Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

DESIGNING AND COMPUTATIONAL STUDY OF SOME NOVEL LAMIVUDINE ANALOGUES AS POTENTIAL HIV-1 REVERSE TRANSCRIPTASE INHIBITORS: ANALYSIS OF THE BINDING INTERACTIONS USING QSAR, MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATION STUDY

SOUMENDRANATH BHAKAT[1], BHAVANIPRASAD VIPPERLA[2*]

[1]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835215, [2*]Corresponding Author, Bioinformatics Division, VIT University; Vellore; Tamilnadu 632014, India. Email: bhavaniprasad_Vipperla@hotmail.com

Received: 25 Nov 2012, Revised and Accepted: 15 Dec 2012

ABSTRACT

Reverse-transcriptase inhibitors (RTIs) are a class of antiretroviral drug used to treat HIV infection. Lamivudine is one of the prominent reverse transcriptase inhibitors. Recent clinical studies have shown that, due to the prominent usage the resistance has increased against lamivudine, hence we modeled three new analogs of lamivudine. The drug likeness and molecular properties of the analogs are analyzed and further used in docking studies. The structure of a catalytic complex of HIV-1 reverse transcriptase is selected for performing the docking studies with the new analogs. Prior to docking, the protein structure is inserted in water and molecular dynamics simulations were performed. The energy minimized structure is obtained from the MD simulations and used in docking studies. The new analogues showed good interactions when compared with the lamivudine prototype. Our multidirectional approach indicates good ligand efficacy in addition to stable binding affinities to HIV-1 reverse transcriptase, and should be potent candidates for HIV-1 reverse transcriptase inhibition.

Keywords: Lamivudine; HIV-1 reverse transcriptase, Structure Activity Relationship (SAR); Docking; Molecular Dynamics

INTRODUCTION

Human immunodeficiency virus (HIV) causes acquired immune deficiency syndrome (AIDS). The reverse transcriptase of HIV-1 is a crucial target of antiviral therapy in the treatment of AIDS. HIV reverse transcriptase is a dimer having two chains, a 64-KD (p66) subunit and a 51-KD (p51) subunit derived from p66 by proteolytic cleavage. The two chains have similar four domain structure and p66 also has a COOH-terminal RNaseH (1, 2).

Reverse-transcriptase inhibitors (RTIs) are a class of antiretroviral drug used to treat HIV infection. 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. RTIs block reverse transcriptase's enzymatic function and prevent completion of synthesis of the double-stranded viral DNA, thus preventing HIV from multiplying. One of the prominent and well tolerated RTI is lamivudine [3-5]. The emergence of resistance in patients against RTI's is a major limitation of antiviral therapy.

Lamivudine (2', 3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI) has been effectively used to treat HIV and chronic Hepatitis B. It has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. Lamivudine which is a Cytidine analogue inhibit both types (1 and 2) of HIV reverse transcriptase. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. Lamivudine inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Lamivudine possess an advantage over other anti HIV agents as it showed no evidence of carcinogenicity or mutagenicity in *in vivo* studies in mice and rats at doses from 10 to 58 times those used in humans. But due to growing resistance of lamivudine it becomes necessary to design some lamivudine derivatives which can act on HIV reverse transcriptase as of the prototype lamivudine.

In this study modern computational tools are used to design and analysis some lamivudine derivatives which can ultimately aimed to overcome the resistance developed by lamivudine.

METHODS

Protein preparation

The 3D structure of our protein is available in Protein Data Bank. The structure of a catalytic complex of HIV-1 reverse transcriptase (PDB ID 1RTD) is selected for performing the docking studies [6]. The selected protein 3D structure is having 8 chains, hence only chain A was isolated from the structure and used for docking by using SPDB Viewer. The possible conformation of the refined protein was obtained using procheck analysis, visualized with the aid of Ramachandran plot by checking the dihedral Phi and Psi angles of amino acid residues. The energy minimization and stabilization are carried on the protein structure using molecular dynamics simulations. The active site of the protein is the binding site or usually a pocket at the surface of the protein that contains residues responsible for substrate specificity which often act as proton donors or acceptors. Identification and characterization of binding site is the key step in structure based drug design. The binding site has been identified by computational and literature reports. The active site region of the protein is identified by CASTp server [7]. These servers analytically furnish the area and the volume at the probable active site of each pocket to envisage the binding site.

