV-1 on a human's immune system cannot be ignored. This is the virus that reduces the power of the immune system to fight against any disease. Of course, many anti-HIV drugs are available, and many computational studies have been done to find out their mechanism of action, but the computational study regarding the chemistry behind the mechanism of action was not done yet. Therefore, the main objective of the study was to clarify the chemistry behind the mechanism of action of commercially available anti-HIV drugs. The drugs taken in the presented study were Entry Inhibitors (EIs) and Non-nucleoside reverse transcriptase inhibitors. First, literature data was evaluated computationally to ensure the reliability of the software used for the presented study. It was found that interaction-based experimental results and computationally evaluated results of the literature data were the same. After that, by following the same procedure, a docking study was done on the drugs taken in the current study. In addition, the residues involved in the interactions of EIs and NNRTIs with their receptors were studied to determine the chemistry that acts behind the action of both. It was found that EIs and NNRTIs work differently. It was also predicted that the derivatization of both drugs could make them more effective and active. Therefore, the presented study will be very helpful in the field of medicinal science.

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1. Introduction

The virus that attacks the human immune system is called the human immunodeficiency virus (HIV). Its treatment must be done immediately, otherwise, it can cause a dangerous disease known as acquired immunodeficiency syndrome (AIDS). More than 20 drugs are functioning against HIV. These drugs can be classified on the basis of their functions. For example, drugs that inhibit the entry of HIV into the cell are called entry inhibitors (EIs), and drugs that attack the main protease of HIV are called protease inhibitors (PIs), while drugs that interrupt the main function of reverse transcriptase enzymes (RTs) are called reverse transcriptase inhibitors (RTIs). RTIs are of two types, (i) Nucleoside reverse transcriptase inhibitors (NRTIs) that block the action of the viral reverse transcriptase enzyme required by HIV for the replication, and (ii) Non-nucleoside reverse transcriptase inhibitors (NNRTIs) that work similarly, but attach to different sites of reverse transcriptase. In the presented study, two types of drugs i.e. EIs (Fostemsavir and maraviroc) and NNRTIs (Doravirine, efavirenz, etravirine, nevirapine and rilpivirine) were selected for the computational evaluation of the chemistry acting behind their mechanism of action. The most interesting point to be kept in mind was the presence of different groups in the chemical structures of the drugs taken in the current study. Because of the variation in the chemical structures of anti-HIV drugs, different mechanisms of action were observed by not only the presented study but also others. Hence, there is a definite target for a definite class of drugs. Of course, the drug and its target should be well known when the mechanism of action is being studied, but the chemistry behind the attachment of the drugs on a particular receptor also plays a key role. This is because the chemistry behind the mechanism of action tells us why the drug has a greater affinity towards only one of the available receptors. Before this, the mechanism of pathogenic attachment with the cell receptor must be understood. The most important thing for the entry of HIV-1 into immunological cells is the use of CD4 as a receptor, and CCR5 as the co-receptor [1,2]. It is because the binding of glycoprotein 120 (gp120) of HIV-1 to the receptor CD4 and a coreceptor CCR5, induces conformational changes that are required by HIV-1 to dissociate its gp120 and to refold gp41 [3]. The entry of HIV-1 into the immune cell involves two steps (i) the association of glycoprotein 120 (gp120) of HIV-1 with CD4 to make the complex of CD4-gp120, and (ii) the formation of a CD4-gp120-CCR5 complex by the attachment of CD4-gp120 with CCR5 [4]. As per mutagenesis studies, the binding sites of CD4-gp120 are the N-terminal

