IJPSR (2019), Volume 10, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 December 2018; received in revised form, 22 March 2019; accepted, 28 March 2019; published 01 September 2019

MOLECULAR DOCKING STUDIES OF A FEW NOVEL PYRIMIDINE DERIVATIVES AS REVERSE TRANSCRIPTASE HIV-1 INHIBITORS

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Keywords:

Pyrimidine, HIV-1, AIDS, Docking, Binding energy, *In-silico*

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ABSTRACT: Anti-HIV drug discovery has been increasingly focusing on HIV-1- reverse transcriptase as a potential therapeutic target. Pyrimidine-based derivatives are studied for their HIV-1 reverse transcriptase inhibition activities using *in-silico* techniques. The library of 19 pyrimidine-based derivatives designed using computer-based molecule designed software and docked with HIV-1 reverse transcriptase enzyme using AutoDock. The reported binding energies for the library of molecules are ranging from -9.43Kcal/mole to -13.19 Kcal/mole, with the precision of ±1 Kcal/mole. It is concluded that the presence of $–CH_2OH$ at R1 position suitable for hydrogen bonding and $–C_6H_5$ group enhance the negative binding energy (ΔG Kcal/mole).

INTRODUCTION: Human Immunodeficiency Virus type-1 (HIV-1) is a member of the retrovirus family. It is a slow, progressive, degenerative and induces a life-threatening disease for the human immune system and is called the acquired immunodeficiency syndrome (AIDS) [1, 2]. Nowadays acquired immunodeficiency syndrome (AIDS) is the fastest growing cause of death in human, especially in the case of young people. Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) converts single-stranded viral RNA into a double-stranded proviral DNA. This is a necessary step in the HIV-1 replication cycle. There are many enzymes in the HIV-1 life cycle that can serve as a novel drug target [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(9).4201-06

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(9).4201-06

RT inhibitors have been the primary therapeutic strategies in AIDS patient treatment; therefore the inhibition of reverse transcriptase (RT) has been one of the important targets in inhibiting the replication of HIV-1 [4, 5]. Inhibitor designing has proved to be a success story from decades and many inhibitors are designed for specific enzymes of HIV-1.

For the treatment of HIV-1, two types of RT inhibitors are used. The first one is nucleoside RT inhibitors (NRTIs), which bind directly to the active site of RT polymerase and terminate DNA synthesis after incorporation into the newly synthesized DNA. The second one is non-nucleoside RT inhibitors (NNRTIs) that bind to the hydrophobic pocket within the polymerase domain of the p66 RT subunit, resulting in inhibition of RT activity [6, 7]. Pyrimidine class of compounds is not exhaustively checked for its inhibition activity against HIV-1 RT. Hence, Pyrimidine class of compounds were designed and checked for their activity against HIV-1 RT. Also, it was aimed to understand which pharmacophore at the proper

location in Pyrimidine can provide a higher inhibition activity.

MATERIALS AND METHODS:

Preparation of Receptor Enzyme: The 3D structure of the target enzyme is most often derived from X-ray crystallography. An X-ray crystal structure of HIV-1 reverse transcriptase with non-nucleoside inhibitors is obtained from the Brookhaven protein data bank [8]. The assigned PDB code is 1VRT. This crystal structure is having Nevirapine, which is the first nonnucleoside reverse transcriptase inhibitor (NNRTI) approved by the American food and drug administration (FDA) [9, 10]. **Table 1** shows the structural details of HIV-1 reverse transcriptase (1VRT) and **Table 2** shows the details of ligand and prosthetic groups present.

TABLE 1: STRUCTURAL DETAILS OF 1VRT [11]

	Chain A	Chain B
Description	HIV 1 Reverse Transcriptase	HIV 1 Reverse Transcriptase
Fragment	Null	Null
Mutation	Null	Null
Formula Weight	64601.0	51434.9
Source Method	Human	Human
Entity Name	HIV-1 RT	HIV-1 RT
RCSB_NAME	HIV-1 Reverse Transcriptase (Subunit P66)	HIV-1 Reverse Transcriptase (Subunit P51)

TABLE 2: LIGAND AND PROSTHETIC GROUPS IN HIV-1 REVERSE TRANSCRIPTASE COMPLEX [11]

ID	Name	Chemical Formula	Weight
NEV	Nevirapine	$C_{15}H_{14}N_4O$	266.3
CYA	Two Oxygen Atoms Bound to SH of Cysteine	O_2	31.9
MG	Magnesium Ion	Mg^{2+}	24.3

