THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

In Silico Drug Designing of HIV-1 Reverse Transcriptase Inhibitors

Rajesh Kumar
Ph.D.Scholar, Shri Venkateshwara University, Uttar Pradesh, India
Dr. Kirshan Pal
Department of Biotechnology,Shri Venkateshwara University, Uttar Pradesh, India
Dr. Anju Pal
Scientist, Medicinal and Aromatic Plants,
K.V.K. Haridwar G.B.Pant University of Ag. & Technology, Pantnagar, Uttarakhand, India

Abstract:
The human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrom (AIDS). HIV attacks the human CD4+ T helper cell lymphocytes, which are a key component of the immune system. The antiretroviral therapy is a major HIV therapy, which bases on blocking the HIV replication by inhibiting the enzyme reverse transcriptase. HIV type-1 nucleoside and non-nucleoside reverse transcriptase (NNRTIs) are antiretroviral key drugs, which target protein domains of the reverse transcriptase. With the objective to design ligands for inhibiting a protein domain of the reverse transcriptase, we identified the active site of the reverse transcriptase and evaluated suitable ligands for this receptor site. Therefore, we applied in silico or computer-aided drug design – a very efficient tool in drug discovery. This enabled us to optimize dynamical and statical parameters and to perform homology modeling, model verification, binding site identification, and docking analysis to identify the ligand "lig_raj8_1" as the best inhibitor of the human reverse transcriptase. This ligand exhibits excellent drug likeness properties for in vitro clinical trials and is highly promising as a lead inhibitor of HIV1.

Keywords:*acquired immune-deficiency syndrome, database virtual screening, docking simulation, human immunodeficiency virus type 1 reverse transcriptase, Insilico drug designing, reverse transcriptase, homology modelling, ligand*

1. Background

Acquired immunodeficiency syndrome (AIDS) is a global most serious pandemic public health challenges [1]. The study of Human immunodeficiency virus (HIV) in humans and animal models in last 31 years suggested that it is a causative agent of AIDS [2–3]. The HIV-1 reverse transcriptase (RT) had played a critical role in the life cycle of HIV and it was, consequently, an interesting target for anti- HIV drug therapy [4]. The inhibition of HIV-1 RT is one of the major and potential targets in the treatment of AIDS [5–6]. There was a large number of drugs elicit anti-HIV-1 activity by inhibiting RT which are available in the market [7, 8]. The natural compounds and their derivatives are rich source of biologically active pharmacophores. They have been used as lead molecules for treatment of HIV as well as other vital *diseases* e.g. fungal and bacterial infections, parasitic *diseases*, Cancer and cardiovascular *disease* (CVD), Huntington's *disease* (HD), Alzheimer's and Parkinson's diseases [9–10]. The further modifications in natural compounds (lead molecules for HIV treatment) might improve their anti-HIV ability [11, 12]. There are two classes of HIV-1 RT inhibitors: non-nucleoside reverse transcriptase inhibitors, NNRTIs [13–14] and nucleoside/nucleotide reverse transcriptase inhibitors, NRTIs [15–16]. The NNRTIs are chemically diverse group of therapeutic compounds. The NNRTIs bind noncompetitively with active site residues (unique allosteric hydrophobic binding pocket) on the enzyme leading to a conformational change. The conformational change in the structure of enzyme results in decreased affinity for the substrate [17, 18]. The NNRTIs are highly potent, selective, and specific with very low cellular toxicity [19]. It was suggested that NNRTIs could not interfere with normal function of host DNA polymerase. The factual advantages of NNRTIs were departed due to fractious resistance displayed by most of the approved NNRTIs. The emergence of drug resistance mutations among the different therapies, NNRTIs become less effective. To overcome this problem, novel NNRTIs were searched by modifying the existing drug classes with appropriate pharmacophores. In this in-silico study, we have developed molecule inhibitor using structure based drug designing. The interaction between reverse transcriptase protein and inhibitor were studied by docking methods using Auto Dockvina. The interactions of reverse transcriptase protein ligand conformations, including hydrogen bonds and the bond lengths were analyzed using Accelrys DS Visualizer software. We hope, this Drug will get success to clear out all the phases of clinical trial and it will be effective drug in the cure of AIDS diseases.