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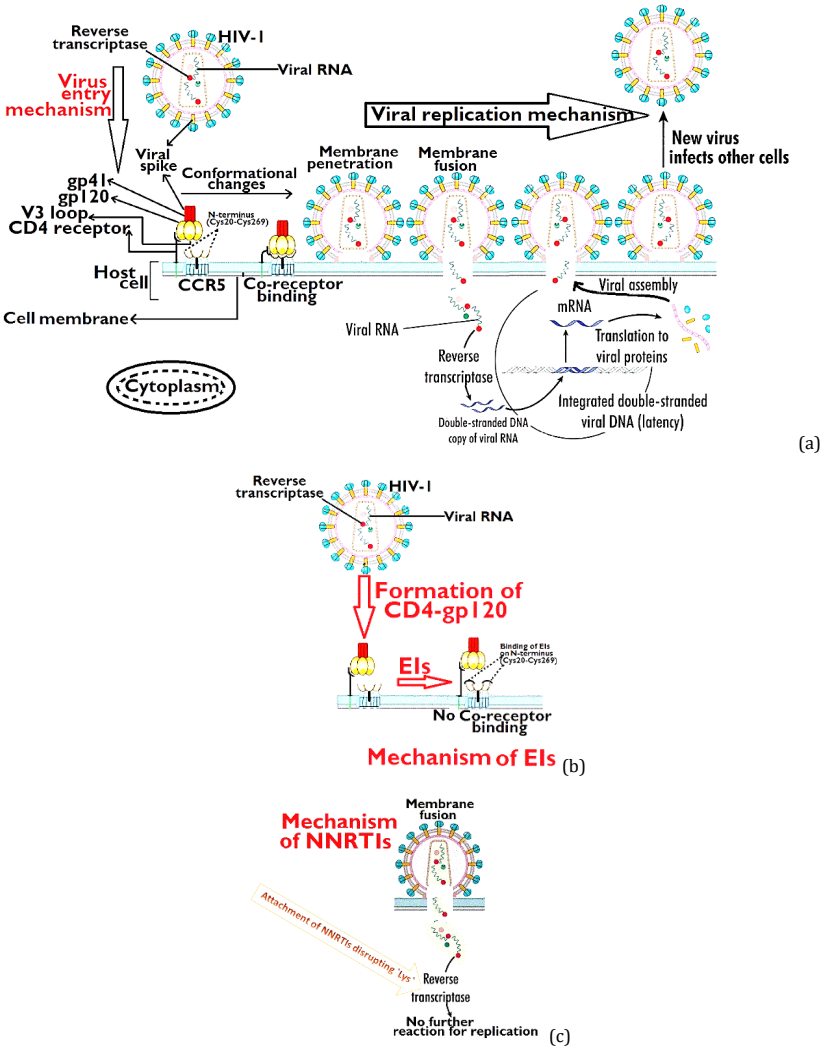

Figure 1. Mechanism of (a) HIV-1 entry, (b) EIs, and (c) NNRTIs.

segment of CCR5 [5,6], and the residues found on the N-terminal segment of CCR5 are Cys20 and Cys269 [4,7]. Furthermore, another study [8,9] has also revealed that the main contacts between CD4-gp120 and CCR5 for the formation of the CD4-gp120-CCR5 complex are the N-terminal of CCR5 (Figure 1a). Their terminus is electrostatic in nature [4].

After entering, HIV-1 replicates with the help of its reverse transcriptase enzyme (Figure 1a). Therefore, two questions arise in the mind regarding the chemistry behind the mechanism of action of EIs and NNRTIs (i) why and where EIs attach during the anti-HIV action, and (ii) how NNRTIs block the main function of the reverse transcriptase enzyme to stop the replication of HIV-1. The presented computational study resolves these questions.

2. Experimental

The software used in the presented study was Marvin Sketch, Autodock tool [10], Discovery studio, Autodock vina [11], PyMOL, and LigPlot [12]. First, the reliability of the software used in the current study was checked and confirmed. For this purpose, the data collected through a literature survey were computationally evaluated. After that, the crystalline structures of reverse transcriptase [13] and CCR5 [14] with pdb codes 2rf2 and 6aky, respectively, were obtained from the protein data bank [https://www.rcsb.org/]. A computational study is very helpful not only in understanding the reaction mechanism [15] but also in drug development [16-18] and the mechanism of action in pharmacokinetics [19]. The currently presented docking study involved the following steps:

2.1. Receptor preparation

The crystalline structures of the receptors obtained from protein data bank, were made pure for the docking purpose using Discovery studio (Figure 2). It is because I did not find a published structure for CCR5 without ligand. Therefore, there were many unwanted things associated with main receptors (RTs and CCR5). Hence, undesirable residues, water molecules, and other nonrequired ligands were removed.

2.2. Ligand preparation

Having prepared the receptor file, the structures of ligands (EIs and NNRTIs) were drawn using MarvinSketch (Figure 3). After that, the confirmation of the 3D structure of the ligands was done with the help of Discovery Studio.