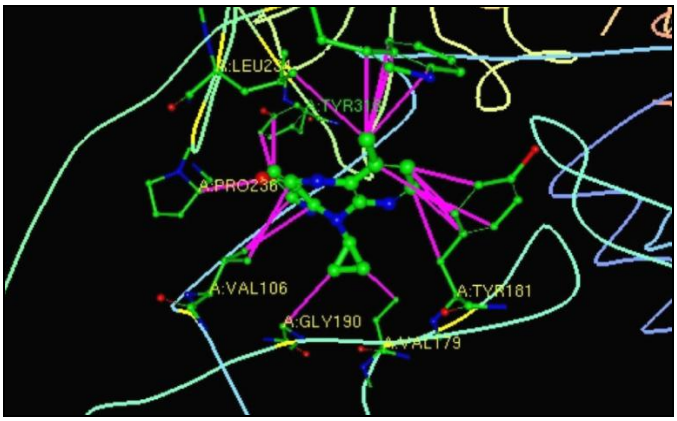


FIG. 1: HYDROPHOBIC INTERACTION BETWEEN NEVIRAPINE WITH 1VRT [12]

The Ligand substrate interaction study reveals that the 1VRT in complex with the original ligand (Nevirapine) shows hydrophobic interaction with amino acids namely 190-GLY, 234-LEU, 236-PRO, 229-TRP, 181-TYR, 318-TYR 106-VAL, and 179-VAL. It is reported in **Fig. 1**. However, it does not show any hydrophilic interaction [12].

In the docking process, the small molecules docked into the binding site at different conformations. The individual poses of each molecule within the binding site ranked and then singled bound conformation selected. The binding site was prepared for docking in such a way that all heteroatom, *i.e.*, non-receptor atoms such as water, ions, etc. were removed. This binding site used for further docking process.

Ligand Design: It has been well established that the heterocyclic rings will play a major role in HIV-1 inhibitors due to their conformational restriction as well as their ability to fill hydrophobic pockets and provide functionality for hydrogen bonding interactions [13]. The Pyrimidine based molecule is the important class of compound. The general structure of the pyrimidine-based lead compound is shown in **Fig. 2**.

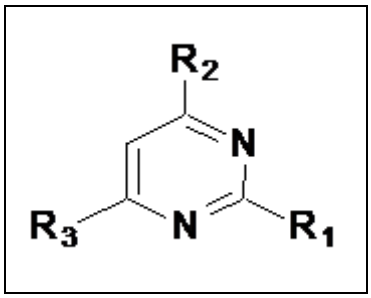


FIG. 2: LEAD COMPOUND: SUBSTITUTED PYRIMIDINE

Design of 2D and 3D Structure of the Ligand: Many computer algorithms are available to draw the 2D structure of compounds and its conversion into three-dimensional structures. The 3D structure of the lead compound must be at global minima and optimized to improve binding to the active site. Optimization is done by the computational method. Lead optimization process involves the systematic modification of the structure of the lead compound by substituting pharmacophore.

The entire pyrimidine-based molecules were designed using Chem Office [14] by substituting different pharmacophore at various positions in the

lead compound. The derivatives designed by substituting at R_1, R_2 and R_3 positions in basic Pyrimidine structure. The major pharmacophores selected for substitution were –SH, -SCH_3, -CH_3, -$C6H_5$, -NH_2, -COC_6H_5, -$CH_2C_6H_5$, -OCH_3, -$C_6H_4OCH_3$, -C_6H_4OH.

From the series of molecules designed, 19 molecules were selected for next process depending on their total energy. The list of designed molecules and calculated LogP by Crippen's and Viswanadhan's methods are shown in **Table 3**.

