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Design, synthesis and evaluation of 2-(1,3-dioxoisoindolin-2-yl)-*N*-phenylacetamides as inhibitors of reverse transcriptase

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ABSTRACT

In the present study, we have designed and synthesized 16 novel 1,3-dioxoisoindole ***4(a-p)*** *derivatives as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors. Lipinski rule of five parameters and molecular parameters like drug likeness and drug scores were calculated for the designed analogs using online servers like Molinspiration and Osiris property explorer. Designed analogs were synthesized using a known synthetic protocol. Synthesized compounds were characterized using FT-IR* ^{1}*H NMR Mass and Elemental analysis. The compounds were evaluated for their HIV-1 Reverse Transcriptase (RT) inhibitor activity by HIV-1 RNA dependent DNA polymerase activity assay at 2 and 20µM concentrations. Among the designed analogs,* ***4a, 4c, 4d, 4h, 4l, 4m*** *and* ***4p*** *showed weak Reverse Transcriptase inhibitory activity at 20µM concentration. For the designed compounds, there was no correlation observed between molecular modeling and in-vitro studies.*

Keywords: NNRTIs, HAART, HIV-1 Reverse Transcriptase, Docking, Molecular Properties, Autodock, Phthalimide.

INTRODUCTION

Acquired Immune Deficiency Syndrome **(**AIDS) is one of the leading causes to death worldwide. According to *UNAIDS*-2012 report, presently 33 millions are living with AIDS and 1.7 millions died in the year 2011 [1]. Availability of potent HAART (Highly Active Anti Retroviral Therapy) reduced number of AIDS related deaths in past few years. Non-Nucleoside Reverse Transcriptase Inhibitors are the key components in HAART, because of their selectivity, high potency and less toxicity when compared to Nucleotide Reverse Transcriptase Inhibitors and Protease Inhibitors [2,3]. Currently United States Food and Drug Administration (USFDA) approved five NNRTIs (Nevirapine, Delavirdine, Efavirenz, Etravirine and Rilpivirine) for the treatment of AIDS. Resistance was developed for First generation NNRTIs, Nevirapine, Delavirdine, Efavirenz. Etravirine and Rilpivirine are Second generation and currently used NNRTIs. High mutation rate of the HIV and rapid emergence of resistance to anti-HIV agents makes the researchers to develop novel anti-HIV agents active against both drug susceptible and resistance strains continuously [4,5].

Many NNRTIs, including α-anilinophenyl acetamide (α-APA) and Tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1*H*)-one (TIBO) derivatives, adopt typical butterfly-like conformations in Non-Nucleoside Inhibitory Binding Pocket (NNIBP) of HIV reverse transcriptase enzyme. Butterfly shape pharmacophore contains one hydrophilic body mainly functional groups like -NH, -C=O and -OH which are able to form hydrogen bonding interactions with

active site aminoacids like K101, K103 and P236. Pharmacophore also contains two hydrophobic wings (wing-1 and wing-2), mainly wings are π-electron containing aromatic ring system, which can form hydrophobic interactions and pi-cationic interactions with amino acids Y181, Y188, W229, F227, V106, P236, L100, L234 and Y318 [6,7].

Compounds containing phthalimide scaffold are reported for anti-inflammatory [8], anticancer [9], antibacterial [10], HIV-1 RT [11,12] and HIV-1 integrase inhibitory [13] activities by various researchers. An extensive perusal of literature revealed that, little work has been done on phthalimides as Non-Nucleoside inhibitors of HIV Reverse Transcriptase. In view of these facts and our continuous interest in the development of novel NNRTIs, we have chosen phthalimide scaffold as one of the hydrophobic wings in butterfly shape pharmacophore. All the newly synthesized compounds were designed based on the derived pharmacophoric model with acetamide moiety as hydrophilic body and phthalimide and substituted aromatic amines as hydrophobic wings (general structure shown in Figure 1).

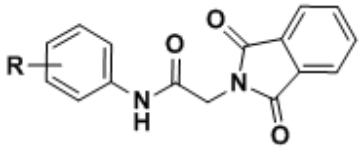

Fig. 1. General structure of designed compounds

MATERIALS AND METHODS

Chemistry

All solvents and reagents purchased from Sigma or Merck companies are used as received without further purification. Solvent system used throughout experimental work for running Thin Layer Chromatography was Ethyl acetate and Hexane Mixture (30:70) in order to monitor the progress of chemical reaction.

