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Original article

Rhodium(II) acetate-catalyzed stereoselective synthesis, SAR and anti-HIV activity of novel oxindoles bearing cyclopropane ring☆

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ABSTRACT

Novel oxindole derivatives bearing substituted cyclopropane ring have been designed on the basis of docking studies with HIV-1 RT using the software DS 2.5 and synthesized as probable NNRTIs against HIV-1 using rhodium(II) acetate-catalyzed stereoselective cyclopropanation reaction. The cyclopropane isomer, having *trans* relationship with respect to carbonyl of lactam moiety and functional group on the cyclopropane ring, was the major product in all cases along with a small amount of *cis* and methylene products. The *trans* isomers interacted well with HIV-1 RT through H-bonding with amino acids, like Lys101, Lys103, His235, Tyr318, constituting the non-nucleoside inhibitor binding pocket (NNIBP) during docking experiments. However, the compounds showed very little activity when subjected to *in vitro* anti-HIV-1 screening using β-galactosidase assay (TZM-bl cells) and GFP quantification (CEM-GFP cells). The very low level of *in vitro* HIV inhibition, in comparison to predicted EC_{50} values on the basis of computational studies, during CEM-GFP screening using AZT as positive control indicated that probably the HIV RT is not the viral target and the molecules work through some different mechanism.

1. Introduction

HIV, the causative agent of AIDS, has been one of the major challenges for almost three decades before the scientific community all over the world. A large number of molecules are currently being developed as antiviral agents targeting various viral enzymes [1–7]. The enzyme HIV reverse transcriptase (HIV RT) is a vital enzyme in the life cycle of HIV and is a prime target for developing anti-HIV drug molecules.

The HIV RT inhibitors used currently are of two types: nucleos(t)ide reverse transcriptase inhibitors (N(t)RTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The N(t)RTIs are competitive inhibitors, interact at the dNTP binding site on HIV RT and act as chain terminators, whereas the NNRTIs are non-competitive inhibitors and bind at the allosteric site of HIV RT located about 10 Å away from the dNTP binding site [8], change the conformation of HIV RT and make it unsuitable for accepting incoming dNTPs required for cDNA synthesis. Till date, four NNRTIs - nevirapine, delavirdine, efavirenz and etravirine, have been approved for clinical use [9,10] (Fig. 1). A combination of NRTIs and NNRTIs with protease inhibitors, integrase inhibitors or fusion inhibitors has led to the development of Highly Active Antiretroviral Therapy (HAART), the current way of treating AIDS patients.

Heterocyclic molecules consisting of cyclopropane functionality have been reported to exhibit interesting biological properties [11–19]. The approved NNRTIs against HIV, viz. nevirapine and efavirenz, too possess cyclopropane moiety as structural unit. Oxindoles have also been studied, in past, for their anti-HIV activity [20,21]. On the basis of molecular modeling studies, we have designed and synthesized some novel oxindole derivatives using $Rh_2(OAc)_4$ as catalyst for stereocontrolled products. The oxindole derivatives have been screened for their anti-HIV activity.

2. Chemistry

The cyclopropane derivatives of oxindoles were synthesized starting from substituted isatin. Isatin was first reacted with tosyl hydrazine to yield tosyl hydrazone, which was then treated with NaOH to get the diazolactams [21]. The diazolactam was treated with olefin(s) in the presence of $Rh_2(OAc)_4$ under N_2 atmosphere to yield *cis* and *trans* cyclopropane products along with a small amount of olefin product when allyl alcohol was used, whereas with allyl chloride no olefin side product was formed (Scheme 1).

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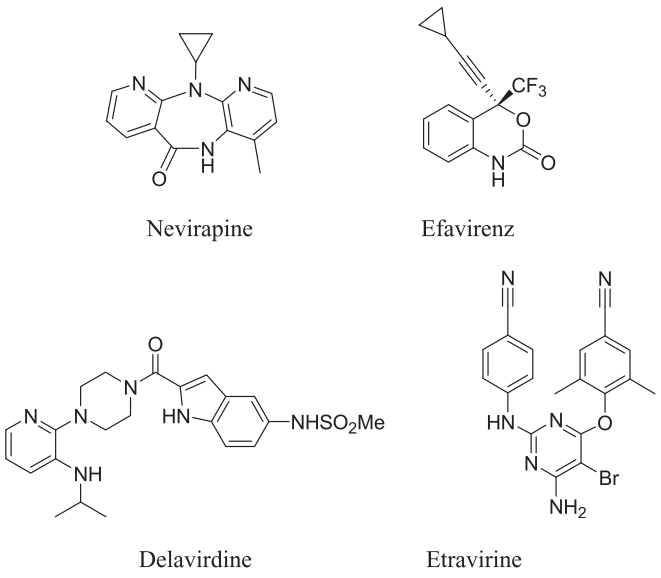


Fig. 1. Approved NNRTIs as drug molecules against HIV-1.

Cyclopropanation reactions of diazolactams using rhodium(II) acetate as catalyst are not so common like that of diazoacetates and diazoketones. The products **3**–**10** were separated by silica gel column chromatography using petroleum ether and ethyl acetate (9:1) as eluent. The products **3a**–**10a** were separated and further analyzed using RP-HPLC (acetonitrile-H_2O (70:30, v/v) as eluent) and characterized using proton NMR spectroscopy.

The *trans* isomer was formed as the major product in all cases along with a small amount of the *cis* and alkene products (Table 1). The formation of *trans* isomer as the major product was confirmed by proton NMR analysis on the basis of higher coupling constant (*J*) values. The formation of *trans* stereoisomer as a major product can be explained using mechanistic model as proposed by Doyle [22–24], which suggests that stereoisomeric pi-complexes are reversibly formed. The R′ group of the alkene favoring an orientation away from the bulky metal, leads to transition states T_t and T_c. Only the transition state leading to *trans* isomer is stabilized because of interaction between developing electrophilic character on carbon of alkene and nucleophilic carbonyl oxygen. The nucleophilicity of carbonyl oxygen is also enhanced because of the presence of nearby *N* in the lactam moiety. This stabilization can only occur in the case of T_t leading to *trans* isomer and not in the case of T_c leading to *cis* isomer. The present results thus derive support from Doyle's interpretation (Scheme 2).