2.3. Docking and data analysis

All PDBQT formatted files of ligands, were docked with their receptors one by one using AutoDock Vina program.

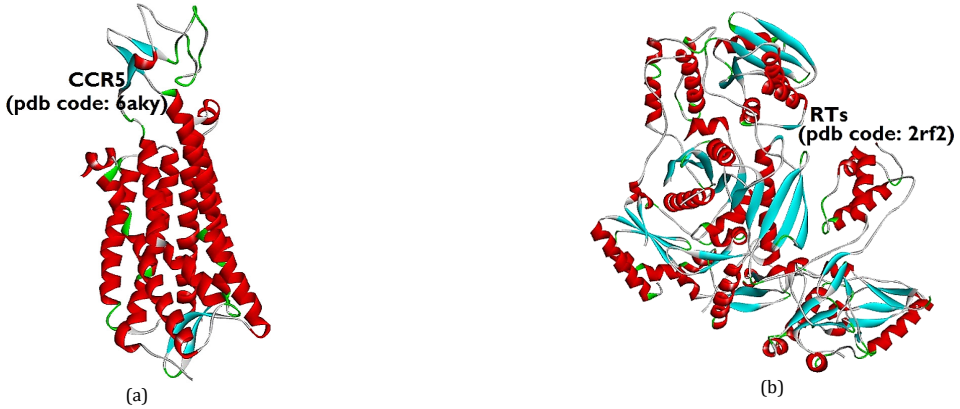

Figure 2. The crystalline structure of computationally cleaned receptors of (a) EIs and (b) NNRTIs.