Designing of Lamivudine derivatives by the Structural-Activity Relationship (SAR) and pharmacophore study

Potent derivatives of lamivudine has been designed by addition of side chains in place of position –R of lamivudine as depicted in Figure.1

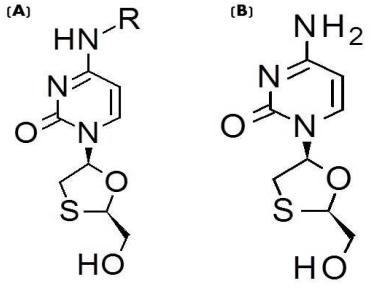

Fig. 1(A): Lamivudine core with –R group at terminal nitrogen (B) Structure of Lamivudine.

Three molecules LMA1, LMA2, LMA3 have been designed by substituting the –R position of lamivudine (In case of lamivudine –R is substituted with –H). The drug likeness score and molecular property of these 3 molecules have been predicted using internet based JAVA dependent MolSoft Drug-Likeness and molecular property prediction tool (http://www.molsoft.com/mprop/). Molecular properties are essential for every stages of drug development from design to synthesis hence predictions of these parameters are important for drug development point of view. It has been observed that all 3 compounds show greater drug likeness score than that of prototype lamivudine which leads us to believe that some of these compounds will show greater or somehow similar activity that of the prototype, lamivudine, which make these 3 molecules possible drug candidates to treat lamivudine resistant HIV (Table I).Topological Polar Surface Area (TPSA)[8] of all the molecules have been determined using Field Align software (http://www.cresset-group.com/products/torch/) developed by Cresset as TPSA which has been used as a predictor for BBB penetration [9-10]. To confirm the drug like properties of these designed lamivudine derivatives molecular docking study of all the designed molecules have performed taking catalytic complex of HIV-1 reverse transcriptase as a target.

Proposed synthesis scheme:

In many cases drug development is hampered because of the suitable synthesis pathway hence synthesis of LMA1, LMA2 and LMA3 has been shown below taking lamivudine as starting element in Figure.2. Preparation of these compounds will follow simple nucleophilic substitution reaction the yield can be varied depending on temperature, solvent and other parameters taking reactants as constant.