Table 1
Yield of *cis-trans* isomers and their HPLC analyses[a]

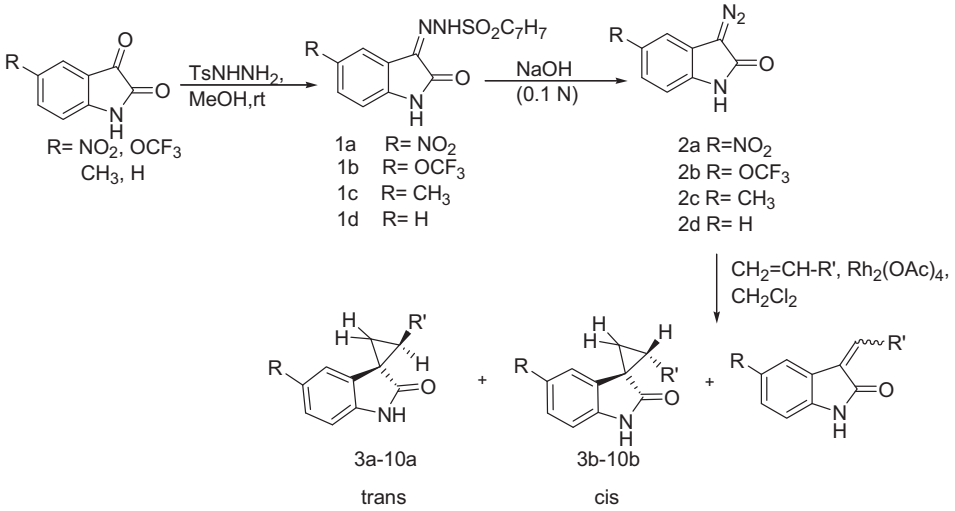
Compound	R	R′	Yield (%)[a]	Retention time (min) on RP-HPLC
3a – *Trans*	$-NO_2$	$-CH_2OH$	45	15.5
3b – *Cis*	–	–	9	16.1
4a – *Trans*	$-NO_2$	$-CH_2Cl$	66	16.0
4b – *Cis*	–	–	8	16.5
5a – *Trans*	$-OCF_3$	$-CH_2Cl$	72	14.8
5b – *Cis*	–	–	11	15.5
6a – *Trans*	$-OCF_3$	$-CH_2OH$	49	14.2
6b – *Cis*	–	–	6	14.8
7a – *Trans*	$-CH_3$	$-CH_2Cl$	53	13.6
7b – *Cis*	–	–	4	14.3
8a – *Trans*	$-CH_3$	$-CH_2OH$	51	13.9
8b – *Cis*	–	–	7	14.3
9a – *Trans*	–H	$-CH_2Cl$	60	14.8
9b – *Cis*	–	–	9	15.7
10a – *Trans*	–H	$-CH_2OH$	57	13.7
10b – *Cis*	–	–	5	14.4

[a] Isolated by silica gel column chromatography using petroleum ether: ethyl acetate (9:1) as eluent and analyzed by RP-HPLC (acetonitrile: H_2O (70:30); flow rate 1 mL/min).