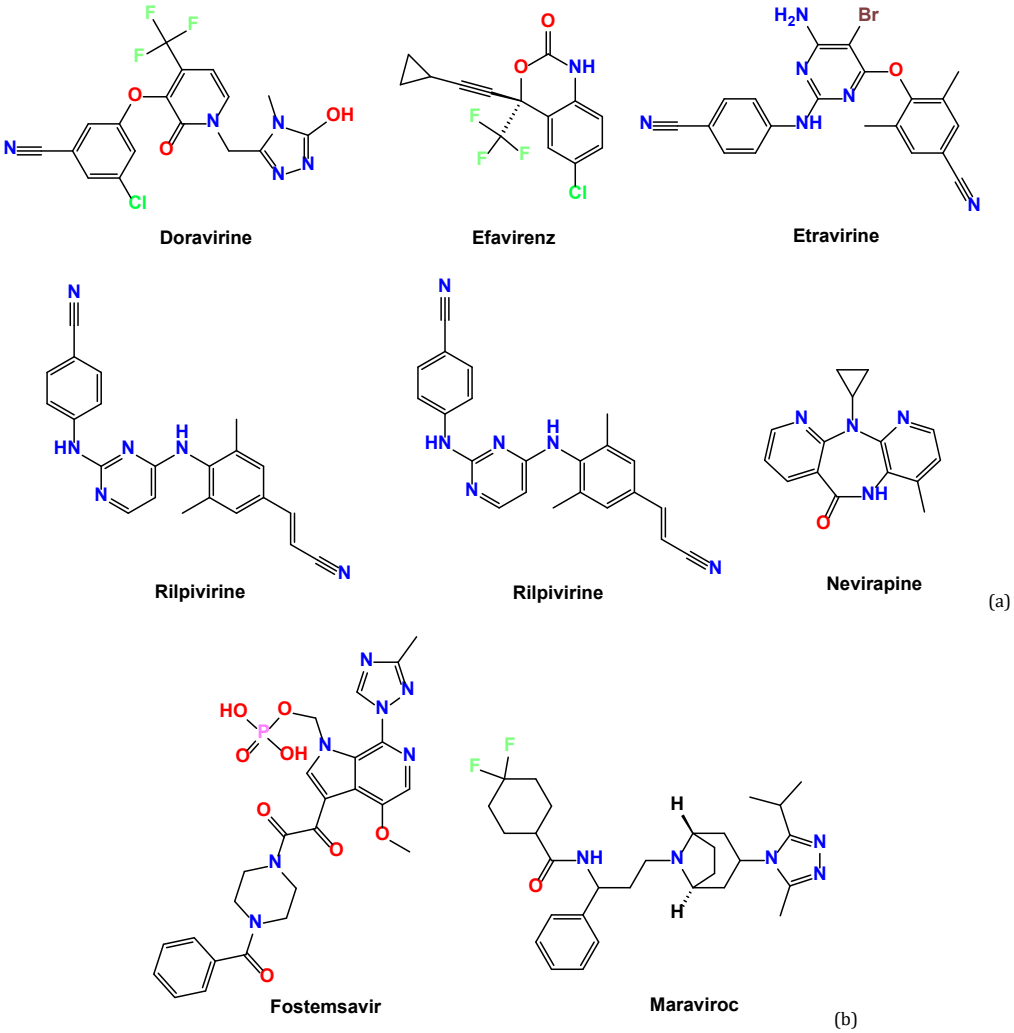

Figure 3. Structures of (a) NNRTIs and (b) EIs.

Vina uses the same PDBQT molecular structure file format as AutoDock Tool. For the docking method, the coordinates of the source were set at x = 30:054, y = 22:75, and z = 4:171. Many autonomous docking runs were applied for each ligand and target to find the lowest free energy of binding confirmation from the largest cluster.

The analysis of the number of hydrogen bonds, the residues involved in hydrogen bonding, mode of interaction, and bond lengths of hydrogen bonds were studied by PyMOL. On the other hand, LigPlot 1.4.5 was used for the study of hydrophobic interactions taking place between drugs and their receptors.

3. Results

It was too good to see the results which were the same as obtained by others [13,20] experimentally using different techniques such as X-ray diffraction technique and Cryo-EM.

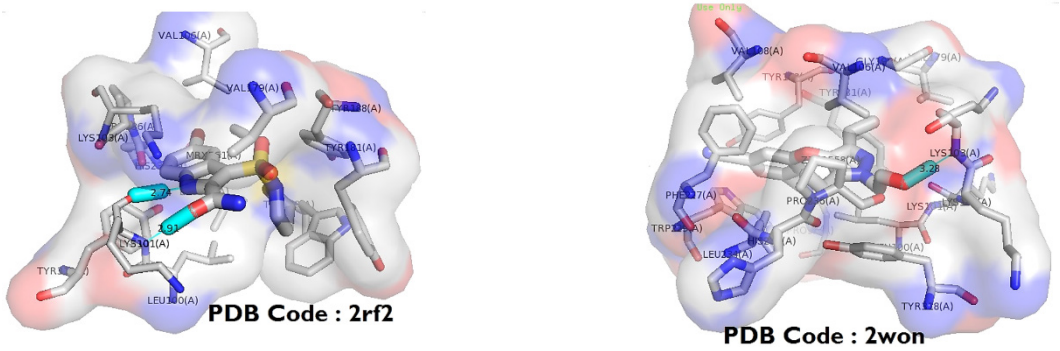

Figure 4. Computationally evaluated experimental data showing the residues of the receptor involved in the hydrophobic interactions.

