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In silico Studies on *N*- (Pyridin-*2-yl*) Thiobenzamides as NNRTIs against Wild and Mutant HIV-1 Strains

Anuradha Singh, **Vishal Kumar Singh**, **Rajesh Verma**, and **Ramendra K. Singh***

Bioorganic Research Laboratory, Department of Chemistry,
University of Allahabad, Allahabad-211002, India

In the present study, keeping the Lipinski's Rule of Five in focus, a series of new *4*-(*4*-benzenesulfonylamino)-*N*-(*5*-substituted-pyridin-*2-yl*)-thiobenzamides bearing different substituents at the *C-4* position of benzenesulfonylamino ring have been designed as NNRTIs of wild-type (WT) and mutant HIV-1 strains. Molecules having drug-like character were further docked into the active domain of wild-type (WT) RT/1 with entry code (PDB: ID 3mec) and K103N/TYR181 mutant RT/2 complex (PDB: ID 3BGR) using Discovery Studio 2.5 software. Analysis of the docking results revealed that all molecules formed hydrogen bonds with active amino acids (Lys101, Lys103, Tyr181, and Tyr318) and exhibited π-stacking interactions with Tyr181, Tyr188, Phe227, and Trp229 present in the NNIBP with both WT and mutant HIV-1 RT. The designed ligands adopted 'horseshoe/seahorse' conformation inside the NNIBP like other second generation NNRTIs and formed more stable complexes (total interaction energy found in the range of (-) 54 – (-) 77 kcal/mol) with HIV-1 RT in comparison to etravirine and rilpivirine (-)61.43 and (-)50.23 Kcal/mol, respectively. Consequently, lower EC_{50} values were predicted for *N*- (Pyridin-*2-yl*) derivatives. Structure-activity relationships (SARs) are discussed in terms of a possible interaction with the RT binding site, depending on the nature of the substituent at ring A and ring C.

INTRODUCTION

Currently, more than 36.9 million people worldwide are infected with HIV-1, which leads to an immune-compromised disorder known as AIDS (UNAIDS 2016). One of the most pressing targets to combat HIV-1 infection is the viral enzyme reverse transcriptase (RT). This multifunctional enzyme converts ss-RNA into the ds-proviral DNA and this provirus gets integrated into the host cell chromosome, successively (Jonckheere et al. 2000; Esposito et al. 2012). The HIV-1 RT is very prone to mutation and exists as a mutant swarm, which ultimately makes it an unsolved puzzle for the scientific community and severely impedes the success of highly active antiretroviral therapy (HAART) (Abram et al. 2014). One of the key components of HAART is NNRTI, which possesses a high chemical diversity and inhibits RT allosterically. The high specificity and low toxicity of NNRTIs make them attractive drug candidates in comparison to NRTIs (De Clercq 2004; Singh et al. 2010; Kumari & Singh 2013). Despite the success of combination therapy, the emergence of drug conflict is still challenging. Thus, the development of new NNRTIs with improved resistance profiles continues to be a dynamic area of study.

X-ray crystallographic studies suggested that Leu100Ile, Lys103Asn, Val106Ala, Val108Ile, Tyr181Cys, Tyr188Leu, Gly190Ala/Ser, or combinations of these mutations are observed in common clinically NNRTI-resistant mutants and among them, Lys103Asn is the most prevalent NNRTI resistance-associated mutation. First-generation NNRTIs relied heavily on π-stacking interactions to Tyr181 and Tyr188, which can easily mutate, conferring resistance. New NNRTIs endeavored to reduce this interaction rely more on interaction with Trp229, Phe227, and Tyr318, which are less prone to mutation. A design feature for second-generation NNRTIs is the innate flexibility between aromatic rings,

*Corresponding author: rksinghsrk@gmail.com

allowing the compound to adopt multiple conformations, which is one explanation for the potent activity against many resistant virus strains (Zhou et. al. 2006; Martins et. al. 2008; Kumari & Singh 2012; Singh et. al. 2015).

