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Design Strategy of Some Novel Tetrahydroquinoline Analogs as Potential Non-Nucleoside Reverse Transcriptase Inhibitors

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Abstract

In our present study, we tried to develop a tetrahydroquinoline based non-nucleoside reverse transcriptase inhibitor by means of an in-silico drug design study. A series of 14 molecules were designed considering the tetrahydroquinoline nucleus as a core moiety. The least energy conformers were generated and docked virtually using AUTODOCK 1.4.6 with the target, reverse transcriptase enzyme. Prior docking operation, the enzyme was fed into Ramachandran plot, thereby; the extent of favorable and unfavorable zone of the enzyme was identified. Most of the molecules have shown a significant docking interaction with promising docking score. Compound (3a) was found to achieve the highest binding energy: of -7.14 Kcal/mole, thus placed itself quite conveniently with the receptor active binding pocket. With the rest of the molecule in the aforementioned series, the binding energy lies within the range of -4.89 to -6.86, which was also very significant in context to effective drug receptor interaction.

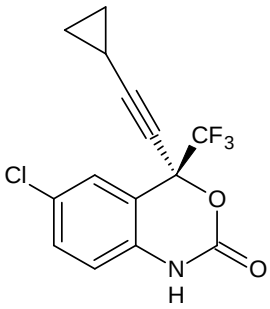
Keywords: Docking, Ramachandran Plot, Tetrahydroquinoline, NNRTIs.

INTRODUCTION

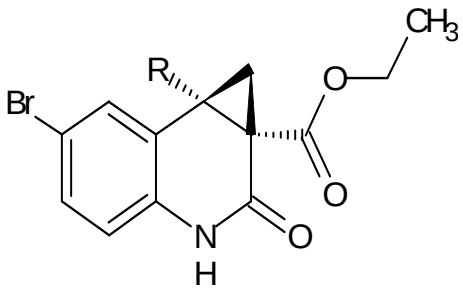
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key elements of multidrug HIV therapy, called HAART (Highly Active Antiretroviral Therapy). HAART consists of combination of nucleoside HIV reverse transcriptase inhibitors (NRTIs), NNRTIs, and protease inhibitors (PIs). **[1]** When HIV infects a cell, reverse transcriptase copies the viral single stranded RNA genome into a double-stranded viral DNA. The viral DNA is then integrated into the host chromosomal DNA, which then allows host cellular processes, such as transcription and translation to reproduce the virus. Reverse Transcriptase Inhibitors block reverse transcriptase's enzymatic function and prevent completion of synthesis of the double-stranded viral DNA, thus preventing HIV from multiplication. **[2]**

Three non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been approved by FDA. They are nevirapine (Viramune), delavirdine (Rescriptor) and efavirenz (Sustiva), and are key

components of the combination therapy. Since significant resistance has been developed against these drugs, there is an urgent need to develop new NNRTIs that would overcome the current drug resistance. Previously, we have reported the discovery of novel NNRTIs via HTS and SAR studies. Among the reported NNRTIs, a number of them share common structural features, such as efavirenz (1). Almost all of them share a fused aromatic core, which consists of a hydrophobic phenyl moiety, and an amide or carbamide moiety. Based on the molecular modeling studies performed **by Ellis *et al* [3]**, the core quinolone fits into the NNRTI binding site and aligns nicely with efavirenz in space. The core quinolone and the core dihydrobenzoxazinone of efavirenz almost overlap in the center of the binding pocket. The halophenyl moieties in both molecule occupy the same hydrophobic pocket. An alkyl R group in quinoline based NNRTIs (2) and the cyclopropylethynyl group in efavirenz could occupy the same hydrophobic pocket. The cyclopropyl moiety in quinoline based NNRTIs and the trifluoromethyl group in efavirenz fill into another small hydrophobic space. **[3]**



(1)



(2)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as TIBO and alpha-APA were the first to have a significant activity against wild-type enzyme of HIV-1, but they had no effect against mutants.

