Docking mode of delvardine and its analogues into the p66 domain of HIV-1 reverse transcriptase: screening using molecular mechanics-generalized born/surface area and absorption, distribution, metabolism and excretion properties

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Delvardine and its structural derivatives are important non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs). In this work, 15 delvardine analogues were studied. A free energy-of-binding (FEB) expression was developed in the form of an optimized linear combination of van der Waal (vdW), electrostatic, solvation and solvent-accessible surface area (SASA) energy terms. The solvation energy terms estimated by generalized born/surface area (GB/SA) play an important role in predicting the binding affinity of delvardine analogues. Out of 15 derivatives, substitution of CH_3 with H at the Y and R positions, as well as substitution of SO_2CH_3 with only CH_2 at the Z position in S2, S8 and S12 analogues, were found to be the most potent (glide score = –7.60, –8.06 and –7.44; pIC50 = 7.28, 7.37 and 7.64) in comparison with the template delvardine (which is used currently as the drug candidate). All the three analogues also passed the absorption, distribution, metabolism and excretion (ADME) screening and Lipinski's rule of 5, and have the potential to be used for second-generation drug development. The work demonstrates that dock molecular mechanics-generalized born/surface area (MM–GB/SA–ADME) is a promising approach to predict the binding activity of ligands to the receptor and further screen for a successful candidate drug in a computer-aided rational drug design.

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1. Introduction

Human immunodeficiency virus (HIV), the causative agent of the deadly disease acquired immunodeficiency syndrome (AIDS) has imperilled the very fabric of human society. The latest statistical report of UNAIDS/WHO (July 2007) revealed that the situation of the disease in India is alarming. India is considered a low-prevalence country with an average infection rate of about 0.36% among the adult population. The mature virus consists of two short strands of ribonucleic acid (RNA) along with the enzymes reverse transcriptase, protease, ribonuclease and integrase. The reverse transcriptase of HIV type 1 (HIV-1 RT) is a unique enzyme, which transcribes a single-stranded viral RNA genome into double-stranded DNA; this is subsequently integrated into the host cell genome by an integrase enzyme (Jacob-Molina and Arnold 1991; Le Grice 1993; Whitecomb and Hughes 1992). HIV-1 RT plays an important role in the duplication of HIV-1 (Arnold and Jacobo-Molina 1991). Thus, it has been one of the important targets for the development of effective drugs against AIDS. RT is a dimer protein consisting of two related chains of 66 kDa (p66)

Keywords. ADME screening; binding affinity; delvardine; docking; Gaussian smooth dielectric constant function; HIV-1 RT; pIC50

Abbreviations used: ADME, absorption, distribution, metabolism and excretion; CoMFA, comparative molecular field analysis; CoMSIA, comparative molecular similarity indices analysis; eMBrAcE, bimolecular association with energetics; E_{lig}, free ligand; $E_{lig–prot}$, protein–ligand complex; $E_{prot,}$ free protein; FEB, free energy-of-binding; NNRTI, non-nucleoside HIV-1 reverse transcriptase inhibitor; pIC_{50}, prediction of biological activity; SASA, solvent accessible surface area

and 51 kDa (p51) (Smerdon *et al* 1994; Garg *et al* 1999; Arnold *et al* 1996; Larder 1993; De Clercq 2002; Ding *et al* 1998; Pedersen and Pedersen 1999). The polymerase and the RNaseH domains are located on the p66 subunit. Several drugs that target this enzyme have been approved for the treatment of AIDS (Huang *et al* 1998). Two categories of inhibitors targeting the enzyme RT have been developed (Huang *et al* 1998). One is a nucleoside analogue which, when incorporated into DNA during synthesis, stops further extension of the strand. The other is a non-nucleoside RT inhibitor (NNRTI), which binds in a hydrophobic pocket located in the palm area of the p66 subdomain of RT (Tantillo *et al* 1994; Kohlstaedt *et al* 1992; Smerdon *et al* 1994; Das *et al* 1996; Jacobo-Molina *et al* 1993).