A 3D pharmacophore model for second-generation NNRTIs should consist of five features: three hydrophobic groups, one hydrogen bond acceptor group, and one hydrogen bond donor group. The H-bond donor and acceptor species bind with two important RT Lysines (Lys101 and/or Lys 103) (Das et. al. 2005; Barreca et al. 2007; La Regina et. al. 2010; Peng et. al. 2013). The researchers used this model for the rational design of NNRTIs having benzenesulfonylamino moiety with thioamide linker and different *5*-substituted-pyridin-*2-yl* substituents, as shown in Figure 1. These molecules have been designed following Lipinski's rule of five and docked into NNIBP of HIV-RT, both wild and mutant type, using DS 2.5 software. The different degrees of potency displayed by the new molecules against wild-type and mutant HIV-1 are also studied using molecular docking approach.

MATERIALS AND METHODS

Compounds and Lipinski's Rule

The researchers analyzed some drug-like properties of *N*- (pyridin-*2-yl*) derivatives on the basis of Lipinski's rule of five using Molinspiration and ChemDraw software (Lipinski et al. 2001). All the filtered molecules were further subjected to molecular modeling analysis.

Molecular Modeling Analysis

The crystal structures of the wild-type (WT) RT/1 with entry code (PDB: ID 3mec) and of the K103N/TYR181 mutant RT/2 complex [PDB: ID 3BGR] retrieved from the Protein Data Bank (www.rcsb.org) have been utilized for modeling (Das et al. 2008; Lansdon et al. 2010). Discovery Studio 2.5 (DS 2.5; Accelrys Software Inc., San Diego; http://www.accelrys.com) was used for molecular modeling analysis.

Receptor Setup

The target proteins (PDB: ID 3MEC) and [PDB: ID 3BGR] were taken, the ligand extracted, the missing hydrogens added, and their positions optimized using the all-atom CHARMm forcefield and the Adopted Basis set Newton-Raphson (ABNR) method available in DS 2.5 protocol until the root mean square (r.m.s.) gradient was less than 0.05 kcal/mol/Å. The minimized protein was defined as the 'receptor' and the method, volume of ligand, was used to define binding site to accommodate all key interacting amino acids in the allosteric site of HIV-1 RT. Further, the 'Input Site Sphere' (radius of 5Å from the center) was defined over the binding site, embedded in binding site module of DS 2.5. Re-minimized protein was used for further docking algorithm (Brooks et al. 1983; Mayo et al. 1990; Momany & Rone 1992; Venkatachalam et al. 2003).

Ligand Setup

A series of new *4*-(*4*-benzenesulfonylamino)-*N*-(*5*-substituted-pyridin-*2-yl*)-thiobenzamides bearing different substituents at the *C-4* position of benzenesulfonylamino

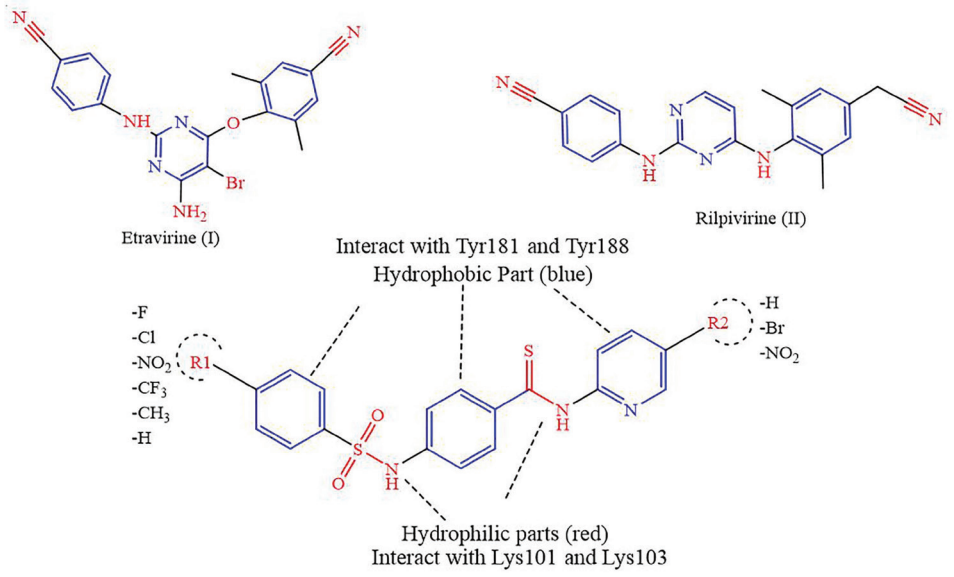

Figure 1. General structure of designed molecules and structural similarities between reported second generation NNRTIs (I-II).

