De-novo design, synthesis and evaluation of novel 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives as HIV-1 reverse transcriptase inhibitors

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Abstract

Background: Acquired Immune Deficiency Syndrome (AIDS) is the advanced stage of infection caused by Human Immunodeficiency Virus (HIV). HIV/AIDS had a great impact on society, both as an illness and as a source of discrimination. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are structurally diverse group of compounds which binds to Reverse Transcriptase (RT) enzyme of HIV. Like other anti-HIV drugs, long-term clinical effectiveness of approved NNRTIs has been hampered due to the rapid development of drug resistance. So, there is an urgent need to discover the NNRTIs, which can be effective against the drug sensitive as well as drug resistant strains of HIV-1 RT.

Results: Two series of novel thirty, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogues (5a-o) and (8a-o) were designed and synthesized as inhibitor of HIV-1 reverse transcriptase. All the synthesized compounds were characterized by infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, mass spectroscopy and evaluated for in-vitro RT inhibitory activity. Among the tested compounds, eighteen compounds exhibited more than 50% inhibition at tested 100 μM concentration, in which two compounds 8h and 8l showed promising inhibition (74.82 and 72.58 %) respectively. The preliminary structure–activity relationship (SAR) of the test compounds and docking studies of the two significantly active compounds 8h and 8l were performed to examine their putative binding with HIV-RT. Predicted physiochemical parameters of the synthesized compounds were within the acceptable range of drugable properties.

Conclusion: The results obtained from this investigation revealed that, the synthesized compounds (5a-o) and (8a-o) showed moderate to promising HIV-1 RT inhibition activity. The overall SAR studies can help in identification of further lead as well as in designing of newer potential inhibitor of HIV-1 RT.

Keywords: Tetrahydroisoquinoline, HIV-1 RT, Docking, NNRTI, Glide, Resistance

Background

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are important components of the preferred combination of antiretroviral therapy for the treatment of HIV infection [1, 2]. Tetrahydroisoquinoline (THIQ) scaffold containing natural products as well as synthetic derivatives have privileged position in the medicinal chemistry and reported for different biological activities like anti-microbial [3], anti-cancer [4], anti-viral [5], anti-HIV [6] and others [7, 8]. Natural THIQs as inhibitors of HIV-1 and its enzyme reverse transcriptase were continuously reported in the literature. For example, michellamine-B (Fig. 1) an alkaloid from Ancistrocladus korupensis was reported for anti-HIV activity [9]. Other THIQ derivatives (Fig. 1) reported in the literature against reverse transcriptase of HIV-1 were chelidoneme, magnoflorine [10], O-methyl psychotrine sulphate [11]. Another series of benzyl THIQ derivatives, isolated from the leaves of Nelumbo nucifera contains R-coclaurine (Fig. 1) as active constituent also showed potent anti-HIV activity [12]. Literature study revealed that, apart from the THIQs obtained from the natural resources, their synthetic
analogues also showed significant potency against HIV-1 RT. In a similar study, two novel derivatives of THIQ (Fig. 2a and b) showed excellent potency against wild strains of HIV-1 by inhibiting RT enzyme [13]. Another study [14] revealed that, compounds having pyrazine ring connected to the tetrahydroisoquinoline via thiaglycynamide linker (Fig. 2c) and its bioisosters (Fig. 2d), exhibited good potency against HIV-1 RT with IC$_{50}$ 4.10 and 1.7 μM respectively. In another study, a series of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines were synthesized and assayed for anti HIV-1 activity, most active compound of the series (Fig. 2e) showed good potency with EC$_{50}$ 16.9 μM [6].

Even though, NNRTIs are structurally diverse compounds, still they contain numerous ubiquitous fragments in their structures and possess a common pharmacophoric model. This model includes an aromatic ring able to participate in pi-pi stacking interactions, amide or thioamide moieties capable of hydrogen bonding and one or more hydrocarbon-rich domain that participate in hydrophobic interactions [15]. So considering these crucial pharmacophoric features of HIV-1 RT inhibitor, we generated a common pharmacophoric model (Fig. 2f). Based upon this model, new tetrahydroisoquinoline prototypes 5 and 8 were designed (Fig. 2). Further using these prototypes, two series of novel thirty compounds 5a-o and 8a-o were synthesized and evaluated for in-vitro RT inhibitory activity. Structure activity relationship (SAR) studies of the test compounds were investigated based upon the in-vitro RT inhibitory potency. Molecular docking studies of most active compound were also carried out in order to know exact binding pattern at the active site of the receptor. These studies may help in further lead identification and designing of more potent molecules against HIV-1 RT.

