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De novo design and *in-silico* studies of novel bis-arylpiperazine derivatives as non-nucleoside inhibitors of HIV-1 reverse transcriptase

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Abstract: In the present study, we have designed some novel bisarylpiperazine derivatives as Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase. Docking studies of the designed analogues were performed by molecular modeling software AutoDock 4.2 using HIV-1 Reverse Transcriptase (PDB ID: 1RT2) as receptor. Lipinski's "Rule of Five (Ro5)" parameters and toxicity parameters were predicted through online servers like Molinspiration and Osiris property explorer. Docking parameters such as binding free energy and predicted inhibitory constant (K_i) values of the designed analogues were compared with standard drugs Efavirenz and co-crystallized ligand TNK-651. Among the designed analogues, **1**, **4**, **8**, **9**, **12**, **15**, **27**, **30**, **37**, **38**, **39** and **43** showed significant and comparable binding free energy and predicted inhibitory constant values as that of standard drugs. These results indicate that, the designed analogues adopt a similar orientation and share the same binding mode as that of some of the classical Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) within the active site of Non-Nucleoside Inhibitory Binding Pocket (NNIBP) of HIV-1 reverse transcriptase.

Keywords: AIDS**;** HIV-1 Reverse Transcriptase; molecular docking; molecular properties; AutoDock; β-carboline

1. Introduction

According to the UNAIDS-2013 report, 2.3 million new Human Immunodeficiency Virus (HIV) infections and 1.7 million people died in the year 2012 with HIV related infections.[1] HIV is a pathogenic lentivirus, belongs to the family of *Lentiviridae* and contains single stranded RNA as genetic material. Number of deaths due to HIV infection is decreasing, because of availability of effective HAART (Highly Active Anti-Retroviral Therapy). HAART includes two Nucleoside / Nucleotide Reverse Transcriptase Inhibitors (NRTIs) and one Protease Inhibitor (PI). An alternative and effective combination is two Nucleoside / Nucleotide Reverse Transcriptase Inhibitors and one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTIs). NNRTIs are the key component in HAART, because of their high potency, selectivity and less toxicity when compared to NRTIs and PIs.[2, 3] Recently, US Food and Drug Administration (FDA) have approved five NNRTIs for the treatment of AIDS. Among the five NNRTIs, Nevirapine, Delavirdine, Efavirenz are the first generation and Etravirine, Rilpivirine are the second generation NNRTIs.[4] However, occurrence of the high mutation rate of the virus and the resulting emergence of resistance makes the researchers to run a never-ending marathon to keep developing new drugs active against both drug sensitive and drug resistance strains of HIV with better therapeutic profile.

Quantitative Structure Activity Relationship (QSAR) and docking methods are two generally used computational methods in Drug Design. QSAR studies establish a statistical relationship between biological activity of compounds and a set of molecular descriptors obtained from their structures.[5] In docking studies, different search algorithms (simulated annealing, genetic algorithm) are being used along with scoring functions (molecular mechanic) to study the binding interactions of the ligands with receptor protein.[6]

Bis-arylpiperazines are a unique class of non-nucleoside inhibitors of HIV-1 reverse transcriptase. This group of compounds was extensively explored in the literature as anti-HIV-1 agents during the development of first generation NNRTIs.[7, 8] Delaviridine, the first generation NNRTI belongs to this class of compounds, but unfortunately these became less effective due to developed resistance. Resistance to first generation NNRTIs was observed majorly due to mutation of active site aminoacids L100I, K101E/Q, K103S, K103N, V106A/M, Y181C, Y188L, M230L, P236L, K238N/T and Y318F.[9] In order to overcome the resistance problems and to delay emergence of resistance, new generation NNRTIs are designed with an aim to establish interactions with conserved aminoacids W229 and F227. In the present study, we have designed some novel bis-arylpiperazines as non-nucleoside inhibitors

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of HIV-1 RT. We have purposely incorporated bulkier β-carboline moiety as one of the aryl fraction with intention to institute interactions with conserved aminoacids and ultimately to overcome the resistance. β-carboline represents a tricyclic pyrido[3,4-*b*]indole ring system present in large number of natural products isolated from different sources like territorial plants,[10] marine sponge,[11] fast food and humans.[12] The β-carboline ring has a privileged position in medicinal chemistry, as compounds containing β-carboline skeleton displayed various biological activities like anticancer,[13] antithrombotic,[14] antimicrobial,[15] antimalarial,[16] antileishmanial,[17] antitubercular[18] and antiviral activity.[19] Some natural as well as synthetic β-carboline derivatives were also exhibited significant anti-HIV activity through multiple mechanisms such as, interfering with the early stages of the HIV life cycle by inhibiting cell to cell transmission or by blocking the TAT–TAR interaction.[20] In the present study, we are reporting, denovo design, docking and predicted *in-silico* molecular properties of some novel 1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl derivatives as Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase.

