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Synthesis, biological activity and docking study of imidazol-5-one as novel non-nucleoside HIV-1 reverse transcriptase inhibitors

Santosh N. Mokale [a], Deepak Lokwani [b], Devanand B. Shinde [b,*]

[a] Dr. Rafiq Zakaria Campus, Y.B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India
[b] Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

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ABSTRACT

A novel series of substituted imidazol-5-ones were designed, synthesized and evaluated for in vitro reverse transcriptase (RT) inhibition activity using reverse transcriptase assay kit (Roche, Colorimetric). It has been observed from in vitro screening that newly synthesized compounds possess RT inhibitory activity. Docking study was performed to study the binding orientation and affinity of synthesized compounds for RT enzyme.

1. Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are structurally diverse group of compounds which binds to the viral enzyme reverse transcriptase (RT) where it interacts with a specific allosteric non-substrate binding pocket site. NNRTIs resistance mutations arise rapidly and most often directly in contact with the NNRTI molecule. Therefore, attempts have been made to develop NNRTIs that are active against resistance mutations. These attempts have led to the identification of a number of compounds that are active against resistance mutations. Currently drugs used to treat AIDS under NNRTIs are Nevirapine, Delaviridine, Efavirenz, Etravirine and Rilpivirine (Fig. 1).[1]

From the literature survey, it was observed that the butterfly-like shape is important factor in the binding of the first generation NNRTIs. Despite their chemical diversity, they assume very similar butterfly-like shape. The butterfly structure has a hydrophilic centre as a 'body' and two hydrophobic moieties representing the 'wings'.

Based on above observation, we had decided to design a new molecular scaffold containing two important cores of Capravirine, that is, imidazole ring, a hydrophilic centre as a 'body', methyl pyridine and substituted ethylidenes/benzylidenes as two hydrophobic moieties representing the 'wings' of butterfly structure (Fig. 2). Thus, we have attempted the synthesis of novel series of 4-substituted ethylidene/benzylidene-2-methyl/phenyl-1-(pyridin-4-yl-methyl)-1*H*-imidazol-5-ones (Fig. 2) for possible reverse transcriptase inhibitor activity.[2–8]

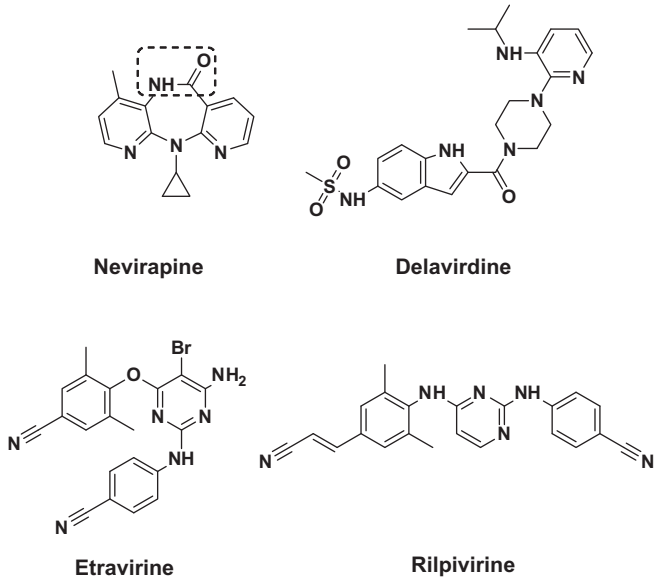

Figure 1. Currently drugs used to treat AIDS under NNRTIs.

* Corresponding author. Tel.: +91 9890409325.
E-mail address: dbssantosh06@rediffmail.com (D.B. Shinde).

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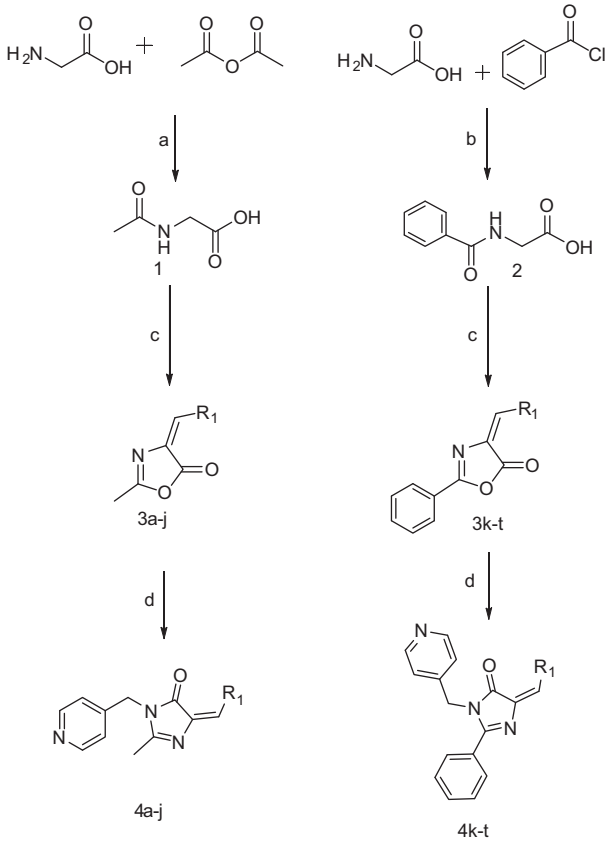


a- Water, b-10%NaOH, c-subtituted aldehude, potassium acetate,acetic anhudride, d-AcOH, pyridin-4-ylmethanamine

Scheme 1.