Crystal structure analysis of HIV-RT enzyme showed that the first generation of drugs (TIBO, alpha-APA, Nevirapine) filled up an allosteric hydrophobic pocket (non nucleoside binding site, NNBS) and bound with the enzyme in a “butterfly-like” mode. One of the “wings” of this butterfly is made of pie-electron-rich moiety (phenyl or allyl substituents) that interacts through pie-pie interactions with a hydrophobic pocket formed mainly by the side chains of aromatic amino acids (Tyr181, Tyr188, Phe227, Trp229, and Tyr318). On the other hand, the other wing is normally represented by a heteroaromatic ring bearing on one side of a functional group capable of donating and/or accepting hydrogen bonds with the main chain of the Lys101 and Lys103. Finally, on the butterfly body a hydrophobic portion fulfills a small pocket formed mainly by the side chains of Lys103, Val106, and Val179 (Figure 1). Upon complexation the NNBS hydrophobic pocket changes its own conformation leading to inactivation of the enzyme itself. Because of the different chemical and structural features of the inhibitors and the side-chain flexibility, the bound NNBS undergo different conformational changes. Moreover, mutations of some amino acid causes a variation of the NNBS pocket properties, thus decreasing affinities of most the inhibitors. **[4]**

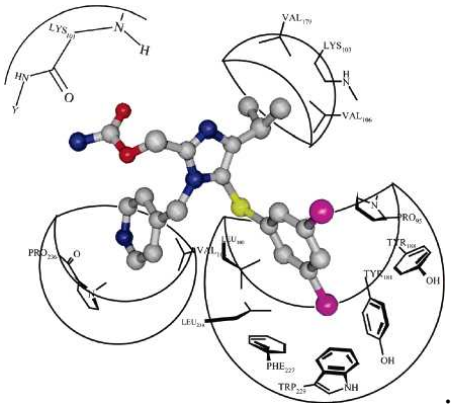


Figure I: Schematic view of the NNBS (Non Nucleoside Binding Site). [4]

Design Strategy

Wide variations in nucleotide and amino acid sequences in HIV-1 make the development of effective anti-AIDS drugs or vaccines, a daunting problems. A successful anti-AIDS drug candidate should be effective against common drug-resistance mutations. Structural understanding of the target enzyme RT (Reverse transcriptase), mode of drug action, and the effects of drug resistance resulting from mutations provide valuable information, which can be used to design more potent NNRTIs. As has already been discussed above, HIV-1 and more specifically the NNIBP (Non-Nucleoside inhibitor Binding Pocket) of RT (Reverse transcriptase) can be changed by mutations; such mutations can interfere with NNRTI binding. **[5]**

As a part of the drug discovery effort, NNRTIs were selected on the basis of their inhibition profiles or chemical compositions, and crystal structure of complexes of HIV-1 RT with these inhibitors were determined. Multiple low energy conformation of the NNRTIs were generated and docked into NNIBPs located in the specific region of the crystal structure.

Till date, various wild and mutant strains for reverse transcriptase have been isolated. Considering various relevant information obtained from different literature sources, we have chosen wild strain of reverse transcriptase with the pdb id 3IOR. One of the key elements found in the co crystallized enzyme is a ligand i.e. RT3601, which bear tetrahydroquinoline as a core. The study will be much scientifically relevant, when a target is isolated along with a ligand. It paved the way to design molecule on the basis of active site of the receptor, where the drug can directly attach. Our designed molecules maintained all the typical characteristics of a ligand moiety to bind with the NNBS (Non-Nucleoside Binding Site) pocket.

The greatest interest in 1,2,3,4-tetrahydroquinolines is due to their diversified therapeutic applications. Some of them are oxaminoquine- a schistosomicide, nicainoprol- an antiarrythmic drug, virantmycin- a novel antibiotic are the best known. Tetrahydroquinoline L-6X9.560 is one of the most potent NMDA antagonists yet found.

Tetrahydroquinolines have also shown their potential as antidepressants, nervous system depressants, potent antiu1cer, cardiovascular, antithrombotic, antiallergenic, antitumor, antirheumatic, immunosuppressant, or antifertility agents. Some tetrahydroquinolines are recognized as high affinity ligands at the glycine site of the NMDA receptor, other as facilitators of noradrenergic transmissions, or myofilament sensitizers without affecting cell Ca^{2+} loading. The wide range of therapeutic benefit have also seen in the treatment of cerebral ischemia and osteoporosis.**[6]**

Considering tetrahydroquinoline as a core moiety is based on the fact that the original ligand which was co crystallized with target holding the same core with it and the wide range of therapeutic application of tetrahydroquinoline in the field of modern drug therapy.