2. Methodology

2.1. Sequence Alignment

Protein sequence has been retrieved from protein database available at National Centre of Biotechnology Information (NCBI) cited at HYPERLINK "http://www.ncbi.nlm.nih.gov/"www.ncbi.nlm.nih.gov/

>gi|255311720

PISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPVFAIKKKDSTKWRKLVDFRELNKRT
QDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPLDEDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKI
LEPFRKQNPDIVIYQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTPDKKHQKEPPFLWMGYELHPDKWTVQPIVLPEK
DSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLTEEAELELAENREILKEPVHGVYYDPSKDLIAEIQKQ
GQGQWTYQIYQEPFKNLKTGKYARMRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWWTEYWQATWIP
EWEFVNTPPLVKLWYQLEKEPIVGAETFYVDGAANRETKLGKAGYVTNRGRQKVVTLTDTTNQKTELQAIYLALQDSGLE
VNIVTDSQYALGIIQAQPDQSESELVNQIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSAGIRKVLFLDGID

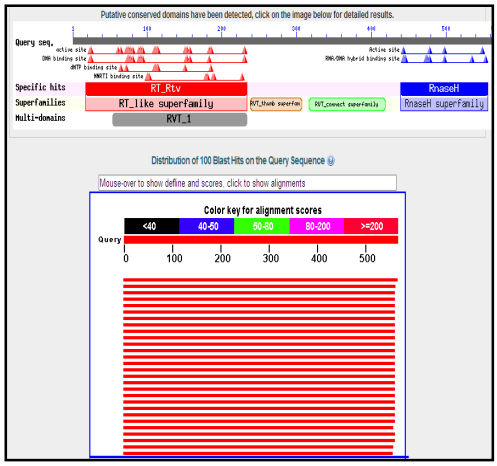

Figure 1: Blast Result

2.2. Protein Homology Modelling

The homology modelling was carried out using the Modeller (HYPERLINK "http://www.salilab.org/modeller/" \n pmc_exthttp://www.salilab.org/modeller/) 9v7 program. The target and the template sequences were aligned using Modeller 9v7, a comparative protein modelling program, was used for homology modelling to generate the 3-D structures of reverse transcriptase protein. Final homology model was selected on the basis of MOLPDF, DOPE score GA341 score.

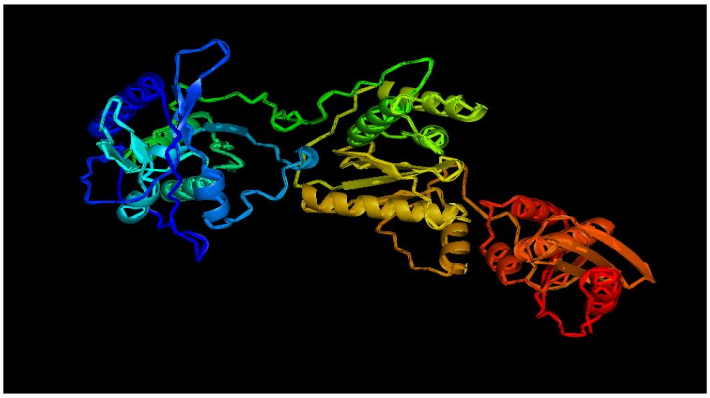

Figure 2: Structure of 2jle A generated by modeller

2.3. Loop Refinement

The alignment between target and template sequence contains gaps. These gaps results for the loops in the 3d structure. So for further refinement of 3d models, loop refinement step was performed by using Modeller (HYPERLINK "http://www.salilab.org/modeller/" \n pmc_exthttp://www.salilab.org/modeller/) 9v7program and the best model was selected on the basis of molpdf value.