TABLE 3: LIST OF NOVEL MOLECULES AND CALCULATED LogP VALUES

S. no.	R1	R2	R3	Chemical Formula	LogP *	LogP**
1	-SH	-C_6H_5	-CH_3	$C_{11}H_{10}N_2S$	3.60	3.28
2	-SH	-CH_3	-C_6H_5	$C_{11}H_{10}N_2S$	3.60	3.28
3	-CH_3	-C_6H_5	-SH	$C_{11}H_{10}N_2S$	4.01	3.75
4	-SH	-$C_6H_4OCH_3$	-CH_3	$C_{12}H_{12}N_2OS$	3.47	3.03
5	-SH	-C_6H_5	-OCH_3	$C_{12}H_{12}N_2OS$	3.48	3.08
6	-SH	-$CH_2C_6H_5$	-OCH_3	$C_{12}H_{12}N_2OS$	3.76	3.33
7	-SCH_3	-C_6H_5	-C6H5	$C_{17}H_{14}N_2OS$	5.25	4.85
8	-SCH_3	-C_6H_4OH	-C6H5	$C_{17}H_{14}N_2OS$	4.86	4.57
9	-SCH_3	-$C_6H_4OCH_3$	-C6H5	$C_{17}H_{14}N_2OS$	5.12	4.60
10	-NH_2	-COC_6H_5	--C6H5	$C_{18}H_{16}N_2OS$	3.54	3.51
11	-$NHCOCH_3$	-C_6H_5	-C6H5	$C_{17}H_{13}N_3O$	3.72	3.61
12	-NH_2	-$COOC_6H_5$	--C6H5	$C_{18}H_{15}N_3O$	3.81	3.70
13	-NH_2	-CH=CHC_6H_5	-CH_3	$C_{17}H_{13}N_3O_2$	4.03	3.74
14	-NH_2	-C_6H_4OH	-C_6H_4OH	$C_{16}H_{14}N_2OS$	3.23	3.41
15	-CH_2OH	-C_6H_5	-C_6H_5	$C_{17}H_{14}N_2O$	4.61	4.70
16	-CH_20H	-C_6H_5	-NH_2	$C_{11}H_{11}N_3O$	2.43	2.53
17	-CH_2OH	-C_6H_5	-C_6H_4OH	$C_{17}H_{14}N_2O_2$	4.22	4.41
18	-CH_2OH	-CH_2CH_3	--C_6H_4Cl	$C_{13}H_{13}ClN_2O$	4.26	4.25
19	-CH_2OH	-SCH_3	-$COOC_6H_5$	$C_{13}H_{12}N_2O_3S$	3.48	3.13

* LogP by Crippen's fragmentation method

** LogP by Viswanadhan's fragmentation method

Validating the Docking Model by Known Drugs: Before docking the set of designed molecules, the model needs validation, and hence, the known drug Nevirapine was docked with the selected enzyme as described in AutoDock manual [15]. The reported binding energy is –9.35874 Kcal/mol. Whereas, 1VRT in complex with the original ligand (Nevirapine) shows hydrophobic interaction with amino acids namely 190-Glycine (GLY), 234-Leucine (LEU), 236-Proline (PRO), 229-Tryptophan (TRP), 181-Tyrosine (TYR), 318-Tyrosine (TYR), 106-Valine (VAL) and 179-Valine (VAL). Hydrophilic interaction between ligand and protein is not reported. This finding is comparable with the ligand interaction study using NGL viewer [12]. **Fig. 3** shows the hydrophobic interaction with protein whereas **Fig. 4** depicting negative result for hydrophilic interaction.

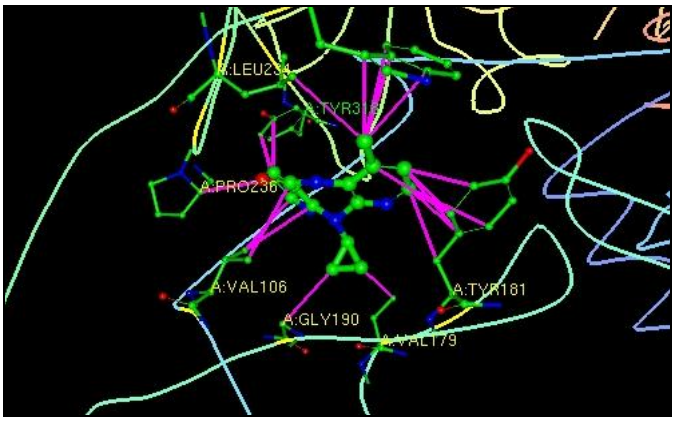


FIG. 3: POSITIVE HYDROPHOBIC INTERACTION OF PROTEIN 1VRT WITH NEVIRAPINE

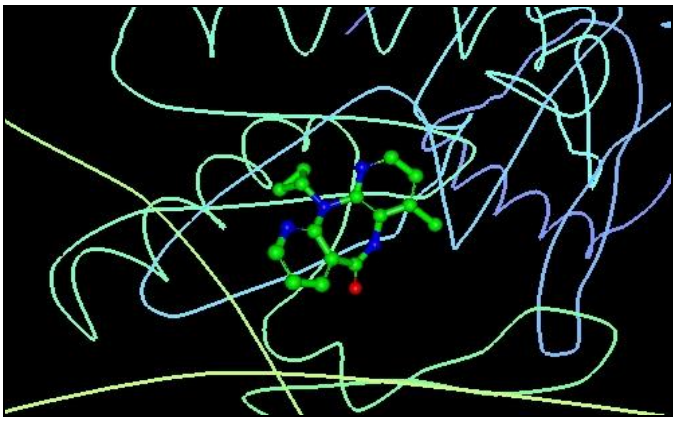


FIG. 4: NEGATIVE RESULTS OF HYDROPHILIC INTERACTION OF PROTEIN 1VRT WITH NEVIRAPINE

The known drug Nevirapine interacts with enzyme and successfully docked. The reported binding energy is negative and the ligand-enzyme complex is stable. The result of the known drug is validating the docking methodology as the drug is already interacting with the selected binding site in vitro. The negative value of free energy is also supporting the validity of docking methodology.