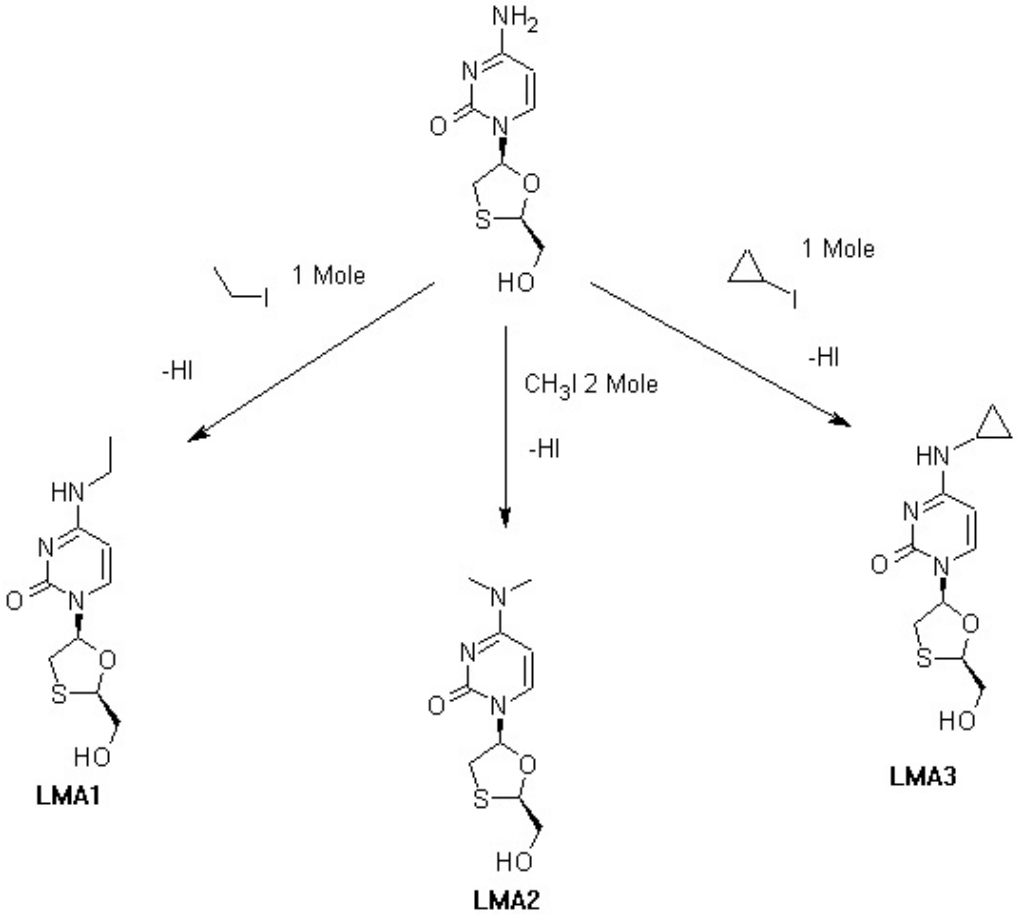

Fig. 2: Proposed Synthesis scheme for synthesis of Designed Molecules

ADME/T & Drug Likeliness prediction

The ADME/T properties of a drug, together with its pharmacological properties are conventionally viewed as part of drug development. The ligands identified were subjected to predict the pharmacokinetic properties using pre-ADMET online server. Structures with unfavorable absorption, distribution, metabolism and elimination have been identified as the major cause of failure of candidate molecules in drug development. So there is an early prediction of ADME properties, with the objective of increasing the success rate of compounds reaching further stages of the development [11-16].

Molecular Dynamics Simulation

GROMACS is a versatile package to perform molecular dynamics, that is simulate the Newtonian equations of motion for systems with hundreds to millions of particles. It is primarily designed for biochemical molecules like proteins, lipids and nucleic acids that have a lot of complicated bonded interactions. This package is used for the stabilization and energy minimization of HIV-1 reverse transcriptase. The OPLS forcefield for all atoms (OPLS-AA) was preferred for the simulation. Water molecules were represented using a simple point charge (SPC216) model. Eight Cl– counter-ions were added by replacing water molecules to ensure the overall charge neutrality of the simulated system. Energy minimization process, position restraint procedure was performed in association with NVT and NPT ensembles [17-20].

An NVT ensemble was adopted at constant temperature of 300 K and with a coupling constant of 0.1ps with time duration of 100ps. After stabilization of temperature an NPT ensemble was performed. In this phase a constant pressure of 1 bar was employed with a coupling constant of 5.0 ps with time duration of 1ns. NPT ensemble was finished after pressure stabilization. The coupling scheme of Berendsen was employed in both of NVT and NPT ensembles The particle mesh Ewald (PME) method for long-range electrostatics, a 14A˚ cutoff for van der Walls interactions, a 12A˚ cutoff for Coulomb interaction with updates every 10 steps, and the Lincs algorithm for

covalent bond constraints were used [21, 22]. A final MD run is performed for 5ns for the HIV-1 reverse transcriptase.

Molecular Docking Analysis

Autodock is used for the Molecular docking studies of the ligands with the receptor protein. Autodock uses binding free energy evaluation to find the best binding mode. Autodock energy values were calculated by the characterization of intermolecular energy (consist of van der Walls energy, hydrogen bonding energy, desolvation energy, and electrostatic energy), internal energy of ligand, and torsional free energy. The docking energy is obtained from the van der Walls energy and hydrogen bonding energy together, while the binding energy is built up from van der Walls energy and desolvation energy. The binding strength and the location of ligand in most of the cases can be decided by the electrostatic interaction between ligands and receptor. The hydrophobic interaction obtained from the docking can affect the agonistic activity to a larger extent [23].

Molecular Imaging & MD Analysis

All the visualizations were carried out using Pymol, VMD tools and graphs were plotted using XMgrace Program [24-26]. The trajectories were analyzed using the inbuilt tool in the GROMACS distribution.

RESULTS AND DISCUSSION

The 3D structure for our protein is available in Protein Data Bank. The structure of a catalytic complex of HIV-1 reverse transcriptase (PDB ID 1RTD) is selected for performing the docking studies. The MD simulations were performed on the obtained HIV-1 reverse transcriptase structure. Initially the system is energy minimized for a period of 1 ns and a position restraints simulation is performed using steepest decent integrator on a stepwise manner. The RMSD for the protein backbone was about 0.3nm after 3ns of MD simulation. The RMSD varied in the first nanosecond time frame but it was quite stable after reaching 1ns of simulation. The average temperature in 3 ns of MD simulation was maintained at 300 K for the investigated system. Therefore, the extracted equilibrium structure at 300 K belonging to the HIV-1 reverse transcriptase was obtained under stable temperature conditions. These facts show that energy conservation was satisfied in MD simulation. After structural refinement of HIV-1 reverse transcriptase model by MD simulation, the quality of the backbone geometry of the developed model was validated (Figure.3). The protein folding process shows good reliability as the protein structure is quite stable during the period of simulation. The average structure was obtained from the MD simulations and using the active sites obtained from CASTp, the ligands are docked flexibly into the HIV-1 reverse transcriptase.

