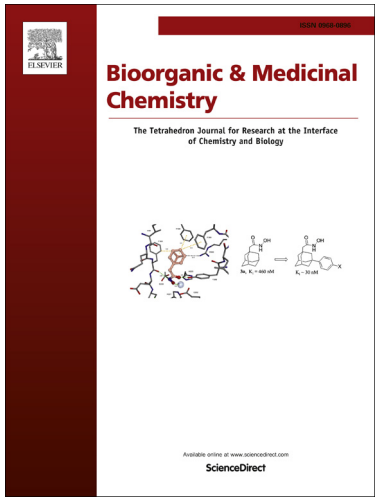
Accepted Manuscript



Rational design and synthesis of novel thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors

Vanangamudi Murugesan, Nandini Makwana, Rahul Suryawanshi, Reshu Saxena, Rajkamal Tripathi, Ramesh Paranjape, Smita Kulkarni, Seturam B. Katti

PII: S0968-0896(14)00272-7
DOI: http://dx.doi.org/10.1016/j.bmc.2014.04.018
Reference: BMC 11513

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 30 January 2014
Revised Date: 9 April 2014
Accepted Date: 10 April 2014

Please cite this article as: Murugesan, V., Makwana, N., Suryawanshi, R., Saxena, R., Tripathi, R., Paranjape, R., Kulkarni, S., Katti, S.B., Rational design and synthesis of novel thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.04.018

Rational design and synthesis of novel thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors

Vanangamudi Murugesan[a], Nandini Makwana[c], Rahul Suryawanshi[c], Reshu Saxena[b], Rajkamal Tripathi[b], Ramesh Paranjape[c], Smita Kulkarni[c,$], Seturam B. Katti[a,$,*]

[a] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, JankiPuram Extension, Sector-10, Lucknow-226031, Uttar Pradesh, India

[b] Toxicology Division, CSIR-Central Drug Research Institute (CDRI), JankiPuram Extension, Sector-10, Lucknow-226031, Uttar Pradesh, India

[c] Department of Molecular Virology, National AIDS Research Institute (NARI), Pune-411026, Maharashtra, India

[$]Equal contribution

[*]Corresponding author:

Dr. Seturam B. Katti, CSIR-CDRI, Lucknow, India

sb_katti@cdri.res.in

Tel: 0522-2772454

Fax: 0522-2771941

Abstract

A series of novel thiazolidin-4-one analogues, characterized by different substitution patterns at positions C-2 and N-3 of the thiazolidin-4-one scaffold for anti-HIV-1 activity has been investigated. Most of the compounds showed anti-HIV-1 activity at micromolar concentrations when tested in TZM-bl cells *in vitro*. Among the thirty-three compounds tested, compound **16** was the most potent inhibitor of HIV-1 replication against HIV-$1_{IIIB,}$ HIV-1_{ADA5}, HIV-1_{UG070} and HIV-1_{VB59} (EC_{50} = 0.02, 0.08, 0.08 and 0.08 µM, respectively) with selectivity index (SI = 6940, 1735, 1692 and 1692) against tested viral strains, respectively. The results of the present study suggested that the substitution of the nitro group at 6' position of the C-2 phenyl ring and 2",6"-dimethylpyridin-2-yl at the N-3 position of thiazolidin-4-one had a major impact on the anti-HIV-1 activity and was found to lower cytotoxicity. The substitution of the heteroaryl ring with bromo group and bicyclic heteroaryl ring at N-3 thiazolidin-4-one was found to lower anti-HIV-1 activity and increase cytotoxicity. The undertaken docking studies thus facilitated the identification of crucial interactions between the HIV-1 RT enzyme and thiazolidin-4-one inhibitors, which can be used to design new potential inhibitors.

Keywords: Thiazolidin-4-ones; Anti-HIV-1 activity; HIV-1 reverse transcriptase; NNRTIs

1. Introduction

The Non-nucleoside reverse transcriptase inhibitors (NNRTIs) play a vital role in the management of HIV infection and are important component of highly active antiretroviral therapy (HAART). They bind allosterically to the hydrophobic pocket located approximately 10 Å from the catalytic site in the palm domain of the p66 subunit, which harbor active sites of the reverse transcriptase (RT) enzyme.[1] Currently, the NNRTI class contains five FDA approved drugs (nevirapine, delavirdine, efavirenz, etravirine, and rilpivirine) that are being used in the management of HIV infection.[2,3] However, these drugs have some common adverse effects which include severe skin reactions, liver toxicity and sleep disorders. The other complication that arises with this class of anti-retroviral drug is the emergence of drug resistance due to rapid mutations in the amino acid residues of the NNRTI binding site.[4-6] Hence, more efforts are needed in developing new anti-HIV-1 agents with higher potency that would help in eradicating the emergence of pan-resistant viral variants. Among the five drugs, etravirine and rilpivirine have been recognized as the most successful NNRTIs developed so far due to their increased potency against HIV-1 wild-type (WT) and the clinically relevant mutant strains.[7] As reported in the earlier paper, substituted pyrimidin-2-yl and pyridin-2-yl rings at the N-3 of thiazolidin-4-one derivatives were effective anti-HIV-1 agents with minimal cytotoxicity.[8,9] Recently, we described a new thiazolidin-4-one series based on rational drug design approach in which the 5-methylisoxazol-3-yl moiety at N-3 of thiazolidin-4-one showed inhibitory activity against HIV-1 reverse transcriptase.[10] Moreover, structural analysis of drugs/compound (rilpivirine, nevirapine and compound N-(4-bromo-1H-pyrazol-3-yl)-2,1,3-benzothiadiazole-4-sulfonamide) demonstrated that fragments such as para-cyanoaniline, pyridyl amide moiety (wing II of nevirapine) and an aryl sulfonamide