**Methods**

**Chemistry**

All solvents and reagents purchased from Sigma or Merck companies were used as received without further purification. Solvent system used throughout experimental work for running TLC was ethyl acetate and hexane mixture (in suitable proportion) in order to monitor the progress of reactions. Melting points were uncorrected and determined in open capillary tubes on a Precision Buchi B530 (Flawil, Switzerland) melting point apparatus containing silicon oil. IR spectra of the synthesized compounds were recorded using FTIR spectrophotometer (Shimadzu IR Prestige 21, India). 1H NMR spectra were recorded on a Bruker DPX-400 spectrometer (Bruker India Scientific Pvt. Ltd., Mumbai) using TMS as an internal standard (chemical shifts in δ). ESMS were recorded on MICROMASS Quattro-II LCMS system (Waters Corporation, Milford, USA).
**In-vitro HIV-1 RT inhibitory activity**

Current study involved the use of enzymatic assay for *in-vitro* screening of compounds against HIV-1 RT, apart from this human being or other animals were not used in the study. Synthesised compounds were evaluated for *in-vitro* HIV-1 RT inhibitory potency using colorimetric assay method (Roche diagnostics) and carried out as described in the kit protocol. Marketed drug efavirenz was used as reference during the study. Test is based upon the colorimetric enzyme immunoassay, which quantitatively determines the retroviral reverse transcriptase activity. 100 μM concentrations of the test compounds were used for *in-vitro* assay. Briefly, the reaction mixture was set with template primer complex, RT enzyme and dNTPs in the lysis buffer with or without inhibitors. Reaction mixture was incubated at 37 °C for 1 h and then 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 optical density (OD) of 405 nm using micro titer plate ELISA reader.

The % inhibition of HIV-1 RT was calculated using the following formula:

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

Docking methodology

In-silico docking studies were performed using Glide 5.9 [16] running on maestro version 9.4, to investigate the exact binding mode of most active compound in NNIBP (Non-Nucleoside Inhibitory Binding Pocket) of HIV-1 RT [17]. Enzyme used for the docking study was wild RT, retrieved from RCSB Protein Data Bank (PDB id: 3MEE) in complex with rilpivirine. Protein preparation wizard of Schrödinger suite was used for preparation of retrieved 3MEE protein. Protein was pre-processed separately by deleting the substrate co-factor as well as the crystallographically observed water molecules (water without H bonds), followed by optimization of hydrogen bonds. After assigning charge and protonation state, finally energy was minimized with Root Mean Square Deviation (RMSD) value of 0.30 Å using Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field [18]. Prepared protein and co-crystallized ligand was employed to build energy grids using the default value of protein atom scaling (1.0 Å) within a cubic box of dimensions, centered on the centroid of the X-ray ligand pose. The structures of the designed analogues were drawn using ChemSketch and converted to 3D structure with the help of 3D optimization tool. By using the LigPrep 2.6 [19] module, the drawn ligands were geometry optimized. Partial atomic charges were computed using OPLS-2005 force field. Finally, 32 poses were included with different tautomeric and steric features for docking studies. Finally, prepared ligands were docked with prepared protein using Glide 5.9 module, in extra precision mode (XP). The best docked pose (with lowest Glide score value) obtained from Glide was analyzed. RMSD value was calculated between the experimental binding mode of ligand rilpivirine as in X-ray co-crystallized pose in 3MEE and Glide re-docked pose to ensure accuracy and reliability of the docking procedure. Physicochemical parameters of the synthesized compounds were in-silico predicted in order to evaluate their drug likeness properties using Qik-prop 3.7 [20] module of Schrödinger and online tool AdmetSAR [21].

**Results and discussion**

**Chemistry**

The synthesis of target compounds 5a-o and 8a-o is outlined in Schemes 1 and 2 respectively. In Scheme 1, reaction of substituted anilines 1a-o and chloroacetyl chloride 2 in DCM, in the presence of triethylamine as a base afforded substituted 2-chloro-N-phenylacetamide (3a-o), which on subsequent treatment with hydrochloride salt of 6,7-dimethoxy tetrahydroisoquinoline (4), in acetonitrile in the presence of potassium carbonate as base afforded the title compounds (5a-o). In Scheme 2, reaction of chloroacetyl chloride (2) and 6,7-dimethoxy tetrahydroisoquinoline (4) in DCM as solvent and triethylamine as base gave intermediate 6. Further, for the synthesis of final compounds of series 8, reaction conditions were standardized using the conventional as well as microwave assisted synthetic approaches. Finally reaction of intermediate 6 with substituted anilines (7a-o) under solvent free, microwave condition in the presence of potassium carbonate as base afforded the final compounds in comparatively better yield than the conventional synthetic approach (8a-o).

All the synthesized compounds were characterized by spectral analysis. The IR spectra of 5a-o showed the expected absorption bands of amide hydrogen (−CONH−) at the region 3200–3500 cm⁻¹ (strong, broad). Likewise compounds 8a-o contained secondary nitrogen (−NH−) which showed absorption peak in the region 3250–3400 cm⁻¹ (strong, sharp). All the synthesized compounds possessed characteristic amide carbonyl (C = O)

![Scheme 1](image-url)
peak at 1630–1690 cm\(^{-1}\) in its expected region. \(^1\)H NMR signals and proton counting of the title compounds were also observed in their expected region. ESI-MS of the synthesized compounds showed the corresponding M + 1 peak.