2. Result and discussion

2.1. Chemistry

The scheme for synthesis of target compounds **4a–t** is outlined in Scheme 1. Glycine was used as starting material to synthesize intermediates **3a–j**. Acetyl glycine (**1**) was synthesized from glycine and further subjected to cyclocondensation with aliphatic or aromatic aldehydes in the presence of acetic anhydride and anhydrous potassium acetate to get 4-(substituted ethylidene/benzylidene)-2-methyloxazol-5-ones (**3a–j**, Table 1). Benzoyl glycine was used to synthesize the intermediates **3k–t**. Benzoyl glycine (**2**) was synthesized from glycine by reacting with benzoyl chloride in alkaline condition and further subjected to cyclocondensation with aliphatic or aromatic aldehydes in the presence of acetic anhydride and anhydrous potassium acetate to get 4-(substituted ethylidene/benzylidene)-2-phenyloxazole-5-ones (**3k–t**, Table 1).

The target compounds 4-substituted ethylidene/benzylidene-2-methyl/phenyl -1-(pyridin-4-yl-methyl)-1*H*-imidazol-5-ones (**4a–t**, Table 2) were obtained by reacting 4-substituted ethylidene/benzylidene-2-methyl/phenyl oxazol-5-ones (**3a–t**) with pyridin-4-methanamine.

2.2. Biological activity

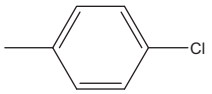
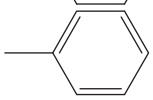
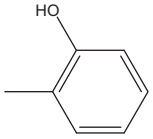
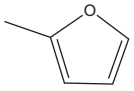
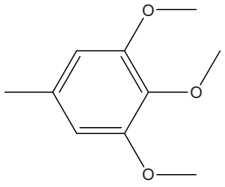
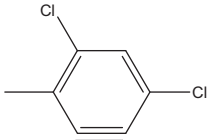
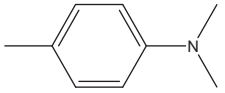
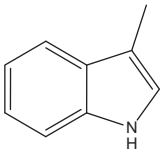
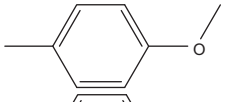
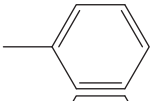
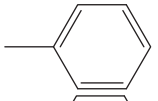
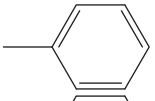
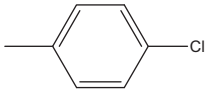
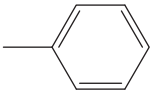
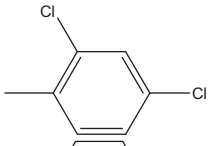
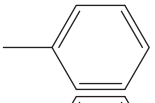
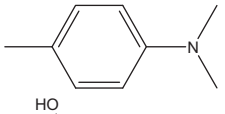
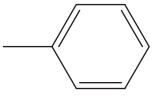
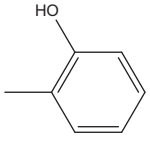
The standard Nevirapine and test compounds (**4a–t**) were powdered finely and suspended in DMSO. The RT inhibition assay was performed by using an RT assay kit (Roche). The procedure for performing RT inhibition assay was carried out as described in the kit protocol. The synthesized derivatives those having % inhibition more than 70% were subjected for determination of IC_{50} value (Table 3).[9]

It has been observed from in vitro screening that newly synthesized compounds possess RT inhibitory activity. Among the synthesized compounds, **4a, 4c, 4d** and **4m** showed a more significant RT inhibitory activity and their IC_{50} values were **3.9, 3.5, 2.5** and **3.8** μM, respectively. The compound in which phenyl group was substituted at $\mathbf{C_2}$ position on the imidazole ring showed lesser RT inhibitory activity in comparison with methyl at $\mathbf{C_2}$ position except **4m**. The hydrophobic groups are important for RT inhibitory activity at $\mathbf{C_4}$. However, while the comparing more and less bulky substituents at this position, the methyl group showed decreased activity. This indicates the ring substituents are required for RT inhibiting activity at $\mathbf{C_4}$ position.