group, respectively seem to be essential moieties for anti-HIV-1 activity since they form hydrophobic and hydrogen bonds interactions with the HIV-1 RT enzyme.[11-13] Further, 3D-QSAR of CoMFA and CoMSIA investigations performed on thiazolidin-4-one derivatives, also suggested that the presence of steric groups at 2' and 6' positions of phenyl ring at C-2 of thiazolidinone system and bulky groups at N-3 of heteroaromatic ring would influence positively the HIV-1 RT inhibitory activity.[14] Based on these results, we designed new thiazolidin-4-one analogues in which a para-cyanophenyl moiety at C-2 or a 4-methylbenzenesulfonamide, isonicotinamide, para-cyanoaniline and pyridylamino portions at N-3 position were installed (Fig.1). Furthermore halogen atoms at 2',6'-positions of phenyl ring were replaced by a nitro or methoxy groups. Additionally, other heteroaryl moieties were introduced at N-3 of thiazolidin-4-one system. Here we report the structural modifications at C-2 and N-3 of thiazolidin-4-one (Fig. 2) which could offer a structurally novel scaffold for further development of a new series of thiazolidin-4-one as NNRTIs.

2. Results and discussion

2.1. Chemistry

The thiazolidin-4-ones (compounds **1-27**) were synthesized by the multicomponent, one-pot reaction conditions with the appropriate amino component and aldehyde in the presence of mercaptoacetic acid in toluene under reflux conditions (**Scheme 1**).[10] Whereas the synthesis of thiazolidin-4-ones (compounds **28-33**) was carried out in two steps. In the first step hydrazones were synthesized by reacting aryl/heteroarylhydrazines and aromatic aldehydes in toluene under reflux protocol and the toluene was removed, in the second step reaction

mixture was heated with large excess of mercaptoacetic acid under neat conditions at 60 °C for 12 hours (**Scheme 2**).[15]

2.2. Anti-HIV-1 evaluation

The C-2 and N-3 modified thiazolidin-4-one compounds were tested against laboratory-adapted HIV-$1_{IIIB,}$ HIV-1_{ADA5} strains and primary isolates (HIV-1_{UG070} and HIV-1_{VB59}) in TZM-bl cells using nevirapine as the reference molecule. The results of anti-HIV-1 activity, cytotoxicity and selectivity index of thiazolidin-4-ones were expressed as EC_{50}, CC_{50}, and SI values, respectively (Table 1 and Table 2). In addition, these compounds were tested for inhibition of HIV-1 reverse transcriptase enzyme, using a RT assay kit, and expressed as the percentage inhibitions at 100 µg/ml (Table 1). The potency of the some thiazolidin-4-ones displayed moderate to potent activities against the four tested HIV-1 strains with EC_{50} values in the range of 11.82–0.02 µM concentrations. Among all the thiazolidin-4-ones, compound **16** was the most potent inhibitor of HIV-1 replication against HIV-$1_{IIIB,}$ HIV-1_{ADA5}, HIV-1_{UG070} and HIV-1_{VB59} (EC_{50} = 0.02, 0.08, 0.08 and 0.08 µM, respectively), with the highest selectivity index (SI = 6940, 1735, 1692 and 1692, respectively). Compound **16** exhibited similar HIV-1_{IIIB} inhibitory activity, when compared with the reference compound nevirapine (EC_{50} = 0.03 µM). But in other primary isolates (HIV-1_{UG070} and HIV-1_{VB59}), compound **16** (EC_{50} = 0.08 µM) showed 2.5 less fold activity than the positive control nevirapine. In addition, the compound **16** exhibited less cytotoxicity (CC_{50} = 138.8 µM in TZM-bl cells) and also showed 91.55% inhibition against HIV-1 RT enzyme. Taking into consideration the substitution at N-3, from the data reported in Table 1 it can be observed that the compounds which present a 4,6-dimethyl pyiridin-2-yl substitution at N-3 show a better antiretroviral activity profile than the corresponding compounds with other heteroaryl moieties (see