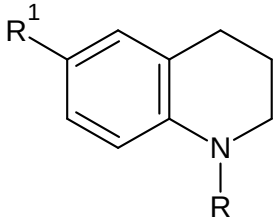
MATERIALS AND METHODS

Experimental work

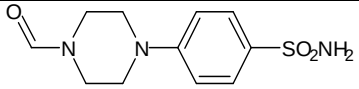
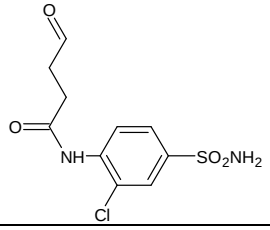
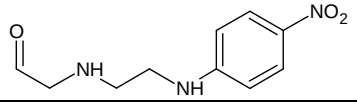
All computational studies were carried out using AUTODOCK 4.0.1(version: 1.4.6) **[7-10]** installed in a single machine running on a 2.0 GHz Intel core2 duo processor with 2GB RAM and 320 GB hard disk with LINUX (Fedora 8) version 6.0.19 as an operating system. The geometry of reverse transcriptase was extracted from the Brookhaven protein data bank **[11]** (entry code: 3I0R) complexed with the irreversible inhibitor RT3601. The Autodock Tools package version 1.4.6 was employed to generate the docking input files and to analyze the docking results. All the non polar hydrogens were merged and the water molecules were removed. For the docking, a grid spacing of 0.389 Å and 80×80×80 number of points was used in *x,y,z* direction. The grid was centered on the mass center of the experimental bound RT3601 coordinates. Autodock generated 10 possible binding conformations, i.e. 10 runs for each docking by using Genetic Algorithm (GA-LS) searches. A default protocol was applied, with an initial population of 150 randomly placed individuals, a maximum number of 2.5 x 10^5 energy evaluations, and a maximum number of 2.7 x 10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

RESULTS AND DISCUSSION

Table I: Docking Output of compound 3(a-n)



(3)

Sl No	R	R^1	Free energy of binding (Kcal/mole)	Interacting residue	Surrounding residue
a		NH_2	-7.14	ASP177	VAL179,LYS101, LYS103, LEU100.
b		CH_3	-6.86	PRO243	ILE244,LYS263, TRP266, GLN242.
c		CH_3	-6.63	VAL314	HIS315,GLU312, LYS311, GLU308.