2.4. Model Optimization and Evaluation

The protein models generated by homology modeling often produce unfavourable bond lengths, bond angles, torsion angles and bad contacts. Therefore, it was essential to minimize the energy to regularize local bond and angle geometry as well as to remove bad

contacts. Energy minimisation were done with the GROMOS96 (Scott *et al*., 1999) force field by implementation of Swiss-PdbViewer (HYPERLINK "http://www.expasy.org/spdbv" \n pmc_exthttp://www.expasy.org/spdbv). After the optimization procedure, the 3D models of reverse transcriptase protein were verified by using PROCHECK (Laskowski *et al.*, 1993) Program of Structural Analysis and Verification Server (SAVES).

The quality of models was also validated by ProSA (Wiederstein *et al*., 2007) (Sippl, 1993) server (HYPERLINK "https://prosa.services.came.sbg.ac.at/prosa.php" \n pmc_exthttps://prosa.services.came.sbg.ac.at/prosa.php), a web server for Protein

2.5. Structure Analysis

MODEL NO.	CORE	ALLOWED	GENER	DISALL	BAD CONTACTS
MODEL NO.1	87.3	12.7	0.0	0.0	2
MODEL NO.2	88.4	11.1	0.0	0.0	1
MODEL NO.3	89.2	10.8	0.0	0.0	0
MODEL NO.4	**91.6**	**8.4**	**0.0**	**0.0**	**0**
MODEL NO.5	90.8	9.2	0.0	0.0	0

Table 1: SAVS Results after Validation

2.6. Active Site Identification

The active sites were revealed on the basis of previous studies. Pocket is the free space in the structure of the protein where a ligand can be fixed or we can say docked. After running the ligsite we got nine pocket and we selected the pocket number seven because the active site residues Asp 110, Asp 185, Asp 186 are present inside the pocket. Finding pocket is very essential in drug discovery. The pocket is large enough so that the inhibitor can easily get into the pocket and the ligand can easily grow inside the pocket during running the ligbuilder.

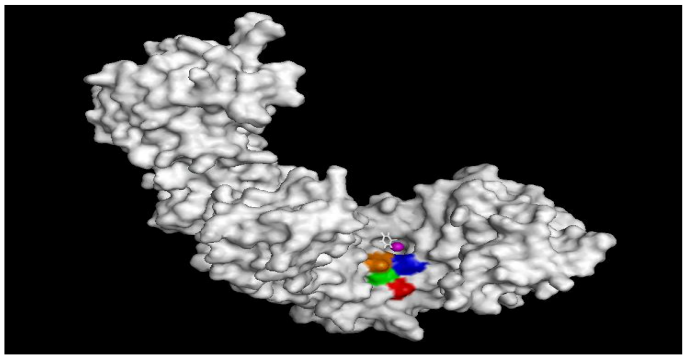

Figure 3: rigid docking of inhibitor and 2jleA

2.7. Rigid Docking

Hex 4.5 was used for the purpose of docking of the lead with the target molecule. The lead compound “reverse transcriptase protein were opened in the Hex (HYPERLINK "http://hex.loria.fr/" \n pmc_exthttp://hex.loria.fr/) and ligand was attached to the residue on the minimum distance position to the active site position .The docking controls were activated with default parameters .The ligand was hence docked to the receptor protein.