Modern approaches to finding new leads for therapeutic targets are increasingly based on 3-dimensional information about receptors. An effective way to predict the binding structure of a substrate in its receptor is docking simulation, which has been successfully used in many applications (Leroy *et al* 2001; Dixon and Blaney 1997). Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, it describes a process by which two molecules fit together in a 3-dimensional space. Combinations of this method with other methods, such as MD simulation, free energy-of-binding calculation, comparative molecular field analysis (CoMFA) (Crammer *et al* 1988) and comparative molecular similarity indices analysis (CoMSIA) (Klebe *et al* 1994; TBohm *et al* 1999) enable insights into biological systems and help rational drug design (Buolamwini *et al* 2002; Nair *et al* 2002).

One of the main goals in drug discovery is the identification of innovative small molecular scaffolds exhibiting high binding affinity and selectivity for the target together with a reasonable absorbtion, distribution, metabolism and excretion (ADME) profile, lead and/or drug likeness. Such chemical entities are likely to be able to enter higher phases of the drug development process. This has resulted in a paradigm shift in identifying the drug likeness properties of lead molecules early in the drug discovery process. Thus, *in vitro* approaches are now widely used to investigate the ADME properties of new chemical entities and, more recently, computational (*in silico*) modelling has been investigated as a tool to optimize selection of the most suitable candidates for drug development (Smith *et al* 2004).

Unfortunately, the crystal structure of delvardine and its analogues complexed with RT have not yet been reported. The need to determine their binding structures in the active site of RT and explore the interactions for these new RT analogues is essential in order to improve the design of second-generation inhibitors. In the present work, delvardine and its 15 other structural derivatives were used to study the binding modes and binding affinities to the receptor. We try to use a flexible docking (Glide) (Halgren *et al* 2004) approach to predict the 'preferable' binding structure of a ligand in RT. To further study the association of the ligands with the receptor, we use the automated mechanism of multi-ligand bimolecular association with energetics (eMBrAcE). It uses traditional MM methods to calculate ligand–receptor interaction energies (*G*ele, *G*vdW) with a Gaussian smooth dielectric constant function method (GB/SA) (Cramer *et al* 1988; Qiu *et al* 1997) for the electrostatic part of solvation energy and solvent-accessible surface for the non-polar part of solvation energy. The approach is simple, fast and straightforward. It benefits the calculation of relative binding affinity needed to evaluate the activity of a large set of molecules in rational drug design. The final screening of delvardine and its analogues for their probable ADME properties are calculated using the Qikprop program (Schrödinger, Inc.) designed by Professor William L Jorgensen *et al* (2000).

2. Materials and methods

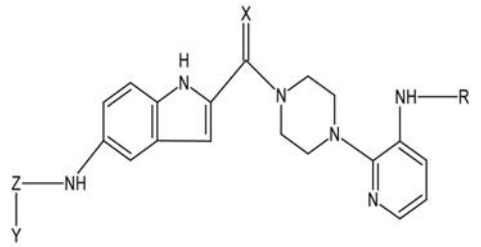
2.1 *Preparation of protein target structure*

The starting coordinates of the HIV-1 RT complex [PDB ID:1REV] was taken from the Protein Data Bank (*www.rcsb.org*) and further modified for Glide docking calculations. For Glide (Schrödinger) calculations, HIV-1 RT complex was imported to Maestro (Schrödinger), the co-crystallized ligands were identified and removed from the structure and the protein was minimized using the protein preparation wizard (shipped by Schrödinger) by applying an OPLS-AA forcefield (Jorgensen 1996) by application of the autoref.pl script. Progressively weaker restraints (tethering force constants 3, 1, 0.3, 0.1) were applied to non-hydrogen atoms only. This refinement procedure is recommended by Schrödinger (technical notes for version 1.8), because Glide uses the full OPLS-AA force field at an intermediate docking stage and is claimed to be more sensitive to geometrical details than other docking tools. Water molecules were removed and H atoms were added to the structure. The most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of His residues, and Chi 'flip' assignments for Asn, Gln and His residues were selected by the protein assignment script shipped by Schrödinger. Minimizations were performed until the average root mean square deviation of the non-hydrogen atoms reached 0.3Å.