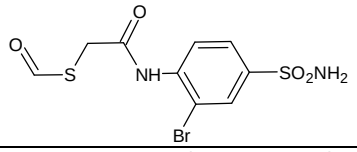
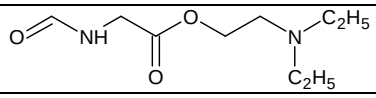
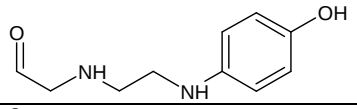
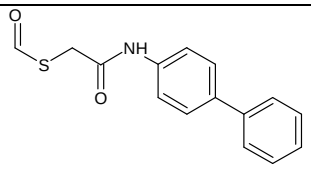
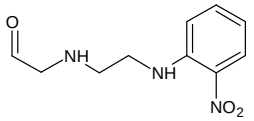
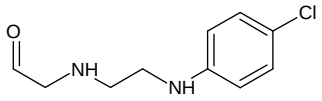
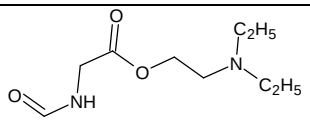
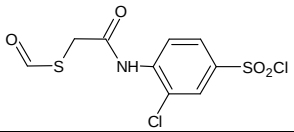
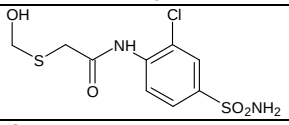
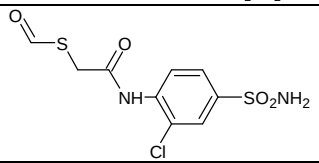
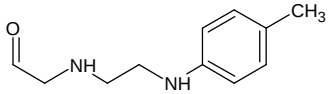
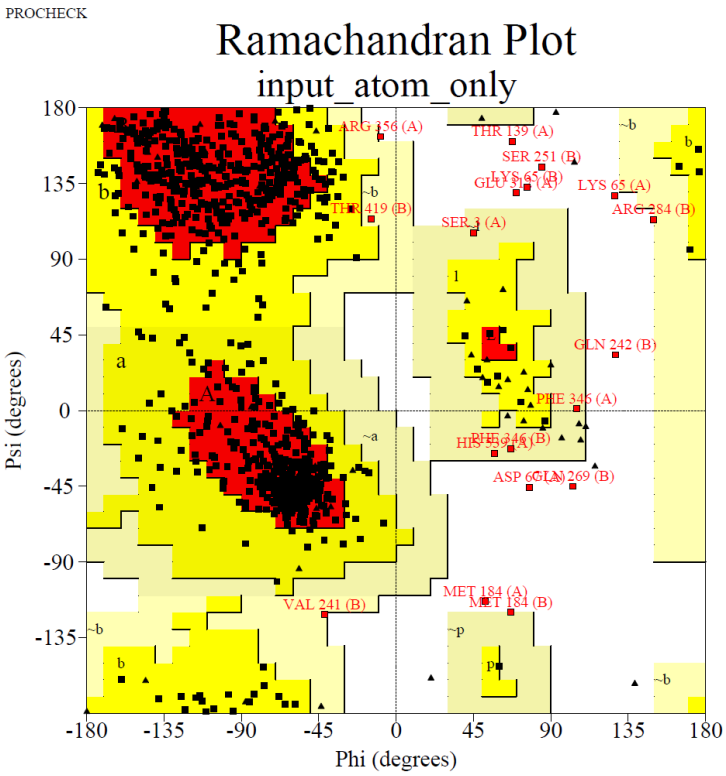
d		CH_3	-6.57	GLU233	ILE244,PRO243, LEU310,LYS311, GLU312, PRO313.
e		NH_2	-6.45	ILE244	VAL245,LEU246, GLU312.
f		CH_3	-6.42	PHE87	LYS154,PRO157, VAL90,MET184, GLN161.
g		CH_3	-6.36	LYS366	HIS361,ALA360 TRP406,TYR501, GLN500.
h		CH_3	-6.04	TRP414 LYS395	TRP410
i		CH_3	-5.53	PHE346	TYR342,GLN340, GLN330,ASN348, VAL317,VAL314, HIS315.
j		CH_3	-5.53	GLU224	PRO226,PRO225, LYS223, LEU228.
k		CH_3	-5.49	GLY335	LYS331,GLN334, GLN512,GLU514, ASP511.
l		CH_3	-5.36	GLU233	ILE 244,LEU246, PRO247.
m		NH_2	-5.26	ASP113	LYS70,ILE37, LYS73,PHE116, LYS46, GLU40.
n		CH_3	-4.89	GLN300	LEU298,PRO294, SER251, ASP250.

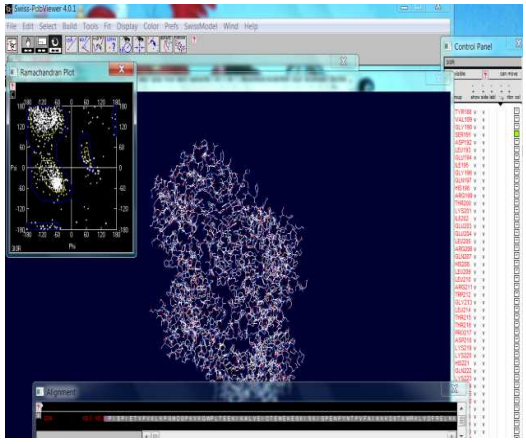
Fig 2: Ramachandran Plot Generated By Procheck On 3i0r with Their Statistics



Plot statistics

Residues in most favoured regions [A,B,L]	657	79.8%
Residues in additional allowed regions [a,b,l,p]	148	18.0%
Residues in generously allowed regions [~a,~b,~l,~p]	6	0.7%
Residues in disallowed regions	12	1.5%
Number of non-glycine and non-proline residues	823	100.0%
Number of end-residues (excl. Gly and Pro)	6	
Number of glycine residues (shown as triangles)	55	
Number of proline residues	66	
Total number of residues	950	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.



There are few amino acids lying in unfavorable environment which can be ignored in overall considerations of the model structures. The distribution of main chain torsion angle psi and phi are examined in Ramachandran plot. The result clearly shows the vast majority of the amino acids are in psi and phi distribution constrained with right handed helices. The remaining residues that fall into the random or configuration geometries are very short segments and primarily in the loop region of the protein. 79.8% of the residue of the model lies within the most favored region indicating good quality model. The backbone of the 118 structures of resolution of at least 2.0 angstroms and R factor not exceeding 20%.