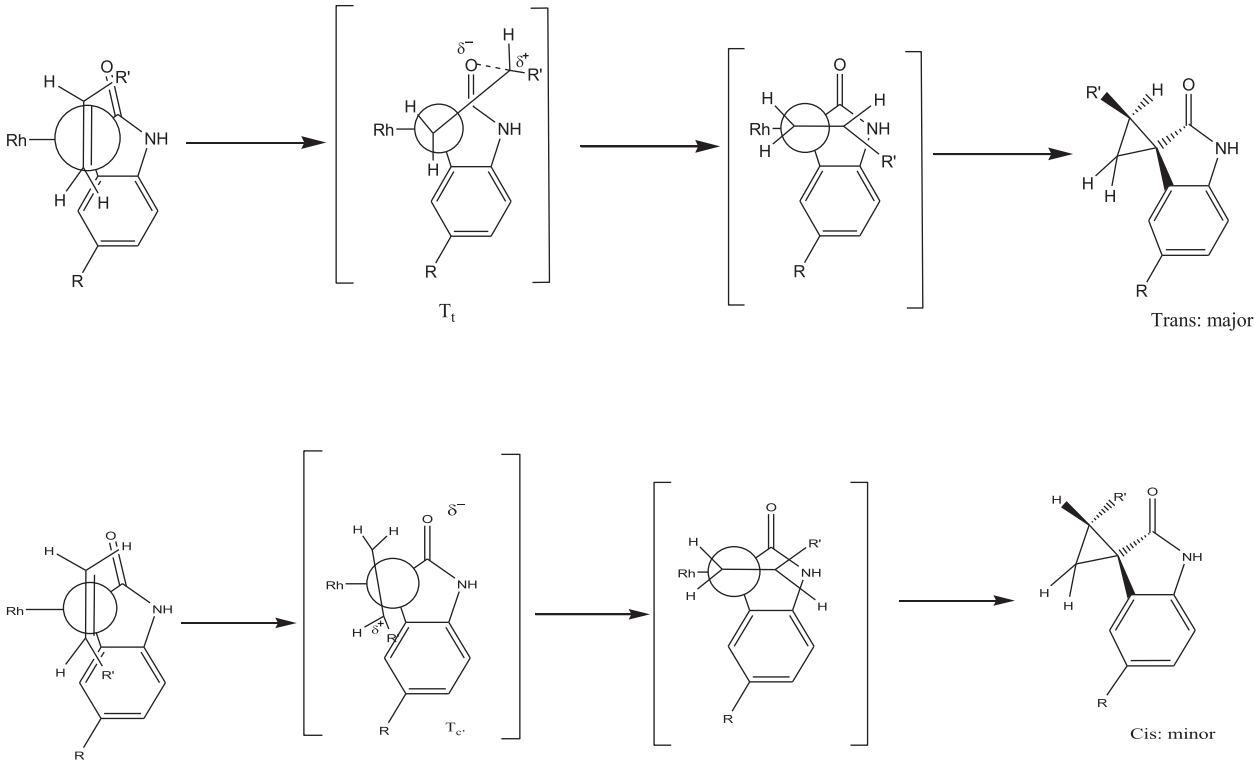
3. Results and discussion

3.1. Molecular modeling and biological evaluation

All molecules designed obeyed the Lipinski's Rule of Five (Table 2). The molecules were docked into the allosteric site of HIV-1 RT to study molecular interactions. Docking studies revealed that when $-CH_2OH$ was present as a substituent on the cyclopropane ring, one H-bond was formed between –OH of the compound and Lys103 as in the case of compound **3a**, whereas in the case of compound **6a**, the H-bond was formed with Lys101. However, in the case of compounds **8a** and **10a**, no H-bond was formed. In the case of **9a**, one H-bond was formed with the NH of the lactam moiety and Lys101 of HIV RT. Both the amino acids, viz. Lys101 and Lys103, lie in



Scheme 1. Synthesis of oxindole derivatives bearing cyclopropane ring using $Rh_2(OAc)_4$ as stereoselective catalyst.



Scheme 2. Mechanism of cyclopropanation showing trans isomer as major product.

the non-nucleoside inhibitor binding pocket (NNIBP) of HIV-1 RT and hence interactions with these amino acids are very crucial. However, when $-CH_2Cl$, a more hydrophobic group, was present as a substituent on the cyclopropane ring, no H-bond was formed between the −Cl and amino acid residues of the NNIBP (Fig. 2).

In the case of compounds **3a** and **7a**, H-bonds were formed between C═O of the oxindole moiety and His235 and Tyr318 of the NNIBP. No such bonds were formed with **4a**, **5a** and **6a.**

To further explore the SAR, the role of substituents at 5-position of benzene ring of oxindole nucleus was analyzed. Four types of derivatives, bearing a nitro group (**3a** and **4a**), a trifluoromethoxy group (**5a** and **6a**), a methyl group (**7a** and **8a**) and hydrogen (**9a** and **10a**) were studied. The nitro group in compound **3a** formed a H-bond with Lys101 in the NNIBP. In the case of compound **4a**, the nitro substituent alone formed seven H-bonds with Lys101 and Tyr181 in the NNIBP. When the nitro group was replaced by trifluoromethoxy group, methyl or hydrogen, no H-bond was formed between these groups and amino acid residues in the NNIBP, as in the case of compounds **5a**–**10a**. But the predicted EC_{50} values of the substituents having trifluoromethoxy group (**5a** and **6a**) were much higher than their counterparts. This may be because of better orientation of these derivatives in the NNIBP. The predicted EC_{50} values (μg/mL) of all derivatives on the basis of their Ludi_3 score, a computational assessment of level of interaction between the molecules and HIV RT, are presented in Table 3, where the EC_{50} value for compound **6a** was the lowest one.

Further, during docking studies, the lowering of docking energy reflected the stability of HIV RT-ligand complexes. The results indicated that most of the ligands moved into a stable position comparable to nevirapine and efavirenz. However, compound **6a** formed most stable complex with lowest docking energy (−50.81 kcal/mol) as shown in Table 4.

Analysis of the anti-HIV screening results (Table 5) confirmed the computational data. All compounds have shown only slight inhibition of HIV growth under *in vitro* condition using TZM-bl and CEM-GFP cell lines. The low level of anti-HIV activity shown by the molecules specially during CEM-GFP screening using AZT as positive control, in contrast to the predicted EC_{50} values on the basis of docking studies with HIV RT, indicated towards the possibility of HIV RT being not the viral target and the molecules adopt some other mechanism to show anti-HIV activity. This observation is also supported by the difference shown in the predicted and experimental EC_{50} values in Table 3 and Table 5, respectively. However, the compound **6a** showed some better result. Because of very low HIV-1 inhibition during preliminary investigations at two different concentrations, i.e., 2 and 20 μg/mL, no further experiments were performed for calculation of EC_{50} and CC_{50} values.

In silico studies revealed that none of the molecules had orientation similar to efavirenz. In the case of efavirenz, three H-bonds were formed, two between NH and CO moieties with Lys101 and one with CO moiety and Lys103 (Fig. 3). Thus, the lactam moiety is crucial for interactions with HIV RT. However, in the present case, only **3a**, **8a** and **9a** formed H-bonds through their CO moiety with His235 and Tyr318. Further, at C-5 position, in the case of efavirenz, Cl is present, whereas in the case of **3a**–**10a**, NO_2, OCF_3, CH_3 and H are present. Thus, it may be assumed that at 5-position on benzene ring smaller electronegative groups are preferred for better orientation of the molecules in the hydrophobic pocket.

Table 2
Oxindole derivatives **3a**–**10a** in the eyes of Lipinski's Rule.