Melting points are uncorrected and were determined in open capillary tubes on a Precision Buchi B530 melting point apparatus containing silicon oil. IR spectra were recorded using a Jasco FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker DPX-400 spectrometer, using TMS as an internal standard (chemical shifts in δ). The ESMS were recorded on MICROMASS Quadro-II LCMS system. Elemental analysis was done on Vario elemental analyzer.

General procedure for synthesis of 2-(1,3-dioxoisoindolin-2-yl)-*N*-phenylacetamide *4:*

To a solution of isoindoline-1,3-dione (**3**) (2 mmol) in acetonitrile, potassium carbonate (6 mmol) and corresponding 2-chloro-*N*-(substituted phenyl) acetamides **2(a-p)** (2 mmol) were added and refluxed for 8h. On completion of the reaction as monitored by TLC, the contents were poured on crushed ice. Resulted precipitate was filtered, dried and recrystallized from ethanol to obtain pure product **4**.

2-(1,3-dioxoisoindolin-2-yl)-*N*-phenylacetamide (4a): White solid, (Yield 84%, MP = 194-196°C); IR (KBr, cm^{-1}): 3325 (N-H), 1778 and 1710 (C=O, isoindole), 1697 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.51 (s, 2H, CH_2), 7.12 (t, J = 7.4 Hz, 1H, ArH), 7.32 (t, J = 7.8 Hz, 2H, ArH), 7.50 (d, J = 8.2 Hz, 2H, ArH), 7.56 (brs, 1H, NH), 7.77 (dd, J1 = 5.5 Hz, J = 3.0 Hz, 2H, ArH), 7.91 (dd, J_1 = 5.4 Hz, J_2 = 3.1 Hz, 2H, ArH); Mass (m/z): 281.4 $(M+H)^+$; Anal calc for $C_{16}H_{12}N_2O_3$ Calcd.: %C, 68.56; H, 4.32; N, 9.99. found: %C 68.20, H 4.75, N 9.60.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(4-methoxyphenyl)acetamide (4b): White solid, (Yield 86%, MP = 167-168°C); IR (KBr, cm^{-1}): 3250 (N-H), 1774 and 1730 (C=O, isoindole), 1697 (C=O, amide), 1249 (C-O-C); Anal calc for $C_{17}H_{14}N_2O_4$ Calcd.: %C, 65.80; H, 4.55; N, 9.03. found: %C, 65.95; H, 4.15; N, 9.45.

2-(1,3-dioxoisoindolin-2-yl)-*N*-p-tolylacetamide (4c): White solid, (Yield 78%, MP = 165-167°C); IR (KBr, cm^{-1}): 3265 (N-H), 1776 and 1726 (C=O, isoindole), 1698 (C=O, amide); Anal calc for $C_{17}H_{14}N_2O_3$ Calcd.: %C, 69.38; H, 4.79; N, 9.52. found: %C, 69.80; H, 4.40; N, 9.80.

***N*-(4-chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4d):** White solid, (Yield 80%, MP = 168-169°C); IR (KBr, cm^{-1}): 3265 (N-H), 1772 and 1730 (C=O, isoindole), 1675 (C=O, amide), 689 (C-Cl); Anal calc for $C_{16}H_{11}ClN_2O_3$ Calcd.: %C, 61.06; H, 3.52; N, 8.90. found: %C, 61.35; H, 3.20; N, 8.55.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(3-methoxyphenyl)acetamide (4e): White solid, (Yield 82%, MP = 160-162°C); IR (KBr, cm^{-1}): 3259 (N-H), 1774 and 1712 (C=O, isoindole), 1703 (C=O, amide), 1234 (C-O-C); Anal calc for $C_{17}H_{14}N_2O_4$ Calcd.: %C, 65.80; H, 4.55; N, 9.03. found:%C, 65.65; H, 4.75; N, 9.25.

2-(1,3-dioxoisoindolin-2-yl)-*N*-m-tolylacetamide (4f): White solid, (Yield 78%, MP = 134-136°C); IR (KBr, cm^{-1}): 3324 (N-H), 1774 and 1726 (C=O, isoindole), 1682 (C=O, amide); Anal calc for $C_{17}H_{14}N_2O_3$ Calcd.: %C, 69.38; H, 4.79; N, 9.52. found:%C, 69.65; H, 4.30; N, 9.20.