compounds **3** and **16**). The 4-6-dimethoxypyrimidin-2-yl substitution also seems to have positive effects on the anti HIV-1 activity (see compounds **17** and **25**-**27**) and in particular on % RT inhibition of compounds **21** and **26** which is 93. 81% and 95.40%, respectively. Furthermore, the presence of bromine atom at 5 position on the 4,6-dimethylpyiridin-2-yl nucleus at N-3 position marginally reduced the potency and subsequently increase the cellular toxicity profile of compound **19** and the previously reported corresponding 5-bromo-4,6-di-me-pyridin-2-yl congeners.[10] Introduction of a bicyclic ring of 9H-purin-6-yl at N-3 of thiazolidin-4-one in compounds **23** and **24** (EC_{50} = 11.82 and 36.33 µM against $HIV\text{-}1_{IIIB}$ and 11.71 and 27.84 µM against $HIV\text{-}1_{ADA5}$ strains, respectively) led to reduced anti-viral potency. These observations suggested that the magnitude of the bicyclic ring did not accommodate in the binding pocket of RT. In the set of 4,6-di-methoxypyrimidin-2-yl congeners at N-3, compounds **17**, **26** and **27** revealed slight reduction of anti-HIV-1 activities than compound **25** because of replacement of the Cl atom with -NO_2 group or F atom at 6' position of the C-2 phenyl ring of thiazolidin-4-one. Based on the results it may be inferred that introduction of -NO_2 group at 6' position of the C-2 phenyl ring of thiazolidin-4-one has a positive influence on the anti-HIV-1 activity (when 4,6-di-Me-pyridin-2-yl moiety placed at N-3). Moreover the compounds **20-22**, having 2',6'-dimethoxyphenyl moiety at C-2 of thiazolidin-4-one, displayed moderate to poor activity against all four HIV-1 strains. The introduction of a para-cyanoaniline in compounds **1**-**8** in place of 2',6'-dihalophenyl group at C-2 of thiazolidin-4-one, leads to a substantial decrease of potency in both enzyme inhibition and anti-viral activity in cell cultures. Additionally, the impact of introducing 3-cyanopyridin-6-yl moiety in compound **9** at the N-3 of thiazolidin-4-one, resulted in low or moderate activities against the four HIV-1 strains and loss of HIV-1 RT inhibition. Further, we evaluated the effect of a

linker (-NH-, -CONH-, and -SO_2NH-) between heteroaryl/aryl rings and the N-3 position of thiazolidin-4-one on anti-HIV-1 activity. Only the NH- linker of pyridyl-2-ylamino moiety led to compounds **28** and **29** which showed good to moderate potency with EC_{50} = 0.99 and 2.10 μM against HIV-1_{IIIB} and 1.85 and 3.79 μM against HIV-1_{ADA5}, 1.17 and 4.66 μM against HIV-1_{UGO70} and 1.70 and 5.31 μM against HIV-1_{VB59} strains respectively. The other linkers mainly -CONH- and -SO_2NH- present in compounds **10**-**15** showed very low potency against all HIV-1 strains with concomitant decrease of the RT inhibition. This indicated that the linker between heteroaryl/aryl rings and N-3 of thiazolidin-4-one core could play a crucial role in the antiviral activity and HIV-1 RT enzyme inhibition.

2.3. Molecular Docking Studies

The molecular interactions studies of newly synthesized thiazolidin-4-ones were explored by using the AutoDock 4.0[16]. Five compounds, **10**, **12**, **16**, **25** and **28**, were docked into the active site of HIV-1 RT non-nucleoside binding site (NNBS). The coordinates of the non-nucleoside binding site were taken from the crystal structure of HIV-1 Reverse Transcriptase (RT) in complex with TMC278 (Rilpivirine) (pdb entry codes: 2ZD1)[17]. Detailed analysis of the binding mode of potent compound **16** (Fig. 3) showed that the 4,6-dimethylpyridin-2-yl moiety along with thiazolidin-4-one scaffold lay in a hydrophobic pocket, which is mainly formed by the side chains of Tyr181, Tyr188, Phe227, and Trp229. The nitro group at 6'-phenyl moiety in compound **16** also formed two hydrogen bonds with the backbone of Gly190 and Val189 in the HIV-1 RT. The 2',6'-dihalophenyl moiety along with thiazolidin-4-one ring of compound **25** and 2',6'-dihalophenyl part of compounds **10**, **12** and **28** (Fig. 4 and 5) exist in the π-box formed by the hydrophobic residues of Tyr181, Tyr188, Phe227, and Trp229. The oxygen atom of the terminal dimethoxy group at pyrimidin-2-yl of compound **25** formed

two hydrogen bonds with the backbone amino groups of Lys101 and Lys103 residues. The carbonyl oxygen atom of the thiazolidin-4-one nucleus and -NH-SO_2- group of compounds **12** and **10** forms single hydrogen bond interactions with the backbone N–H of Lys101 residue. In the compound **28**, the pyridyl-2yl ring makes close hydrophobic contacts with the aliphatic isobutyl side chain of Leu100 and Leu234. The docked binding energy (Table 3), of compounds **10, 12, 16, 25** and 2**8** displayed moderate to strong binding energy values (binding energy = -8.23, -7.98, -10.08, -8.96 and -9.67 kcal/mol, respectively). The thiazolidin-4-ones (**16** and 2**8**) form stable complexes with the RT enzyme, because of various hydrogen bonds and hydrophobic interactions with the RT enzyme. Molecular modeling results could be used as a support for the development of novel and more active thiazolidin-4-ones against the HIV-1 RT target.