2. Result and Discussion

2.1. Molecular docking studies

HIV-1 Reverse Transcriptase enzyme co-crystallized with TNK 651 ligand (pdb code: 1RT2, Ramachandran plot, **Figure 1.**) was used as target receptor.[21] Co-crystallized ligand TNK-651 and standard drug Efavirenz are used for calibration and validation of the docking process.

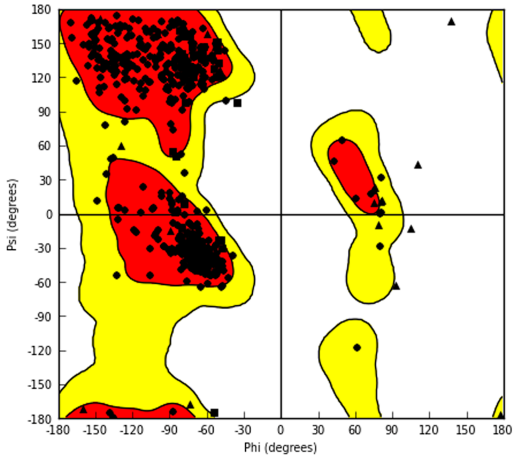

Figure 1. Ramachandran plot of HIV reverse transcriptase (PDB: 1RT2)

Initially, co-crystallized ligand TNK-651 was extracted and re-docked into hydrophobic binding pocket of RT in order to validate the docking calculations. It was evident that, redocked pose of the ligand was almost superimposed with that of the co-crystallized ligand (**Figure 2.**) with acceptable root mean square deviation of 0.56 Å. Then standard drug efavirenz was subjected to docking study in the same manner as that of TNK-651 in order to check reliability and reproducibility of the docking parameters used for the study. Interaction of efavirenz with the active site amino acid residues of HIV-1 RT was shown in **Figure 3.**

Docking is an approach to rational drug design that seeks to predict the binding mode and binding free energy of ligand-receptor complex. It not only gives an idea about how the ligand is going to bind with the receptor but also up to what extent conformational changes can be brought in the receptor structure upon binding with ligand. The docking studies of the designed analogues, TNK-651 and Efavirenz (**Figure 4**) were performed using molecular modeling software AutoDock 4.2 in order to identify the enzyme inhibitory potential against HIV-1 RT (1RT2). In the present study, two docking parameters such as binding free energy (Kcal/mole) and predicted inhibitory constant (K_i) values were determined and are shown in **Table 1**.

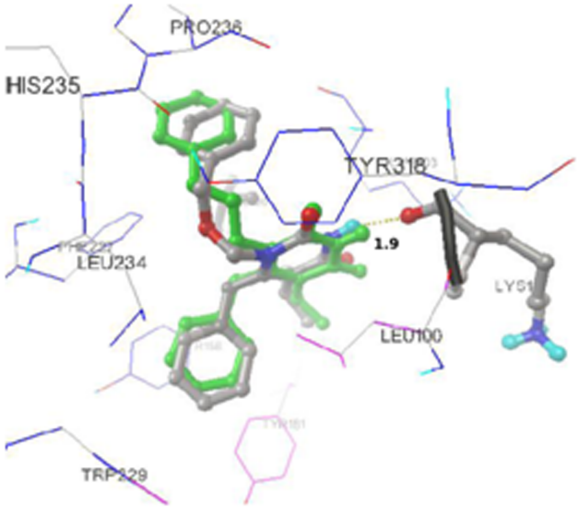

Figure 2. Re-docked mode of TNK 651(Green) superimposed with the co-crystallized ligand (Grey) in the NNIBP of HIV-1 RT (1RT2). Ligand is shown as ball & stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen Bond Interaction represented as yellow dotted line.