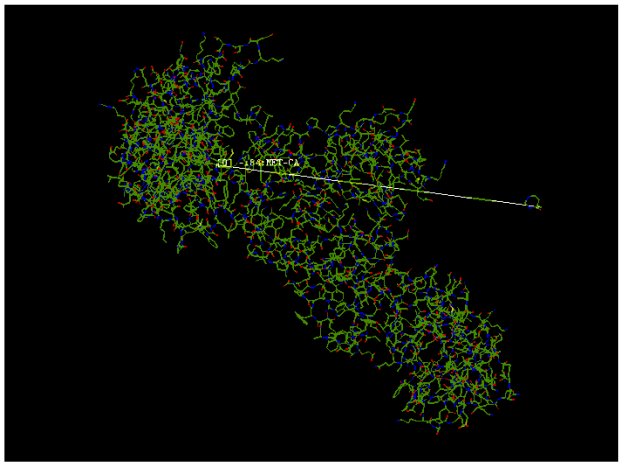

Figure 4: structure of hex result

2.8. Generation and Optimization of Ligand

Ligbuilder was used for the generation of the ligands. The conformation of the pre-placed "seed" ensuring the binding affinity decides the manner that ligands would be grown with Ligbuilder software. Novel ligands had been developed with Ligbuilder (HYPERLINK"http://mdl.ipc.pku.edu.cn/drug_design/work/ligbuilder.html"\n pmc_exthttp://mdl.ipc.pku.edu.cn/drug_design/work/ligbuilder.html) v1.2 software. We developed 200 novel ligands for the ireverse transcriptase protein. Virtual screening, an insilico tool for drug discovery, has been widely used for lead identification in drug discovery programs. Out of 200 novel ligands generated, 10 ligands were selected on the basis of maximum binding affinity measured in kcal/mol. The selected 10 ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" and other drug like properties of valid structures using OSIRIS Property Explorer(HYPERLINK"http://www.organicchemistry.org/prog/peo/"npmc_exthttp://www.organicchemistry.org/prog/peo/), Mol soft: Drug- Likeness and molecular property explorer (HYPERLINK "http://www.molsoft.com/mprop/" \n pmc_exthttp://www.molsoft.com/mprop/). On the basis of binding affinity and drug like properties, one ligand that passed all of the screening tests was taken for further molecular docking study.

2.9. Protein-Ligand Docking

The docking of ligands to the reverse transcriptase protein was performed using Auto Dock Vina software. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the software, polar hydrogen atoms were added to the reverse transcriptase protein and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 20 × 20 × 20 points was used around the catalytic triad to cover the entire enzyme binding site and accommodate ligands to move freely. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interactions of complex reverse transcriptase protein ligand conformations, including hydrogen bonds and the bond lengths were analyzed using Accelrys DS Visualizer software ("HYPERLINK"http://accelrys.com/"http://accelrys.com HYPERLINK """).