***N*-(3-chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4g):** White solid, (Yield 76%, MP = 134-136°C); IR (KBr, cm^{-1}): 3234 (N-H), 1774 and 1726 (C=O, isoindole), 1697 (C=O, amide), 678 (C-Cl); Anal calc for $C_{16}H_{11}ClN_2O_3$ Calcd.: %C, 61.06; H, 3.52; N, 8.90. found: %C, 61.45; H, 3.20; N, 8.75.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(2-methoxyphenyl)acetamide (4h): White solid, (Yield 78%, MP = 170-172°C); IR (KBr, cm^{-1}): 3336 (N-H), 1774 and 1728 (C=O, isoindole), 1703 (C=O, amide), 1242(C-O-C); Anal calc for $C_{17}H_{14}N_2O_4$ Calcd.: %C, 65.80; H, 4.55; N, 9.03. found: %C, 65.50; H, 4.70; N, 9.30.

***2*-(1,3-dioxoisoindolin-2-yl)-*N*-o-tolylacetamide (4i):** White solid, (Yield 74%, MP = 142-144°C); IR (KBr, cm^{-1}). 3265 (N-H), 1772 and 1705 (C=O, isoindole), 1694 (C=O, amide); Anal calc for $C_{17}H_{14}N_2O_3$ Calcd.: % C, 69.38; H, 4.79; N, 9.52. found: %C, 69.55; H, 4.60; N, 9.10.

***N*-(2-chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4j):** White solid, (Yield 76%, MP = 132-134°C); IR (KBr, cm^{-1}): 3302 (N-H), 1784 and 1702 (C=O, isoindole), 1676 (C=O, amide), 697 (C-Cl); Anal calc for $C_{16}H_{11}ClN_2O_3$ Calcd.: %C, 61.06; H, 3.52; N, 8.90. found: %C, 61.45; H, 3.20; N, 8.75.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(4-nitrophenyl)acetamide (4k): Yellow solid, (Yield 86%, MP = 165-167°C); IR (KBr, cm^{-1}): 3282 (N-H), 1772 and 1712 (C=O, isoindole), 1686 (C=O, amide), 1542, 1322 (C-NO_2); Anal calc for $C_{16}H_{11}N_3O_5$ Calcd.: %C, 59.08; H, 3.41; N, 12.92. found: %C, 59.30; H, 3.72; N, 12.50.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(3-nitrophenyl)acetamide (4l): Yellow solid, (Yield 78%, MP = 192-194°C); IR (KBr, cm^{-1}): 3338 (N-H), 1774 and 1710 (C=O, isoindole), 1693 (C=O, amide), 1537, 1327 (C-NO_2); Anal calc for $C_{16}H_{11}N_3O_5$ Calcd.: %C, 59.08; H, 3.41; N, 12.92. found:%C, 59.45; H, 3.90; N, 12.80.

2-(1,3-dioxoisoindolin-2-yl)-*N*-(2-nitrophenyl)acetamide (4m): Yellow solid, (Yield 76%, MP = 152-154°C); IR (KBr, cm^{-1}): 3328 (N-H), 1764 and 1724 (C=O, isoindole), 1698 (C=O, amide), 1531, 1336 (C-NO_2); Anal calc for $C_{16}H_{11}N_3O_5$ Calcd.: %C, 59.08; H, 3.41; N, 12.92. found: %C, 59.25; H, 3.70; N, 12.45.

***N*-(2,4-dimethylphenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4n):** White solid, (Yield 82%, MP = 206-208°C); IR (KBr, cm^{-1}): 3364 (N-H), 1768 and 1710 (C=O, isoindole), 1682 (C=O, amide); Anal calc for $C_{18}H_{16}N_2O_3$ Calcd.: %C, 70.12; H, 5.23; N, 9.09. found: %C, 70.30; H, 5.60; N, 9.35.

***N*-(3,4-dimethylphenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4o):** White solid, (Yield 80%. MP = 204-206°C); IR (KBr, cm^{-1}): 3286 (N-H), 1778 and 1708 (C=O, isoindole), 1697 (C=O, amide); Anal calc for $C_{18}H_{16}N_2O_3$ Calcd.: %C, 70.12; H, 5.23; N, 9.09. found: %C, 70.35; H, 5.40; N, 9.40.

***N*-(3-chloro-2-methylphenyl)-2-(1,3-dioxoisoindolin-2-yl)acetamide (4p):** White solid, (Yield 74%, MP = 178-180°C); IR (KBr, cm^{-1}): 3259 (N-H), 1774 and 1726 (C=O, isoindole), 1695 (C=O, amide), 694 (C-Cl); Anal calc for $C_{17}H_{13}ClN_2O_3$ Calcd.: %C, 62.11; H, 3.99; N, 8.52. found: %C, 62.35; H, 4.20; N, 8.83.