2.3. Docking methodology

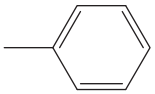
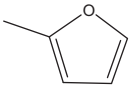
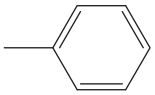
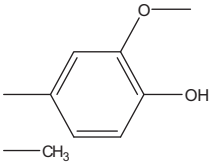
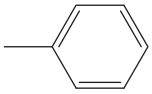
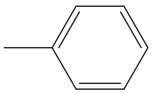
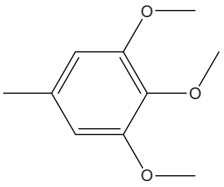
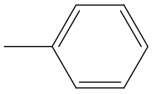
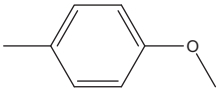
Molecular docking studies were performed using Glide v5.6 (Schrödinger, LLC). The coordinates for reverse transcriptase enzyme were taken from RCSB Protein Data Bank (PDB Id. 1FKP) and prepared for docking using protein preparation wizard. Water molecules in the structures were removed and termini were capped by adding ACE and NMA residue. The bond orders and formal charges were added for hetero groups and hydrogens were added to all atoms in the structure. Side chains that were not close to the binding cavity and do not participate in salt bridges were neutralized. After preparation, the structures were refined to optimize the hydrogen bond network using OPLS_2005 force field. This

Table 1
Characterization data for 4-substituted ethylidene/benzylidene-2-methyl/phenyl oxazol-5-one (**3a–t**)

Compd	R	R_1	Molecular formula	Molecular weight	Yield (%)	Melting point* (°C)
3a	CH_3		$C_{11}H_8ClNO_2$	221.64	78.14	142
3b	CH_3		$C_{11}H_9NO_2$	187.19	74.25	87
3c	CH_3		$C_{11}H_9NO_3$	203.19	62.45	158
3d	CH_3		$C_9H_7NO_3$	177.16	57.18	124
3e	CH_3		$C_{14}H_{15}NO_5$	277.27	69.86	136
3f	CH_3		$C_{11}H_7Cl_2NO_2$	256.08	82.67	168
3g	CH_3		$C_{13}H_{14}N_2O_2$	230.26	43.94	102
3h	CH_3		$C_{13}H_{10}N_2O_2$	226.23	86.49	164
3i	CH_3	$-CH_3$	$C_6H_7NO_2$	125.13	59.11	96
3j	CH_3		$C_{12}H_{11}NO_3$	217.22	57.43	182
3k			$C_{19}H_{17}ClO_4$	345	74.25	167
3l			$C_{16}H_{10}Cl\ NO_2$	283	78.14	157
3m			$C_{16}H_9Cl_2NO_2$	318	82.67	153
3n			$C_{18}H_{16}N_2O_2$	292	47.49	215
3o			$C_{16}H_{11}NO_3$	265	43.94	165

(*continued on next page*)

Table 1 (*continued*)

Compd	R	R_1	Molecular formula	Molecular weight	Yield (%)	Melting point* (°C)
3p			$C_{18}H_{12}N_4O_2$	316.31	40.22	87
3q			$C_{17}H_{13}NO_4$	295	69.86	177
3r			$C_{11}H_9NO_2$	187	57.18	144
3s			$C_{19}H_{17}NO_5$	339	45.82	107
3t			$C_{17}H_{13}NO_3$	279	62.45	150

* Melting points are uncorrected.

helps in reorientation of side chain hydroxyl group. The minimization was terminated when the energy converged or the RMSD reached a maximum cutoff of 0.30 Å. Grids were then defined around refined structure by centering on ligand using default box size. The extra precision (XP) docking mode for compounds, optimized by Ligprep, was performed on generated grid of protein structure.[10]

2.3.1. Interpretation of docking studies

While performing docking study, the hydrogen bonding with Lys 101 was selected as constraints for the specificity of binding of compounds in activity site of RT enzyme as reported in literature[11] and as detected by LIGPLOT.[12] The docking pose of active compounds showing higher inhibition was compared with that of standard. In docking study, both standard Efavirenz (Fig. 3a) and Nevirapine (Fig. 3b) and compound **4d** showed the hydrogen bonding with Lys 101. It was observed that –C═O group of imidazole ring of compound **4d** formed the hydrogen bonding with –NH groups of Lys 101. Further the hydrogen bonding of –C═O group of imidazole and –NH of Lys 101 was found in hydrophobic space of enzyme which increases the affinity of the compounds towards the enzyme (Fig. 4a). This indicates the importance of central imidazole ring for anti-HIV activity. Fig. 4b shows the visualization of the compound **4d** covered by hydrophobic space of the enzyme. This confirms the importance of both $–CH_3$ groups attached to imidazole and pyridine ring to occupy the hydrophobic space of the enzyme.

3. Conclusions

We have designed, synthesized and evaluated of 4-substituted ethylidene/benzylidene-2-methyl/phenyl-1-(pyridin-4-yl-methyl) -1*H*-imidazol-5-ones for reverse transcriptase inhibition activity. All the title compounds possess RT inhibitory activity. The docking studies were performed for newly synthesized molecules which indicate that imidazole ring is important for RT inhibition activity. The –C═O group of imidazole ring help to form the hydrogen bonding with in hydrophobic pocket of enzyme and thus increases the affinity of compounds toward RT enzyme inhibition. It is further concluded that compounds with imidazole ring having methyl group substitution on $\mathbf{C_2}$ position and combine hydrophobic group like furan ring substitution on $\mathbf{C_4}$ position showed higher activity. This may form the possible butterfly-like conformation of 1,4 substitutions on imidazole ring required for RT inhibition. The presence of phenyl group at $\mathbf{C_2}$ position may be lead to change the conformation of butterfly structure, hence only small group like $–CH_3$ at $\mathbf{C_2}$ position shows the higher activity.