Inhibition of Designed Molecules: The inhibition of designed molecules was performed using AutoDock [15, 16] which is a widely used tool for understanding the possibilities of inhibition of enzyme with small molecules. The docking procedure is systematically explained in the AutoDock manual [15]. **Table 4** depicts the hardware and software used for executing the docking process. The calibration of the installed system and software were achieved using the standard procedure as described in Autodock user manual [15].

TABLE 4: COMPUTER SYSTEM AND SOFTWARE USED FOR THE DOCKING PROCESS

Computer Hardware CPU	CPU: AMD Athlon 3.0 Mother Board: ASUS RAM: 4 GB, 444 MHz.
Operating system	Linux: Fedora core 5
Computer Hardware	4 GB, 444 MHz
Software	Automated Docking of Flexible Ligands to Receptors) Version 4.0

In the present study, docking was performed using a Non-genetical algorithm (NON-GA) and Genetic Algorithm (GA). GA is the most intelligent search algorithm and provides a more realistic interaction between ligand and enzyme. **Table 5** depicts the parameters set for the Non-genetic and Genetic algorithm used for docking. The process of docking is systematically explained in the user manual of AutoDock [15] and used as it is.

TABLE 5: CONTROL PARAMETERS SET FOR DOCKING

Parameters for genetic algorithm docking	
Population size = 100	Local search maximum iteration =20
Maximum generation = 5000	Converged when RMSD population fitness < 1 Kcal/Mole
Elitism Number = 5	Grid dimensions = 67×77×61
Crossover rate: 0.8	Total number of grid points = 314699
Mutation rate: 0.2	RMSD calculation: 2A with defined
Local search rate = 0.06	rotatable bonds and active torsions set to fewest atoms

Binding Energy Calculation: The docking process output is binding energy (ΔG in Kcal/Mole) between substrate and ligand. Docking algorithm makes use of force field equations and parameters to calculate the binding energy. It is the sum of intermolecular interactions between substrate and ligand which is represented by equation 1.

$$E_{Dock} = E_{vdw} + E_{H\text{-}bond} + E_{Electrostatic} + E_{Internal} \quad \ldots\ldots\ldots(1)$$

Set of Pyrimidine class of compounds as shown in **Table 3** were docked with IVRT binding site. Obtained binding energy for each molecule is depicted in **Table 6**.

TABLE 6: RESULTS OF INHIBITION STUDY FOR PYRIMIDINE BASED MOLECULES WITH 1VRT

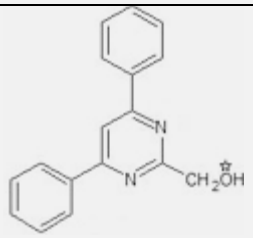
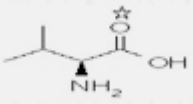
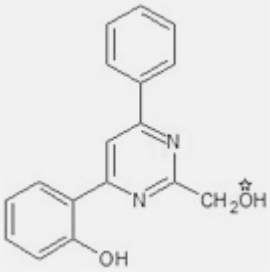
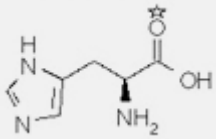
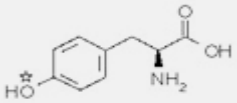
Molecule no.	R_1	R_2	R_3	Binding Energy ΔG in Kcal/ Mole.	
				GA Docking	Non-GA Docking
1	-SH	$-C_6H_5$	$-CH_3$	-10.25	-10.11
2	-SH	$-CH_3$	$-C_6H_5$	-10.18	-10.01
3	$-CH_3$	$-C_6H_5$	-SH	-10.03	-9.97
4	-SH	$-C_6H_4OCH_3$	$-CH_3$	-9.43	-9.39
5	-SH	$-C_6H_5$	$-OCH_3$	-9.60	-9.52
6	-SH	$-CH_2C_6H_5$	$-OCH_3$	-10.32	-10.21
7	$-SCH_3$	$-C_6H_5$	$-C_6H_5$	-13.19	-13.05
8	$-SCH_3$	$-C_6H_4OH$	$-C_6H_5$	-12.73	-12.66
9	$-SCH_3$	$-C_6H_4OCH_3$	$-C_6H_5$	-12.70	-12.47
10	$-NH_2$	$-COC_6H_5$	$-C_6H_5$	-12.37	-12.24
11	$-NHCOCH_3$	$-C_6H_5$	$-C_6H_5$	-12.79	-12.71
12	$-NH_2$	$-COOC_6H_5$	$-C_6H_5$	-11.96	-11.92
13	$-NH_2$	$-CH=CHC_6H_5$	$-CH_3$	-11.53	-11.41
14	$-NH_2$	$-C_6H_4OH$	$-C_6H_4OH$	-10.97	-10.86
15	$-CH_2OH$	$-C_6H_5$	$-C_6H_5$	-12.74	-12.67
16	$-CH_2OH$	$-C_6H_5$	$-NH_2$	-10.42	-10.35
17	$-CH_2OH$	$-C_6H_5$	$-C_6H_4OH$	-13.03	-12.92
18	$-CH_2OH$	$-CH_2CH_3$	$-C_6H_4Cl$	-11.35	-10.46
19	$-CH_2OH$	$-SCH_3$	$-COOC_6H_5$	-10.57	-9.67