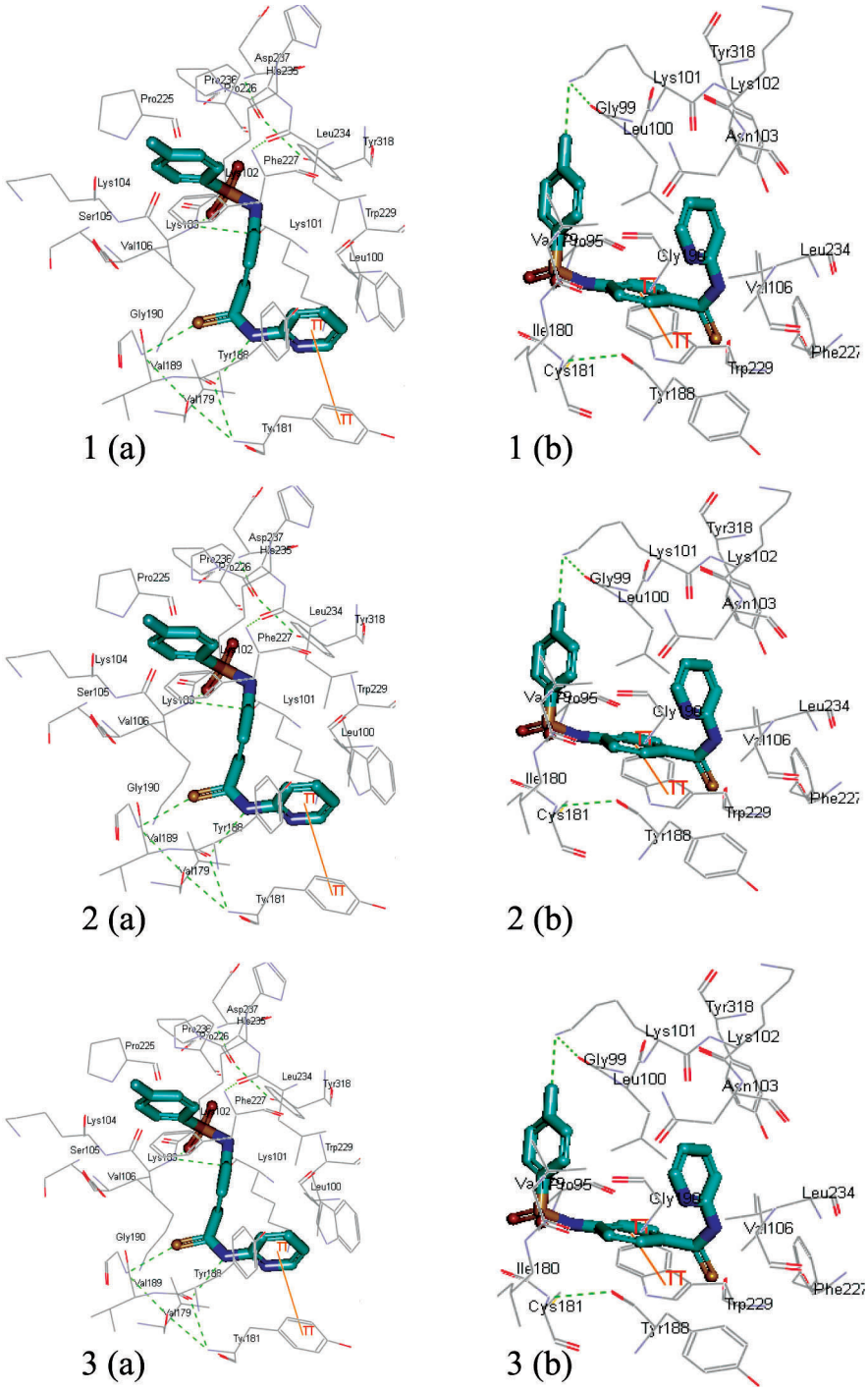

Figure 2. (a) Docked conformations of compound 1, 2, and 3 into allosteric site of wild-type HIV-1 RT showing 'seahorse' conformation; and (b) compound 1, 2, and 3 into allosteric site of mutant-type HIV-1 RT showing 'horseshoe' conformation.

ring (Figure 1; ring A) was prepared using 'build-and-edit' module of DS 2.5. A molecular dynamics (MD) approach consisting of simulated annealing chain was used to conformational search of ligands. The lowest energy conformation was taken to be the most probable conformation.