2.2 *Preparation of compound libraries*

The coordinates of delvardine were obtained from the drug bank (ID: APRD00149) (*http://redpoll.pharmacy.ualberta.ca/drugbank*). The other inhibitors for the target

Table 1. Structures and HIV-1 RT inhibitory activity of delvardine-derived compounds used in the work



Compound	X	Y	Z	R
S1(delvardine)	O	CH_3	SO_2CH_3	CH_3
S2	O	CH_3	SO_2CH_3	H
S3	S	CH_3	SO_2CH_3	CH_3
S4	S	CH_3	SO_2CH_3	H
S5	S	H	SO_2CH_3	CH_3
S6	S	H	SO_2CH_3	H
S7	O	H	SO_2CH_3	CH_3
S8	O	H	SO_2CH_3	H
S9	O	CH_3	CH_2	CH_3
S10	O	CH_3	CH_2	H
S11	O	H	CH_2	CH_3
S12	O	H	CH_2	H
S13	S	CH_3	CH_2	CH_3
S14	S	CH_3	CH_2	H
S15	S	H	CH_2	CH_3
S16	S	H	CH_2	H

protein HIV-1 RT, p66 domain were built using delvardine as a template. The analogue library was generated by modifying the respective functional groups with stearically and conformationally allowed substituents using the reagent database and a combinatorial design module (Schrödinger) (table 1). Each structure was assigned an appropriate bond order using the ligprep script shipped by Schrödinger. The inhibitors were converted to mae format (Maestro, Schrodinger, Inc.) and optimized by means of the MMFF94 force field using a default setting (Hayes *et al* 2004).

2.3 *Glide docking and scoring function*

Glide calculations were performed with Impact version v18007 (Schrödinger, Inc.) (Halgren *et al* 2004; Krovat *et al* 2005; Friesner *et al* 2004). It performs grid-based ligand docking with energetics and searches for favourable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein (Halgren *et al* 2004). Schrödinger recommends the performance of test calculations with different scaling factors for the van der Waal radii of the receptor and ligand atom, because steric repulsive interactions might otherwise be overemphasized, leading to rejection of overall correct binding modes of active compounds. After ensuring that the protein and ligands were in the correct form for docking, the receptor-grid files were generated using a grid-receptor generation program. To soften the potential for non-polar parts of the receptor, we scaled van der Waal radii of receptor atoms by 1.00 Å with a partial atomic charge of 0.25. A grid box of size 56 x 56 x 56 Å with coordinates X = 3.4860, Y = –36.804 and Z = 22.3858 was generated at the centroid of the active site consisting of residues Pro-95, Leu-100, Lys-101, Lys 103, Val-106, Val-179, Tyr-181, Tyr-188, Trp-229 (Ren *et al* 1995) and the size of ligands to be docked was selected from the workspace. The ligands were docked with the active site using the 'xtra precision' Glide algorithm. Glide generates conformations internally and passes these through a series of filters. The first places the ligand centre at various grid positions of a 1 Å grid and rotates it around the three Euler angles. At this stage, crude score values and geometrical filters weed out unlikely binding modes. The next filter stage involves a grid-based force field evaluation and refinement of docking solutions including torsional and rigid body movements of the ligand. The OPLS-AA force field is used for this purpose. A small number of surviving docking solutions can then be subjected to a Monte Carlo procedure to try and minimize the energy score. The final energy evaluation is done with GlideScore and a single best pose is generated as the output for a particular ligand.

$$\text{GScore} = a * \text{vdW} + b * \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

where, vdW => van der Waal energy; Coul => Coulomb energy; Lipo => lipophilic contact term; HBond => hydrogen-bonding term; Metal => metal-binding term; BuryP => penalty for buried polar groups; RotB => penalty for freezing rotatable bonds; Site => polar interactions at the active site; and the coefficients of vdW and Coul are: a = 0.065, b = 0.130.