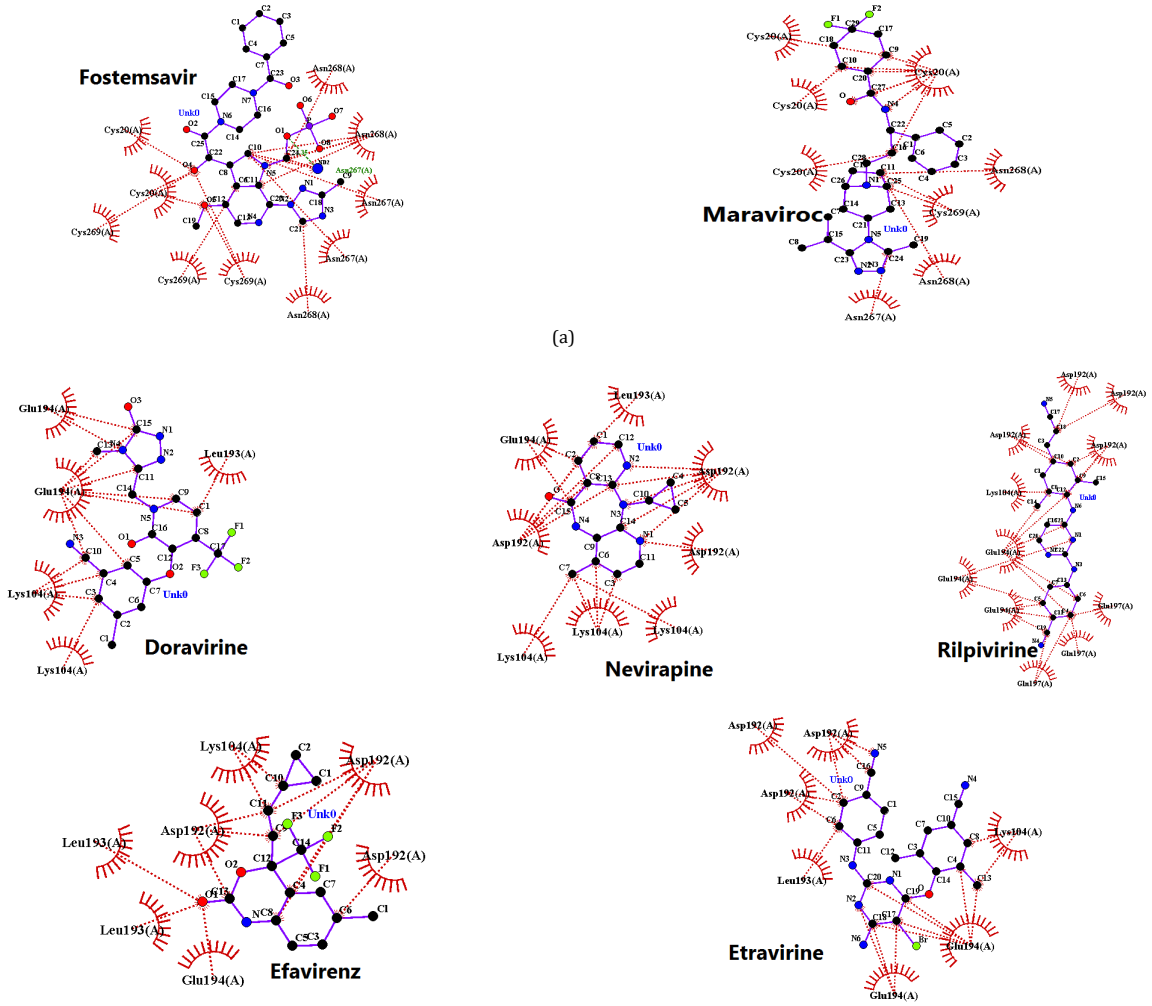

Figure 5. Docking results showing the binding pockets of (a) CCR5 for EIs and (b) RTs for NNRTIs.

For example, indole-3-sulfonamides [13] and lersivirine [20] were found as potent NNRTIs whose interaction-based experimental data available on protein data bank with pdb codes: 2rf2 and 2won, respectively, was evaluated computationally. It was observed that the computationally evaluated data showed the same residues of both the drugs with reverse transcriptase enzyme (Figure 4) as found experimentally in indole-3-sulfonamides [13] and lersivirine [20]. Hence, the results were satisfactory and supported the reliability of the software used in the current study.

The docking results gave a new thought to rethink. These results were evidenced by the experimental consequences of others. In the case of EIs (Fostemsavir & Maraviroc), it was perceived that fostemsavir and maraviroc attack on the N-terminus of CCR5 i.e. C20 (Cys20) and C269 (Cys269). Fostemsavir formed one hydrogen bond with the N-terminus of CCR5, while maraviroc also formed one hydrogen bond (Figure 5a). The binding affinities of fostemsavir and maraviroc for CCR5 were -2.4 and -2.3, respectively (Table 1).

Table 1. Drug-receptor binding affinities and residues involved in interactions *.