3. Conclusions

In this study, we have discussed the preliminary structure-activity relationships of C-2 and N-3 modified thiazolidin-4-one derivatives against four HIV-$1_{IIIB,}$ HIV-1_{ADA5}, HIV-1_{UG070} and HIV-1_{VB59} strains along with HIV-1 RT enzyme assay. Amongst these, compound **16** was found to be the most promising inhibitor of HIV-1 replication against HIV-$1_{IIIB,}$ HIV-1_{ADA5}, HIV-1_{UG070} and HIV-1_{VB59} (EC_{50} = 0.02, 0.08, 0.08 and 0.08 µM, respectively), with highest selectivity index SI = 6940, 1735, 1692 and 1692, respectively. The overall SAR information and binding model analysis of thiazolidin-4-one analogues could serve as potential lead to further development of novel and potent NNRTIs.

4. Experimental protocol

4.1. General

All chemicals and solvents were purchased from Sigma Aldrich and Merck, respectively. Melting points (Mp) were determined by a Complab apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Perkin Elmer Spectrum RX1 spectrometer in potassium bromide (4000-450 cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a NMR Spectrometer Bruker DRX-300 using $CDCl_3$ as a solvent at 300 and 75 MHz, respectively. The chemical shifts are reported as parts per million (δ ppm) from tetramethylsilane (TMS) as an internal standard. The following abbreviations are used to describe peak patterns: s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet), q (quadruplet), and br (broad). Coupling constants are given in hertz. HRMS and ESI-MS were performed using a Waters Agilent 6520- Q-TofMS/MS system and JEOL-AccuTOF JMST100LC instruments. Elemental analyses were carried out on CARLO-ERBA EA1108 C, H, N elemental analyzer and values were in the acceptable limits of the calculated values. Analytical thin-layer chromatography (TLC) was carried out on Merck's precoated silica-gel plates 60 F_{254} and spots were visualized by irradiation with UV light (254 nm) and/or by iodine vapours. Column chromatography separations were performed on silica gel (230-400 mesh) as the stationary phase using hexane-ethyl acetate as mobile phase.

4.2. General synthetic procedure for the compounds from (hetero) aromatic amine/tosylhydrazine/isonicotinohydrazide (compounds 1-27)

The appropriate (hetero) aromatic amine/tosylhydrazine/isonicotinohydrazide (1.0 mmol) and 2,6-disubstituted benzaldehyde/4-cyanobenzaldehyde (1.5 mmol) were stirred in dry toluene

under reflux condition followed by addition of mercaptoacetic acid (2.0 mmol). The reaction mixture was refluxed with a Dean-Stark trap for 24-48 hours until the complete consumption of (hetero) aromatic amine. The reaction mixture was concentrated to dryness under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was successively washed with 5% aqueous citric acid, water, 5% aqueous sodium hydrogen carbonate, and then finally with brine. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to get a crude product that was purified by column chromatography on silica-gel (230-400 mesh) using hexane–ethyl acetate as eluents. The structures of all synthesized compounds were characterized by means of TLC, IR, ESI-MS, ^{1}H NMR, ^{13}C NMR, elemental analyses and HRMS.

4.2.1. 4-(3-(5-methylisoxazol-3-yl)-4-oxothiazolidin-2-yl)benzonitrile (1)

White solid, yield: 98.7%. Mp: 95-96 °C; IR (KBr): ν_{max} C=O 1708, CN 2229 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.66 (d, 2H, J = 8.2 Hz, $H_{2,6}$-PhH), 7.41 (d, 2H, J = 8.2 Hz, $H_{3,5}$-PhH), 6.84 (s, 1H, H_4-isoxazol-3-yl-H), 6.34 (s, 1H, CH), 3.98 (d, 1H, J = 16.2 Hz, CH_2), 3.79 (d, 1H, J = 16.3 Hz, CH_2), 2.39 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6 (C=O), 170.5 (isoxazol-3-yl-C5), 157.2 (isoxazol-3-yl-C3), 145.6, 132.8, 126.3, (Ph-C), 118.3 (CN), 112.3 (Ph-C), 95.4 (isoxazol-3-yl-C4), 61.3 (C2), 34.3 (C5), 12.6 (CH_3); MS (ESI): *m/z* 285.1 (M^+). Anal. Calcd for $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73. Found: C, 58.90; H, 3.91; N, 14.77.

4.2.2. 4-(3-(5-bromo-4,6-dimethylpyridin-2-yl)-4-oxothiazolidin-2-yl)benzonitrile (2)

White solid, yield: 94.6 %. Mp: 155-156 °C; IR (KBr): ν_{max} C=O 1710, CN 2222 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.93 (s, 1H, H_3-pyridin-2-yl), 7.61 (d, 2H, J = 8.3 Hz, $H_{2,6}$-PhH), 7.42 (d, 2H, J = 8.2 Hz, $H_{3,5}$-PhH), 6.81 (s, 1H, CH), 4.05 (dd, 1H, J = 16.1 Hz, 0.9 Hz, CH_2),

3.86 (d, 1H, J = 16.1 Hz, CH_2), 2.41 (d, 6H, J = 1.0 Hz, CH_3 at $C_{4,6}$-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7 (C=O), 155.3, (pyridin-2-yl-C6), 149.5 (pyridin-2-yl-C4), 147.7, 147.0 (pyridin-2-yl-C2 & Ph-C4), 132.5 (Ph-C2 & C6), 126.6, (Ph-C3 & C5), 120.0 (pyridin-2-yl-C5),118.3 (CN), 115.4 (pyridin-2-yl-C3), 111.9 (Ph-C1), 62.2 (C2), 34.0 (C5), 25.0, 23.7 (CH_3 at $C_{4,6}$-pyridin-2-yl); MS (ESI): *m/z* 389.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{17}H_{14}BrN_3OS+H^+$ *m/z* 388.0119, Found 388.0122.