Molecular Docking study

Docking studies of all the derivatives **4(a-p)** were performed using molecular modeling software autodock 4.2 [14], installed on a single machine running on a 3.4 GHz pentium processor with windows XP SP2 as the operating system. HIV-1 RT enzyme [pdb code: 1rt2 (shown in Figure 2)] was taken from the RCSB, used as target protein [7]. Target protein pdb was further refined by removal of water molecules and by adding polar hydrogens and kollmann charges. For the docking, a grid spacing of 0.375 A° and 63 × 63 × 63 number of points were used. The grid was centered on the active site. The auto grid program generated separate grid maps for all atom types of the ligand structures and one for electrostatic interactions. PRODRG online server was used to generate the energy

minimized conformations of the ligands in pdb format [15]. Energy minimized conformation of ligands were subjected to calculation of Gasteiger-Huckel charges and saved in default format of Autodock. Autodock generated 50 possible binding conformations i.e., 50 runs for each docking by using LGA search. Default protocol was applied, with initial population of 150 randomly placed individuals, a maximum number of 2.5 * 10^5 energy evaluations and 2.7 * 10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

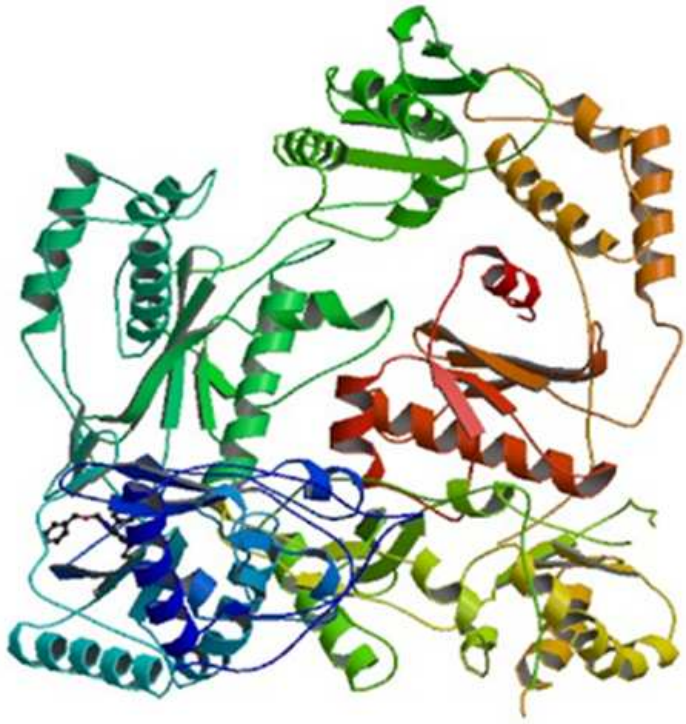

Fig. 2. Structure of HIV-1 Reverse Transcriptase (Ref: 7**)**

Validation of docking

Initially the receptor was docked with extracted ligand TNK 651 in order to validate the docking calculations, reliability and reproducibility of the docking parameters for the study. It was evident that the docked pose of the re-docked ligand was almost superimposed with that of the co crystallized ligand (Figure 3) with RMSD value of 0.5. Then docking was performed with the standard drug efavirenz with 1rt2 for validation and the mode of interaction was shown in Figure 4.

The binding free energies (docking score) and predicted inhibitory constant (Ki) values of the designed analogs were compared with binding free energies and inhibitory constants of the co-crystallized ligand TNK-651 and standard drug efavirenz. Binding free energies and predicted inhibitory constant values of TNK-651, efavirenz and designed analogs were given in Table 1. Docking studies of designed compounds showed satisfactory results, hydrophilic body of designed analogs exhibited Hydrogen bonding interactions with aminoacids of receptor protein 1rt2. Hydrogen bonding interactions of compound **4p** with LYS 103 was shown in Figure 5. All the designed analogs showed similar kind of orientation in the Non-Nucleoside Inhibitory Binding Pocket (NNIBP) of receptor protein. Orientation of some designed compounds (having significant binding free energy) in NNIBP of receptor was shown in Figure 6.