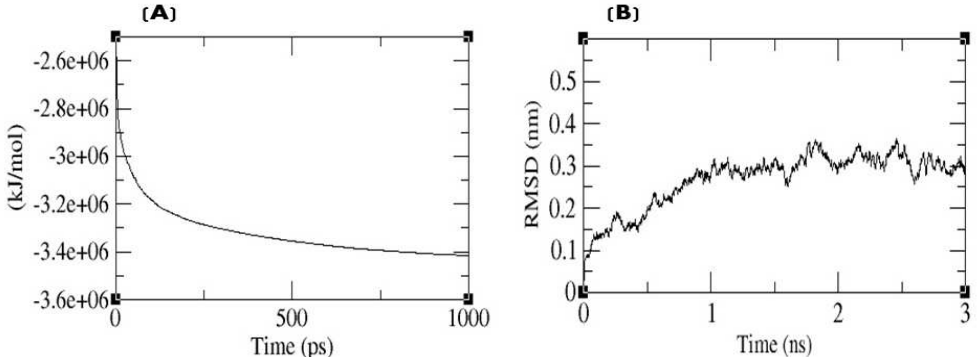

Fig. 3: (A).The variation of the total energy in investigated system during MD simulation (B).Time dependence of the RMSDs (Å) of 1rtd for the backbone atoms in the 3 ns MD simulation

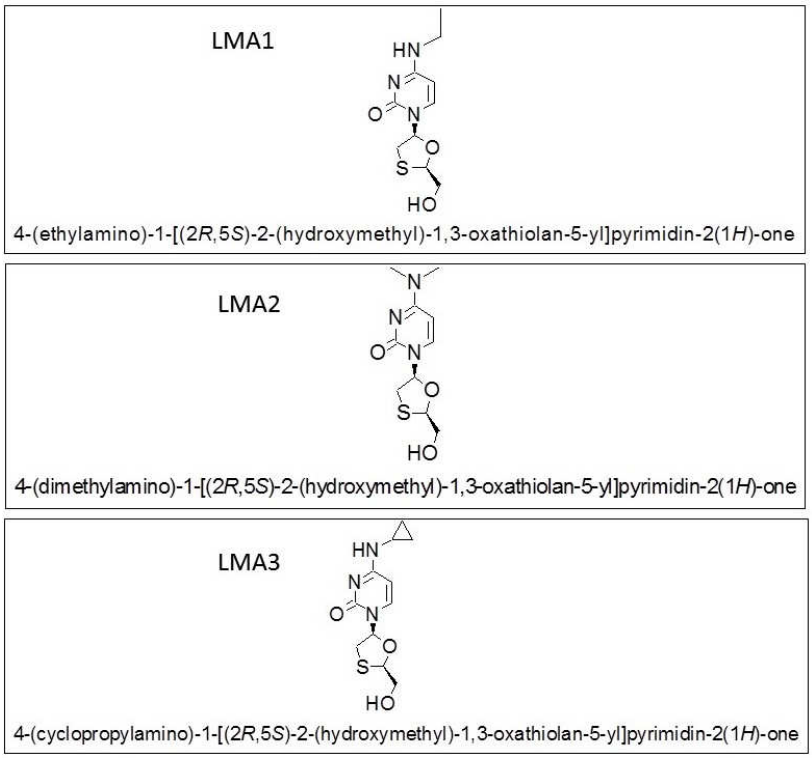

Fig. 4: The structure of all three designed lamivudine derivatives

The drug-likeness and the molecular properties of the designed ligands (Figure.4) are analyzed using Molsoft program and the designed ligands presented better drug-likeness values than the lamivudine prototype. In this study we also verified whether the designed ligands are satisfying the Lipinski rule of five, which indicates if a chemical compound could be an orally active drug in humans. Our results showed that all the designed ligands have fulfilled this rule which can be seen in Table I and II. Field point display of all designed molecules have been viewed using Cresset's Field Align software as these parameters are important prerequisites of computer aided drug design (Figure.5).

Table I: Molecular and Drug likeness Property of all the designed molecules taking lamivudine as prototype.