Receptor (pdb code)	Nature of drugs	Drugs	Binding affinity (kcal/mol)	Number of H-bonds	Residues involved in H-bond (Bond length in Å)	Receptor: drug residues hydrophobic interaction
CCR5 (6aky)	EIs	Fostemsavir	-2.4	1	.107/A/Asn267/ND2 & O of phosphate group (3.3 Å)	Cys20:O4, O5 Cys269:O4, O5, C6 Asn268:C10, C11, C21, C24 Asn267:C10
		Maraviroc	-2.3	1	.627/A/Cys20/N & O of -CONH- group (3.5 Å)	Cys20:C9, C10, C16, C20, C27, N4, O Cys269:C11, C25 Asn268:C11 Asn267:C24
RTs (2rf2)	NNRTIs	Doravirine	-3.2	2	.107/A/Gln197/NE2 & N of -N= group (3.5 Å) .107/A/Gln197/NE2 & N of -N= group (3.5 Å)	Lys104:C3, C4, C10 Glu194:C1, C5, C9, C10, C11, C13, C15 Leu193:C1
		Efavirenz	-3.2	2	.647/A/Glu194/OE2 & O of -COO- group (3.2 Å) .647/A/Glu194/OE1 & O of -COO- group (3.4 Å)	Lys104:C10, C11 Glu194:O1 Leu193:O1 Asp192:C4, C8, C9, C10, C11, N5
		Etravirine	-3.2	2	.647/A/Glu194/OE2 & N of -N= group (3.4 Å) .647/A/Asp192/OD1 & N of -C≡N group (3.6 Å)	Lys104:C8, C13 Glu194:C4, C13, C17, C18, C19, C20 Leu193:C6 Asp192:C2, C6, C16, N5
		Nevirapine	-3.3	1	.647/A/Glu194/OE2 & O of -CONH- group (3.4 Å)	Lys104:C3, C6, C7 Glu194:C2, C15 Leu193:C1 Asp192:C13, C14, N1, N2, N3
		Rilpivirine	-3.7	2	.647/A/Glu194/OE2 & H of -NH- group (2.7 Å) .647/A/Glu194/OE2 & N of -N= group (3.2 Å)	Lys104:C8 Glu194:C4, C5, C6, C11, C12, C14, C21, N1 Asp192:C2, C9, C10, C18

* RTs: Reverse transcriptase, NNRTIs: Non-nucleoside reverse transcriptase inhibitors; CCR5: Chemokine receptor 5.

Besides, the common residues of fostemsavir and maraviroc involved in the hydrophobic interactions with CCR5 were Cys20, Cys269, Asn267, and Asn268 (Figure 6a). In the case of NNRTIs (Doravirine, efavirenz, etravirine, nevirapine, and rilpivirine), it was perceived that NNRTIs attack on that side of the receptor where the residue "Lys" is present specifically. The number of hydrogen bonds formed by NNRTIs with RTs enzyme was two in the case of doravirine, efavirenz, etravirine, rilpivirine, and one in the case of nevirapine (Figure 5b). Another observable point was the binding affinities of NNRTIs with RTs. The binding affinities of doravirine, efavirenz, etravirine towards RTs enzyme were -3.2 kcal/mol, while those of nevirapine and rilpivirine were -3.3 and -3.7 kcal/mol, respectively (Table 1). Besides, the common residues of NNRTIs involved in the hydrophobic interactions with RTs enzyme were Lys 104, Glu194, Leu193, Asp192 (Figure 6b).

4. Discussion

The main findings of the current study were fully supported by the literature data. Using both things, the chemistry behind the mechanisms of action of EIs and NNRTIs was made understandable and acceptable. The attack of EIs (taken in the presented study) on the N-terminus of CCR5 clearly showed that they interfere in the formation of the CD4-gp120-CCR5 complex. As per the introductory part of the current article [4-7], the binding of CD4-gp120 with CCR5 takes place on the N-terminus (Cys20-Cys269) which is necessary for the formation of CD4-gp120-CCR5 complex so that HIV-1 can enter the cell. On the other hand, as per the docking results, EIs also attach to the N-terminus (Cys20-Cys269) of CCR5. Hence, a competition takes place between EIs and CD4-gp120 for the attachment with the N-terminus (Cys20-Cys269) of CCR5. The attachment of maraviroc (EI) in the hydrophobic pocket of CCR5, and the competition taking place between maraviroc and CD4-gp120 has already been experimentally confirmed [4], but the question, why maraviroc fit itself to the N-terminus (Cys20-Cys269) of CCR5, was unsolved. The presented work resolves this question by keeping the hydrophobicities of both the drugs (EIs) into consideration as the evidence that supports the attachment of EIs with N-terminus (Cys20-Cys269) of CCR5. Actually, the presence of aromatic rings in the structures of EIs makes them hydrophobic. On the other hand, the binding sites (Cys20-Cys269) of CCR5 are also hydrophobic [21]. Hence, the attachment of maraviroc with the N-terminus of CCR5 takes part faster than CD4-gp120. As the glycoprotein (gp-120) of HIV-1 is hydrophilic [22], the attachment of gp-120 with the N-terminus of CCR5 does not take part faster than maraviroc. It is because hydrophilic compound does not have greater affinity towards hydrophobic complex, but hydrophobic compound does, as per universal truth. Hence, due to this competition, the formation of CD4-gp120-CCR5 complex does not take part, and HIV-1 becomes unable to enter the cell (Figure 1b). Besides, another notable point was the binding affinities of both drugs. As per the docking results, the binding affinity of fostemsavir towards CCR5 was greater than that of maraviroc (Table 1), which shows that fostemsavir binds with CCR5 more tightly as compared to maraviroc. Hence, fostemsavir would be a more effective drug than maraviroc. The difference in the binding affinities of both drugs can also be correlated to their chemical structures. The presence of more aromatic rings in the structure of fostemsavir makes it more hydrophobic in nature, and the receptor is also hydrophobic in nature. Hence, fostemsavir showing more hydrophobicity attach with the hydrophobic N-terminus (Cys20-Cys269) of CCR5 more rapidly and tightly as compared to maraviroc. Thus, the chemistry behind the mechanism of inhibitory action of EIs for the entry of HIV-1 into the cell is resolved.