2.4 *MM and free energy of binding*

For the calculation of free energy of binding (FEB) of the ligands with RT, only the Glide-XP docking results and only the best scoring pose for each ligand were taken into consideration. eMBrAcE developed by Schrödinger was used for the physics-based rescoring procedure (Guvench *et al* 2002). For each ligand, the protein–ligand complex ($E_{lig–prot}$), the free protein (E_{prot}), and the free ligand (E_{lig}) were all subjected to energy minimization in implicit solvent (water) using the OPLS_2001 force field with a

constant dielectric electrostatic treatment of 1.0 (Wu *et al* 2003; Todorov *et al* 2003). It uses traditional MM methods to calculate ligand–receptor interaction energies (*Gele, GvdW, Gsolv*) by a GB/SA method (Reynolds 1995) for the electrostatic part of solvation energy and solvent-accessible surface for the non-polar part of solvation energy. A conjugate gradient minimization protocol with default values was used in all minimization. eMBrAcE minimization calculations were performed using an energy difference mode, in which the calculation is performed first on the receptor, then on the ligand and finally on the complex, taking as input the complexes obtained after docking analysis (Glide outputs). The energy difference is then calculated using the equation:

$$\blacktriangle E = Ecomplex - Eligand - Eprotein$$

2.5 *ADME screening*

The QikProp program (Duffy and Jorgensen 2000) was used to obtain the ADME properties of the analogues. It predicts both physically significant descriptors and pharmaceutically relevant properties. All the analogues were neutralized before being used by Qikprop. The neutralizing step is essential, as QikProp is unable to neutralize a structure and no properties will be generated in the normal mode. The program was processed in normal mode, and predicted 44 properties for the 48 molecules, consisting of principal descriptors and physiochemical properties with a detailed analysis of the log P (Octanol/Water), QP%, and log HERG. It also evaluates the acceptability of the analogues based on Lipinski's rule of 5 (Lipinski 2001), which is essential for rational drug design.

3. Results and discussion

3.1 *Docking delvardine and its analogues*

To study the molecular basis of interaction and affinity of binding of delvardine and its analogues, all the ligands were docked into the active site of RT. The docking result of these ligands is given in table 2. The ranking of ligands was based on the Glide score. All the 16 ligands accepted poses with the receptors (1REV). The difference in Glide score among all the 16 ligands was also very small (±0.774). The minimum RMSD (1.86Å) after superposition of the docked delvardine analogues in 1REV revealed that the binding mode of these inhibitors in RT is considered to be essentially similar. The result demonstrates that docking simulation can dock all the delvardine analogues into the same binding site as well (figure 1). By superposing the scaffold structure of all the analogues (RMSD varies from 0.07 to 1.86Å), it is

Table 2. The docking results of delvardine and its analogues in the original crystal structure of RT (1REV) using Glide-xp

Rank	Ligand No.	No. of poses generated	Glide score	Δscore	RMSD (Å)	Emodel** (kcal/mol)	CvdW* (kcal/mol)
1	S8	100	–8.06	$-0.25_{(S8–S11)}$	$0.75_{(S8–S11)}$	–58.9	–38.6
2	S11	100	–7.81	$-0.07_{(S11–S15)}$	$0.17_{(S11–S15)}$	–47.0	–36.8
3	S15	100	–7.74	$-0.14_{(S15–S2)}$	$0.44_{(S15–S2)}$	–53.0	–37.4
4	S2	100	–7.60	$-0.09_{(S2–S9)}$	$0.22_{(S2–S9)}$	–66.0	–42.3
5	S9	100	–7.51	$-0.07_{(S9–S12)}$	$0.19_{(S9–S12)}$	–54.9	–37.2
6	S12	100	–7.44	$-0.17_{(S12–S13)}$	$0.57_{(S12–S13)}$	–49.7	–36.0
7	S13	100	–7.27	$-0.2_{(S13–S1)}$	$0.62_{(S13–S1)}$	–50.4	–38.2
8	S1 (delvardine)	100	–7.07	$0.0_{(S1–S10)}$	$0.07_{(S1–S10)}$	–66.7	–46.7
9	S10	100	–7.07	$-0.25_{(S10–S3)}$	$0.94_{(S10–S3)}$	–51.0	–36.0
10	S3	100	–6.82	$-0.13_{(S3–S16)}$	$0.38_{(S3–S16)}$	–62.1	–45.0
11	S16	100	–6.69	$-0.05_{(S16–S5)}$	$0.19_{(S16–S5)}$	–42.8	–29.3
12	S5	100	–6.64	$-0.36_{(S5–S6)}$	$1.16_{(S5–S6)}$	–62.0	–44.2
13	S6	100	–6.28	$-0.01_{(S6–S14)}$	$0.09_{(S6–S14)}$	–59.4	–39.6
14	S14	100	–6.27	$-0.66_{(S14–S4)}$	$1.86_{(S14–S4)}$	–42.7	–31.8
15	S4	100	–5.61	$-0.21_{(S4–S7)}$	$0.71_{(S4–S7)}$	–52.4	–36.4
16	S7	100	–5.40	–	–	–59.2	–41.1
Mean ± SD	–	–	–6.95 ± 0.774	–	–	–54.89 ± 7.52	–38.54 ± 4.59