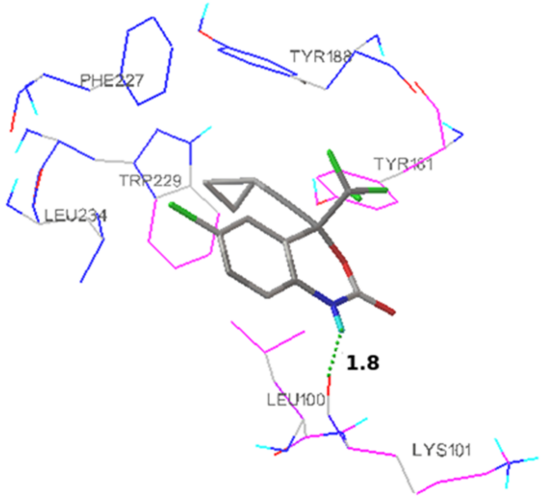

Figure 3. Binding mode of Efavirenz in the NNIBP of HIV-1 RT (1RT2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen Bond Interactions represented as yellow dotted line.

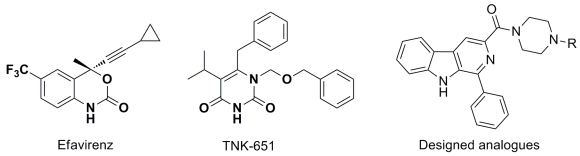

Figure 4. Structure of standard drugs and designed ligands

Table 1. Docking results of the designed analogues

Code	R	Wild type HIV-1 RT (1RT2)		Code	R	Wild type HIV-1 RT (1RT2)	
		Binding free energy (Kcal/mole)	K_i (nM)			Binding free energy (Kcal/mole)	K_i (nM)
Efavirenz	**-**	**-12.96**	**0.35 nM**	**25**	-2,4CH_3ph	-9.87	58.11
TNK 651	**-**	**-13.52**	**0.11 nM**	**26**	-3,4CH_3ph	-7.87	1710
1	*-ph*	*-10.71*	*14.05*	***27***	*-2,6CH_3ph*	*-11.06*	*7.83*
2	-CH_2ph	-9.07	226.3	**28**	-2,4OCH_3ph	-7.74	2130
3	-4CH_3ph	-9.89	56.66	**29**	-3,4OCH_3ph	-9.02	245.5
4	*-3CH_3ph*	*-10.67*	*15.1*	***30***	*-2,6OCH_3ph*	*-10.97*	*9.15*
5	-2CH_3ph	-9.14	199.18	**31**	-2,4Clph	-9.95	53.2
6	-4OCH_3ph	-10.01	46.2	**32**	-3,4Clph	-7.64	2510
7	-3OCH_3ph	-9.59	93.13	**33**	-2,6Clph	-8.76	381.9
8	*-2OCH_3ph*	*-11.24*	*5.75*	**34**	-2,4Fph	-9.09	216.82
9	*-4Clph*	*-10.62*	*16.31*	**35**	-3,4Fph	-9.29	154.8
10	-3Clph	-10.25	30.85	**36**	-2,6Fph	-9.93	52.29
11	-2Clph	-9.59	93.12	***37***	*-2,4Brph*	*-11.47*	*3.88*
12	*-4Fph*	*-10.65*	*15.73*	***38***	*-3,4Brph*	*-10.61*	*16.78*
13	-3Fph	-8.23	923.69	***39***	*-2,6Brph*	*-11.54*	*3.47*
14	-2Fph	-10.01	45.81	**40**	-3,4DNO_2ph	-9.16	192.5
15	*-4NO_2ph*	*-11.1*	*7.3*	**41**	-2,4OHph	-9.07	224.8
16	-3NO_2ph	-8.9	299	**42**	-3,4OHph	-9.62	88.71
17	-2NO_2ph	-9.09	215.03	***43***	*-2,6OHph*	*-10.76*	*12.93*
18	-2,3Clph	-10.23	31.49	**44**	4-pyrimidyl	-9.71	75.85
19	-4OHph	-9.43	121.98	**45**	2-pyrimidyl	-8.48	603.9
20	-3OHph	-8.11	1013	**46**	2-pyrollyl	-9.46	115.73
21	-2OHph	-9.46	115.7	**47**	3-pyrollyl	-9.59	92.7
22	4-pyridyl	-8.47	614.3	**48**	2-furayl	-8.4	692.9
23	3-pyridyl	-8.03	1003	**49**	3-furayl	-9.07	223.8
24	2-pyridyl	-9.36	137.2	**50**	3-pyrazolyl	-9.32	148.2

free energy of the individual compounds was calculated using the following formula,

Binding Energy = $[(1) + (2) + (3) - (4)]^3$

where, 1 denotes final intermolecular energy [vanderwalls energy + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol)], 2 denotes final total internal energy [Internal Energy Ligand + Internal Energy Receptor (kcal/mol)], 3 denotes torsional free energy (kcal/mol) and 4 denotes unbound system's energy (kcal/mol).