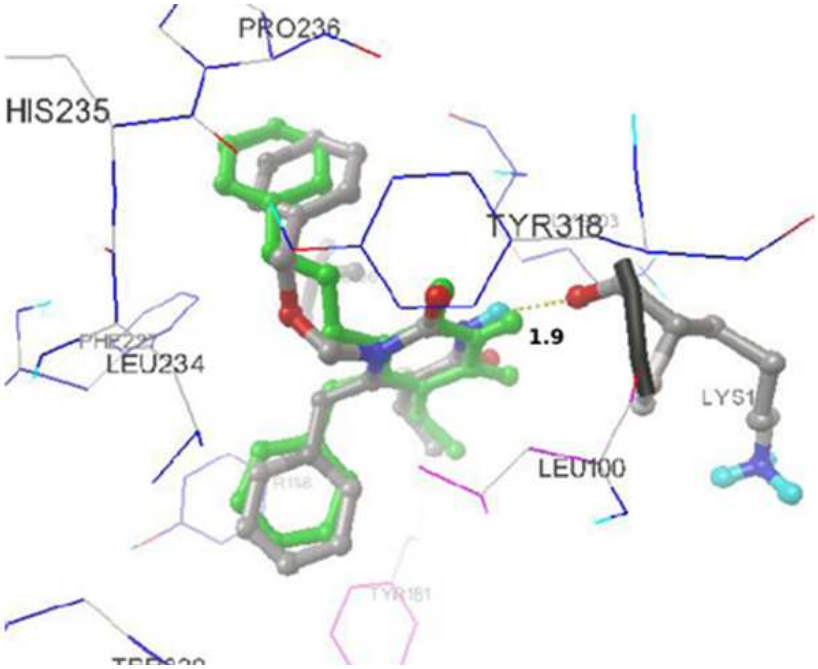

Fig. 3. Redocked mode of TNK 651 (3) (Green) superimposed with the co-crystallized ligand (Grey) in the NNIBP of HIV-1 RT (1rt2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen Bond Interaction (1.9 Å) with LYS 103 amino acid residue of Reverse Transcriptase is shown as dotted spheres. Rest of the protein is suppressed for clarification purposes

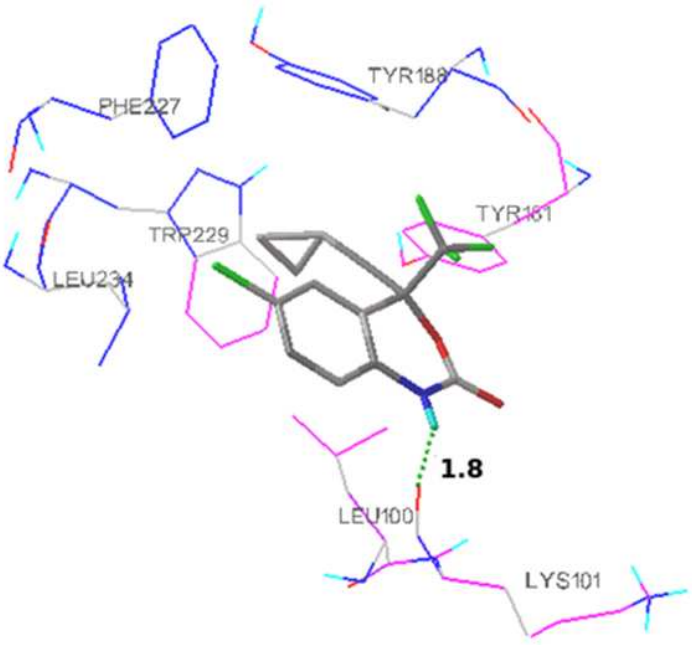

Fig. 4. Binding mode of standard drug Efavirenz in the NNIBP of HIV-1 RT (1rt2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen Bond Interactions (1.8 Å) with LYS 101 amino acid residues of Reverse Transcriptase respectively are shown as dotted spheres. Rest of the protein is suppressed for clarification purposes

Table 1 Binding free energies and predicted inhibitory constant values of the compounds