*CvdW = Coul + vdW is the non-bonded interaction energy between the ligand and the receptor.
**Emodel is a specific combination of GScore, CvdW and the internal torsional energy of the ligand conformer.

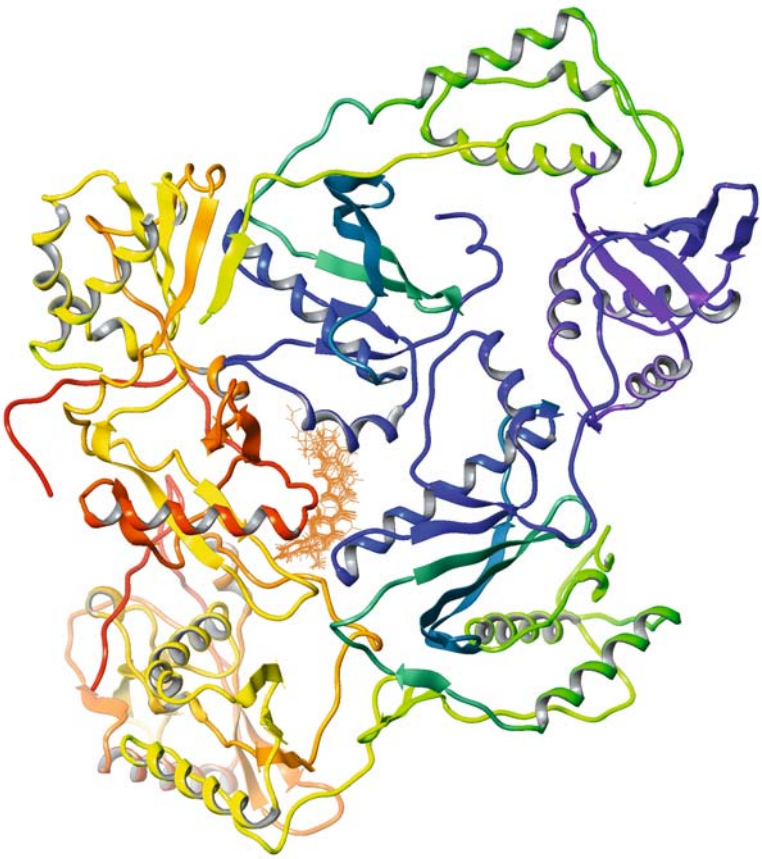


Figure 1. Binding of delvardine and its analogues into the p66 domain of HIV-1 RT.

seen that these analogues bind in the same orientation and similar position in terms of the common structure (tetra-ring part). As these molecules have the same backbone structure of the tetra-ring, it is obvious that they bind in a similar pattern in the active site of RT. All the 15 delvardine analogues were found to be good binders with RT (docking score –6.95 ± 0.774). The docking score using Glide varied from –5.40 to a minimum of –8.06 with delvardine having a docking score of –7.07. This proved that its analogues could be potential drugs for second-generation drug development.