4.2.3. 4-(3-(4,6-dimethylpyridin-2-yl)-4-oxothiazolidin-2-yl)benzonitrile (3)

White solid, yield: 95.8 %. Mp: 145-146 °C; IR (KBr): ν_{max} C=O 1690, CN 2228 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.75 (s, 1H, H_3-pyridin-2-yl), 7.59 (d, 2H, J = 8.2 Hz, $H_{2,6}$-PhH), 7.43 (d, 2H, J = 8.2 Hz, $H_{3,5}$-PhH), 6.88 (s, 1H, H_5-pyridin-2-yl), 6.71 (s, 1H, CH), 4.05 (d, 1H, J = 16.0 Hz, CH_2), 3.87 (d, 1H, J = 16.0 Hz, CH_2), 2.32, 2.24 (s, 6H, CH_3 at $C_{4,6}$-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0 (C=O), 156.2 (pyridin-2-yl-C6), 149.6 (pyridin-2-yl-C4), 147.1 (pyridin-2-yl-C2 & Ph-C4), 132.4 (Ph-C2 & C6), 126.8 (Ph-C3 & C5), 121.46 (pyridin-2-yl-C5), 118.4 (CN), 114.1 (pyridin-2-yl-C3), 111.6 (Ph-C1), 62.4 (C2), 34.1 (C5) 23.6, 21.2 (CH_3 at $C_{4,6}$-pyridin-2-yl); MS (ESI): *m/z* 310.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{17}H_{15}N_3OS+H^+$ *m/z* 310.1014, Found 310.1045.

4.2.4. 4-(3-(4,6-dimethoxypyrimidin-2-yl)-4-oxothiazolidin-2-yl)benzonitrile (4)

White solid, yield: 93.1 %. Mp: 138-137 °C; IR (KBr): ν_{max} C=O 1734, CN 2224 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.64 (d, 2H, J = 8.2 Hz, $H_{2,6}$-PhH), 7.46 (d, 2H, J = 8.2 Hz, $H_{3,5}$-PhH), 6.57 (s, 1H, H_5-pyrimidin-2-yl), 5.78 (s, 1H, CH), 3.99 (d, 2H, J = 16.1 Hz, CH_2), 3.80 (s, 6H, OCH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5 (C=O), 169.7 (pyrimidin-2-yl-C4 & C6), 155.1 (pyrimidin-2-yl-C2), 147.0 (Ph-C4), 132.7 (Ph-C2 & C6), 126.0 (Ph-C3 & C5), 118.2 (CN), 112.0 (Ph-C1), 86.7 (pyrimidin-2-yl-C5), 62.4 (C2), 54.2

(OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 33.7 (C5); MS (ESI): *m/z* 343 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{14}N_4O_3S+H^+$ *m/z* 343.0865, Found 343.0891.

4.2.5. 4-(3-(4,6-dimethylpyrimidin-2-yl)-4-oxothiazolidin-2-yl)benzonitrile (5)

Pale Yellow solid, yield: 84.9 %. Mp: 161-162 °C; IR (KBr): ν_{max} C=O 1710, CN 2227 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.59 (dd, 4H, J = 8.3 Hz, 13.9 Hz, $H_{2,3,5,6}$-PhH), 6.78 (s, 1H, H_5-pyrimidin-2-yl), 6.69 (s, 1H, CH), 4.03 (dd, 1H, J = 16.1 Hz, 1.0 Hz, CH_2), 3.89 (d, 1H, J = 16.0 Hz, CH_2), 2.39 (s, 6H, CH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (C=O), 168.3 (pyrimidin-2-yl-C4 & C6), 156.2 (pyrimidin-2-yl-C2), 146.0 (Ph-C4), 132.4 (Ph-C2 & C6), 127.3 (Ph-C3 & C5), 118.3 (CN), 117.5 (pyrimidin-2-yl-C5), 112.1 (Ph-C1), 62.6 (C2), 34.0 (C5), 23.8 (CH_3 at $C_{4,6}$-pyrimidin-2-yl); MS (ESI): *m/z* 311.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{14}N_4OS+H^+$ *m/z* 311.0967, Found 311.0978

4.2.6. 4-(3-(4-methylpyrimidin-2-yl)-4-oxothiazolidin-2-yl)benzonitrile (6)

Pale Yellow solid, yield: 86.8 %. Mp: 163-164 °C; IR (KBr): ν_{max} C=O 1716, CN 2230 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.49 (d, 1H, J = 5.0 Hz, H_6-pyrimidin-2-yl), 7.60 (dd, 4H, J = 8.3 Hz, 23.3 Hz, $H_{2,3,5,6}$-PhH), 6.92 (d, 1H, J = 5.0 Hz, H_5-pyrimidin-2-yl), 6.69 (s, 1H, CH), 4.04 (d, 1H, J = 16.1 Hz, CH_2), 3.90 (d, 1H, J = 16.1 Hz, CH_2), 2.43 (s, 3H, CH_3 at C_4-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (C=O), 168.9 (pyrimidin-2-yl-C4), 157.8 (pyrimidin-2-yl-C2), 156.5 (pyrimidin-2-yl-C6), 146.1 (Ph-C4), 132.6 (Ph-C2 & C6), 127.1 (Ph-C3 & C5), 118.4 (CN), 117.9 (pyrimidin-2-yl-C5), 112.2 (Ph-C1), 62.6 (C2), 34.0 (C5), 24.6 (CH_3 at C_4-pyrimidin-2-yl); MS (ESI): *m/z* 297.0 ($M+H^+$). Anal. Calcd for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.91. Found: C, 60.72; H, 4.11; N, 18.88