4. Experimental

Melting points were determined on SRS OPTIMELT and were uncorrected. ^{1}H NMR spectra were recorded on a BRUKER AVANCE II 400 spectrometer (400 MHz) with TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on Time of flight mass spectrometer. FT-IR spectra were recorded on JASCO FT-IR 4000 using KBr powder. The Labsystem Multiscane Microplate reader was used to determine absorbance of the sample to calculate the % inhibition.

4.1. Synthesis of acetyl glycine from glycine (1) [13]

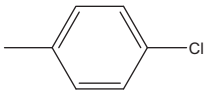
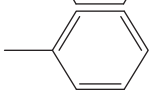
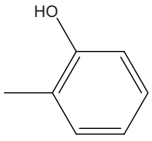
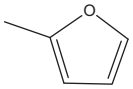
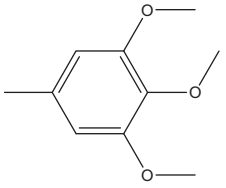
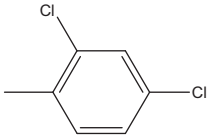
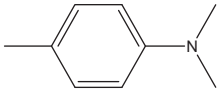
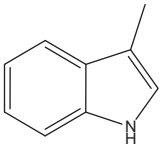
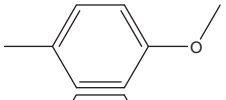
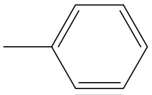
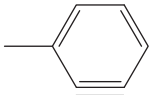
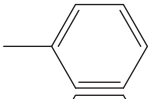
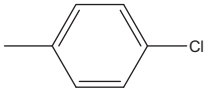
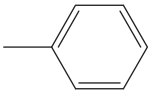
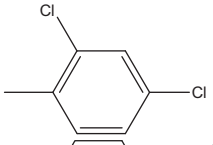
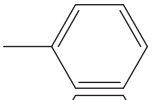
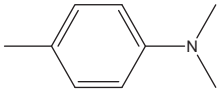
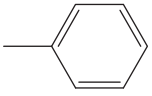
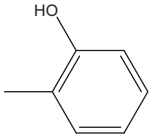
Glycine (0.5 mol) and 150 mL of water was transferred to 500-mL conical flask. The resultant solution was stirred vigorously until the solid has almost completely dissolved. To this solution acetic anhydride (1 mol) was added in one portion and again stirred vigorously for 15–20 min. The resultant solution was cooled in a refrigerator overnight. The precipitate was collected by filtration, washed with ice-cold water and dried at 100 °C. The resultant residue was recrystallized from 40 mL of boiling water.

Yield: 65.21%; MP: 206 °C.

4.2. Synthesis of benzoyl glycine from glycine (2) [13]

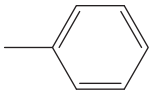
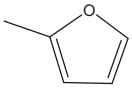
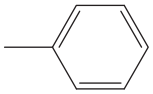
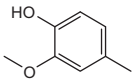
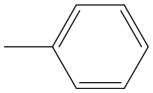
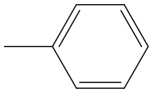
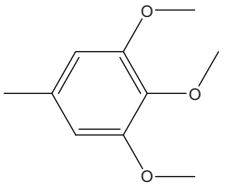
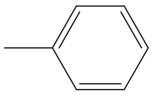
Glycine (1 mol) was dissolved in 750 ml of 10% sodium hydroxide solution. To this, benzoyl chloride (1.15 mol) in five portions was added with stirring until benzoyl chloride completely reacted. The solution was transferred to beaker and rinsed the conical flask

Table 2
Characterization data for of 4-substituted ethylidene/benzylidene-1-(4-hydroxy-6-methyl pyrimidin-2-yl)-2-methyl/phenyl-1*H*-imidazol-5(4*H*)-one derivatives (**4a**–**t**)

Compd	R	R_1	Molecular formula	Molecular weight	Yield (%)	Melting point* (°C)
4a	CH_3		$C_{17}H_{14}ClN_3O$	311	61	165–167
4b	CH_3		$C_{17}H_{15}N_3O$	277	65	204–207
4c	CH_3		$C_{17}H_{15}N_3O_2$	293	68	204–206
4d	CH_3		$C_{15}H_{13}N_3O_2$	267	56	300
4e	CH_3		$C_{20}H_{21}N_3O_4$	367	64	334–336
4f	CH_3		$C_{17}H_{13}Cl_2N_3O$	346	61	214–216
4g	CH_3		$C_{19}H_{20}N_4O$	320	69	210
4h	CH_3		$C_{19}H_{16}N_4O$	316	56	246–248
4i	CH_3	$-CH_3$	$C_{12}H_{13}N_3O$	215	58	193–195
4j	CH_3		$C_{18}H_{17}N_3O_2$	307	62	210
4k			$C_{22}H_{17}N_3O$	339	69	178–180
4l			$C_{22}H_{16}ClN_3O$	337	68	188–190
4m			$C_{22}H_{15}Cl_2N_3O$	408	56	124–126
4n			$C_{24}H_{22}N_4O$	382	71	204–206
4o			$C_{22}H_{17}N_3O_2$	355	62	188–190

(*continued on next page*)

Table 2 (*continued*)

Compd	R	R_1	Molecular formula	Molecular weight	Yield (%)	Melting point* (°C)
4p			$C_{20}H_{15}N_3O_2$	329	53	<350
4q			$C_{23}H_{19}N_3O_3$	385	63	186–188
4r		–CH_3	$C_{17}H_{15}N_3O$	277	52	130–132
4s			$C_{25}H_{23}N_3O_4$	429	61	136–138
4t			$C_{23}H_{19}N_3O_2$	369	65	150–152

* Melting points are uncorrected.