Compound	Formula	Mol. Wt.	H-acceptors	H-donors	logP	TPSA
3	$C_{11}H_{10}N_2O_4$	234.21	6	2	0.54	95.15
4	$C_{11}H_9N_2O_3$	252.65	5	1	1.84	74.92
5	$C_{12}H_9ClF_3NO_2$	234.21	3	1	3.48	38.33
6	$C_{12}H_{10}F_3NO_3$	273.21	4	2	1.89	58.56
7	$C_{12}H_{12}ClNO$	203.24	2	1	2.44	29.10
8	$C_{12}H_{13}NO_2$	221.68	3	2	1.21	40.33
9	$C_{11}H_{11}NO_2$	189.08	2	1	2.19	29.10
10	$C_{11}H_{10}ClNO$	207.66	3	2	1.95	49.35

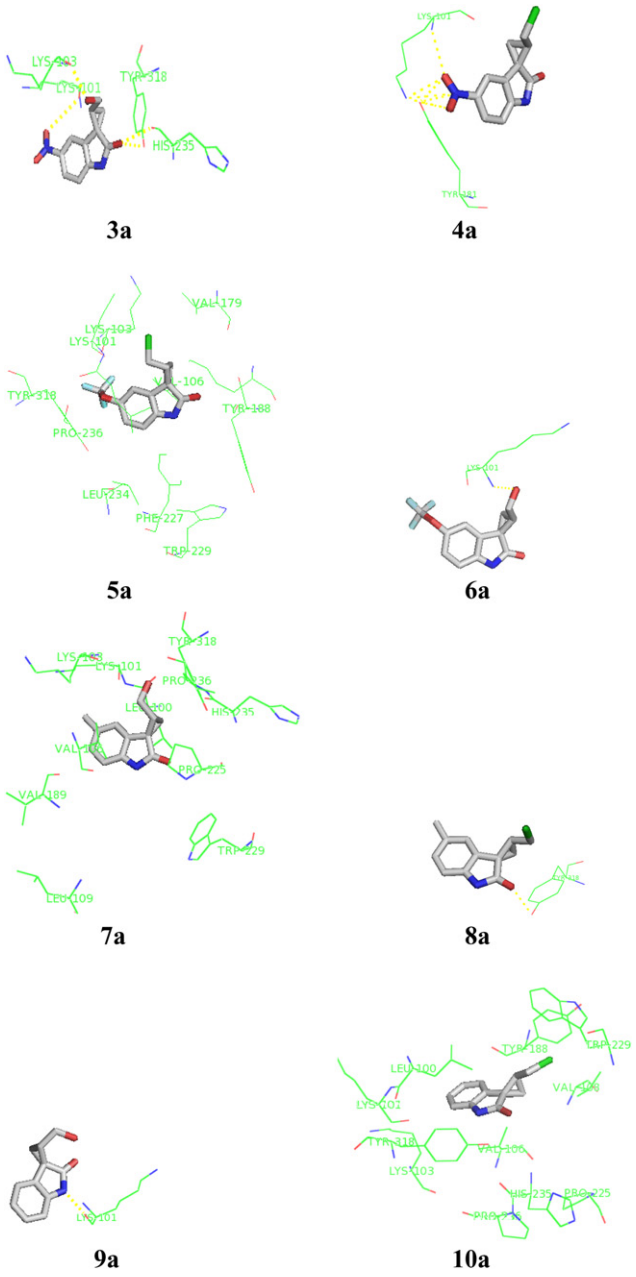


Fig. 2. Binding of **3a**–**10a** into allosteric site of wild-type HIV-1 RT (only main surrounding residues are shown in green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

In summary, we have carried out $Rh_2(OAc)_4$ catalyzed stereoselective synthesis of novel oxindoles with cyclopropane moiety as probable NNRTIs against HIV-1 on the basis of molecular modeling studies using the software Discovery Studio 2.5. The compounds showed very low anti-HIV activity under *in vitro* conditions using TZM-bl and CEM-GFP cell lines. The compound **6a** expressed a little better result. It was assumed that although the compounds showed some anti-HIV activity, HIV RT was not the target of the molecules under study on the basis of *in vitro* experimental and computational results. All molecules, however, showed almost nil or very little cytotoxicity, which indicated towards the possibility of exploring the potential of oxindoles as anti-HIV molecules.

Table 3
Ludi_3 score and predicted EC_{50} of **3a**–**10a** along with number of H-bonds formed with NNIBP.

Compound	R, R′	Ludi_3 Score[a]	Predicted EC_{50} value (μg/mL)	No. of H-bonds formed
3a	NO_2, CH_2OH	488	3.0	4
4a	NO_2, CH_2Cl	420	15.9	7
5a	OCF_3, CH_2Cl	515	1.6	0
6a	OCF_3, CH_2OH	530	1.4	1
7a	CH_3, CH_2Cl	483	3.1	1
8a	CH_3, CH_2OH	418	14.4	0
9a	H, CH_2OH	453	5.3	1
10a	H, CH_2Cl	416	13.7	0

[a] Higher score predicts better interaction and thus better EC_{50} value.

5. Experimental

5.1. Chemistry

Silica gel G for TLC was obtained from E. Merck India Ltd. All reactions were performed in oven-dried flasks and solvents used were anhydrous. Melting points determined using an electrothermal apparatus are uncorrected. HPLC analysis was carried out on 6 AD Binary Gradient Shimadzu HPLC systems. Mass spectra were recorded on Micromass Quattro II. Fluorescence detection was done using FLUOstar Optima, BMG Labtech, Germany. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 MHz Fourier transform spectrometer and using an internal deuterium lock. Chemical shifts are quoted in parts per million downfield from tetramethylsilane.