3.2 *Calculated free energy of binding versus activity*

For calculation of FEB and prediction of biological activity (pIC_{50}) only the docking complex with best conformation (with the lowest Glide score out of 5 poses) was taken into consideration. The FEB was calculated using eMBrAcE (Schrödinger) after a minimization was performed on a docked ligand in which atoms within 7.5Å from the ligand were free to move (other atoms were fixed). The interaction energy includes an implicit solvation (H_2O) term. A vdW, solvation and electrostatic energy as well as solvent accessible surface area (SASA) were calculated for each minimized complex (table 3). A scheme similar to linear response was used to develop an FEB relationship based on these energies which in turn was used to predict the pIC_{50} of the delvardine analogues. Theoretically, FEB can be partitioned into several components: vdW, electrostatic, solvation and entropy energy terms. The contribution of entropy is the most difficult to calculate. However, several methods (Wang *et al* 2001) have been suggested to estimate the entropy contribution. In relatively rigid molecules, entropy is relatively small and is normally ignored or cancelled in the calculation of relative free energy. Further, in rational drug design, the calculation of relative rather than absolute FEB is important. The plot of the FEB and log ($1/pIC_{50}$) reveals a significant relationship ($R^2 = 0.9925$) between these two parameters (figure 2). The linear trend in the plot indicated that the docking calculation produced reasonable binding modes. Based on a correlation study, it is seen that solvation energy has most significant correlation to the pIC_{50} (R^2=34.4%) followed by electrostatic energy (Gele) (R^2=30.1%), SASA (R^2 = 12.7%) and vdW energy (R^2 = 4.4%). It indicates that in the binding of delvardine analogues, solvation energy terms estimated by GB/SA may

Table 3. Calculated energies and estimated free energy of binding (FEB) of delvardine and its analogues (kJ/mol)

Ligand	Gvdw	Gele	Gsolv	SASA	FEB[1]	pIC_{50}[2]
S1(delvardine)	22.18	8.76	–8.67	786.262	–22.27	8.92
S2	18.80	3.83	–4.45	774.019	–18.18	7.28
S3	19.46	105.27	–94.36	701.185	–30.37	12.17
S4	–4.66	11.74	–2.62	692.67	–22.46	9.00
S5	17.15	–0.60	–0.22	740.73	–20.33	8.15
S6	11.99	3.05	1.08	751.346	–21.96	8.80
S7	14.77	99.06	–90.75	721.798	–23.08	9.25
S8	–6.25	20.84	–5.19	733.829	–18.40	7.37
S9	16.28	10.11	–2.95	710.58	–23.44	9.39
S10	3.79	90.91	–88.89	709.452	–20.81	8.34
S11	17.30	94.86	–86.22	655.227	–25.94	10.40
S12	24.53	–10.41	4.96	658.306	–19.08	7.64
S13	21.80	–7.32	7.13	668.428	–21.61	8.66
S14	11.84	95.20	–80.24	644.712	–26.80	10.74
S15	–2.54	74.41	–68.28	662.706	–20.59	8.25
S16	20.50	–7.41	10.60	684.069	–23.69	9.49
Mean ± SD	12.93 ± 9.95	37.02 ± 46.06	–31.81 ± 42.97	705. 9 ± 43.18	22.44 ± 3.21	8.99 ± 1.29

(i) Calculated free energy of binding (FEB) is calculated from optimized linear combination of ΔGele ΔGvdW, ΔGsolv, and SASA from regression.

(ii) Predicted pIC_{50} is estimated from ΔGcald using the following relationship:

ΔGbinding = RTlnKdissociated ≈ RTlnIC_{50} = –RTpIC_{50},

where 300 Kelvin is used in the work for temperature T.