**In-vitro HIV-1 RT screening**

*In-vitro* studies of the test compounds (5a-o), showed weak to moderate activity against HIV-1 RT (Table 1). Among the tested compounds, five compounds (5d, 5f, 5h, 5n and 5o) showed more than 50% enzyme inhibition at tested 100 \(\mu\)M concentration. Substitutions with electron donating groups especially at the para position of the phenyl ring enhanced the potency against HIV-1 RT, for example \(p\)-methyl substituted compound 5d (52.46% inhibition) exhibited 1.5 times more potent as compared to un-substituted one (5a) (34.32% inhibition). Similarly, \(p\)-methoxy substituted compound 5f is more potent (56.23% inhibition) as compared to the \(m\)-substituted compound 5e (44.21% inhibition). Substitution with electron withdrawing group also altered potency against HIV-1 RT, halogen groups like chloro substituted compounds (5h, 5i and 5j) followed the pattern in decreasing order of potency ortho > meta > para. Similarly compound 5g having \(p\)-fluoro substitution decreased the potency, while substitution of phenyl ring with \(p\)-nitro group (5k) slightly enhanced the potency. Compound 5l with strong electron withdrawing group (m-trifluoromethyl) also showed less potency against HIV-1 RT. Upon di-substitution at phenyl ring, good enhancement in the potency was observed (compounds 5m, 5n and 5o). Compound 5n (3,4-dimethyl substitution) showed highest potency (58.12% inhibition) among the tested 5a-o series of compounds.

Majority of compounds in 8a-o series showed more than 50% inhibition of HIV-1 RT at 100 \(\mu\)M tested concentration except compounds 8a and 8d (Table 1). Substitutions with electron-donating groups at phenyl ring like methoxy (compounds 8a, 8b and 8c) showed moderate potency against RT. Compounds substituted with electron withdrawing groups like fluoro (8d and 8e), potency was not changed much may be due to its very small size and very strong electron withdrawing nature. However substitution with other electron withdrawing groups like chloro, bromo, nitro and aceto (8f, 8g, 8h, 8i, 8j, 8k, 8l, 8m and 8n) on the phenyl ring increased the potency. Compounds having chloro substitution at the ortho and meta position (8f and 8g) did not show any significant difference in potency, but substitution at para position (8h) significantly enhanced the potency (74.82% inhibition of HIV-RT). Like chloro substituted compounds almost similar pattern of potency was exhibited by the bromo substituted compounds (8i, 8j and 8k). Substitution with cyano, an electron withdrawing group at the para position (8l) also significantly increased the potency. Apart from this, compound 8m and 8n having aceto and trifluoromethyl group at the meta position showed moderate to good potency (54.75 and 66.74% inhibition) respectively against RT. Further, dimethyl substituted compound (8o) found to be more potent as compared to the single methoxy substituted compounds (8a, 8b and 8c). Among all the tested compounds, analogue (8h) exhibited highest potency (74.82% inhibition of HIV-1 RT).

**Table 1**  *In-vitro* HIV-1 RT inhibition results of the test compounds

| Compound | R      | % RT Inhibition | Compound | R      | % RT Inhibition |
|----------|--------|----------------|----------|--------|----------------|
| 5a       | H      | 34.32          | 8a       | 2-OCH₃ | 45.31          |
| 5b       | 2-CH₃  | 36.23          | 8b       | 3-OCH₃ | 51.32          |
| 5c       | 3-CH₃  | 39.51          | 8c       | 4-OCH₃ | 57.45          |
| 5d       | 4-CH₃  | 52.46          | 8d       | 3-F    | 48.37          |
| 5e       | 3-OCH₃ | 44.21          | 8e       | 4-F    | 53.93          |
| 5f       | 4-OCH₃ | 56.23          | 8f       | 2-Cl   | 61.38          |
| 5g       | 4-F    | 28.45          | 8g       | 3-Cl   | 63.74          |
| 5h       | 2-Cl   | 52.34          | 8h       | 4-Cl   | 74.82          |
| 5i       | 3-Cl   | 37.26          | 8i       | 2-Br   | 63.38          |
| 5j       | 4-Cl   | 33.65          | 8j       | 3-Br   | 60.46          |
| 5k       | 4-NO₂  | 42.78          | 8k       | 4-Br   | 68.63          |
| 5l       | 3-CF₃  | 30.64          | 8l       | 4-CN   | 72.58          |
| 5m       | 2,6-dimethyl | 48.36 | 8m       | 3-Aceto | 54.75 |
| 5n       | 3,4-dimethyl | 58.12 | 8n       | 3-CF₃   | 66.74 |
| 5o       | 2-CH₃, 5-S-CI | 53.76 | 8o   | 2,5-dimethyl | 63.64 |