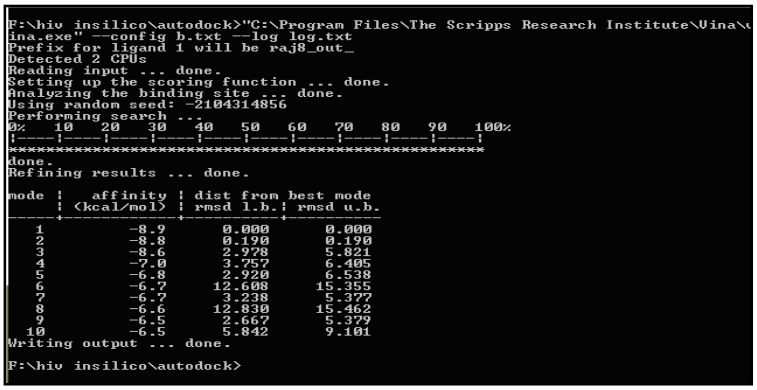

Figure 5: Running Auto dock for 2jleA

3. Discussion

The sequence of the reverse transcriptase protein was retrieved from NCBI Database .The similarity searches was performed by protein- protein blast. The 100% similarity was found in reverse transcriptase protein (2jleA), was used as template for protein homology modelling .The predicted 3D structure of 2jleA protein was generated by Modeller and the structure with the lowest DOPE scores were selected . The alignment between target and template sequence contains gaps .So loop refinement step was also performed by using Modeller. The best models were selected on the basis of molpdf value. The modeller generated models statistically analyzed by structure analysis and validation server (SAVS).The structure submitted were validated and zero bad contacts was used for the further process at lead target prediction .The final protein structures selected after analysis in SAVS. The Rigid docking was performed by HEX in which protein and ligand were opened in docking software and was attached to the residue on the minimum distance position to the active site position .The ligand was hence docked to the receptor protein. The Ligbuilder tool was used for the inhibitor generation. After generation of the lead molecule it was then screened for its activity and its drug likeness. Web based tools like Mo inspiration, and OSIRIS property explorer were used for this purpose. Mo inspiration uses sophiscated Bayesian statistics to compare structures of the representative ligands active on the particular target site. In OSIRIS we draw chemical structures to calculate various drug relevant properties. The binding pattern analyzed by AUTODOCK, is used to predict small molecule to the receptors of known 3D structure. The ligand and target protein were given as input and the flexible docking was performed. The negative and low value of ΔGbind indicates strong favourable bonds between reverse transcriptase protein and the novel ligand indicating that the ligand was in its most favourable conformations. The interactions of complex reverse transcriptase protein-ligand conformations, including hydrogen bonds, sigma and pi bonds were analyzed using Accelrys DS Visualizer software (" HYPERLINK "http://accelrys.com/" \n pmc_exthttp://accelrys.com").

4. Conclusion

This enabled us to optimize dynamical and statical parameters and to perform homology modelling, model verification, binding site identification, and docking analysis to identify the ligand "lig_raj8_1" as the best inhibitor of the human reverse transcriptase. This ligand exhibits excellent drug likeness properties for in vitro clinical trials and is highly promising as a lead inhibitor of HIV1.

5. References

i. R Tian, et al. Virologica Sinica. 2007;22:476. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449371/" \l "R01"Ref list]
ii. SJ O'Brien, JJ Goedert. Curr Opin Immunol. 1996;8:613. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/8902385" \n pmc_extPubMed]
iii. TR Frieden, et al. MMWR Morb Mortal Wkly Rep. 2011;60:689. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/21637182" \n pmc_extPubMed]
iv. H Jonckheere. Med Res Rev. 2000;20:129. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/10723025" \n pmc_extPubMed]
v. A Jacobo-Molina, E Arnold. Biochemistry. 1991;30:6351. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/1711368" \n pmc_extPubMed]
vi. J Wang, et al. J Am Chem Soc. 2001;123:5221. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/11457384" \n pmc_extPubMed]
vii. R Pauwels, et al. Curr Opin Pharmacol. 2004;4:437. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/15351347" \n pmc_extPubMed]
viii. E De Clercq. Nature Rev Drug Discov. 2007;6:1001. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/18049474" \n pmc_extPubMed]
ix. RD Yedery, KV Reddy. Eur J Contracept Reprod Health Care. 2005;10:32. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/16036297" \n pmc_extPubMed]
x. B Zhao. Neurochem Res. 2009;34:630. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/19125328" \n pmc_extPubMed]
xi. D Yu, et al. Med Res Rev. 2007;27:108. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/16888749" \n pmc_extPubMed]
xii. SK Chauthe, et al. Bioorg Med Chem. 2010;18:2029. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/20137956" \n pmc_extPubMed]
xiii. RA Koup, et al. J Infect Dis. 1991;163:966. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/1708400" \n pmc_extPubMed]
xiv. OS Pedersen, EB Pedersen. Antiviral Chem Chemother. 1999;10:285. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/10628805" \n pmc_extPubMed]
xv. H Mitsuya, et al. Proc Natl Acad Sci USA. 1985;82:7096. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pmc/articles/PMC391317/"PMC free article] [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/2413459" \n pmc_extPubMed]
xvi. ED Clercq. Med Res Rev. 1993;13:229. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/7683360" \n pmc_extPubMed]
xvii. R Esnouf, et al. Nat Structural Biology. 1995;2:303. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/7540935" \n pmc_extPubMed]
xviii. H David, H Bill. Rev Med Virol. 2002;12:31. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/11787082" \n pmc_extPubMed]
xix. FW Bell, et al. J Med Chem. 1995;38:4929. [HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/8523406" \n pmc_extPubMed]