Docking and Scoring

The LigandFit docking protocol was used to generate docked poses of ligand parallel to the shape of the binding site. The docked poses were evaluated with a set of scoring functions using default settings. While minimization, the atoms of ligand and the binding site residues (within 5

Å) of the receptor were kept flexible. The ligand pose, corresponding to the highest Dockscore, was taken as the best docked pose. Furthermore, the stability of protein-ligand complex was assessed through the protocol 'Calculate Binding Energies' as described earlier (Böhm 1994, 1998).

Validation

Docking was validated with known inhibitors – nevirapine, etravirine and rilpivirine; the root mean square deviation (RMSD) of the best docked pose of the molecules reported herein was less than 2 Å, which is supposed to be an appropriate condition for developing NNRTIs. Two structures overlap very well with positional RMSD of 1.5 Å (Singh et al. 2016; Yadav et al. 2017).

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RESULTS AND DISCUSSIONS

Drug-like Properties

The researchers used an *in silico* structure-based approach to developing some new potent NNRTIs containing benzenesulfonylamino moiety with thioamide linker and different *5*-substituted-pyridin-*2-yl* substituents. In the first step, drug-likeness of designed compounds was analyzed on the basis of Lipinski's rule of five. Molecules showing more than one type of violations out of 40 molecules taken all together are supposed to have problems with bioavailability, hence rejected. In the present study (Table 1), all nineteen molecules showed the allowed values for the properties analyzed and exhibited drug-like characteristics.

Table 1: Physicochemical descriptors for compounds 1-19.

Name	R_1	R_2	MW	Log P	TPSA	HD	HA	N-violations
1	-F	-H	387.46	3.83	71.08	2	5	0
2	-F	-Br	466.35	4.81	71.08	2	5	0
3	-F	$-NO_2$	432.45	3.96	116.91	2	8	0
4	-Cl	-H	403.92	4.35	71.08	2	5	0
5	-Cl	-Br	482.81	5.33	71.08	2	5	1
6	-Cl	$-NO_2$	432.84	3.47	133.98	2	9	0
7	-Br	-H	448.36	4.48	71.08	2	5	0
8	-Br	$-NO_2$	493.36	4.61	116.91	2	8	0
9	$-NO_2$	-H	414.46	3.63	116.91	2	8	0
10	$-NO_2$	-Br	493.36	4.61	116.91	2	8	0
11	$-NO_2$	$-NO_2$	459.46	3.76	162.73	2	11	1
12	$-CH_3$	-H	383.49	4.12	71.08	2	5	0
13	$-CH_3$	-Br	462.39	5.10	71.08	2	5	1
14	$-CH_3$	$-NO_2$	428.49	4.25	116.91	2	8	0
15	$-CF_3$	-H	437.46	4.57	71.08	2	5	0
16	$-CF_3$	$-NO_2$	482.46	4.69	116.91	2	8	0
17	-H	-H	369.47	3.67	71.08	2	5	0
18	-H	-Br	448.36	4.65	71.08	2	5	0
19	-H	$-NO_2$	414.46	3.80	116.91	2	8	0
Nevirapine			266.30	1.38	63.58	1	5	0
Etravirine			435.28	5.02	120.65	3	7	1
Rilpivirine			354.41	4.91	97.42	2	6	0

MW, molecular weight; H-A, number of H bond acceptors; H-D , number of H bond donors; TPSA, total polar surface area

Docking and Scoring

The anti-HIV potential of the designed compounds was assayed according to the computational modeling analysis and results are presented in Table 2, 3, 4, and 5. Docking parameters used were like hydrogen bond, pi-pi (hydrophobic), and non-polar pi-cation (non-covalent) interactions for the *in silico* studies (Gallivan & Dougherty 1999). The docking analysis revealed a predominant orientation of the ligand within the binding pocket of protein, and the conformation with the highest dock score value for each molecule was chosen for further analysis.