In the same way, the mechanism of NNRTIs was also resolved with the help of the docking results and literature data. As per docking results, all NNRTIs drugs taken in the presented study showed their attachment with different residues. The most important residue that was common in the case of all NNRTIs, and plays a key role in the synthesis of viral protein, was Lys (Lysine) specifically. A huge association between lysine and HIV-1 RNA replication has been found. The synthesis of viral protein needs lysine too much, and it may increase the risk of high viral load, subsequent acceleration of immune-suppression and HIV progression [23].

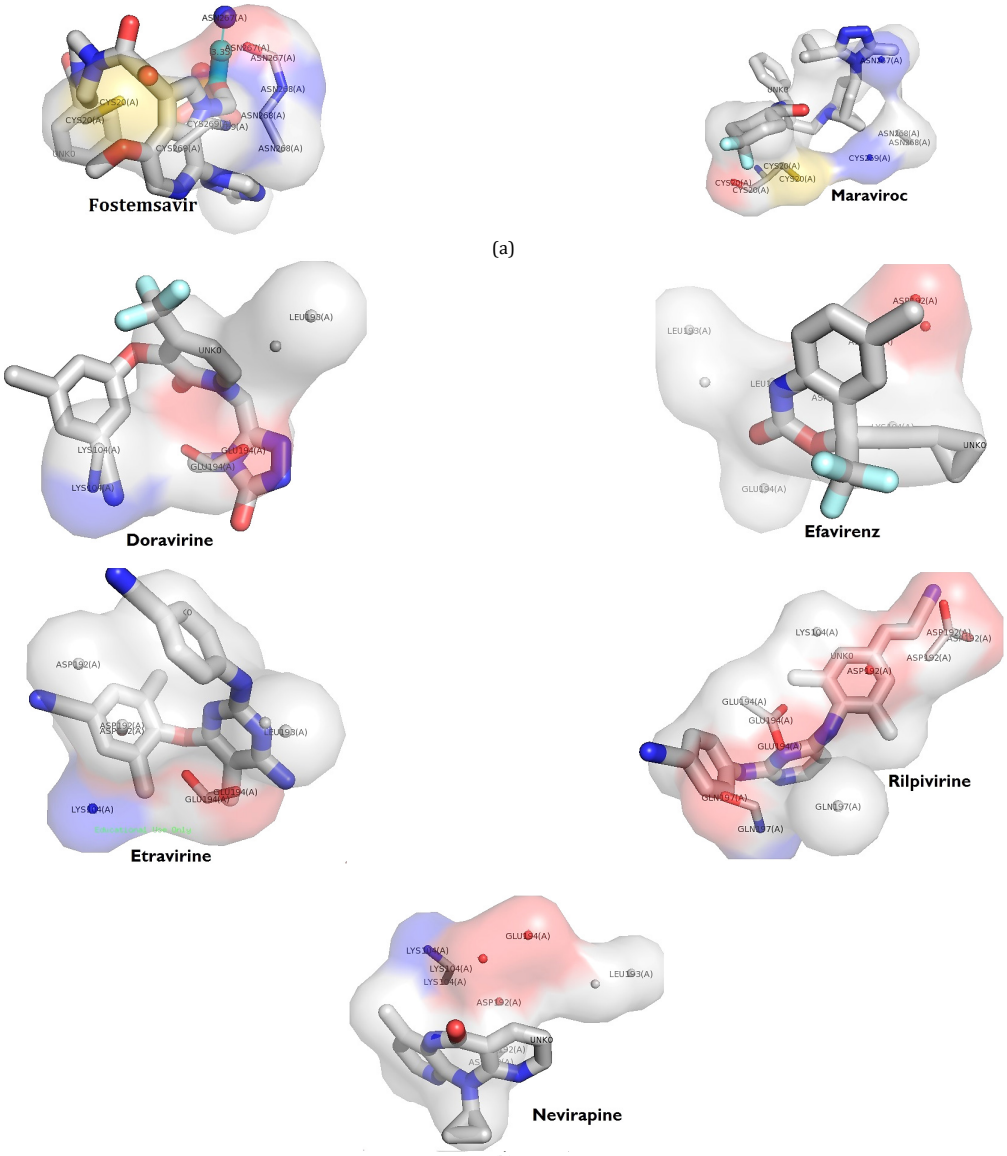

Figure 6. The hydrophobic interactions of (a) EIs with CCR5, and (b) NNRTIs with RTs.

The role of lysine in the synthesis of viral protein is not common in all types of viruses such as herpesvirus [24], but its crucial role was found especially in HIV-1 [23] and reovirus [25]. The attack of NNRTIs (Doravirine, efavirenz, etravirine, nevirapine, rilpivirine) on the most important residue (Lys) of RTs clearly showed that they interfere in the main function of 'Lys' during viral protein synthesis. Hence, due to the disruption in the function of the crucial residue (Lys), HIV-1 does not synthesize its protein and becomes unable to replicate itself (Figure 1c). Moreover, the involvement of the same residue i.e. Lys of RTs enzyme with the newly synthesized NNRTIs [13,20] (Figure 4) other than the drugs taken in the current study, was also found. In Figure 4, the sky-blue color shows the hydrogen bond formed by the newly synthesized NNRTIs [13,20] with Lys (lysine). Hence, this evidence can also be included to prove the attachment of NNRTIs on that side of RTs enzyme where the residue playing a key role in the protein synthesis and replication, is present. Besides, another notable point was the binding affinity of NNRTIs. As per the docking results, the binding affinity of rilpivirine towards RTs was greater than that of other NNRTIs, it shows that rilpivirine binds with RTs more tightly as compared to other NNRTIs. Hence, rilpivirine would be a more effective drug than NNRTIs. Thus, the mechanism of inhibitory action of NNRTIs (doravirine, efavirenz, etravirine, nevirapine, rilpivirine) for the replication of HIV-1 in the cell, is resolved.