S. No	Compound Code	R	Binding Free Energy (Kcal/mole)	Inhibitory Constant (Ki) (nM)
1.	Efavirenz	-	-12.02	1.56
2.	TNK-651	-	-11.88	1.95
3.	4a	H	-10.41	23.55
4.	4b	4-OCH_3	-9.87	58.16
5.	**4c**	**4-CH_3**	**-10.54**	**18.76**
6.	4d	4-Cl	-10.16	35.48
7.	4e	3-OCH_3	-10.34	26.44
8.	**4f**	**3-CH_3**	**-10.53**	**19.19**
9.	4g	3-Cl	-10.77	12.76
10.	4h	2-OCH_3	-10.32	27.46
11.	4i	2-CH_3	-10.28	29.21
12.	**4j**	**2-Cl**	**-10.61**	**16.79**
13.	4k	4-NO_2	-10.29	28.52
14.	**4l**	**3-NO_2**	**-10.68**	**14.89**
15.	**4m**	**2-NO_2**	**-10.54**	**18.70**
16.	**4n**	**2,4- diCH_3**	**-10.64**	**15.96**
17.	4o	3,4- diCH_3	-10.48	20.73
18.	**4p**	**3-Cl, 2-CH_3**	**-10.86**	**11.00**

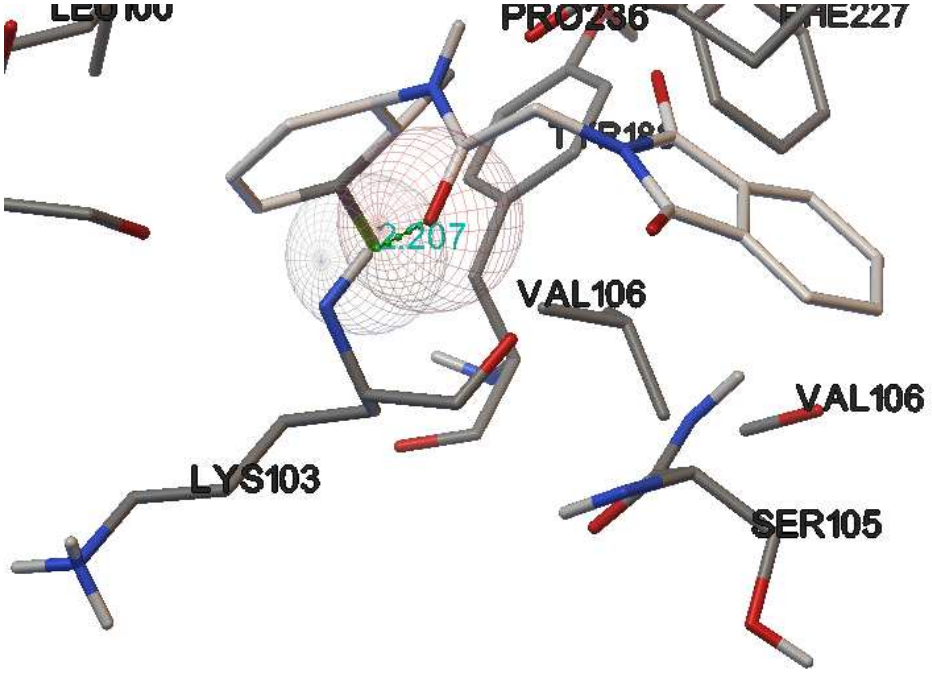

Fig. 5. Binding mode of compound 4p in the NNIBP of HIV-1 RT (1rt2). Ligand and the amino acid residues interacting with the ligands are shown as ball and sticks model. Hydrogen Bond Interactions (2.207 Å) with LYS 103 amino acid residues of Reverse Transcriptase are shown as spheres. Rest of the protein is suppressed for clarification purposes