*Data are indicated as percentage of inhibition of HIV-1 RT at 100 \(\mu\)M concentration*
Docking studies
In order to predict the exact binding mode and to know the interactions of most active compounds (8h and 8l) within NNIBP of HIV-1 RT, docking studies were carried out on X-ray coordinates of HIV-1 RT enzyme (PDB ID: 3MEE). To evaluate the accuracy and reliability of the docking procedure, co-crystallized native ligand rilpivirine was removed from its binding site of 3MEE and again subjected to dock into the same binding pocket (Fig. 3). As a result, the value of RMSD obtained between experimental binding mode as in X-ray and re-docked pose for rilpivirine was 0.8, which suggested that docking procedure could be relied onto predict the exact binding mode of the designed compounds.

Docking studies of the reference drug efavirenz showed that, its cyclopropyl ring extents towards deep hydrophobic pocket surrounded by amino acids Tyr-188, Tyr-181, Leu-100 and Trp-229 and chloro phenyl ring showed hydrophobic interaction with Tyr-318 and Val-106 residues (Fig. 4). Apart from hydrophobic interaction, NH of the efavirenz form strong hydrogen bond interaction with the C = O of the Lys-101. These strong hydrophobic and hydrophilic interactions of efavirenz with receptor may accounts for good in-silico activity.

However, analysis of best docked pose of two most active compounds 8h and 8l revealed that their 6,7-dimethoxytetrahydroisoquinoline nucleus form strong hydrophobic interaction with amino acids Tyr-188, Tyr-181 and Trp-229 (Figs. 5 and 6). Other hydrophobic wing of 8h (p-chloro phenyl) showed hydrophobic interactions with Val-106 and Tyr-318 (Fig. 5b), while second wing of 8h (p-cyano phenyl) exhibited hydrophobic interactions predominantly with Tyr-318 (Fig. 6b). Moreover NH of both ligands form hydrogen bond interaction with Lys-101, which is generally considered crucial for the RT inhibition activity and additionally enhanced the binding affinity of ligands 8h and 8l with HIV-1 RT. Substitution at the para position of phenyl especially with electron withdrawing groups of moderate size like chloro and cyano in 8h and 8l respectively enhanced the hydrophobic area of contact between the ligand-receptor complex and may be responsible for their significant in-vitro activity.

Predicted in-silico drug likeness properties
ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicological) parameters play very crucial role in the discovery and development of novel drugs. Currently available drugs in the market possess a balance of desirable ADMET properties and intrinsic potency. Lipinski’s rule of five (RO5) helps to predict the presence of drug-like physicochemical properties in a given compound, these properties affect as drug’s pharmacokinetics (ADMET) in the human body. Drug candidates that comply with the Lipinski rule of five have less failure rate during the clinical trial.

So in our study, physicochemical parameters of the synthesized compounds were in-silico generated in order to evaluate their drug likeness properties using Qik-prop module of Schrödinger and online tool AdmetSAR (Table 2). Results obtained in this study revealed that, the in-silico generated parameters of all synthesized derivatives were within the acceptable range of drug-likeness. General range of physicochemical parameters for drug likeness varies as: Mol. wt. (150–650), H bond donor (≤5), H bond acceptor (≤10) Log Po/w (−0.4 to 5.6), Log S (−6.5 to −0.5), Log BB (−3.0 to 1.2), % Human oral absorption (≥80 % is high and ≤25 % is poor).

Experimental
General procedure for the synthesis of substituted 2-chloro-N-phenylacetamide (3a-o)
Chloroacetyl chloride 2 (1.68 g, 1.5 mmol) was added dropwise to the ice cooled stirring solution of substituted anilines (1a-o) (1.5 mmol) in DCM, in the presence of triethyl amine (4.5 mmol, 0.46 g) as base. Reaction was further stirred at rt for 2 to 3 h, progress of the reaction was monitored with TLC. After completion of reaction, DMC layer was washed with distilled water, dried over sodium sulphate and evaporated on rotary evaporator to yield the intermediates (3a-o).

General procedure for the synthesis of substituted 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-phenyl acetamide (5a-o)
Substituted 2-chloro-N-phenylacetamide (intermediates 3a-o, 1 mmol) was added to the stirring reaction mass of 4, in acetonitrile in the presence of...
potassium carbonate as base. Reaction mass was further allowed to reflux for 5 to 7 h, progress of the reaction was monitored by the TLC (Scheme 1). After completion of reaction, solvent acetonitrile was evaporated on rotary evaporator; water was added to the reaction mixture and extracted twice with equal volume (25 ml) of ethyl acetate. Combined ethyl acetate layer was washed with brine water and evaporated on rotary evaporator to afford the final compounds 5a-o.