5.1.1. General procedure for synthesis of compounds *3a*–*10a*

Synthesis of desired oxindole derivatives through cyclopropanation of diazolactams started from the corresponding isatin, which was reacted with tosyl hydrazine (1 equiv) in dry methanol. The reaction mixture was allowed to stand overnight, **1**(**a**, **b**) obtained as a precipitate was filtered and washed with cold methanol and dried. **1**(**a**, **b**) was then treated with 0.1 N NaOH (1 equiv) and the mixture was allowed to stand at room temperature for 24 h. The diazoketone **2**(**a**, **b**) obtained as a precipitate was then filtered and air-dried. For cyclopropanation reaction, olefin (allyl chloride or allyl alcohol) (10 equiv) in dry dichloromethane and $Rh_2(OAc)_4$ (0.001 equiv) were taken in a nitrogen-flushed round bottomed flask, and to this, **2**(**a**, **b**) dissolved in dry dichloromethane was added slowly over 1 h with continuous stirring under N_2 atmosphere. Completion of reaction was confirmed by TLC, which showed disappearance of **2**. The reaction mixture was then filtered, concentrated and the products **3**–**10** were isolated from the reaction mixture by silica gel column chromatography using petroleum ether and ethyl acetate (9:1, *v*/*v*) as eluent. The separation of the

Table 4
Discovery Studio 2.5 docking results showing the stability of HIV-1 RT – ligand complexes.

Ligand	Docked energy (kcal/mol)		
	Van der Waals	Electrostatic	Total
3a	−33.37	−14.05	−47.42
4a	−34.34	−14.76	−49.10
5a	−36.71	−5.98	−42.69
6a	−35.18	−15.63	−50.81
7a	−37.67	−4.49	−42.16
8a	−31.92	−7.01	−38.93
9a	−29.21	−4.68	−33.89
10a	−30.67	−2.41	−33.08
Nevirapine	−38.53	−7.46	−45.99
Efavirenz	−39.84	−4.14	−43.98

Table 5
Cytotoxicity and anti-HIV Activity of compounds **3a**–**10a**.

Compound	Cell viability (%) by MTT assay								Anti-HIV activity	
	Using TZM-bl cells				Using CEM-GFP cells				Using TZM-bl cells	Using CEM-GFP cells
	10 μg	20 μg	50 μg	100 μg	10 μg	20 μg	50 μg	100 μg	% Inhibition at 2 μg (β- galactosidase assay)	% Inhibition at 20 μg (GFP quantification)
3a	100	107	104	109	119	127	122	116	5	03
4a	102	102	101	92	101	118	103	76	6	04
5a	91	95	66	60	94	74	37	02	9	03
6a	90	93	62	60	91	72	33	03	10	07
7a	93	91	88	87	92	89	85	81	8	05
8a	102	100	95	93	105	101	100	100	6	03
9a	99	98	92	89	96	95	92	90	4	06
10a	93	92	87	86	91	87	83	88	7	02

isomers **3a**–**10a** was done using C18 column (Phenomenex Luna C18(2), 250 × 4.6 mm^2, flow rate of 1 mL/min) on RP-HPLC (acetonitrile - H_2O (70:30, *v*/*v*) as eluent) and the isomers were characterized using proton NMR spectroscopy.

*5.1.1.1. (1R, 2R)-2-(Hydroxymethyl)-5′-nitrospiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**3a**).* Mp 140–142 °C; R_f = 0.43 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.34–7.78 (m, 3H, Ar-H), 8.02 (s, 1H, CONH), 3.53 (d, 2H, CH_2), 1.76–1.52 (m, 1H, CH), 1.27 (d of d, *J* = 10.1, 5.7 Hz, 1H), 0.9 (d of d, *J* = 7.1, 5.7 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) 234.06 (100); Anal. Calcd for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.32; H, 4.39; N, 11.84.

*5.1.1.2. (1R, 2S)-2-(Hydroxymethyl)-5′-nitrospiro [cyclopropane-1,3′-indol]-2′(1′H)-one (**3b**).* Mp 141–143 °C; R_f = 0.41 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.04–7.82 (m, 3H, Ar-H), 7.92 (s, 1H, CONH), 3.49 (d, 2H, CH_2), 1.55–1.35 (m, 1H, CH), 1.25 (d of d, *J* = 6.7, 5.8 Hz, 1H), 1.00 (d of d, *J* = 9.5, 5.8 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) 234.06 (100); Anal. Calcd for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N, 11.96 Found: C, 56.32; H, 4.39; N, 11.84.

*5.1.1.3. (1R, 2R)-2-(Chloromethyl)-5′-nitrospiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**4a**).* Mp 149 °C; R_f = 0.47 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32–7.89 (m, 3H, Ar-H), 8.02 (s, 1H, CONH), 3.39 (d, 2H, CH_2), 1.67–1.42 (m, 1H, CH), 1.26 (d of d, *J* = 10.2, 5.9 Hz, 1H), 0.92 (d of d, *J* = 7.3, 5.9 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) *m*/*z* 252.03 (100); Anal. Calcd for $C_{11}H_9ClN_2O_3$: C, 52.29; H, 3.59; Cl, 14.03; N, 11.09. Found: C, 52.21; H, 3.65; Cl, 14.05; N, 11.02.

*5.1.1.4. (1R, 2S)-2-(Chloromethyl)-5′-nitrospiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**4b**).* Mp 148–150 °C; R_f = 0.45 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.12–7.94 (m, 3H, Ar-H), 7.87 (s, 1H, CONH), 3.34 (d, 2H, CH_2), 1.53–1.31 (m, 1H, CH), 1.24 (d of d, *J* = 6.8, 5.7 Hz, 1H), 1.02 (d of d, *J* = 9.8, 5.8 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) 252.03 (100); Anal. Calcd for $C_{11}H_9ClN_2O_3$: C, 52.29; H, 3.59; Cl, 14.03; N, 11.09. Found: C, 52.21; H, 3.65; Cl, 14.05; N, 11.02.

*5.1.1.5. (1R, 2R)-2-(Chloromethyl)-5′-(trifluoromethoxy)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**5a**).* Mp 150–153 °C; R_f = 0.41 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.1(s, 1H, CONH), 7.65–6.82 (m, 3H, Ar-H), 3.36 (d, 2H, CH_2), 1.76–1.52 (m, 1H, CH), 1.27 (d of d, *J* = 10.1, 5.7 Hz, 1H), 0.94 (d of d, *J* = 7.2, 5.6 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) 291.03 (100); Anal. Calcd for $C_{12}H_9ClF_3NO_2$: C, 49.42; H, 3.11; Cl, 12.16; F, 19.54; N, 4.80 Found: C, 49.31; H, 3.21; Cl, 12.09; F, 19.63; N, 4.84.