5. Conclusion

Based on results and discussion, it can be concluded that EIs act as a competitor drug that competes with gp-120 of HIV-1 for the attachment with CCR5. As per the docking results supported by literature data, the affinity of EIs for CCR5 was found greater than that of gp-120. It was just because of the hydrophobic nature of EIs and their receptors. Therefore, EIs can be made more effective if the derivatization of these drugs is done by introducing those groups that may increase the hydrophobicity of EIs. Hence, the derivatization of EIs to increase their hydrophobic nature may play an important role in making them a better competitor. On the other hand, as per docking results, NNRTIs attack on the most important residue (Lys) of HIV-1. This is the residue that is required by HIV-1 for its replication, as per literature data. Therefore, it is clear that both the types

of drugs taken in the current study, attack on different targets, and act with different mechanisms on them

Disclosure statement

Conflict of interest: The author declares that he has no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.
Human and animal rights: No animals/humans were used for the studies that are the basis of this research

CRediT authorship contribution statement

Conceptualization: Mohammad Suhail; Methodology: Mohammad Suhail; Software: Mohammad Suhail; Validation: Mohammad Suhail; Formal Analysis: Mohammad Suhail; Investigation: Mohammad Suhail; Resources: Mohammad Suhail; Data Curation: Mohammad Suhail; Writing - Original Draft: Mohammad Suhail; Writing - Review and Editing: Mohammad Suhail; Visualization: Mohammad Suhail; Funding acquisition: Mohammad Suhail; Supervision: Mohammad Suhail; Project Administration: Mohammad Suhail.

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