Fig 3: Stereo and molecular surface view of the docking conformation of compound 3a (ball and stick model) in the active site of 3I0R. The residues (colored line model with three letter codes) interacting with compound 3a are shown. The rest of the protein structure was suppressed for clarification purposes.

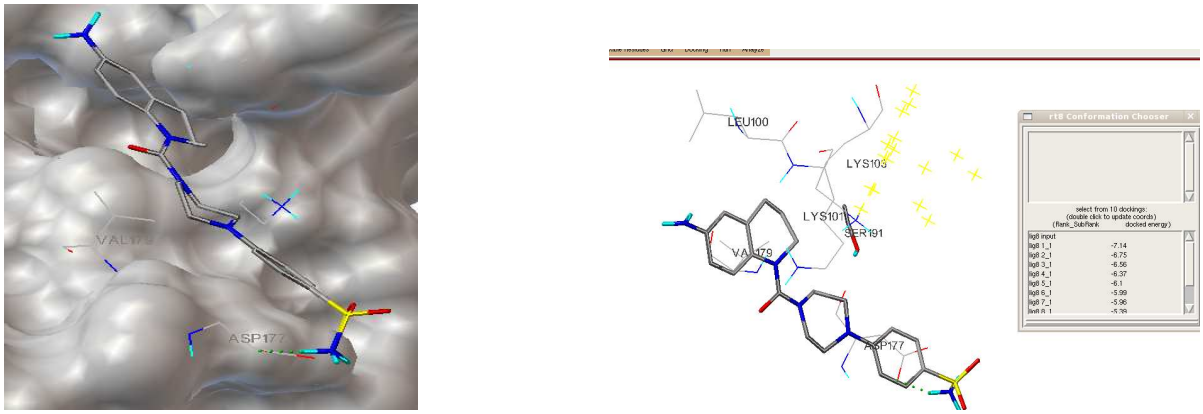


Fig 4: Stereo and molecular surface view of the docking conformation of compound 3b (ball and stick model) in the active site of 3I0R. The residues (colored line model with three letter codes) interacting with compound 3b are shown. The rest of the protein structure was suppressed for clarification purposes.

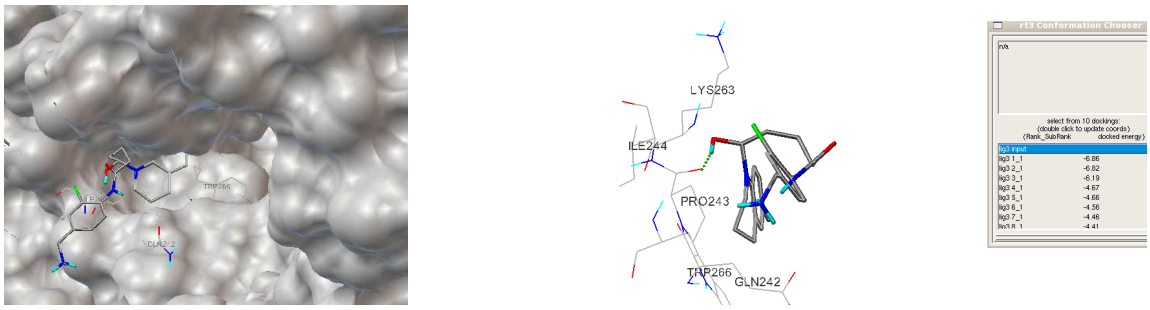


Fig 5: Stereo and molecular surface view of the docking conformation of compound 3d (ball and stick model) in the active site of 3I0R. The residues (colored line model with three letter codes) interacting with compound 3d are shown. The rest of the protein structure was suppressed for clarification purposes.