*5.1.1.6. (1R, 2S)-2-(Chloromethyl)-5′-(trifluoromethoxy)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**5b**).* Mp 152–153 °C; R_f = 0.38 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.9 (s, 1H, CONH), 7.45–6.62 (m, 3H, Ar-H), 3.32 (d, 2H, CH_2), 1.52–1.32 (m, 1H, CH), 1.23 (d of d, *J* = 6.8, 5.7 Hz, 1H), 1.03 (d of d, *J* = 9.8, 5.8 Hz, 1H); MS (EI^+,): *m*/*z* (%) 291.03 (100); Anal. Calcd for $C_{12}H_9ClF_3NO_2$: C, 49.42; H, 3.11; Cl, 12.16; F, 19.54; N, 4.80 Found: C, 49.31; H, 3.21; Cl, 12.09; F, 19.63; N, 4.84.

*5.1.1.7. (1R, 2R)-2-(Hydroxymethyl)-5′-(trifluoromethoxy)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**6a**).* Mp 121–122 °C; R_f = 0.41 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.2 (s, 1H, CONH), 7.75–6.96 (m, 3H, Ar-H), 3.53 (d, 2H, CH_2), 1.78–1.54 (m, 1H, CH), 1.28 (d of d, *J* = 10.1, 5.7 Hz, 1H), 0.93 (d of d, *J* = 7.3, 5.7 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) 273.06 (100); Anal. Calcd for $C_{12}H_{10}F_3NO_3$: C, 52.75; H, 3.69; F, 20.86; N, 5.13 Found: C, 52.24; H, 3.69; F, 20.98; N, 5.28.

*5.1.1.8. (1R, 2S)-2-(Hydroxymethyl)-5′-(trifluoromethoxy)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**6b**).* Mp 121–123 °C; R_f = 0.40 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.0 (s, 1H, -CONH), 7.47–6.67 (m, 3H, Ar-H), 3.47 (d, 2H, CH_2), 1.55–1.35 (m, 1H, CH), 1.25 (d of d, *J* = 6.7, 5.8 Hz, 1H), 1.00 (d of d, *J* = 9.5, 5.8 Hz, 1H); MS (EI^+, 70 eV): *m*/*z* (%) = 273.06 (100); Anal. Calcd for $C_{12}H_{10}F_3NO_3$: C, 52.75; H, 3.69; F, 20.86; N, 5.13 Found: C, 52.24; H, 3.69; F, 20.98; N, 5.28.

*5.1.1.9. (1R, 2R)-2-(Chloromethyl)-5′-methylspiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**7a**).* Mp 210–212 °C; R_f = 0.39 (DCM-MeOH,

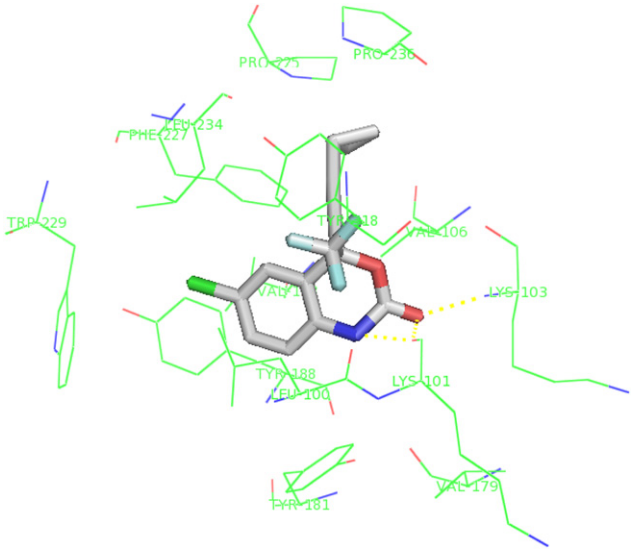


Fig. 3. Binding of efavirenz into allosteric site of wild-type HIV-1 RT.

9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.12 (s, 1H, −CONH), 7.64–6.8 (m, 3H, Ar-H), 3.36 (d, 2H, $-CH_2$), 2.33(s, 3H, CH_3), 1.74–1.54 (m, 1H, −CH), 1.27 (d of d, J = 10.1, 5.7 Hz, 1H), 0.96 (d of d, J = 7.2, 5.6 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 221.06 (100); Anal. Calcd for $C_{12}H_{12}ClNO$: C, 65.02; H, 5.46; Cl, 15.99; N, 6.32 Found: C, 64.99; H, 5.43; Cl, 15.95; N, 6.30.

*5.1.1.10. (1R, 2S)-2-(Chloromethyl)-5′-methylspiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**7b**).* Mp 208–210 °C; R_f = 0.37(DCM-MeOH, 9.8:0.2); ^{1}H NMR (44 MHz, DMSO-d_6): δ = 7.98 (s, 1H, -CONH), 7.44–6.68 (m, 3H, Ar-H), 3.32 (d, 2H, $-CH_2$), 2.42(s, 3H, CH_3), 1.54–1.34 (m, 1H, −CH), 1.24 (d of d, J = 6.8, 5.7 Hz, 1H), 1.11 (d of d, J = 9.8, 5.8 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 221.06 (100); Anal. Calcd for $C_{12}H_{12}ClNO$: C, 65.02; H, 5.46; Cl, 15.99; N, 6.32 Found: C, 64.99; H, 5.43; Cl, 15.95; N, 6.30.