4.2.7. 4,4'-(4-oxothiazolidine-2,3-diyl)dibenzonitrile (7)

Yellow solid, yield: 98.4 %. Mp: 152-153 °C; IR (KBr): ν_{max} C=O 1703, CN 2223 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.65-7.58 (m, 3H, $H_{2,3,6}$-Ph-4'-yl), 7.41 (d, 3H, J = 8.4 Hz, $H_{2,3,6}$-Ph-4-yl), 7.14-7.03 (m, 1H, H_5-Ph-4'-yl), 6.85 (d, 1H, J = 7.5 Hz, H_5-Ph-4-yl), 6.22 (s, 1H, CH), 4.00 (d, 1H, J = 16.0 Hz, CH_2), 3.90 (d, 1H, J = 16.1 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (C=O), 143.9, 141.1, 133.1, 127.1, 124.2, (Ph-C & C'), 117.9, 117.9 (di-CN), 113.1, 110.1 (Ph-C & C'), 63.7 (C2), 33.7 (C5); MS (ESI): *m/z* 305.2 (M^+). Anal. Calcd for $C_{17}H_{11}N_3OS$: C, 66.87; H, 3.63; N, 13.76. Found: C, 66.90; H, 3.61; N, 13.74.

4.2.8. 6-(2-(4-cyanophenyl)-4-oxothiazolidin-3-yl)nicotinonitrile (8)

White solid, yield: 88.3 %. Mp: 129-130 °C; IR (KBr): ν_{max} C=O 1708, CN 2231 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.55 (d, 1H, J = 8.8 Hz, H_2-pyridin-6-yl), 8.47 (d, 1H, J = 1.6 Hz, H_4-pyridin-6-yl), 8.01 (dd, 1H, J = 8.8 Hz, 2.2 Hz, H_5-pyridin-6-yl), 7.64 (d, 2H, J = 8.3 Hz, $H_{3,5}$-PhH), 7.39 (d, 2H, J = 8.2 Hz, $H_{2,6}$-PhH), 6.81 (s, 1H, CH), 4.06 (d, 1H, J = 16.5 Hz, CH_2), 3.87 (d, 1H, J = 16.5 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5 (C=O), 152.6 (pyridin-6-yl-C2), 150.9 (pyridin-6-yl-C6), 146.2 (Ph-C4), 141.2 (pyridin-6-yl-C4), 132.8 (Ph-C3 & C5), 126.2 (Ph-C2 & C6), 118.2, 116.2 (di-CN), 115.1 (pyridin-6-yl-C5), 112.2 (Ph-C1), 106.1 (pyridin-6-yl-C3) 62.0 (C2), 33.7 (C5); MS (ESI): *m/z* 306.2 (M^+). Anal. Calcd for $C_{16}H_{10}N_4OS$: C, 62.73; H, 3.29; N, 18.29. Found: C, 62.78; H, 3.25; N, 18.34.

4.2.9. 6-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-yl)nicotinonitrile (9)

White solid, yield: 85.9 %. Mp: 119-120 °C; IR (KBr): ν_{max} C=O 1712, CN 2225 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.50-8.46 (m, 2H, $H_{2,4}$-pyridin-6-yl), 7.96-7.92 (m, 1H, H_5-pyridin-6-yl), 7.54 (s, 1H, CH), 7.36-7.33 (m, 1H, H_4-PhH), 7.19-7.09 (m, 2H, $H_{3,5}$-PhH), 4.23 (dd, 1H, J = 16.1 Hz, 1.6 Hz, CH_2), 3.98 (d, 1H, J = 16.1 Hz, CH_2); ^{13}C NMR (75 MHz,

$CDCl_3$): δ 172.0 (C=O), 152.9 (pyridin-6-yl-C2), 150.6 (pyridin-6-yl-C6), 140.9 (pyridin-6-yl-C4), 135.5 (Ph-C1), 134.2, 132.8 (Ph-C2 & C6), 130.5, 129.4 (Ph-C3 & C5), 128.8 (Ph-C4), 116.4 (CN), 115.3 (pyridin-6-yl-C5), 105.8 (pyridin-6-yl-C3) 59.0 (C2), 35.5 (C5); MS (ESI): *m/z* 350.0 (M^+). Anal. Calcd for $C_{15}H_9Cl_2N_3OS$: C, 51.44; H, 2.59; N, 12.00. Found: C, 51.41; H, 2.60; N, 12.03.