In docking studies, binding free energy value is indirectly proportional to enzyme inhibitory activity. Compounds showing less binding energy have higher enzyme inhibitory activity and vice versa. In the present study, the designed analogues showed binding energy values in the range of -11.54 to -7.64. Among the designed analogues, **1**, **4**, **8**, **9**, **12**, **15**, **27**, **30**, **37**, **38**, **39** and **43** showed significant binding free energy values of -10.71, -10.67, -11.24, 10.62, -10.65, -11.1, -11.06, -10.97, -11.47, -10.61, -11.54 and -10.76 kcal/mol respectively against HIV-1 reverse transcriptase enzyme (1RT2) with that of standard drug Efavirenz and TNK-651 (-12.96 and -13.52 kcal/mol respectively). The overlay view of these analogues was showed in **Figure 5**.

Observation of receptor-ligand complex reveals that, like other NNRTIs, the designed analogues adopt similar kind of orientation in NNIBP of HIV-1 RT. These ligands mainly displayed Van derWaals and electrostatic interactions such as pi-pi interactions, pi-cationic interactions with active site amino acid residues like Y181, Y188, F227, W229, H235 and Y318. It is more important that, the designed analogues exhibited desired electrostatic interactions with conserved aminoacids like F227 and W229 (**Figure 6**). Interactions with these conserved aminoacids may useful; to display potency against resistance strains and to delay the emergence of resistance. In the designed compounds amide carbonyl oxygen also showed hydrogen bonding interactions with L100, K103S, S105 and V106 (**Figure 7**). These interactions played vital role in the determination of binding free energy and stability of receptor-ligand complex.

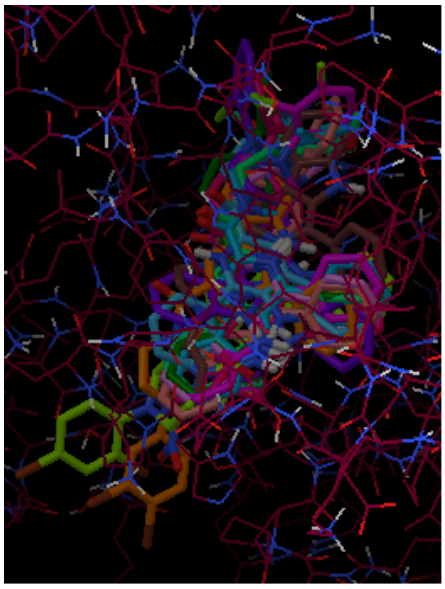

Figure 5. Overlay stereo view of best twelve compounds in the Non-Nucleoside Inhibitory Binding Pocket of HIV-1 RT (1RT2).

In addition, another docking parameter, predicted inhibitory constant (K_i) was also determined. The inhibitory constant is a measure of compound inhibitory potency of a biological or biochemical function. Inhibitory constant is directly proportional to the binding energy of receptor-ligand complex. In the present study, the designed analogues showed inhibitory constant values in the range of 3.47 nM to 2510 nM. Among the designed analogues, **1**, **4**, **8**, **9**, **12**, **15**, **27**, **30**, **37**, **38**, **39** and **43** showed significant inhibitory constant values of 14.05, 15.1, 5.75, 16.31, 15.73, 7.3, 7.83, 9.15, 3.88, 16.78, 3.47 and 12.93 nM respectively against HIV-1 reverse transcriptase enzyme (1RT2) with that of standard drug Efavirenz and TNK-651 (352.14 and 114.27 pM respectively).