*5.1.1.11. (1R, 2R)-2-(Hydroxymethyl)-5′-methylspiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**8a**).* Mp 220–223 °C; R_f = 0.42(DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.16 (s, 1H, −CONH), 7.82–6.94 (m, 3H, Ar-H), 3.56 (d, 2H, $-CH_2$), 2.48 (s, 3H, CH_3), 1.76–1.54 (m, 1H, -CH), 1.29 (d of d, J = 10.1, 5.7 Hz, 1H), 0.94 (d of d, J = 7.3, 5.7 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 203.09 (100); Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89 Found: C, 70.91; H, 6.42; N, 6.86.

*5.1.1.12. (1R, 2S)-2-(Hydroxymethyl)-5′-methylspiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**8b**).* Mp 221–222 °C; R_f = 0.39(DCM-MeOH, 9.8:0.2); ^{1}H NMR (44 MHz, DMSO-d_6): δ = 8.0 (s, 1H, −CONH), 7.49–6.68 (m, 3H, Ar-H), 3.48 (d, 2H, $-CH_2$), 2.44 (s, 3H, CH_3), 1.54–1.36 (m, 1H, −CH), 1.26 (d of d, J = 6.7, 5.8 Hz, 1H), 1.02 (d of d, J = 9.5, 5.8 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 203.09 (100); Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89 Found: C, 70.91; H, 6.42; N, 6.86.

*5.1.1.13. (1R, 2R)-2-(Hydroxymethyl)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**9a**).* Mp 200–202 °C; R_f = 0.38(DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.14 (s, 1H, −CONH), 7.92–7.22 (m, 4H, Ar-H), 3.39 (d, 2H, $-CH_2$), 1.67–1.42 (m, 1H, −CH), 1.26 (d of d, J = 10.2, 5.9 Hz, 1H), 0.92 (d of d, J = 7.3, 5.9 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 189.08 (100); Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40 Found: C, 69.81; H, 5.82; N, 7.34.

*5.1.1.14. (1R, 2S)-2-(Hydroxymethyl)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**9b**).* Mp 199–201 °C; R_f = 0.37(DCM-MeOH, 9.8:0.2); ^{1}H NMR (44 MHz, DMSO-d_6): δ = 7.98 (s, 1H, −CONH), 7.82–7.04 (m, 4H, Ar-H), 3.34 (d, 2H, $-CH_2$), 1.53–1.31 (m, 1H, −CH), 1.24 (d of d, J = 6.8, 5.7 Hz, 1H), 1.02 (d of d, 9.8, 5.8 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 189.08 (100); Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40 Found: C, 69.81; H, 5.82; N, 7.34.

*5.1.1.15. (1R, 2R)-2-(Chloromethyl)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**10a**).* Mp 189–190 °C; R_f = 0.43(DCM-MeOH, 9.8:0.2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.12 (s, 1H, −CONH), 7.88–7.16 (m, 4H, Ar-H), 3.53 (d, 2H, $-CH_2$), 1.76–1.52 (m, 1H, −CH), 1.27 (d of d, J = 10.1, 5.7 Hz, 1H), 0.9(d of d, J = 7.1, 5.7 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 207.05 (100); Anal. Calcd for C, 63.62; H, 4.85; Cl, 17.07; N, 6.75 Found: C, 63.56; H, 4.82; Cl, 17.06; N, 6.7.

*5.1.1.16. (1R, 2S)-2-(Chloromethyl)spiro[cyclopropane-1,3′-indol]-2′(1′H)-one (**10b**).* Mp 188–190 °C; R_f = 0.42 (DCM-MeOH, 9.8:0.2); ^{1}H NMR (44 MHz, DMSO-d_6): δ = 8.02 (s, 1H, −CONH), 8.78–7.02 (m, 4H, Ar-H), 3.49 (d, 2H, $-CH_2$), 1.55–1.35 (m, 1H, −CH), 1.25 (d of d, J = 6.7, 5.8 Hz, 1H), 1.00 (d of d, J = 9.5, 5.8 Hz, 1H); MS (EI$^+$, 70 eV): m/z (%) = 207.05 (100); Anal. Calcd for C, 63.62; H, 4.85; Cl, 17.07; N, 6.75 Found: C, 63.56; H, 4.82; Cl, 17.06; N, 6.7.

5.2. Biological evaluation

5.2.1. Anti-HIV screening using TZM-bl cell lines

The molecules were tested for their anti-HIV activity employing TZM-bl cell lines. TZM-bl cells (2.0×10^5) were seeded in 12-well plates and cultured overnight at 37 °C in humidified atmosphere of 5% CO_2. Infective HIV-1 virus isolate at a multiplicity of infection (MOI) of 0.025 was treated at 37 °C for 1 h with respective compounds in a total reaction volume of 100 μL. After incubation, the virus with various treatments was added to TZM-bl cells growing in 12-well plates and was further incubated at 37 °C for 4 h in the humidified atmosphere of 5% CO_2. After 4 h, medium was removed, cells washed twice with serum free DMEM medium and fresh growth medium (DMEM + 10% FCS) was added. Various compounds were added again in the respective wells and plates were further incubated at 37 °C for 48 h. After incubation, the cells were washed twice with phosphate buffer saline and fixed in 1% glutaraldehyde for 10 min. The cells were stained with 1 mL of staining solution at 37 °C for 24 h. The blue stained cells were counted under the microscope. Inhibition was calculated by taking the number of blue foci in experimental group divided by number of blue foci in infected cells in absence of any test extract/AZT multiplied by hundred. Percent inhibition was calculated by subtracting above value from hundred.