Table 3
HIV-RT inhibitory activity

Compd code	% Inhibition	IC_{50} (μM)	Compd code	% Inhibition	IC_{50} (μM)
4a	69.83	3.9	**4l**	47	
4b	60.70		**4m**	70.01	3.8
4c	74.16	3.5	**4n**	36.81	
4d	92.66	2.5	**4o**	43.22	
4e	67		**4p**	62.47	
4f	62.84		**4q**	50.20	
4g	58.13		**4r**	61.52	
4h	58.69		**4s**	57.75	
4i	64.32		**4t**	50.01	
4j	59.21		Nevirapine	99.92	
4k	54.35				

with a little water. A few gram of crushed ice was added in the solution. Then concentrated hydrochloric acid was added slowly with stirring until the mixture was acidic. The crystalline precipitate of benzoyl glycine was filtered, washed with carbon tetrachloride and then with cold water. The solid product was collected, dried and recrystallized in boiling water.

Yield: 94.51%; MP: 86 °C

4.2.1. General procedure for synthesis of 4-(substituted ethylidene/benzylidene)-2-methyloxazol-5-ones from acetyl glycine (3a–j)

A mixture of aldehyde (1 mol), acetyl glycine (**1**, 1 mol), acetic anhydride (3 mol) and anhydrous potassium acetate (1 mol) was heated on an electric hot plate with constant shaking till the mixture liquefied. Then the content of RBF was further heated on water bath for 2 h. To this, 100 mL of ethanol was added slowly and allowed the mixture to stand overnight. The crystalline product was separated by filtration, washed with 25 mL of ice-cold alcohol and then finally washed with 25 mL of boiling water and recrystallized using suitable solvent.

4.2.2. General procedure for synthesis of 4-(substituted ethylidene/benzylidene)-2-phenyloxazole-5-ones from benzoyl glycine (3k–t)

A mixture of aldehyde (1 mol), benzoyl glycine (**2**, 1 mol), acetic anhydride (3 mol) and anhydrous potassium acetate (1 mol) was heated on an electric hot plate with constant shaking till the mixture liquefied. Then the content of RBF was further heated on water bath for 2 h. To this 100 mL of ethanol was added slowly and allowed the mixture to stand overnight. The crystalline product was separated by filtration, washed with 25 mL of ice-cold alcohol and then finally washed with 25 ml of boiling water and recrystallized using suitable solvent.

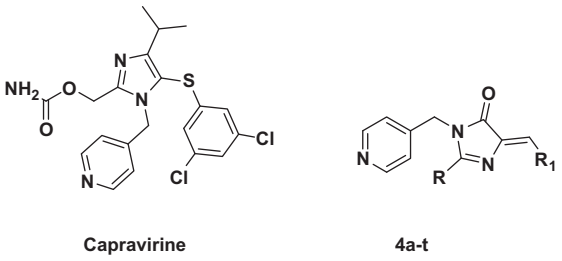


Figure 2. General structure of synthesized compounds.

4.3. General procedure for synthesis of 4-substituted ethylidene/benzylidene-2-methyl/phenyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5-one (4a–t)

A mixture of 4-substituted ethylidene/benzylidene-2-methyl/phenyl-1,3-oxazol-5-one(1 mol), pyridin-4-ylmethanamine (1 mol),