Molecule ID	Drug Likeness Model Score**	MolLogP**	MolLogS(mg./L)**	TPSA***	Number of Stereo Centre**	Rule of Five violation**
Lamivudine Prototype	1.05	-1.26	2289.86	88.2	2	0
LMA1	1.16	0.35	1955.97	74.2	2	0
LMA2	1.23	-0.71	3975.81	65.4	2	0
LMA3	1.30	0.17	1885.47	74.2	2	0

** calculated using MolSoft Drug-Likeness and molecular property prediction tool

*** calculated using Cresset's Field Align software

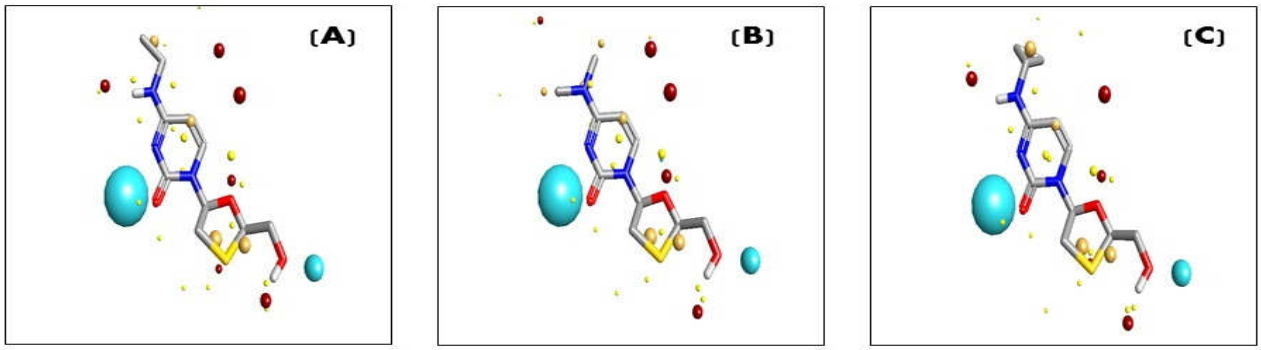

Fig. 5: Field point display of (A) LMA1, (B) LMA2, (C) LMA3.

Moreover, we used PreADMET server to study the absorption, distribution, metabolism, excretion and toxicological properties of all three designed compounds comparing them with the lamivudine prototype. Interestingly, all three designed ligands presented a low *in-silico* toxicity risk profiles, good intestinal absorption and blood-brain barrier penetration, similar to the prototype which is depicted in Table II.

Toxicity of all designed molecules have been predicted using PreADMET server and compared with the prototype drug lamivudine (Table III). It is observed that in Ames test all designed molecules show mutagenic character including the prototype drug lamivudine but lamivudine shows positive result in carcinogenicity prediction in mouse and negative result in carcinogenicity prediction in rat but the designed molecule LMA1 shows negative result in both of the above parameters which is also true with the designed molecule LMA3 which predicts that both LMA1 and LMA3 will have lower carcinogenic risk than that of prototype lamivudine. But as lamivudine showed no evidence of carcinogenicity or mutagenicity in *in vivo* studies in mice and rats at doses from 10 to 58 times those used in humans hence it can be predicted that both LMA1 and LMA3 will also show no evidence of carcinogenicity and mutagenicity in in vivo studies in higher doses than that of lamivudine.

Table II: ADMET properties of the Ligand molecules obtained from PreADMET server.

S. No.	Ligand Name	Donor HB	Acceptor HB	Mol. Wt (g/mol)	Blood-Brain Barrier penetration	%human intestinal absorption	Plasma protein binding
1	Lamivudine Prototype	3	5	229.0	0.087	75.92	6.53
2	LMA1	2	5	257.0	0.04	85.78	16.75
3	LMA2	1	5	257.0	0.01	90.96	18.51
4	LMA3	2	5	269.0	0.06	87.63	34.18

*permissible ranges are as follows: mol wt.: (130–725); donor hb: (0.0–6.0); accept hb: (2.0–20.0); QPlogPo/w: (-2.0 to 6.5); QPlogBB: (<0.1 low, 2-0.1 medium, >2.0 high absorption to cns); %human intestinal absorption: 70-100 % well absorbed, 20-70% moderately absorbed, 0-20 poorly absorbed, %plasma protein binding: >90 strongly bound, <90% weakly bound.

Table III: Predicted toxicity of the molecules using PreADMET server

Molecule ID	Ames Test	Carcinogenicity(Mouse)	Carcinogenicity(Rat)
Prototype Lamivudine	Mutagen	Positive	Negative
LMA1	Mutagen	Negative	Negative
LMA2	Mutagen	Positive	Negative
LMA3	Mutagen	Negative	Negative