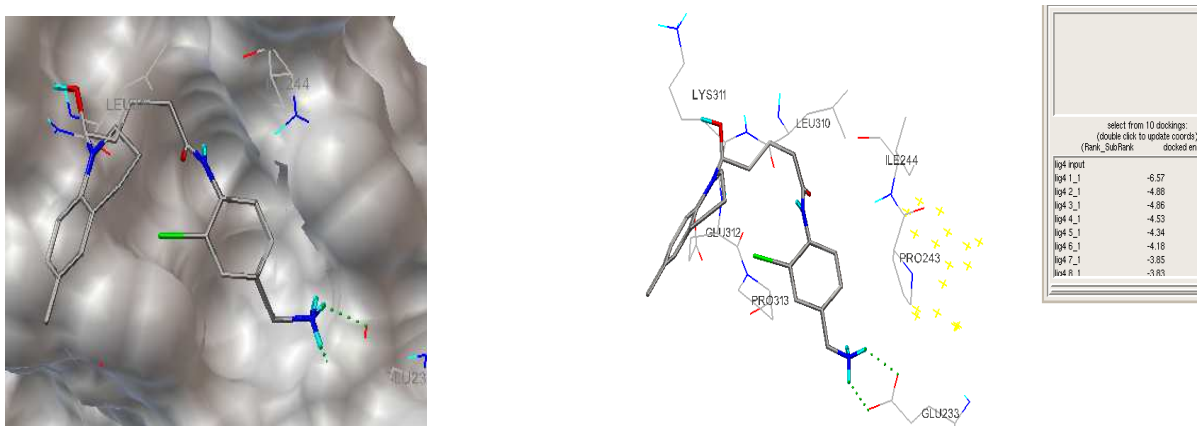
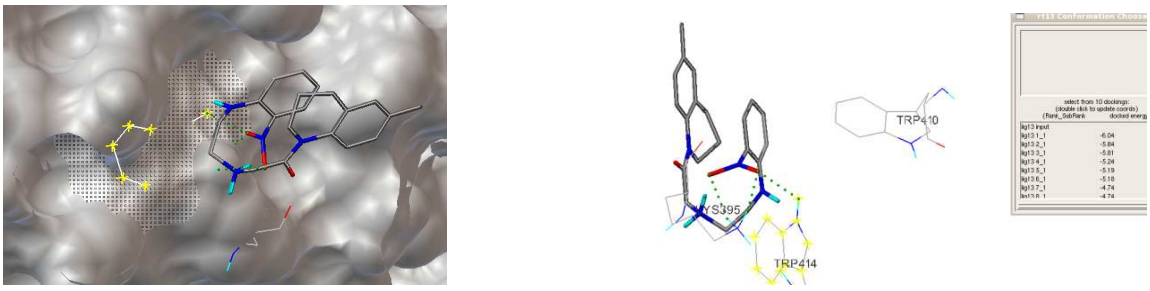


Fig 6: Stereo and molecular surface view of the docking conformation of compound 3e (ball and stick model) in the active site of 3I0R. The residues (colored line model with three letter codes) interacting with compound 3e are shown. The rest of the protein structure was suppressed for clarification purposes.

Fig 7: Stereo and molecular surface view of the docking conformation of compound 3h (ball and stick model) in the active site of 3I0R. The residues (colored line model with three letter codes) interacting with compound 3h are shown. The rest of the protein structure was suppressed for clarification purposes.



In the **figure 3:** compound **3a** shows a favorable docking interaction with ASP177 and surrounding residue was found to be VAL179, LYS101, LYS103, LEU100. The molecular surface view clearly shows that, the ligand placed itself in a significant manner in the non nucleoside binding pocket and the orientation of the molecule inside the pocket resembles to a butterfly, which was profusely desired as supported by the literature. **[5]**

Compound **3b, 3d, 3e and 3h** have shown to have snugly fitted within the receptor binding pocket, thus maintaining the butterfly like structure. They gained a significant docking score. The interacting residue of those corresponding molecules have scripted in table-I.

CONCLUSION

Though docking study cannot affirm the effectiveness of a drug in reality, but it is a part and parcel of novel drug design, which has already gained a huge amount of kudos globally. It can give an array of hope in the drug discovery process. Considering the pros and cons of the particular study, we have developed a series of molecule. It has been well identified from our present study that the interacting partner and surrounding residues of the highest energy conformer, i.e. compound 3a, have a close resemblance with that of the ligand originally co crystallized with the enzyme.

Most of the compounds have shown a butterfly like orientation around the active binding site, which was supposed to be happened as supported by the literature. The surrounding residue

alignment of the designed molecule shows the exact residue alignment as per need of the NNBS pocket. Hence, the present work enforce us to optimize those suitable compounds by means of synthesis, if further optimized through biological evaluation may prove to be as effective as novel selective and potent Non- Nucleoside Reverse Transcriptase inhibitors for the treatment of AIDS.

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