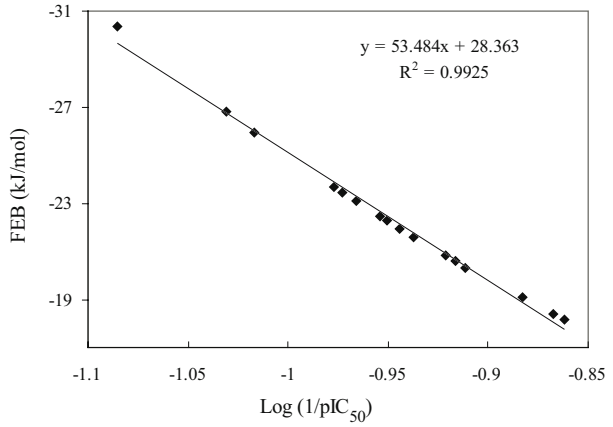


Figure 2. Linear regression plot between FEB and log ($1/pIC_{50}$) of 16 HIV1-RT inhibitors used in the study.

be a major driving force in their binding and contribute to their activity. The calculated FEB among the ligands varies between –18.18 and –30.37 kJ/mol and the overall difference is also very small (± 3.21 kJ/mol). This shows that all these molecules bind in RT with high affinity and showed activity (pIC_{50}) between 7.28 and 12.17. The ligand delvardine, having an FEB of –22.27 kJ/mol and a pIC_{50} of 8.92 proved to be less potent than its analogues S2, S8 and S12.

3.3 *Predicted ADME properties*

We analysed 44 physically significant descriptors and pharmaceutically relevant properties of NNRTIs and their analogues, among which were molecular weight, H-bond donors, H-bond acceptors, log P (octanol/water), log P MDCK, log Kp (skin permeability), humoral absorption and their position according to Lipinski's rule of 5 (tables 4 and 5). Lipinski's rule of 5 is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a

drug's pharmacokinetics in the human body, including its ADME. However, the rule does not predict if a compound is pharmacologically active (Lipinski 2001). In this study, out of 16 ligands, 15 structures showed allowed values for the properties analysed and exhibited drug-like characteristics based on Lipinski's rule of 5. Only the analogue S5 did not show drug-like characteristics.

3.4 *Biological indications of docking structure and ADME screening*

Natural and prepared compounds (table 1) were evaluated using docking, the MM–GB/SA approach and their binding energy with RT. The docking results showed that structurally homologous inhibitors bind in a very similar position and orientation in RT, which suggests that homologous inhibitors have similar binding patterns in and modes of interaction with RT, and have a similar inhibitory mechanism. The most potent inhibitor should have the best interaction with RT.

The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of RT, as is evident from the superposition of all the 16 analogues in the figure. The calculated FEB of the 15 delvardine analogues in RT-based docked structures demonstrates a linear correlation ($R^2 = 0.9925$) with their log ($1/pIC_{50}$) value. This confirms that the structural modification implemented in this study is significantly related to their activity. Also, this proved the reasonability and reliability of the docking results. It can be seen that substitution of functional groups at positions X, Y, Z and R leads to an increase in the binding affinity of modified analogues which is even more intense than that of delvardine. For example, substitution of O, CH_3, SO_2CH_3 and H in S2 ; O, H, SO_2CH_3 and H in case of S8; and O, H, CH_2 and H in S12 analogues at X, Y, Z and R positions in the scaffold structure of delvardine obtained the best results with docking scores = –7.60, –8.06, –7.44; FEB = –18.18, –18.40, –19.08 kJ/mol and pIC_{50} = 7.28, 7.37, 7.64, respectively. Further, ADME screening provided a peer analysis for the final selection of potential candidates from the compound library generated. Based on the overall analysis we can conclude that the analogues S2, S8 and S12 (with Glide score: –7.7 ± 0.32, RMSD: 0.42–0.63 Å and pIC_{50}: 7.43 ± 0.188; table 6) are the most potent analogues that could be used for second-generation drug development. All the three analogues exhibited effective binding in the active site of RT, showed optimal pIC_{50} values and even qualified Lipinki's rule of 5. The combined approach of docking–MM–GB/SA–ADME screening used in this work has the power to express the binding affinity of a large set of ligands in the receptor as well as validate them as potential candidates for second-generation drug discovery.