5.2.2. Anti-HIV screening using CEM-GFP cell lines

CEM-GFP (5×10^6) cells were taken and washed in polybrene (2 μg/mL) containing RPMI-1640 medium. Cells were resuspended in 0.5 mL of RPMI-1640 medium containing 1 μg/mL polybrene and were infected with HIV-1 NL4.3 virus isolate at a multiplicity of infection (MOI) of 0.05 for 4 h with intermittent mixing. After infection, cells were washed twice with serum free RPMI-1640 media and were resuspended in 25 mL of complete medium. Aliquots of 2.0×10^5 cells/mL were then seeded in 24 well culture plates. Compounds were added to the respective wells in duplicate. Zidovudine (10 μM, AZT, a known antiretroviral drug and reverse transcriptase inhibitor) was used as a positive reference control and 50% ethanol and distilled water were used as a vehicle negative control. Plate was kept for incubation at 37 °C in humidified atmosphere of 5% CO_2 for 5 days. On the 5th day 0.4 mL of cell suspension was removed from each well and 1 mL of fresh complete media was added. Test compounds and controls were added in their respective wells and plate was further incubated at 37 °C in humidified atmosphere of 5% CO_2. On 8th day, cells were lysed with 150 μL of Promega cell culture lysis buffer and the lysate was centrifuged at 14,000 *g* for 10 min at 4 °C. The supernatant was transferred to black optiplate and absorbance read at an excitation wavelength of 485 nm and emission at 520 nm using fluorimeter. The results are expressed in percentage inhibition, calculated by taking the GFP fluorescence in experimental group (i.e., in the presence of test extract/AZT) divided by GFP fluorescence in infected cells in the absence of test extract/AZT multiplied by hundred. Percent inhibition was obtained by subtracting the above value from hundred.

5.2.3. MTT cell cytotoxicity assay

Cytotoxicity of compounds was assayed using MTT cell cytotoxicity assay employing TZM-bl cells. The MTT assay is based on the reduction of the yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells (live cells) to a blue colored formazan, which can be measured spectroscopically. Briefly, 3×10^3 TZM-bl cells were seeded in a 96 well plate in the absence or presence of various concentrations of test compounds and incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 48 h. After

incubation, 20 μL of MTT reagent at a concentration of 5 mg/mL was added to each well and incubated at 37 °C for 3 h. After the incubation, 100 μL of MTT solvent (20% SDS and 50% dimethyl formamide in 1 × PBS) was added in each well. The absorbance (OD) was read at 570 nm with reference filter at 690 nm (690 nm reading was used as blank). The % cell viability was calculated from the equation,

% Viability = {[(OD drug treated cultures) − (OD untreated virus control cultures)] / [(OD uninfected cultures) − (OD untreated virus control cultures)]} × 100%

5.3. Molecular modeling

The computational studies were carried out with the X-ray crystal structure of HIV RT complexed with a ligand (PDB: ID 3E01). All computational studies were done using Discovery Studio 2.5 (Accelrys Ltd., UK through Apsara Innovations Pvt Ltd, Bangalore, India).

5.3.1. Receptor setup

The target protein (PDB: ID 3E01) was taken, the ligand was extracted, hydrogens were added and their positions were optimized using the all-atom CHARMm forcefield [25,26] and the Adopted Basis set Newton Raphson (ABNR) method available in DS 2.5 protocol until the root mean square (rms) gradient was less than 0.05 kcal/mol/Å. The minimized protein was defined as the receptor using the binding site module of DS 2.5. The binding site was defined from the volume of ligand method, which was modified to accommodate all the important interacting residues in the allosteric site of HIV RT. The Input Site Sphere was defined over the binding site, with a radius of 5 Å from the center of the binding site. The protein, thus characterized, was taken as the target receptor for the docking procedure.

5.3.2. Ligand setup

Using the built-and-edit module of DS 2.5, various ligands were built, all-atom CHARMm forcefield parameterization was assigned and then minimized using the ABNR method. A conformational search of the ligand was carried out using a stimulated annealing molecular dynamics (MD) approach. The ligand was heated to a temperature of 700 K and then annealed to 200 K. Thirty such cycles were carried out. The transformation obtained at the end of each cycle was further subjected to local energy minimization, using the ABNR method. The 30 energy-minimized structures were then superimposed and the lowest energy conformation occurring in the major cluster was taken to be the most probable conformation.

5.3.3. Docking and scoring

Docking of the HIV RT with different ligands was carried out with CDOCKER protocol of DS 2.5. CDOCKER [27,28] is a grid-based MD-simulated, annealing-based algorithm that uses CHARMm. The receptor (protein) was held rigid, while the ligands were flexible during the refinement. The basic strategy involves the generation of several initial ligand orientations in the allosteric site of the target protein followed by MD-based simulated annealing, and final refinement by minimization.

Input Site Sphere parameters specify a sphere around the center of the binding site, where CDOCKER experiment was performed. The center of the Input Site Sphere was used for initial ligand placement. Ten replicas for each ligand were generated and randomly distributed around the center of the allosteric site. Each of these was subjected to MD-based simulated annealing and final refinement by minimization, leading to 10 minimized docked poses.

The docked complexes were further refined by the Ligand Minimization protocol, during which the side-chain atoms within the Input Site Sphere were free to move. A Smart Minimizer algorithm with 1000 steps and r.m.s. gradient of 0.5 kcal/mol/Å was used for ligand minimization.

The final step in docking was the scoring of the refined docked poses. This was done using the Score Ligand Poses protocol of DS 2.5. The Ludi Energy Estimate [29–31] was used for the refined poses. The ligand pose corresponding to the highest Ludi score [32] was taken as the best-docked pose. Visualization was done using PyMol software.

5.3.4. Validation of the docking methodology

The docking methodology was tested on nevirapine, a well-known marketed NNRTI, which was docked into the allosteric site of HIV-1 RT after extracting the ligand from the crystal structure. The Ludi_3 score was found to be 718 and the corresponding K_d value was 0.06 μM. This is in good agreement with the observed K_d value of 0.05 μM.

5.3.5. Calculation of H-acceptors, H-donors, TPSA and logP values

H-acceptors, H-donors and TPSA were calculated using Molinspiration software and logP values using ChemDraw software.

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