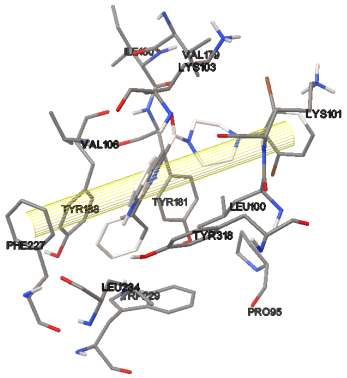

Figure 6. pi-pi interaction of compound **39** with conserved aminoacid residue F227.

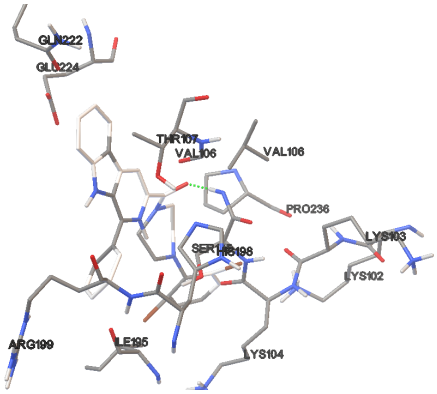

Figure 7. Hydrogen bonding interactions of compound **37** with aminoacid residue S105

2.2. Molecular parameters

Molecular properties like Molecular weight (MW), CLogP, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), drug likeness (DL) and toxicity risks like mutagenic, tumorigenic, irritant and effect on sexual reproduction, drug score (DS) were predicted for the designed analogues using online tools, OSIRIS property explorer (http://www.organic-chemistry.org/prog/peo/) and Molinspiration chemoinformatics (http://www.molinspiration.com/). Predicted molecular properties of the designed analogues were reported in **Table 2**.

Table 2. Predicted molecular parameters of the designed molecules

Code	MW	CLogP	HBA	HBD	DL	DS	Code	MW	CLogP	HBA	HBD	DL	DS
1	432	5.22	5	1	8.43	0.24	**26**	460	5.79	5	1	3.46	0.15
2	446	5.34	5	1	7.96	0.26	**27**	460	5.79	5	1	8.58	0.15
3	446	5.5	5	1	5.37	0.21	**28**	492	4.96	7	1	4.72	0.30
4	446	5.54	5	1	7.17	0.29	**29**	492	4.96	7	1	4.79	0.30
5	446	5.5	5	1	7.64	0.21	**30**	492	4.96	7	1	7.87	0.30
6	462	5.12	6	1	5.23	0.19	**31**	500	6.31	5	1	8.06	0.20
7	462	5.12	6	1	6.52	0.14	**32**	500	6.31	5	1	6.46	0.20
8	462	5.12	6	1	7.94	0.24	**33**	500	6.31	5	1	8.24	0.20
9	466	5.84	5	1	7.27	0.19	**34**	468	5.3	5	1	6.12	0.27
10	466	5.84	5	1	7.58	0.19	**35**	468	5.3	5	1	3.67	0.27
11	466	5.84	5	1	7.75	0.15	**36**	468	5.3	5	1	4.98	0.27
12	450	5.28	5	1	5.44	0.22	***37***	*588*	*6.55*	5	1	5.67	0.13
13	450	5.2	5	1	4.81	0.30	***38***	*588*	*6.55*	5	1	4.55	0.16
14	450	5.28	5	1	6.25	0.22	***39***	*588*	*6.55*	5	1	6.00	0.16
15	477	5.09	8	1	-4.55	0.11	***40***	*522*	3.25	*11*	1	-3.09	0.16
16	477	4.18	8	1	2.27	0.31	**41**	464	4.41	7	1	7.39	0.31
17	477	4.18	8	1	0.75	0.28	**42**	464	4.41	7	1	6.66	0.39
18	500	6.45	5	1	6.94	0.15	**43**	464	4.41	7	1	7.75	0.39
19	448	4.75	6	2	7.28	0.36	**44**	434	3.89	7	1	7.35	0.43
20	448	4.75	6	2	7.42	0.36	**45**	434	3.89	7	1	8.62	0.44
21	448	4.75	6	2	7.81	0.36	**46**	421	4.17	6	1	7.58	0.42
22	433	4.15	6	1	6.64	0.33	**47**	421	3.82	6	1	7.56	0.48
23	433	4.1	6	1	7.6	0.44	**48**	422	4.58	6	1	6.95	0.36
24	433	4.63	6	1	8.39	0.28	**49**	422	4.23	6	1	7.10	0.41
25	460	5.79	5	1	4.37	0.12	**50**	422	3.34	7	1	8.28	0.49