Different scoring functions (i.e. PLP1, PLP2, PMF, Lig_Internal_Energy, binding energy, Ludi_3, dock score) were used to analyze binding affinity of the protein-ligand complex. Higher PLP scores indicated stronger receptor-ligand binding (Gehlhaarl et al. 1995). It is worthwhile to mention that for wild-type strain majority of title compounds showed comparable PLP scores to the reference ligands; however, compounds 3 (117.34), 6 (110.27), 8 (118.22), 13 (116.21), 15 (111.15), and 16 (118.22) exhibited PLP1 score higher than the etravirine (107.62). For mutant type strain, all compounds showed lower PLP scores than the reference ligands (128.89).

Table 2: Docking results showing the stability of HIV-1 RT – ligand complexes.

Name	Total I.E (Kcal\ mol) with HIV-1 RT- ligand complexes (wild type)	Total I.E (Kcal\ mol) with HIV-1 RT- ligand complexes (mutant type)
1	-72.18	-58.28
2	-66.43	-57.90
3	-68.49	-62.56
4	-77.39	-50.06
5	-63.64	-47.80
6	-55.50	-57.72
7	-62.63	-63.10
8	-68.10	-59.82
9	-77.87	-48.24
10	-65.02	-59.49
11	-70.83	-64.99
12	-73.01	-57.97
13	-63.28	-50.90
14	-67.32	-58.79
15	-70.07	-65.46
16	-69.83	-58.16
17	-74.23	-54.77
18	-54.20	-53.12
19	-65.35	-53.24
Nevirapine	- 45.99	-40.48
Etravirine	-61.43	-54.87
Rilpivirine	-50.23	-49.65

The PMF scoring function well correlated with the binding free energy of protein-ligand complexes (Zhao et al. 2008). It was found that for wild-type strain, only compounds 2 (71.77), 6 (67.04), and 9 (75.75) showed lower PMF scores than the reference (80.9) and for mutant strain, most of the compounds showed lower PMF scores than the reference (91.5).

High negative ligand internal energy (i.e., sum of a van der Waals (vdW) term and an optional electrostatic term) defines the efficiency of ligand and it was found that all title hybrid analogs demonstrated significantly high negative values as compared to that of the reference for both wild and mutant strains (Bindewald & Skolnick 2005).

The dockScore function was used to evaluate aspirant ligand poses and higher dock score predicted better interaction (Jain 2006). The entire range of hybrid compounds 1-19 showed higher dock score ranging from 55.94 to 64.75, which suggested that all molecules have a similar mode of binding and significant binding affinity towards NNIBP receptor domain to that of the etravirine (55.05) in the case of the wild-type strain. In the case of mutant strain, dockscore ranged from 49.73 to 59.64 (Table 3).

The Ludi_2 value was used to predict the binding free energy (ΔG). The lower ΔG value of molecules reflected their high binding affinity with protein. The binding energy of the title molecules as well as of reference was analyzed from their corresponding docked conformation in the receptor. Results illustrated that for both wild type and mutant strains, the binding energy of compounds was similar to that of the reference (Muegge & Martin 1999; Lagos et al. 2008).

SAR Analysis

All molecules adopted 'horseshoe' conformation when docked with mutant HIV-1 RT. With wild-type HIV-1 RT, the molecules 3, 6, 8, 14, 15, and 18 showed 'horseshoe' conformation and the molecules 1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 16, and 17 adopted 'seahorse' conformation (Figure 2). In wild-type strain, ring A of compounds 2, 7, 11, 13, and 19 plus ring C of compounds 1, 4, 9, 10, 12, and 17 are embedded deep into a cylindrical tunnel having hydrophobic amino acids, which connects the NNRTI binding pocket to DNA/RNA binding cleft and favoured interaction with the more conserved amino acids Trp229 and Tyr318. In compounds 3, 5, 6, 8, 14, 15, 16, 17, and 18, the conformation of molecule aligned inside the NNIBP is organized in such a way that both rings A and C are flanked

Table 3. Scoring of ligands and stability of HIV-1 RT-ligand complexes (wild type).