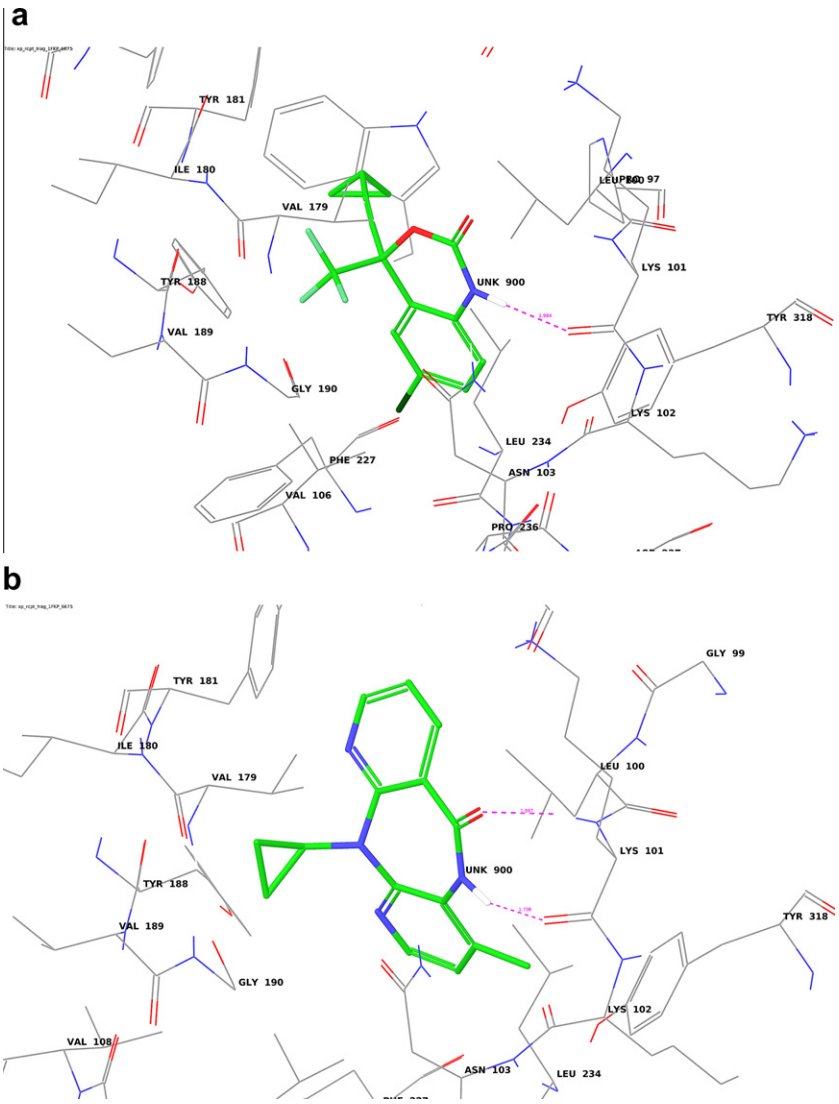


Figure 3. Docking pose of standards (a) Efavirenz and (b) Nevirapine at active binding site of RT enzyme. Hydrogen bonding with amino acid is shown in pink dotted lines.

25 mL glacial acetic acid and potassium acetate (1 mol) was placed in a 250 mL RBF equipped with a reflux condenser and heated on heating mantel for 10–12 h. The completion of reaction was monitor on TLC plate. After completion of reaction, the reaction mixture was poured in ice. The precipitates were collected and recrystallized from suitable solvent.

The spectral characterizations of synthesized derivatives are given below.

4.3.1. 4-(4-Chlorobenzylidene)-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4a)

IR (KBr): 3052, 2950, 2900, 1728, 1638, 1572, 1464, 876, 658 cm^{-1};

^1H NMR δ: 8–8.5 (m, 2H), 7.8-7.5 (m, 7H), 4.8 (s, 2H), 2.3 (s, 3H);

MS: *m/z* 312 (M+1);

4.3.2. 4-Benzylidene-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4b)

IR (KBr): 3057, 2961, 2877, 1730, 1638, 1575, 1454, 878 cm^{-1};

^1H NMR δ: 8.2–8.5 (m, 2H), 7.8-7.5 (m, 8H), 4.7 (s, 2H), 2.1 (s, 3H);

MS: *m/z* 278 (M+1);

4.3.3. 4-(2-Hydroxybenzylidene)-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4c)

IR (KBr): 3052, 2959, 1734, 1650, 1602, 1443, 1381, 888 cm^{-1};

^1H NMR δ: 8.2–8.4 (m, 3H), 7.5-6.5 (m, 6H), 5.7 (s, 1H), 4.5 (s, 2H), 2.5 (s, 3H);

MS: *m/z* 294; (M+1);

4.3.4. 4-(Furan-2-ylmethylene)-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4d)

IR (KBr): 3563, 3052, 2959, 1734, 1650, 1602, 1443, 888 cm^{-1};

^1H NMR δ: 8.2–7.8 (m, 3H), 7.3-7.5 (m, 5H), 4.2 (s, 2H), 2.3 (s, 3H);

MS: *m/z* 268(M+1);

4.3.5. 2-Methyl-1-(pyridin-4-ylmethyl)-4-(3,4,5-trimethoxybenzylidene)-1*H*-imidazol-5(4*H*)-one (4e)

IR (KBr): 3057, 2877, 1730, 1638, 1575, 1454, 878 cm^{-1};

^1H NMR δ: 8.2–8.1 (m, 2H), 7.8–7.5 (m, 5H), 4.5 (s, 2H), 3.5 (s, 9H), 2.3 (s, 3H);

MS: *m/z* 368 (M+1);

4.3.6. 4-(2,4-Dichlorobenzylidene)-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4f)

IR (KBr): 3050, 2959, 1744, 1658, 1612, 1447, 1386, 875 cm^{-1};