4.2.10. N-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-yl)-4-methylbenzenesulfonamide(10)

Pale yellow solid, yield: 89.1%. Mp: 140-142 °C; IR (KBr): ν_{max} C=O 1699, -NH- 3399, -N-SO_2- 1327, 1220 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.08, 7.99 (s, 2H, $H_{2,6}$-Ph-SO_2-NH-), 7.93 (d, 2H, J = 8.2 Hz, $H_{3,5}$-Ph-SO_2-NH-), 7.35 (t, 3H, J = 7.0 Hz, $H_{3,4,5}$-PhH), 7.23 (s, 1H, CH), 4.17 (d, 1H, J = 15.14 Hz, CH_2), 4.13 (d, 1H, J = 15.0 Hz, CH_2), 2.44 (s, 3H, CH_3 at C_4-Ph-SO_2-NH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.3 (C=O), 146.7, 142.9, 138.3, 137.3, 137.2, 133.6, 132.1, 130.4, 128.4, 123.1, 122.9, 117.7, (Ph-C), 51.9 (C2), 35.2 (C5), 23.7 (CH_3); MS (ESI): *m/z* 417.9 (M^+). Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3S_2$: C, 46.05; H, 3.38; N, 6.71. Found: C, 46.00; H, 3.32; N, 6.79.

4.2.11.N-(2-(2-chloro-6-fluorophenyl)-4-oxothiazolidin-3-yl)-4 methylbenzenesulfonamide (11)

Pale yellow solid, yield: 87.3%. Mp: 139-141 °C; IR (KBr): ν_{max} C=O 1703, -NH- 3451, -N-SO_2- 1323, 1222 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.04 (d, 2H, J = 7.1 Hz, $H_{2,6}$-Ph-SO_2-NH), 7.93 (d, 2H, J = 8.2 Hz, $H_{3,5}$-Ph-SO_2-NH-), 7.36 (d, 3H, J = 7.9 Hz, $H_{3,4,5}$-PhH), 7.03 (t, 1H, J = 8.2, CH), 4.17 (d, 1H, J = 15.1 Hz, CH_2), 4.12 (d, 1H, J = 15.2 Hz, CH_2), 2.44 (s, 3H, CH_3 at C_4- Ph-SO_2-NH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.8 (C=O), 162.4, 147.2, 140.6, 138.6, 134.4, 134.2, 132.6, 130.9, 115.1, 115.0, 114.8, 114.6, 52.9 (C2), 34.3 (C5), 24.6

(CH_3); MS (ESI): *m/z* 400.4 (M^+). Anal. Calcd for $C_{16}H_{14}ClFN_2O_3S_2$: C, 47.94; H, 3.52; N, 6.99. Found: C, 47.98; H, 3.45; N, 6.82.

4.2.12. N-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (12)

Yellow solid, yield: 91.2%. Mp: 139-141 °C; IR (KBr): ν_{max} -NH- 3342, thiazolidinone C=O 1719, amide C=O 1647 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.47 (s, 2H, $H_{2,6}$-isonicotinamide), 8.39 (br, 1H, CONH), 7.39-7.35 (m, 2H, $H_{3,5}$-isonicotinamide), 7.23-7.14 (m, 3H, $H_{3,4,5}$-PhH), 7.06 (s, 1H, CH), 4.08 (dd, 1H, J = 15.6 Hz, 1.8 Hz, CH_2), 3.91 (d, 1H, J = 15.6 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7 (thiazolidinone C=O), 167.9 (amide C=O), 145.8, 135.1, 135.0, 134.6, 132.1, 131.0, 130.6, 130.4, 130.2, 128.8, 128.6 (aromatic-C), 57.9 (C2), 32.3 (C5); MS (ESI): *m/z* 368.1 (M^+). Anal. Calcd for $C_{15}H_{11}Cl_2N_3O_2S$: C, 48.93; H, 3.01; N, 11.41. Found: C, 48.98; H, 3.09; N, 11.38.

4.2.13. N-(2-(2-chloro-6-fluorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (13)

Yellow solid, yield: 93.7%. Mp: 125-126 °C; IR (KBr): ν_{max} -NH- 3348, thiazolidinone C=O 1709, amide C=O 1666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.98 (br, 1H, CONH), 8.74 (s, 1H, H_2-isonicotinamide), 7.36-7.29 (m, 2H, $H_{6,3}$-isonicotinamide), 6.98-6.85 (m, 4H, H_5-isonicotinamide & $H_{3,4,5}$-PhH), 6.51 (s, 1H, CH), 4.11 (d, 1H, J = 15.5 Hz, CH_2), 3.82 (d, 1H, J = 15.6 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7 (thiazolidinone C=O), 163.1 (amide C=O), 159.7, 133.4, 131.3, 131.1, 126.0, 124.0, 123.8, 115.6, 115.3 (aromatic-C), 74.7 (C2), 32.0 (C5); MS (ESI): *m/z* 351.2 (M^+). Anal. Calcd for $C_{15}H_{11}ClFN_3O_2S$: C, 51.21; H, 3.15; N, 11.94. Found: C, 51.29; H, 3.07; N, 11.97.