Name	DS	-PLP1	-PLP2	-PMF	LIG	Ludi_2	ΔG	Ludi_3	Predicted EC_{50} (μM)
1	57.53	98.17	97.62	98.63	-6.17	505	-7.1	786	0.14×10^{-7}
2	61.55	83.01	80.5	71.77	-5.72	551	-7.8	850	0.03×10^{-7}
3	58.99	117.34	93.72	95.64	-5.21	486	-6.8	777	0.17×10^{-7}
4	58.84	102.9	102.91	94.96	-6.3	515	-7.3	681	1.54×10^{-7}
5	58.69	99.73	103.59	99.99	-6.48	564	-8.0	624	5.75×10^{-7}
6	62.02	110.27	101.41	67.04	-5.53	532	-7.5	641	3.89×10^{-7}
7	58.80	85.65	90.47	92.74	-6.32	506	-7.1	725	0.56×10^{-7}
8	60.34	118.22	98.35	99.47	-5.46	508	-7.2	743	0.37×10^{-7}
9	56.23	98.59	95.95	75.75	-6.39	511	-7.2	788	1.31×10^{-7}
10	64.75	91.3	74.43	93.41	-6.29	592	-8.3	827	0.05×10^{-7}
11	57.53	98.17	97.62	98.63	-8.07	454	-6.4	693	1.17×10^{-7}
12	58.30	101.41	94.94	84.55	-5.69	557	-7.9	801	0.09×10^{-7}
13	59.58	116.21	108.08	82.15	-5.82	572	-8.0	775	0.17×10^{-7}
14	60.10	121.12	102.91	112.05	-8.07	492	-6.9	694	1.14×10^{-7}
15	57.59	111.15	102.3	100.73	-6.04	467	-6.6	684	1.44×10^{-7}
16	60.34	118.22	98.35	99.47	-5.46	518	-7.3	672	1.90×10^{-7}
17	57.23	97.59	94.95	74.75	-6.29	492	-6.9	778	0.16×10^{-7}
18	58.70	90.45	80.37	106.11	-6.25	545	-7.7	660	2.51×10^{-7}
19	64.75	91.3	74.43	93.41	-6.86	494	-7.0	779	0.16×10^{-7}
Nevirapine	40.42	80.06	73.22	83.18	-0.11	470	-6.6	557	2.7×10^{-6}
Etravirine	55.05	107.62	100.08	80.9	-6.46	501	-7.1	766	2×10^{-8}
Rilpivirine	51.61	123.79	116.37	105.15	-7.17	592	-8.3	768	2×10^{-8}

DS, Dock score; -PLP, Piecewise Linear Potential; PMF, Potential of Mean Force; LIG, Lig-Internal_Energy (kcal⁄ mol) ; ΔG, Binding energy (kcal⁄ mol)

Table 4. Scoring of ligands and stability of HIV-1 RT-ligand complexes (mutant type).

Name	DS	-PLP1	-PLP2	-PMF	LIG	Ludi_2	ΔG	Ludi_3	Predicted EC_{50} (μM)
1	51.74	74.27	72.91	85.83	-6.92	417	-5.9	557	0.2×10^{-5}
2	56.62	81.93	80.99	94.69	-6.27	444	-6.3	563	0.2×10^{-5}
3	56.17	77.96	77.56	95.22	-5.93	443	-6.2	566	2×10-5
4	49.73	74.15	70.74	88.64	-7.01	430	-6.0	463	2.3×10^{-5}
5	56.02	69.31	65.72	90.41	-6.03	428	-6.0	582	0.1×10^{-5}
6	57.35	79.58	66.71	92.06	-5.91	432	-6.1	570	0.2×10^{-5}
7	54.85	68.47	67.46	82.21	-5.42	446	-6.1	550	0.3×10^{-5}
8	59.64	78.93	77.78	92.14	-8.01	424	-6.0	547	0.3×10^{-5}
9	50.90	71.36	67.63	83.55	-8.21	444	-6.3	570	0.2×10^{-5}
10	56.88	74.55	69.65	69.29	-7.42	455	-6.5	517	0.6×10^{-5}
11	56.88	65.13	57.91	92.76	-7.02	354	5.0	537	0.4×10^{-5}
12	50.19	68.91	69.96	73.39	-7.14	425	-6.0	549	0.3×10^{-5}
13	53.45	68.45	75.22	90.39	-6.54	404	-5.7	490	1.2×10^{-5}
14	57.83	67.95	62.7	79.25	-7.57	439	-6.2	534	0.4×10^{-5}
15	51.12	65.09	65.68	85.55	-4.45	465	-6.5	549	0.3×10^{-5}
16	55.56	62.69	60.73	91.83	-6.58	428	-6.0	551	0.7×10^{-5}
17	51.11	73.76	73.02	79.41	-6.95	409	-5.8	619	0.1×10^{-5}
18	53.16	61.51	58.33	55.31	-5.14	409	-5.8	457	2.7×10^{-5}
19	55.18	77.07	75.54	91.90	-6.54	385	-5.5	455	2.8×10^{-5}
Nevirapine	47.76	76.01	69.6	67.71	-5.88	388	-5.50	629	5.1×10^{-1}
Etravirine	60.78	128.8	118.11	91.5	-8.5	522	-7.40	742	3.8×10^{-2}
Rilpivirine	64.3	138.3	126.31	110.9	-9.08	578	-8.20	779	1.6×10^{-8}