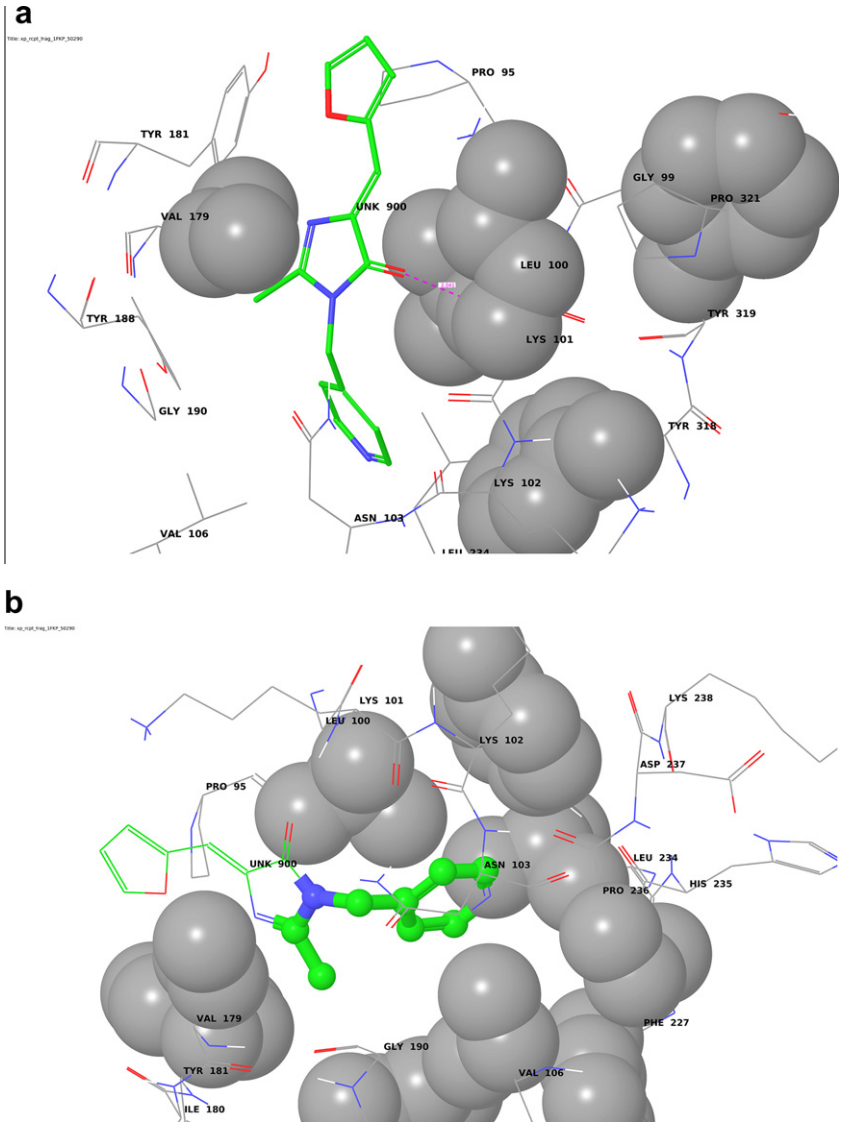


Figure 4. Docking pose of compound **4d** at active binding site of RT (a) visualization of hydrogen bonding of compound **4d** with Lys 101 in hydrophobic space (b) visualization of hydrophobic space around compound **4d**. Hydrogen bonding with amino acid is shown in pink dotted lines.

^{1}H NMR δ: 8.5–8.3 (m, 3H), 7.8–7.5 (m, 5H), 4.4(s, 2H), 2.4 (s, 3H);
MS: *m/z* 347 (M+1);

4.3.7. 4-(4-(Dimethylamino) benzylidene)-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4g)

IR (KBr): 3042, 2897, 1735, 1638, 1575, 1454, 864, 762, 658 cm^{-1};
^{1}H NMR δ: 8.5–8.6 (m, 2H), 7.8–7.5 (m, 7H), 4.8 (s, 2H), 3.7 (s, 6H), 2.5 (s, 3H);
MS: *m/z* 321 (M+1);

4.3.8. 4-((1*H*-Indol-3-yl) methylene)-2-methyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4h)

IR (KBr): 3052, 2959, 1734, 1650, 1602, 1443, 888 cm^{-1};
^{1}H NMR δ: 10.2 (s, 1H), 8.5–8.6 (m, 2H), 7.8–6.5 (m, 8H), 4.8 (s, 2H), 2.3 (s, 3H);
MS: *m/z* 317 (M+1);

4.3.9. 4-Ethylidene-2-methyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4i)

IR (KBr): 3353, 3053, 2972, 1750, 1662, 1618, 1444, 1380, 868 cm^{-1};
^{1}H NMR: δ 8.2 (s, 2H), 7.5–6.8 (m, 3H), 4.8 (s, 2H), 2.6 (s, 3H), 2.1 (s, 3H);
MS: *m/z* 216 (M+1);

4.3.10. 4-(4-Methoxybenzylidene)-2-methyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4j)

IR (KBr): 2984, 2877, 1735, 1632, 1585, 1441, 884 cm^{-1};
^{1}H NMR δ: 8.6 (s, 2H), 7.8–7.5 (m, 7H), 4.5 (s, 2H), 3.5 (s, 3H), 2.5 (s, 3H);
MS: *m/z* 308 (M+1);

4.3.11. 4-Benzylidene-2-phenyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4k)

IR (KBr): 3050, 1790, 1651, 1596, 1489, 886 cm^{-1};
^{1}H NMR: δ 8.5 (s, 2H), 7.5–6.8 (m, 13H), 4.9(s, 2H);
MS: *m/z* 340 (M+1);