DL: Drug likeness; DS: Drug score; HBA: No. of Hydrogen Bond Acceptor; HBD: No. of Hydrogen Bond Donor; MW: Molecular weight

Among the fifty designed analogues, four molecules **37**, **38**, **39** were violated from Molecular weight, CLogP and the compound **40**, violated molecular weight, number hydrogen bond acceptor parameters of Lipinski's Ro5. Only single violation from Lipinski's Ro5 is accepted, any molecules violating more than one time from these parameter may have oral bioavailability problems. Hence to avoid risk factor, we have screened off **37**, **38**, **39** and **40** for further studies like synthesis and evaluation of HIV-1 RT inhibitory potency.

3. Experimental

3.1. Molecular docking studies

The docking studies of the designed analogues were performed using molecular modeling software AutoDock 4.2 installed on a single machine running on a 3.4 GHz pentium processor with windows XP SP2 as the operating system. Target protein, HIV-1 RT enzyme [pdb code: 1RT2] was taken from the RCSB protein data bank and Ramachandran plot of prepared protein 1RT2 (**Figure 1**.) was obtained from Discovery studio viewer. Target protein's pdb structure was further refined by removal of water molecules, by adding polar hydrogens and kollmann charges. For the docking study, a grid spacing of 0.375 Å and 63×63×63 number of points were used. The grid was centered on the co-crystallized ligand. The autogrid-4.2 program generated separate grid maps for all atom types of the ligand structures and one for electrostatic interactions. PRODRG online server (http://davapc1.bioch.dundee.ac.uk/prodrg/) was used to generate the energy minimized conformations of the ligands in pdb format.[22] Energy minimized conformation of ligands were subjected to calculation of Gasteiger-Huckel charges and saved in default format of Autodock. Autodock generated 50 possible binding conformations i.e., 50 runs for each docking by using LGA search. Default protocol was applied, with initial population of 150 randomly placed individuals, a maximum number of $2.5*10^5$ energy evaluations and $2.7*10^4$ generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

3.2. Molecular parameters

Molecular properties of ligands plays vital role in the determination of their pharmacokinetic profile, hence *in-silico* molecular properties prediction of any new chemical entity is growing interest in medicinal chemistry.[23] Lipinski's rule of 5 is used to predict pharmacokinetic properties such as absorption, distribution, metabolism and excretion by their molecular properties like ClogP (≤5), Molecular weight (≤500), number of hydrogen bond acceptors (≤5), number of hydrogen bond donors (≤10). Molecules violating more than one of these rules may have problem with oral bioavailability.[24] Drug likeness is a qualitative concept used in drug design for how "drug like" a substance is with respect to factors like bioavailability and toxicity. Around 80% of the marketed drugs have positive drug likeness value (http://www.organic-chemistry.org/prog/peo/). The drug score is a handy value and is useful to judge the compound's overall potential to qualify as drug (**Table 2**.). Drug score (ds) of a molecule is mainly depends up on the drug likeness, cLogP, logS, molecular weight and toxicity risks of the molecule. It is calculated from the following formula,

$$ds = \prod \left(\frac{1}{2} + \frac{1}{2} si\right). \prod ti$$

ds=drug score, Si=contribution directly calculated from cLogP, logS, molecular weight and drug likeness, ti=contribution directly calculated from toxicity risks.

4. Conclusion

In the present study, we have designed fifty novel bisarylpiperazines as HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors. Docking study of the designed analogues was performed using molecular modeling software AutoDock 4.2. Lipinski's Ro5 parameter, drug likeness and drug score of the designed analogues were predicted using online tools, OSIRIS property explorer and Molinspiration cheminformatics. Among the designed analogues, compound **1**, **4**, **8**, **9**, **12**, **15**, **27**, **30**, **37**, **38**, **39** and **43** showed significant binding free energy and predicted inhibitory constant values as that of standard drugs. Even though compounds like **37**, **38** and **39** showed significant binding free energy and predicted inhibitory constant values, they have screened off for next level of study because of their predicted poor pharmacokinetic profile. From this study concluded that, bulky hydrophobic group substitutions on phenyl ring attached to piperazine are very much important to form stable receptor-ligand complex as well as for better inhibitory potency. Synthesis, characterization and evaluation of *in-vitro* HIV-1 reverse transcriptase inhibition activity of these lead compounds identified from this *in-silico* study is under progress.

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