Synthesis of 2-chloro-1-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)ethanone (6)
Chloroacetyl chloride 2 (2.24 g, 20 mmol) was added dropwise to the ice cooled stirring solution of starting material 4 (4.6 g, 20 mmol) in DCM, in a round-bottom flask using triethyl amine (7.0 ml, 50 mmol,) as base (Scheme 2). Reaction mixture was further stirred at rt for 2.5 h until completion as per TLC. After completion of reaction, DMC layer was washed with distilled water once and then with brine water and dried over sodium
sulphate and finally evaporated on rotary evaporator to afford the titled compounds.

**General procedure for the synthesis of substituted 1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-(phenylamino) ethanone using Microwave irradiation (8a-o)**

2-methoxyaniline (7a) (0.12 g, 1 mmol) and chloro intermediate 6 (0.27 g, 1 mmol) was taken in microwave reaction vial in presence of potassium carbonate as base (0.347 g, 2.5 mmol) (Scheme 2). The microwave oven model Cata RI was programmed to 300 W at 100 °C for 2 to 4.5 min. The reaction was monitored using TLC. After completion of reaction, ice water was added to the reaction mixture which resulted the precipitation of the product. The solid product was filtered off and washed with excess cold water and dried to afford product 8a. Similar apporoach was followed for the synthesis of rest of the compounds (8b-o).

**The spectral characterization of the synthesized derivatives**

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-(3-methoxyphenyl)acetamide (5e)

Yield 76 %; white solid, MF: C_{20}H_{24}N_{2}O_{4}; MW: 356.18; MP: 139–141 °C; IR (KBr, v, cm⁻¹): 3277, 2922, 2829, 1685, 1597, 1523, 1465, 1355, 1139, 1005; ¹H NMR (400 MHz, CDCl₃) δ 9.17 (s, 1H), 7.42 (d, J = 11.7 Hz, 2H), 6.55 (s, 1H), 3.88 (d, J = 12.1 Hz, 6H), 3.76 (s, 2H), 3.32 (s, 2H), 2.91 (d, J = 3.1 Hz, 3H), 2.33 (s, 3H); MS: m/z 357 (M + 1).

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-m-tolylacetamide (5c)

Yield 78% ; white solid, MF: C_{20}H_{24}N_{2}O_{3}; MW: 340.18; MP: 131–133 °C; IR (KBr, v, cm⁻¹): 3286, 3010, 2949, 2833, 1683, 1519, 1489, 1220, 1143, 1024; ¹H NMR (400 MHz, CDCl₃) δ 9.17 (s, 1H), 7.40 (d, J = 11.8 Hz, 2H), 7.23 (t, J = 7.7 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.88 (d, J = 12.3 Hz, 6H), 3.74 (d, J = 17.9 Hz, 2H), 3.33 (s, 2H), 2.98 – 2.80 (m, 4H), 2.36 (s, 3H); MS: m/z 341 (M + 1).

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-p-tolylacetamide (5d)

Yield 81 %; white solid, MF: C_{20}H_{24}N_{2}O_{3}; MW: 340.18; MP: 151–153 °C; IR (KBr, v, cm⁻¹): 3267, 2943, 2827, 2779, 1685, 1517, 1462, 1253, 1196; ¹H NMR (400 MHz, CDCl₃) δ 9.14 (s, 1H), 7.51 – 7.42 (m, 2H), 7.15 (d, J = 8.2 Hz, 2H), 6.67 (s, 1H), 6.55 (s, 1H), 3.88 (d, J = 12.1 Hz, 6H), 3.76 (s, 2H), 3.32 (s, 2H), 2.91 (d, J = 3.1 Hz, 3H), 2.33 (s, 3H); MS: m/z 341 (M + 1).

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-n-tolylacetamide (5b)

Yield 76 %; white solid, MF: C_{20}H_{24}N_{2}O_{3}; MW: 340.18; MP: 131–133 °C; IR (KBr, v, cm⁻¹): 3286, 3010, 2949, 2833, 1683, 1519, 1489, 1220, 1143, 1024; ¹H NMR (400 MHz, CDCl₃) δ 9.33 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.25 (s, 1H), 7.17 (d, J = 7.2 Hz, 1H), 7.06 (td, J = 7.5, 1.2 Hz, 1H), 6.66 (s, 1H), 6.54 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.79 (s, 2H), 3.38 (s, 2H), 2.95 (d, J = 3.8 Hz, 4H), 2.20 (s, 3H); MS: m/z 341 (M + 1).