RESULTS: The obtained binding energy (ΔG) of all molecules ranges from -9.39 Kcal/mole to -13.19 Kcal/mole. It is found out that there is little difference in the value of binding energy obtained by GA and Non-GA docking. GA method shows more conformations than Non-GA method. Molecule number 7, 8, 9, 11, 15 & 17 docked with higher negative binding energy compared to other molecules.

From docking results, it is observed that molecule number 5, 12, 15, 16, 17, 18 & 19 make hydrogen bonds in the complex. Whereas, it is observed that molecule number 15 and 17 show more negative docking values along with some hydrogen bonds. Molecule number 15 and 17 form two hydrogen bonds with 179-Valine (VAL), 190-Glycine (GLY) and 235-Histidine (HIS), 318-Tyrosine (TYR) respectively. **Table 7** depicts the detailed information about the hydrogen bonding by these two molecules.

Comparing the docking results with Nevirapine, it is reported that molecule number 15 and 17 also show the interaction with 179-VAL, 190-GLY, 229-TRP, and 318-TYR. Whereas, the binding energy values for molecule number 15 and 17 is higher by 3 to 4 Kcal/mole. This shows that molecule number 15 and 17 shows approximately 30% higher interaction with 1VRT as compared to Nevirapine. Also, both molecules show two hydrogen bonds having 2.4 A^o to 2.8 A^o.

TABLE 7: PYRIMIDINE BASED MOLECULES DOCKED WITH 1VRT SHOWING POSSIBLE HYDROGEN BONDS, BOND DISTANCE, AND BINDING ENERGY VALUES

Molecule number	H-bond position in the molecule	H-bond position in the amino acid	H-bond distance in A°	Docking energy ΔG in kcal/mol.
15		O of 179 VAL  N of 190 GLY 	2.81A° 2.4 A°	-12.74
17		O of 235 HIS  O of 318 TYR 	2.8 A° 2.6 A°	-13.03

* represents the location of hydrogen bond

CONCLUSION: The docking studies indicated that in most of the compounds form a hydrogen bond with residues 179-Valine (VAL), 229-Tryptophan (TRP), and 188-Tyrosine (TYR), 318-Tyrosine (TYR), 190-Glycine (GLY), 235-Histidine (HIS) and 101-Lysine (LYS).

Out of these -*OH* group attached to a phenyl ring in amino acids 188-TYR and 318-TYR found prominent for hydrogen bonding.-CH_2OH group at R_1 in 2, 4, 6 substituted pyrimidine position is found to be favorable for hydrogen bonding. The decrease in binding energy of pyrimidine-based molecule studied here is most likely due to the phenyl ring. Molecule number 15 and 17 can further be studied for biological activity.

The present result supports the fact that the binding pocket of 1VRT is favoring hydrophobic interactions. If the molecule makes a hydrogen bond with 179-VAL, 190-GLY, 229-TRP or 318-TYR, then possibilities of having higher negative binding energy are more. Hence, a further increase in hydrophobic groups in ligand will help in making the successful HIV-1 RT inhibitor.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: The author(s) declare that there is no conflict of interest.

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How to cite this article:
Halmare RS and Ganatra SH: Molecular docking studies of a few novel pyrimidine derivatives as reverse transcriptase HIV-1 inhibitors. Int J Pharm Sci & Res 2019; 10(9): 4201-06. doi: 10.13040/IJPSR.0975-8232.10(9).4201-06.