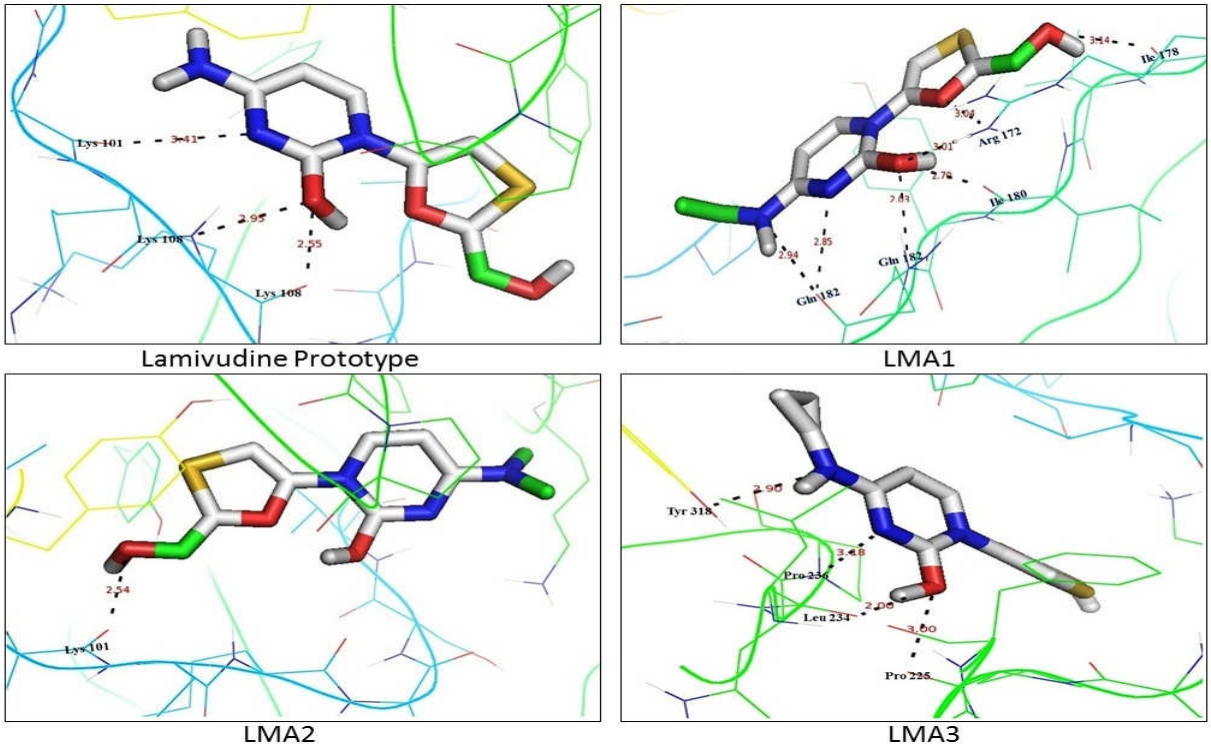

Fig. 6: The H-Bond interactions spotted in black dotted lines and their bond lengths of each ligand with HIV-1 reverse transcriptase visualized in Pymol.

The designed ligands were subjected to molecular docking studies using Autodock software. The docked complex is visualized using Pymol and the hydrogen bond interactions and their bond lengths are calculated as shown in Figure.6 and Table-IV.

The obtained results of the molecular docking of HIV-1 reverse transcriptase with the four ligands showed good binding energies and inhibition constants (Table IV). Hydrophobic interactions often provide a very important contribution to the binding affinity for ligands. All the four ligands have shown significant number of Hydrogen bonds between the receptor and the ligands. In the case of lamivudine prototype ligand all the H-bonds are formed with Lys amino acid, two of them accounting for Lys108 and one with Lys101.

Among the three designed analogues LMA1 showed the best binding energy and also highest number of H-bond interactions. In the case of LMA1, seven H-bond interactions have been obtained with Gln182 accounting for the H-bonds, Arg172 accounting for two H-bonds and Ile178, 180 accounting for one each. In case of LMA2, a lone H-bond is formed with Lys101 amino acid. In case of LMA3, four H-bonds have been formed with Pro225, Pro236, Leu234, Tyr318 accounting for one each H-bond. Docking analysis showed that hydrogen bonding govern the binding affinities between the ligand compounds and HIV-1 reverse transcriptase (27, 28). Based on the binding energies, H-bond interactions and Inhibition constant values LMA1 is considered the best derivative of lamivudine prototype. The general binding pose is depicted in Figure. 7.