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-phenylacetamide (5a)

Yield 73 %; white solid, MF: C_{19}H_{22}N_{2}O_{3}; MW: 326.16; MP: 151–154 °C; IR (KBr, v, cm⁻¹): 3288, 3001, 2821, 2775, 1693, 1517, 1423, 1228, 1139, 1105; ¹H NMR (400 MHz, CDCl₃) δ 9.21 (s, 1H), 7.59 (dd, J = 8.6, 1.1 Hz, 2H), 7.39 – 7.31 (m, 2H), 7.14 (d, J = 7.4 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.88 (d, J = 12.2 Hz, 6H), 3.77 (s, 2H), 3.34 (s, 2H), 2.92 (s, 4H); MS: m/z 327 (M + 1).
Table 2  *In-silico* predicted physiochemical parameters of the test compounds

| Compound code | Mol. wt. | Mol. Vol. | H bond donor | H bond acceptor | Log Po/w | Log S | Log BB | Human oral absorption (%) |
|---------------|---------|-----------|--------------|-----------------|----------|------|--------|--------------------------|
| 5a            | 326.39  | 1099.45   | 1            | 5               | 3.12     | -3.62| 0.19   | 98.23                    |
| 5b            | 340.42  | 1135.79   | 1            | 5               | 3.3      | -3.75| 0.23   | 97.35                    |
| 5c            | 340.42  | 1160.54   | 1            | 5               | 3.4      | -4.21| 0.17   | 96.65                    |
| 5d            | 340.42  | 1160.72   | 1            | 5               | 3.43     | -4.21| 0.17   | 97.78                    |
| 5e            | 356.42  | 1174.76   | 1            | 6               | 3.21     | -3.83| 0.11   | 97.21                    |
| 5f            | 356.42  | 1175.85   | 1            | 6               | 3.21     | -3.85| 0.11   | 95.16                    |
| 5g            | 344.38  | 1116.84   | 1            | 5               | 3.36     | -4.01| 0.30   | 96.45                    |
| 5h            | 360.83  | 1137.53   | 1            | 5               | 3.59     | -4.18| 0.40   | 97.32                    |
| 5i            | 360.83  | 1144.61   | 1            | 5               | 3.62     | -4.36| 0.37   | 96.15                    |
| 5j            | 360.83  | 1144.83   | 1            | 5               | 3.62     | -4.38| 0.35   | 95.68                    |
| 5k            | 371.39  | 1173.43   | 2            | 6               | 2.40     | -3.78| -0.01 | 78.41                    |
| 5l            | 394.39  | 1199.11   | 1            | 5               | 4.11     | -5.08| 0.45   | 94.32                    |
| 5m            | 354.44  | 1187.59   | 1            | 5               | 3.66     | -4.14| 0.30   | 95.41                    |
| 5n            | 354.44  | 1211.12   | 1            | 5               | 3.70     | -4.62| 0.17   | 97.16                    |
| 5o            | 374.86  | 1194.24   | 1            | 5               | 3.92     | -4.63| 0.44   | 98.24                    |
| 5p            | 356.42  | 1171.02   | 1            | 6               | 3.27     | -4.02| -0.33 | 95.55                    |
| 5q            | 356.42  | 1169.84   | 1            | 6               | 3.22     | -4.13| -0.38 | 96.36                    |
| 5r            | 356.42  | 1170.15   | 1            | 6               | 3.22     | -4.14| -0.38 | 96.64                    |
| 5s            | 344.38  | 1111.12   | 1            | 5               | 3.37     | -4.29| -0.20 | 97.80                    |
| 5t            | 344.38  | 1111.09   | 1            | 5               | 3.37     | -4.29| -0.20 | 96.45                    |
| 5u            | 360.83  | 1130.02   | 1            | 5               | 3.53     | -4.51| -0.16 | 98.12                    |
| 5v            | 356.42  | 1139.08   | 1            | 5               | 3.62     | -4.66| -0.14 | 97.86                    |
| 5w            | 360.83  | 1139.13   | 1            | 5               | 3.62     | -4.66| -0.14 | 98.64                    |
| 5x            | 405.29  | 1137.62   | 1            | 5               | 3.60     | -4.61| -0.14 | 99.16                    |
| 5y            | 405.29  | 1147.99   | 1            | 5               | 3.70     | -4.77| -0.13 | 98.20                    |
| 5z            | 405.29  | 1148.04   | 1            | 5               | 3.70     | -4.77| -0.13 | 97.56                    |
| 5\(\alpha\)  | 351.40  | 1160.41   | 1            | 6               | 2.37     | -4.87| -1.14 | 86.13                    |
| 5m            | 368.43  | 1216.51   | 1            | 6               | 2.60     | -4.12| -0.95 | 90.86                    |
| 5n            | 394.39  | 1193.18   | 1            | 5               | 4.11     | -5.36| -0.04 | 98.54                    |
| 5o            | 354.44  | 1209.19   | 1            | 5               | 3.80     | -5.05| -0.22 | 99.22                    |
| 5p            | 315.67  | 880.03    | 1            | 3               | 3.52     | -4.86| 0.10  | 94                       |

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-(4-methoxyphenyl)acetamide (5f)

Yield 84 %; white solid, MF: C\(_{20}\)H\(_{24}\)N\(_2\)O\(_4\); MW: 356.18; MP: 153–155 °C; IR (KBr, v, cm\(^{-1}\)): 3317, 2957, 2920, 2821, 1688, 1615, 1517, 1467, 1253, 1134, 1103; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.09 (s, 1H), 7.51 (s, 1H), 7.48 (s, 1H), 6.89 (d, \(J=2.2\) Hz, 1H), 6.87 (d, \(J=2.2\) Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.76 (s, 2H), 3.32 (s, 2H), 2.92 (s, 4H); MS: m/z 357 (M + 1).