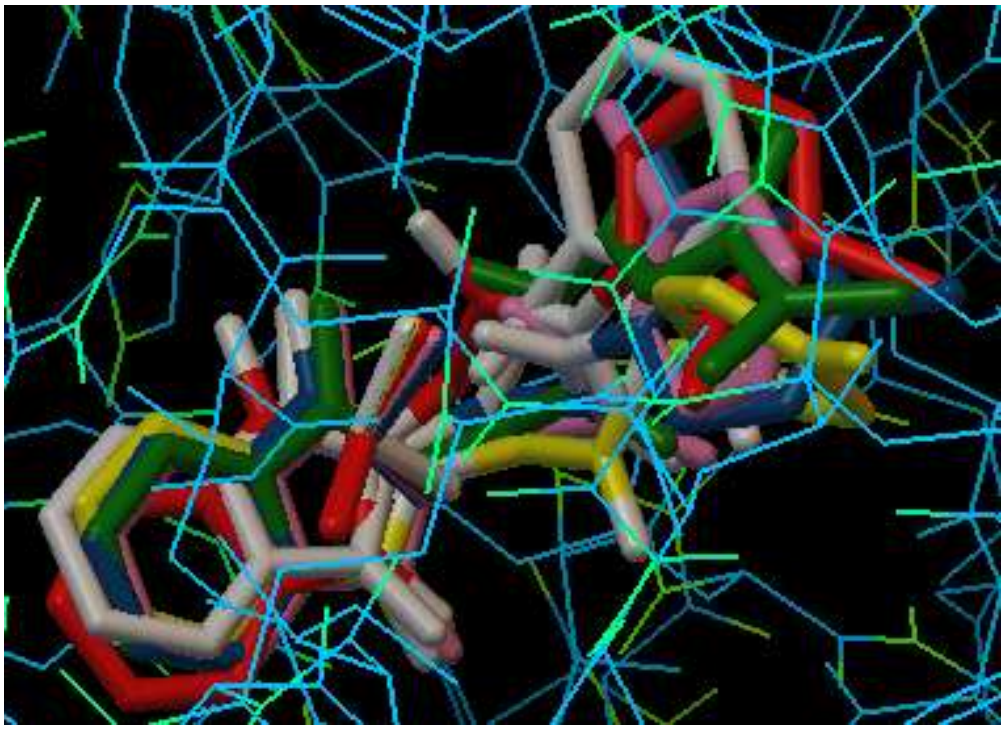

Fig. 6. Overlay stereoview of 4c (yellow), 4j (pink), 4l (green), 4m (white), 4n (red) and 4p (blue) in the Non-Nucleoside inhibitory binding pocket of HIV-1 RT (1rt2)

Molecular parameters

Lipinski rule of five parameters like ClogP, Molecular weight, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), number of atoms, number of rotatable bonds (nrotb) and total polar surface area (TPSA) were derived through online servers Molinspiration [16]. All the calculated values were given in Table 2.

Table 2 Predicted Molecular parameters of the synthesized compounds

Compound Code	CLogP	TPSA	N atoms	M. Wt	No. of HBA	No. of HBD	nrotb
4a	2.33	68.17	21	280.28	5	1	3
4b	2.38	77.40	23	310.39	6	1	4
4c	2.77	68.17	22	294.31	5	1	3
4d	3.01	68.17	22	314.72	5	1	3
4e	2.36	77.40	23	310.39	6	1	4
4f	2.75	68.17	22	294.31	5	1	3
4g	2.98	68.17	22	294.31	5	1	3
4h	2.33	77.40	23	310.39	6	1	4
4i	2.72	68.17	22	294.31	5	1	3
4j	2.96	68.17	22	294.31	5	1	3
4k	2.29	113.99	24	325.28	8	1	4
4l	2.26	113.99	24	325.28	8	1	4
4m	2.24	113.99	24	325.28	8	1	4
4n	3.15	68.17	23	308.34	5	1	3
4o	3.15	68.17	23	308.34	5	1	3
4p	3.36	68.17	23	308.34	5	1	3

In-vitro HIV-1 RT inhibitory activity

All the synthesized compounds **4(a-p)** were evaluated for HIV-1 RT inhibitory activity at concentrations of 2 and 20µM by using HIV-1 RT RNA Dependent DNA Polymerase Activity Assay [17]. HIV-1 RT inhibitory activity results were shown in Table 3. Rilpivirine was used as standard drug in the assay.

HIV-1 RNA Dependent DNA polymerase Activity Assay

Poly(rA)/oligo(dT) was used as a template for the RNA-dependent DNA polymerase reaction by HIV-1 RT, either wt or carrying the mutations. For the activity assay, 25 µl final reaction volume contained TDB buffer (50mM Tris-HCl (pH 8.0), 1mM dithiothreitol (DTT), 0.2 mg/ml bovine serum albumin (BSA), 2% glycerol), 10 mM $MgCl_2$, 0.5 mg of poly(rA):oligo(dT)10:1 (0.3 mM 3' –OH ends), 10 mM ^{3}[H]-dTTP (1Ci/mmol) and finally, introduced into tubes containing aliquots of different enzyme concentrations (5 to 10 nM RT). After incubation at 37°C for indicated time, 20µL from each reaction tube were spiked on glass fibre filters GF/C and, immediately, immersed in 5% ice-cold trichloroacetic acid (TCA) (AppliChem GmbH, Darmstadt). Filters were washed three times with 5% TCA and once with ethanol for 5 minutes, then dried and finally, added with EcoLume scintillation cocktail (ICN, Research Products Division, Costa Mesa, CA USA), to detect the acid-precipitable radioactivity by PerkinElmer Trilux MicroBeta 1450 Counter.