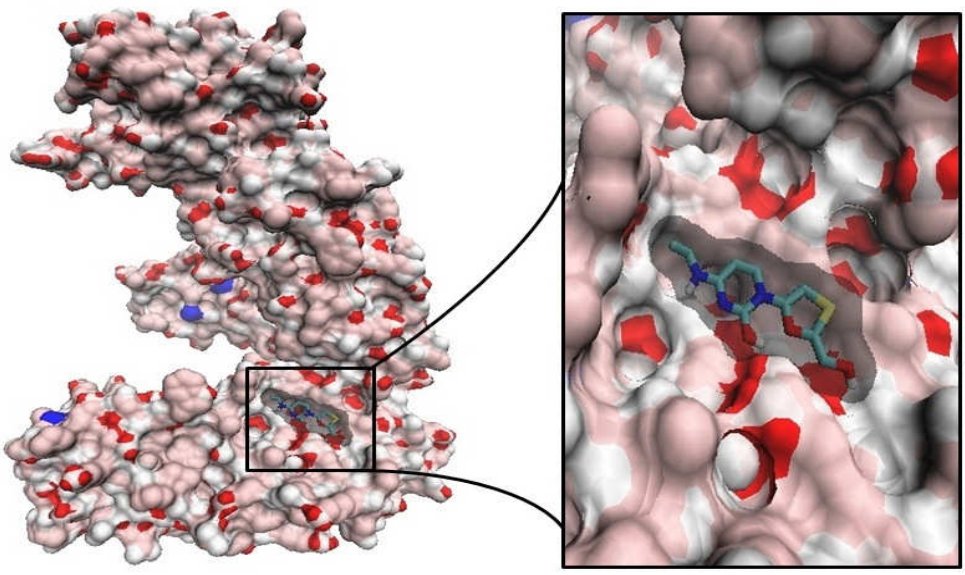

Fig. 7: Molecular surface of HIV-1 reverse transcriptase showing the ligand in binding cavity.

Table IV: H-bond Interactions and Bond length obtained for Lead ligand with HIV-1 reverse transcriptase.

S. No.	Name of the Ligand	Binding energy (K.Cal/Mol)	Inhibition constant (298.15 K) Ki (μM)	H-Bond interactions	Bond length (A°)
1	Lamivudine Prototype	-6.61	14.2	(Lys 103) O – OH	2.5
				(Lys 103) HN – O	2.9
				(Lys 101) O – N	3.4
2	LMA1	-6.72	30.28	(Gln 182) HN – O	2.0
				(Ile 180) O – OH	2.7
				(Gln 182) O – NH	2.8
				(Gln 182) O -- OH	2.9
				(Arg 172) NH -- O	3.0
				(Arg 172) NH -- OH	3.0
				(Ile 178) O -- OH	3.1
3	LMA2	-6.68	12.79	(Lys 101) O – OH	2.5
4	LMA3	-6.64	13.65	(Leu 234) O – OH	2.0
				(Tyr 318) OH – N	2.9
				(Pro 225) O – NH	3.0
				(Pro 236) N-- OH	3.1

CONCLUSION

Lamivudine is a potent nucleoside analog reverse transcriptase inhibitor which is being effectively used to treat HIV. Using an *in silico* structure-based approach, we have designed potent derivatives by addition of side chains in place of position –R of lamivudine. The drug likeness score and molecular property of these 3 molecules have been studied and used for the docking studies with HIV-1 reverse transcriptase. The ideal structure was retrieved for HIV-1 reverse transcriptase and the structure was stabilized and energy minimized by performing molecular dynamics simulations. The stabilized structure obtained from MD simulations was used in molecular docking studies to explore the potential binding mechanisms of lamivudine inhibitors. Docking studies show that the lamivudine analogs were having better binding orientations when compared to the Lamivudine prototype, LMA1 showed the best inhibitory activity of all and even LMA3 showed better activity than that of prototype. The above results suggest that the designed lamivudine analogs are a potent inhibitor of HIV-1 reverse transcriptase, and are a suitable lead compounds for the development of new drugs against HIV-1 reverse transcriptase.