N-(2-chlorophenyl)-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetamide (5g)

Yield 64 %; white solid, MF: C\(_{20}\)H\(_{22}\)ClN\(_2\)O\(_3\); MW: 344.18; MP: 109–111 °C; IR (KBr, v, cm\(^{-1}\)): 3298, 2993, 2889, 2833, 1701, 1519, 1436, 1230, 1139, 1103; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.97 (s, 1H), 8.48 (dd, \(J=8.3, 1.5\) Hz, 1H), 7.37 (dd, \(J=8.0, 1.4\) Hz, 1H), 7.32 (dd, \(J=7.5, 1.0\) Hz, 1H), 7.10 – 7.01 (m, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.79 (s, 3H), 3.69 (s, 3H), 3.64 (s, 3H), 3.22 (s, 2H), 2.92 (s, 4H); MS: m/z 345 (M + 1).
N-(3-chlorophenyl)-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetamide (5j)

Yield 67 %; white solid, MF: C<sub>20</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>3</sub>; MW: 354.19; 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.11 (s, 1H), 7.35 (s, 2H), 7.09 (d, J = 7.9 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.90 (s, 3H), 3.87 (3H), 3.76 (2H), 3.33 (2H), 2.92 (d, J = 9.1 Hz, 6H); MS: m/z 355 (M + 1).

N-(3-chlorophenyl)-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetamide (5k)

Yield 74 %; white solid, MF: C<sub>20</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>; MW: 394.15; 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.11 (s, 1H), 7.35 (s, 2H), 7.09 (d, J = 7.9 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.90 (s, 3H), 3.87 (3H), 3.76 (2H), 3.33 (2H), 2.92 (d, J = 9.1 Hz, 6H); MS: m/z 371 (M + 1).

N-(3-chlorophenyl)-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetamide (5l)

Yield 68 %; white solid, MF: C<sub>19</sub>H<sub>21</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>; MW: 356.17; 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.38 (d, J = 8.5 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.89 (s, 3H), 3.87 (3H), 3.76 (2H), 3.33 (2H), 2.92 (d, J = 9.1 Hz, 6H); MS: m/z 355 (M + 1).

N-(5-chloro-2-methylphenyl)-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acacetamide (5m)

Yield 77 %; white solid, MF: C<sub>21</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>; MW: 354.19; 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.11 (s, 1H), 7.35 (s, 2H), 7.09 (d, J = 7.9 Hz, 1H), 6.67 (s, 1H), 6.55 (s, 1H), 3.90 (s, 3H), 3.87 (3H), 3.76 (2H), 3.33 (2H), 2.92 (d, J = 9.1 Hz, 6H); MS: m/z 355 (M + 1).
2-(4-chlorophenylamino)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) ethanone (8h)
Yield 75 %; white solid, MF: C_{19}H_{21}ClN_{3}O_{3}; MW: 360; MP: 115–116 °C; IR (KBr, v, cm⁻¹): 3360, 2927, 2906, 2833, 1658, 1600, 1511, 1433, 1265, 1226, 1124; ¹H NMR (400 MHz, CDCl₃) δ 7.16 (d, J = 8.7 Hz, 2H), 6.69 – 6.63 (m, 2H), 6.59 (dd, J = 8.9, 2.3 Hz, 2H), 4.74 (s, 1H), 4.57 (s, 1H), 3.95 (d, J = 2.9 Hz, 2H), 3.92 – 3.87 (m, 7H), 3.68 (s, 1H), 2.90 (t, J = 5.9 Hz, 1H), 2.84 (s, 1H); MS: m/z 361 (M + 1).

2-(2-bromophenylamino)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) ethanone (8b)
Yield 68 %; white solid, MF: C_{19}H_{21}BrN_{2}O_{3}; MW: 404.07; MP: 109–110 °C; IR (KBr, v, cm⁻¹): 3797, 2999, 2931, 2837, 1649, 1517, 1433, 1442, 1257, 1228, 1207; ¹H NMR (400 MHz, CDCl₃) δ 7.50 (dd, J = 9.4, 4.5 Hz, 1H), 7.22 (s, 1H), 6.69 – 6.64 (m, 2H), 6.64 – 6.53 (m, 2H), 4.76 (s, 1H), 4.58 (s, 1H), 4.06 (s, 1H), 3.93 (t, J = 5.9 Hz, 1H), 3.91 – 3.83 (m, 7H), 3.70 (t, J = 5.9 Hz, 1H), 2.91 (t, J = 5.7 Hz, 1H), 2.85 (t, J = 5.7 Hz, 1H); MS: m/z 405 (M + 1).