DS, Dock score; -PLP, Piecewise Linear Potential; PMF, Potential of Mean Force; LIG, Lig-Internal_Energy (kcal⁄ mol); ΔG, Binding energy(kcal⁄ mol)

Table 5. Docking interaction of ligand with receptor in ligand-receptor docked complex.

Name	Interaction with WT HIV-1 RT						Interaction with MT HIV-1 RT					
	No. of HB	Amino acid in HB	D (A°)	No. of π-B	Amino acid in π-B	D (A°)	No. of HB	Amino acid in HB	D (A°)	No. of π-B	AA in π-B	D (A°)
1	3	Lys103 Gly190 Tyr188	2.8 3.0 3.1	1	Tyr181	4.7	1	Lys101	2.7	2	Trp229	4.7 5.3
2	1	Lys103	3.0	1	Tyr181	5.0	1	Lys101	1.9	1	Phe227	4.6
3	2	Lys101 Lys103	3.0 3.2	3	Tyr181 Tyr318 Tyr188	6.0 6.0 4.4	3	Cys181 Gln182 Ile180	2.6 2.2 1.9	4	Tyr 188 Phe227 Trp229 Tyr 183	3.7 6.0 4.0 5.1
4	2	Lys103 Tyr318	2.8 3.0	1	Tyr181	4.8	1	Ile180	2.0	0	--	--
5	2	Lys101 Tyr188	2.8 3.0	1	Tyr181	5.0	1	Thr165	2.5	0	--	--
6	2	Lys101	3.2 2.8	1	Tyr181		1	Asn103	2.0	2	Phe227 Tyr 318	5.0 4.5
7	1	Lys103	3.0	--	--	--	1	Lys101	1.6	0	--	--
8	1	Pro236	2.9	1	Tyr188	4.7	1	Asn103	2.1	2	Phe227 Tyr 318	4.4 4.7
9	3	Lys103 Tyr318 Gly190	2.7 3.0 3.1	--	--	--	2	Thr165 Gln182	2.0 2.1	0	--	--
10	4	Lys101 Tyr318 Gly190 Tyr188	2.9 3.1 2.7 2.9	1	Tyr181	4.0	2	Trp229 Ile180	2.5 1.9	0	--	--
11	2	Pro236 Val106	3.1 2.8	2	Tyr181 Trp229	4.1 6.5	2	Lys101 Asn103	1.8 2.1	2	Phe227 Tyr 318	4.6 4.5
12	2	Lys103 Gly190	2.7 3.0	--	--	--	1	Ile180	2.0	0	--	--
13	1	Pro236	2.9	1	Tyr181	4.7		--	--	1	Lys101	4.4
14	1	Gly190	3.1	--	--		2	Thr165 Asn103	1.9 1.9	1	Phe227	5.0
15	1	Tyr318	2.6	1	Tyr181	4.5	2	Lys101	3.0 2.5	0	--	--
16	1	Lys103	2.9	2	Tyr181 Tyr318	4.4 5.5	1	Asn103	2.0	1	Phe227	4.9
17	4	Lys103 Tyr318 Tyr188 Gly190	2.7 3.0 3.1 3.1	1	Tyr181	4.8	1	Ile180	2.0	0	--	--
18	0	--	--	1	Tyr181	5.7	1	Ile180	1.9	0	--	--
19	2	Tyr318	3.0 2.8	1	Tyr181	4.3	1	Asn103	2.0	1	Tyr 318	4.8
Nevirapine												
Etravirine	1	Lys101	3.0	2	Tyr181 Tyr318	6.0 5.0	2	Lys101 Asn103	2.8 3.0	--	--	--
Rilpivirine	1	Lys101	2.2	--	--	--	1	Asn103	3.0	--	--	--