RESULTS AND DISCUSSION

Chemistry

Designed analogs were synthesized using appropriate synthetic protocol shown in Scheme 1. In the first step, 2-chloro-*N*-(substituted phenyl)acetamide (**2a-p**) analogs were synthesized by treating substituted anilines **(1a-p)** with 2-chloroacetyl chloride in dichloromethane and triethylamine as base. 2-chloro-*N*-(substituted phenyl)acetamide (**2a-p**) intermediates were then treated with Phthalimide (**3**) in presence of base potassium carbonate and acetonitrile as solvent to yield titled compounds as final products **4(a-p)** [18, 19]. Synthesized compounds were isolated as pure compound and characterized by FTIR, ^{1}H NMR, Mass and Elemental analysis data.

Reaction condition: a; TEA, DCM, rt, 30min, b; K_2CO_3, Acetonitrile, Reflux, 8 hr.

Scheme 1

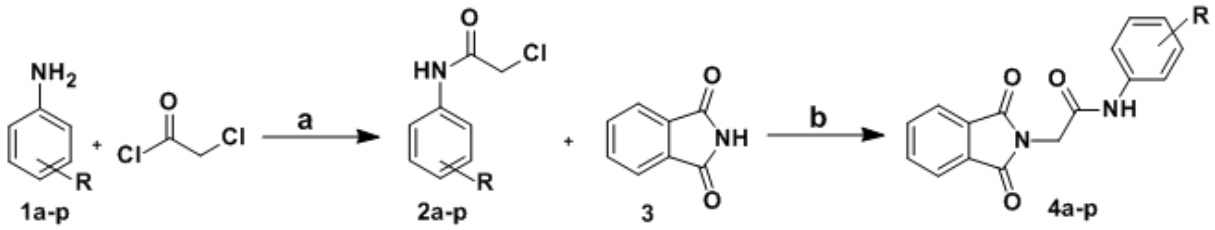

Table 3 HIV-1 RT inhibitory activity of the synthesized compounds

S. No	Comp. Code	%RT inhibition	
		2µM	20µM
1.	4a	NA	20
2.	4b	NA	NA
3.	4c	NA	10
4.	4d	NA	10
5.	4e	NA	NA
6.	4f	NA	NA
7.	4g	NA	NA
8.	4h	NA	20
9.	4i	NA	NA
10.	4j	NA	NA
11.	4k	NA	NA
12.	4l	NA	10
13.	4m	NA	10
14.	4n	NA	NA
15.	4o	NA	NA
16.	4p	NA	20

** NA indicates Not Active*

Molecular Docking

Among the designed analogs, **4c, 4f, 4j, 4l, 4m, 4n** and **4p** showed significant binding free energy values (-10.54, -10.53, -10.61, -10.68, -10.54, -10.64 and -10.86 Kcal/mole respectively) and predicted inhibitory (Ki) constant values (18.76, 19.19, 16.79, 14.89, 18.70, 15.96 and 11.00 nM respectively). Observed binding free energy and

predicted inhibitory constant values of designed analogs are comparable with that of standard drug Efavirenz (-12.02 Kcal/mole and 1.56 nM) and TNK-651 (-11.88 Kcal/mole and 1.95 nM). Docking results encourage us towards their synthesis and evaluation of *in-vitro* RT inhibition assay.

***In-vitro* RT inhibition assay**

All the synthesized compounds **4(a-p)** were evaluated for HIV-1 RT inhibitory activity at concentrations 2 and 20µM by HIV-1 RT RNA Dependent DNA Polymerase Activity Assay. Among these compounds, **4a, 4c, 4d, 4h, 4l, 4m** and **4p** showed weak HIV-1 RT inhibitory activity at 20µM concentration. None of the compounds showed HIV-1 RT inhibition at 2µM concentration (Table 3).

CONCLUSION

All the designed 2-(1,3-dioxoisoindolin-2-yl)-*N*-phenylacetamide **4(a-p)** analogs were synthesized according to the protocol given in scheme-1. Synthesized compounds were characterized by FT-IR, ^{1}H NMR, Mass and Elemental analysis. All compounds were evaluated for HIV-1 Reverse Transcriptase inhibitor activity at 2 and 20 µM concentrations. Among these synthesized compounds **4a, 4c, 4d, 4h, 4l, 4m** and **4p** showed weak HIV-1 RT inhibitor activity at 20µM concentration. There was no correlation observed between molecular modeling and *in-vitro* studies for these synthesized compounds.

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