2-(2-chlorophenylamino)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) ethanone (8f)
Yield 68 %; white solid, MF: C_{19}H_{21}ClN_{2}O_{3}; MW: 360; MP: 104–105 °C; IR (KBr, v, cm⁻¹): 3797, 2999, 2933, 2835, 1665, 1517, 1485, 1415, 1257, 1228, 1209, 1111; ¹H NMR (400 MHz, CDCl₃) δ 7.31 (dd, J = 7.9, 1.4 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 6.68 (s, 2H), 6.66 – 6.58 (m, 2H), 4.76 (s, 1H), 4.59 (s, 1H), 4.03 (d, J = 1.9 Hz, 2H), 3.93 (s, 1H), 3.89 (d, J = 3.2 Hz, 6H), 3.70 (t, J = 5.9 Hz, 1H), 2.91 (t, J = 5.7 Hz, 1H), 2.85 (t, J = 5.9 Hz, 1H); MS: m/z 361 (M + 1).

2-(3-chlorophenylamino)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl) ethanone (8g)
Yield 84 %; white solid, MF: C_{19}H_{21}ClN_{2}O_{3}; MW: 360; MP: 136–137 °C; IR (KBr, v, cm⁻¹): 3367, 3053, 2954, 2933, 2833, 1656, 1593, 1514, 1392, 1261, 1228, 1116; ¹H NMR (400 MHz, CDCl₃) δ 7.14 – 7.06 (m, 1H), 6.71 (dd, J = 7.9, 1.2 Hz, 1H), 6.67 (d, J = 3.2 Hz, 2H), 6.63 – 6.60 (m, 1H), 6.56 (dt, J = 8.2, 2.2 Hz, 1H), 4.73 (d, J = 15.0 Hz, 1H), 4.57 (s, 1H), 3.97 – 3.92 (m, 2H), 3.89 (dd, J = 8.1, 5.4 Hz, 7H), 3.68 (t, J = 5.9 Hz, 1H), 2.90 (t, J = 5.9 Hz, 1H), 2.84 (t, J = 5.8 Hz, 1H); MS: m/z 361 (M + 1).

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethylamino)benzonitrile (8l)
Yield 68 %; white solid, MF: C_{20}H_{21}N_{2}O_{3}; MW: 351.16; MP: 146–147 °C; IR (KBr, v, cm⁻¹): 3358, 2985, 2902, 2833, 2208, 1643, 1517, 1433, 1396, 1285, 1224, 1124; ¹H NMR (400 MHz, CDCl₃) δ 7.48 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 4.6 Hz, 2H), 6.65 – 6.62 (m, 2H), 5.60 (s, 1H), 4.75
In summary, we designed and synthesized novel series of 2-(3-acetylphenylamino)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethanone (8m) containing electron donating group at para position of phenyl ring significantly increased the potency against HIV-1 RT. Docked poses of compounds 8h and 8l in the NNIBP of RT showed strong hydrophobic and hydrophilic interaction, may be responsible for their significant in-vitro activity. Predicted physicochemical parameters of the synthesized compounds were within the acceptable range of drugable properties. Thus overall SAR studies can help in identification of further lead as well as in the designing of newer potential inhibitor of HIV-1 RT.

Conclusion

In summary, we designed and synthesized novel series of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-N-phenylacetamide and 1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-(phenylamino)ethanone as HIV-1 RT inhibitors. In-vitro screening of the synthesized compounds against HIV-1 RT exhibited weak to significant inhibitory activity. Compounds of series 8 exhibited more significant HIV-1 RT inhibition as compared to the compounds of series 5. Among the tested compounds, eighteen compounds (5d, 5f, 5h, 5n, 5o, 8b, 8c, 8e, 8f, 8g, 8h, 8i, 8j, 8k, 8l, 8m, 8n and 8o) exhibited more than 50% inhibition at tested 100 μM concentration and two compounds 8h and 8l showed promising inhibition (74.82 and 72.58%) respectively. SAR analysis of compounds 5a-o demonstrated that, substitution of phenyl ring with electron donating group at para position and electron withdrawing especially chloro at ortho position resulted in enhanced potency. Among the compounds 8a-o, substitution with electron withdrawing group of moderate to large size at para position of phenyl ring significantly increased the potency against HIV-1 RT. Overall SAR studies can help in identification of further lead as well as in the designing of newer potential inhibitor of HIV-1 RT.
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