H-B ,Hydrogen bond; D, Distance (A°) ; D-A , Donor Atom; A-A, Acceptor Atom; π - B , Pi bond

by amino acids forming a hydrophobic tunnel. Thus, both rings A and ring C are considered as important moieties for enhancing activity. Ring B served as the central moiety in compounds and played an important role in the stabilization within HIV-1 RT. The smaller substituent – at the *C-5* position of *N*-pyridin-*2-yl* (Ring C) – is crucial for achieving high potency. It can be concluded that bulky substituent at position *C-5* on ring C collides within the protein pocket, resulting in some conformational changes that lower the potency of the molecules. The rotation around the sulfonamide and thiobenzamide linkages provides additional conformational flexibility, allowing some molecules of the series to tolerate the HIV-RT conformational changes. Smaller substituents like –F, –H and $–NO_2$ are beneficial on Ring A.

SAR studies on all inhibitors revealed that sulfonamide linkage played a pivotal role in the stabilization of protein-ligand complex. As discussed previously, the sulfonamides containing H-bond acceptors directly interacted with a Lys103 backbone of HIV-1 RT, which was similar to interactions shown by many "second generation inhibitors." In the case of compounds 4, 9, and 12, sulfonamide linkage directly interacted with a Lys103 backbone of HIV-1 RT; in the case of compounds 1, 5, 7, and 10, sulfonamide linkage interacted with Lys101. The SAR analysis showed that the combined action of the substituent at the *C-5* position of *N*-pyridine- (Ring C) and *C-4* position of the benzenesulfonylamino (Ring A) influenced the anti-HIV-1 of the newly designed molecules against wild-type and mutant HIV-1 strain.

It has previously been reported that the Lys103Asn mutation introduces a network of hydrogen bonds that stabilize the conformation of RT, which interferes with the binding of an NNRTI. Although the Lys103Asn mutation usually causes resistance, there are NNRTIs (e.g., etravirine and rilpivirine) that interact specifically with Asn103 and are, therefore, effective against the Lys103Asn mutants. The interaction of compounds 6, 8, 11, 14, and 16 with Asn103 was conserved in the mutant strain. In the wild-type, HIV-1 RT complex, all compounds except compound 7, 9, 12, and 14 interacted with Tyr181;in the mutant strain, all compounds (except compound 3) exhibited broken interaction with Tyr181. Thus, on the basis of information about the binding site and SAR results, the researchers designed new target compounds with diverse substituents on both the ring A and C, which could penetrate the hydrophobic cylindrical tunnel of the binding site and reached the conserved amino acids.

CONCLUSION

Though docking study cannot affirm the effectiveness of a drug in reality, the technique is essentially part and parcel of novel drug design, which has already gained considerable acceptance globally. It provides an assortment of hope in the drug discovery process. Using this process, the researchers observed that most of the compounds have shown horse-shoe like orientation around the allosteric binding site, which was supposed to corroborate with information from the literature. The surrounding alignment of the designed molecules showed the exact residue alignment as per the need of the NNIBP pocket. The *in silico* studies further revealed high binding or stabilization energy, in turn of HIV-RT (wild type and mutant)-ligand complexes, which indicated towards lower EC_{50} values dressed for the molecules. The molecules can be further obtained by using more appropriate substitution at R_1 and R_2 position using simulations for better results. In conclusion, the researchers have utilized molecular modeling to design novel *N*- (Pyridin-*2-yl*) thiobenzamide based HIV-1 non-nucleoside RT inhibitors, showing considerable potency against wild and mutant type HIV-1 strains.

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