REFERENCES

1. Huifang Huang, Rajiv Chopra, Gregory L. Verdine, Stephen C. Harrison. Structure of a Covalently Trapped Catalytic Complex of HIV-1 Reverse Transcriptase: Implications for Drug Resistance. Science-1998; 282:1669.
2. Yung-chi Cheng, Ginger E. Dutschman, Kenneth F. Bastow, M.G.Sarngadharan, Robert Y. C. Ting. Human Immunodeficiency Virus reverse transcriptase. General properties and its interactions with nucleoside triphosphate analogs. The journal of biological chemistry. Vol. 262, No. 5, pp. 2187-2189 1987.
3. Zixin Hu and Daniel R. Kuritzkes, Interaction of Reverse Transcriptase (RT) Mutations Conferring Resistance to Lamivudine and Etravirine: Effects on Fitness and RT Activity of Human Immunodeficiency Virus Type 1. Journal of virology; Nov. 2011; p. 11309–11314.
4. J. W. Erickson and S. K. Burt. Structural Mechanisms of HIV Drug Resistance Annual Rev. Pharmacol. Toxicol 1996. 36, 545.
5. Diana Finzi, Monika Hermankova, Theodore Pierson, Lucy M. Carruth, Christopher Buck, Richard E. Chaisson et al. Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy. SCIENCE; VOL. 278; 1997.
6. Huang H, R Chopra, GL Verdine, SC Harrison. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science. 1998; 282(5394):1669-75.
7. Joe Dundas, Ouyang Z, Tseng J, Binkowski A, Turpaz Y, Liang J. CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic Acid Research, 2006. 34:W116-W118.
8. Peter Ertl, Bernhard Rohde, Paul Selzer. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. Journal of Medicinal Chemistry 43, 3714-3717, 2000.
9. D.M. Garrido, Corbett DF, Dwornik KA, Goetz AS, Littleton TR, McKeown SC et al. Synthesis and activity of small molecule GPR40 agonists. Bioorg. Med. Chem. Lett. 16 (2006) 1840–1845.
10. S.C.McKeown, Corbett DF, Goetz AS, Littleton TR, Bigham E, Briscoe CP et al. Solid phase synthesis and SAR of small molecule agonists for the GPR40 receptor. Bioorg. Med. Chem. Lett. 2007 1584–1589.
11. S.K.Lee, G.S.Chang, I.H.Lee, J.E.Chung, K.Y.Sung, K.T.No, "The PreADME: PC-BASED PROGRAM FOR BATCH PREDICTION OF ADME PROPERTIES", EuroQSAR 2004, 2004, 9.5-10, Istanbul, Turkey.
12. S.K.Lee, I.H.Lee, H.J.Kim, G.S.Chang, J.E.Chung, K.T.No, "The PreADME Approach: Web-based program for rapid prediction of physico-chemical, drug absorption and drug-like properties", EuroQSAR 2002 Designing Drugs and Crop Protectants: processes, problems and solutions, 2003, pp. 418-420. Blackwell Publishing, Massachusetts, USA. .
13. Veber. DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 2002, 45, 2615.
14. Irvine J. D, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, Grove JR. MDCK cells: A tool for membrane permeability screening. J. Pharm. Sci. 1999, 88, 28.
15. Lipinski, C. A, Lombardo, F.; Dominy, B. W. and Feeney, P J. Adv. Drug Deliv. Rev. 1997, 23, 3.2.
16. von Grotthuss, M., et al. Ligand-Info, searching for similar small compounds using index profiles. Bioinformatics 19: 1041-1042, 2003.
17. Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: a message-passing parallel molecular dynamics implementation. Comp. Phys. Comm. 91: 43–56, 1995.
18. Berendsen HJC, Grigera JR, Straatsma TP. 1997. The missing term in effective pair potentials. J. Phys. Chem. 91: 6269–6271.
19. van Gunsteren WF, Billeter SR, Eising AA, Huenenberger PH, Krueger P, Mark AE, Scott WRP, Tironi IG. 1996. Bimolecular simulation: GROMOS96 manual and user guide; BIOMOS: Zurich, Groningen.
20. Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. 1996. LINCS: a linear constraint solver for molecular simulations. J. Comp. Chem. 18: 1463–1472.
21. Darden T, York D, Pedersen L. 1993. Particle mesh Ewald: an N_log (N) method for Ewald sums in large systems. J. Chem. Phys. 98: 10089–10092.
22. Kholmurodov K, Smith W, Yasuoka K, Darden T, Ebisuzaki T. 2000. A smooth-particle mesh Ewald method for DL_POLY molecular dynamics simulation package on the Fujitsu VPP700. J. Comput. Chem. 21: 1187–1191.
23. Morris, G. M., Goodsell, D. S., Halliday, R.S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. (1998), Automated Docking Using a

Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. J. Computational Chemistry, 19: 1639-1662.
24. DeLano WL. Pymol. Schrodinger LLC, 2009, Portland, OR.
25. Humphrey, W., Dalke, A. & Schulten, K. (1996). VMD: visual molecular dynamics. J. Mol. Graphics, 14, 33–38.
26. Turner, P.J. XMGRACE, Version 5.1.19. Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton, OR. 2005.
27. K. Anbarasu, s. Jayanthi. In silico studies on *Neisseria meningitidis* dihydropteroate synthase and pterin based inhibitors for meningitis. IJPPS; 2012; vol 4, issue 3.
28. Juni ekowati, Bimo A. Tejo, Shigeru Sasaki, Kimio Highasiyama, Sukardiman, Siswandono. Structure modification of ethyl *p*-methoxycinnamate and their bioassay as chemopreventive agent against mice's fibrosarcoma. IJPPS; Vol 4, suppl 3, 2012.