4.3.12. 4-(4-Chlorobenzylidene)-2-phenyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4l)

IR (KBr): 3063, 2986, 1754, 1582, 1651, 1446, 865 cm^{-1};
^{1}H NMR: δ 8.4 (s, 2H), 7.5–6.8 (m, 12H), 4.8 (s, 2H);
MS: *m/z* 374 (M+1);

4.3.13. 4-(2,4-Dichlorobenzylidene)-2-phenyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4m)

IR (KBr): 3063, 2986, 1754, 1582, 1651, 1446, 865 cm^{-1};
^1H NMR: δ 8.4 (s, 2H), 7.5–6.8 (m, 11H), 4.7 (s, 2H);
MS: *m/z* 409(M+1);

4.3.14. 4-(4-(Dimethylamino) benzylidene)-2-phenyl-1-(pyridin -4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4n)

IR (KBr): 3052, 2920, 1725,1606,1648,1449,891 cm^{-1};
^1H NMR: δ 8.6(s, 2H), 7.5–6.8 (m, 12H), 4.5(s, 2H), 3.8 (s, 6H);
MS: *m/z* 383(M+1);

4.3.15. 4-(2-Hydroxybenzylidene)-2-phenyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4o)

IR (KBr): 3563, 3055, 2962, 1746, 1650, 1604, 1443, 878 cm^{-1};
^1H NMR: δ 8.5 (s, 2H), 8.1–7.8 (m, 12H), 5.5 (s, 1H), 4.8 (s, 2H);
M.S: *m/z* 3356 (M+1);

4.3.16. 4-(Furan-2-ylmethylene)-2-phenyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4p)

IR (KBr): 3055, 2962, 1746, 1650, 1604, 1443, 878 cm^{-1};
^1H NMR: δ 8.5 (s, 2H), 8.1–7.8 (m, 11H), 4.5 (s, 2H);
M.S: *m/z* 330 (M+1);

4.3.17. 4-(4-Hydroxy-3-methoxybenzylidene)-2-phenyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4q)

IR (KBr): 3563, 3052, 1734, 1650, 1602, 1443, 1381, 884 cm^{-1};
^1H NMR: δ 8.5 (s, 2H), 7.8–7.5 (m, 12H), 5.5 (s, 1H), 4.8 (s, 2H), 3.8 (s, 3H);
MS: *m/z* 386 (M+1);

4.3.18. 4-Ethylidene-2-phenyl-1-(pyridin-4-ylmethyl)-1*H*-imidazol-5(4*H*)-one (4r)

IR (KBr): 2984, 2877, 1735, 1632, 1585, 1441, 884 cm^{-1};
^1H NMR: δ 8 .7 (s, 2H), 7.8–7.3 (m, 8H), 4.6 (s, 2H), 2.3 (s, 3H);
MS: *m/z* 278 (M+1);

4.3.19. 2-Phenyl-1-(pyridin-4-ylmethyl)-4-(3,4,5-trimethoxy benzylidene)-1*H*-imidazol-5(4*H*)-one (4s)

IR (KBr): 3004, 2959, 1715, 1603, 1650, 1443, 888 cm^{-1};
^1H NMR δ: 8.6 (s, 2H), 7.8–7.5 (m, 10H), 4.5 (s, 2H), 3.5 (s, 9H);
MS: *m/z* 430 (M+1);

4.3.20. 4-(4-Methoxybenzylidene)-2-phenyl-1-(pyridin-4-yl methyl)-1*H*-imidazol-5(4*H*)-one (4t)

IR (KBr): 3023, 2917, 1730, 1575, 1646, 1447, 863 cm^{-1};
^1H NMR δ: 8.6 (s, 2H), 7.8–7.5 (m, 12H), 4.5 (s, 2H), 3.5 (s, 3H);
MS: *m/z* 370 (M+1).

4.4. In vitro anti-HIV activity

The standard Nevirapine and **test** compounds (**4a–t**) were powdered finely and suspended in DMSO. Finally 20 μg sample was used for in vitro assay. The RT inhibition assay was performed by using an RT assay kit (Roche). The procedure for assaying RT inhibition was performed as described in the kit protocol. Briefly, the reaction mixture consists of template primer complex, dNTPs and reverse transcriptase (RT) enzyme in the lysis buffer with or without inhibitors. After 1-h incubation at 37 °C, the reaction mixture was transferred to streptavidine-coated microtitre plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidine. The unbound dNTPs were washed using wash buffer and anti-DIG-POD was added to the MTP. The DIG-labeled dNTPs incorporated in the template were bound to anti-DIG-POD Antibody. The unbound anti-DIG-POD was washed and the peroxide substrate (ABST) was added to the MTP. A colored reaction product was produced during the cleavage of the substrate catalyzed by a peroxide enzyme. The absorbance of the sample was determined at O.D. Four hundred and five nanometers using micro titer plate ELISA reader. The % inhibition was calculated using following formula.

$$\%\ \text{inhibition} = 100 - \left(\frac{\text{OD at 405nm with inhibitor}}{\text{OD at 405nm without inhibitor}} \times 100\right)$$

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