Table 4. Principal descriptors calculated for delvardine and its analogues by Qikprop simulation

Ligand No.	Molecular weight	Molecular volume	*VDW PSA	HB donors	HB acceptors	Rotatable bonds
S1	472.62	1424.55	93.80	3	9	5
S2	456.56	1397.23	112.91	3	10	5
S3	430.54	1242.57	108.13	3.5	9	4
S4	414.48	1217.85	127.12	3.5	10	4
S5	422.59	1349.75	58.89	3	5.5	5
S6	406.53	1354.48	72.89	3	6.5	5
S7	458.59	1320.88	96.60	3.8	9	5
S8	408.56	1325.67	54.82	3	5.5	4
S9	392.50	1278.08	74.46	3	6.5	4
S10	442.53	1296.75	118.29	3.8	10	5
S11	366.48	1148.19	69.019	3.5	5.5	3
S12	400.45	1151.71	129.94	4.3	10	4
S13	416.51	1175.84	110.17	4.3	9	4
S14	350.42	1122.25	87.16	3.5	6.5	3
S15	364.44	1168.95	90.45	3.5	6.5	4
S16	380.51	1201.74	72.14	3.5	5.5	4
Mean ± SD	411.52 ± 35.51	1261.03 ± 95.44	92.29 ± 23.82	3.45 ± 0.44	7.75 ± 1.88	4.25 ± 0.68

*VDW PSA, vdW polar SASA.

Table 5. Physiochemical descriptors calculated for delvardine and its analogues by Qikprop simulation

Ligand No.	QP logPo/w	QP (%)	Log HERG	QPP Caco	QPP MDCK	Rule of 5
S1	3.79	81.61	–6.25	559.71	586.04	0
S2	2.71	71.45	–6.22	221.15	98.95	0
S3	2.39	73.37	–6.06	232.03	226.28	0
S4	1.35	63.32	–6.10	90.56	37.71	0
S5	5.24	100	–5.94	3159.64	3740.7	1
S6	4.47	95.26	–6.22	1671.73	862.12	0
S7	3.07	77.93	–5.77	414.28	591.76	0
S8	5.15	100	–6.13	3510.45	4045.9	0
S9	3.98	94.16	–5.98	1553.72	796.52	0
S10	1.99	66.81	–5.70	151.96	85.41	0
S11	3.70	95.41	–6.03	1408.29	1470.2	0
S12	0.90	60.8	–5.97	67.98	38.87	0
S13	1.89	70.53	–5.96	169.39	221.70	0
S14	2.74	87.68	–6.05	668.75	320.24	0
S15	2.89	82.47	–5.91	495.19	231.43	0
S16	3.99	93.43	–6.1	1325.25	1460.2	0
Mean ± SD	3.14 ± 1.27	82.14 ± 13.36	–6.02 ± 0.15	981.25 ± 1070.63	925.88 ± 1245.99	–

QP logPo/w, QP log P for octanol/water.
QP %, % human oral absorption in GI (+–20%) (<25% is poor).
log HERG, HERG K+ channel blockage (concern below –5).
QPP Caco, apparent Caco-2 permeability (nm/second) (<25 poor, >500 great).
Qpp MDCk, apparent MDCK permeability (nm/second) (<25 poor, >500 great).
Rule of 5, Lipinski rule of 5 violations.

Table 6. Docking result, predicted IC_{50} and RMSD values among the three potent analogues

Ligand No.	Glide score	E(kcal/mol)	ΔE	RMSD (Å)	pIC_{50}
S2	–7.60	–66.0	$-7.1_{(S2–S8)}$	$0.55_{(S2–S8}$	7.28
S8	–8.06	–58.9	$-9.2_{(S8–S12)}$	$0.63_{(S8–S12)}$	7.37
S12	–7.44	–49.7	$-16.3_{(S2–S12)}$	$0.42_{(S2–S12}$	7.64
Mean ± SD	–7.7± 0.32	–58.2 ± 8.17	–	–	7.43 ± 0.188

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