4.2.14. N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (14)

Yellow solid, yield: 90.8%. Mp: 136-137 °C; IR (KBr): ν_{max} -NH- 3348, thiazolidinone C=O 1712, amide C=O 1656 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 9.06 (s, 1H, CONH), 8.29 (d,

2H, J = 8.7 Hz, $H_{2,6}$-isonicotinamide), 8.22 (d, 2H, J = 8.7 Hz, $H_{3,5}$-PhH), 7.76 (d, 2H, J = 8.7 Hz, $H_{3,5}$-isonicotinamide), 7.57 (d, 2H, J = 8.7 Hz, $H_{2,6}$-PhH), 6.19 (s, 1H, CH), 3.95 (d, 1H, J = 16.1 Hz, CH_2), 3.86 (d, 1H, J = 16.1 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0 (thiazolidinone C=O), 150.65 (amide C=O), 149.0, 148.1, 146.1, 139.4, 128.6, 128.3, 127.4, 125.1, 124.4, 123.9 (aromatic-C), 63.5 (C2), 31.3 (C5); MS (ESI): *m/z* 345.1 ($M+H^+$). Anal. Calcd for $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 52.25; H, 3.59; N, 16.31.

4.2.15. N-(2-(2,6-dimethylphenyl)-4-oxothiazolidin-3-yl)isonicotinamide (15)

White solid, yield: 88.3%. Mp: 155-157 °C; IR (KBr): ν_{max} -NH- 3342, thiazolidinone C=O 1703, amide C=O 1626 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.50 (s, 1H, CONH), 7.15-6.96 (m, 7H, $H_{2,3,5,6}$-isonicotinamide & $H_{3,4,5}$-PhH), 6.70 (s, 1H, CH), 3.89 (s, 2H, CH_2), 2.19 (s, 6H, di-CH_3 at $C_{2,6}$-Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.0 (thiazolidinone C=O), 151.8 (amide C=O), 137.9, 137.2, 135.7, 132.4, 131.2, 131.1, 129.3, 129.1, 128.8, 128.5 (aromatic-C), 59.1 (C2), 31.9 (C5), 20.6, 20.4 (CH_3 at $C_{2,6}$-Ph); MS (ESI): *m/z* 327.1 (M^+). Anal. Calcd for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83. Found: C, 62.31; H, 5.20; N, 12.88.

4.2.16. 2-(2-chloro-6-nitrophenyl)-3-(4,6-dimethylpyridin-2-yl)thiazolidin-4-one (16)

White solid, yield: 77.2%. Mp: 108-109 °C; IR (KBr): ν_{max} C=O 1695cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.86 (s, 1H, H_5-PhH), 7.65 (d, 1H, J = 6.3 Hz, H_3-PhH), 7.48 (d, 1H, J = 7.6 Hz, H_4-PhH), 7.30, 7.25 (s, 2H, $H_{3,5}$-pyridin-2-yl), 6.64 (s, 1H, CH), 4.23 (d, 1H, J = 15.8 Hz, CH_2), 3.99 (d, 1H, J = 15.8 Hz, CH_2), 2.28 (s, 3H, CH_3 at C_6-pyridin-2-yl), 2.16 (s, 3H, CH_3 at C_4-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1 (C=O), 156.1 (pyridin-2-yl-C6), 149.6, 149.49 (pyridin-2-yl-C4 & Ph-C6), 135.6 (pyridin-2-yl-C2), 133.8, 132.8. 128.6, 122.3, 121.2 (Ph-C), 112.8 (pyridin-2-yl-C3 & C5), 57.1 (C2). 35.9 (C5) 23.1, 21.2 (CH_3 at $C_{4,6}$-pyridin-2-

yl); MS (ESI): *m/z* 364.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{14}ClN_3O_3S+H^+$ *m/z* 364.0523, Found 364.0585.

4.2.17. 2-(2-chloro-6-nitrophenyl)-3-(4,6-dimethoxypyrimidin-2-yl)thiazolidin-4-one (17)

White solid, yield: 80.7%. Mp: 110-111 °C; IR (KBr): ν_{max} C=O 1742cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.70-7.58 (m, 2H, $H_{3,5}$-PhH), 7.37 (t, 2H, J = 8.0 Hz, H_4-PhH, H_5-pyrimidin-2-yl), 5.75 (s, 1H, CH), 4.23 (d, 1H, J = 16.3 Hz, CH_2), 3.99 (dd, 1H, J = 14.6 Hz, 2.7 Hz, CH_2), 3.71 (s, 6H, di-OCH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.3 (C=O, pyrimidin-2-yl-C4 & C6), 155.1 (pyrimidin-2-yl-C2), 150.1, 133.6, 133.5, 128.7, 122.8 (Ph-C), 86.9 (pyrimidin-2-yl-C5), 57.6 (OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 54.1 (C2). 29.6 (C5); MS (ESI): *m/z* 397.1 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{15}H_{13}ClN_4O_5S+H^+$ *m/z* 397.0373, Found 397.0382.

4.2.18. 3-(5-bromo-4,6-dimethylpyridin-2-yl)-2-(2-chloro-6-nitrophenyl)thiazolidin-4-one (18)

White solid, yield: 72.6%. Mp: 113-114 °C; IR (KBr): ν_{max} C=O 1698cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.03 (s, 1H, H_5-PhH), 7.68 (d, 1H, J = 7.9 Hz, H_3-pyridin-2-yl), 7.50 (d, 1H, J = 7.7 Hz, H_3-PhH), 7.33 (d, 1H, J = 8.0 Hz, H_4-PhH), 7.16 (s, 1H, CH), 4.23 (d, 1H, J = 17.3 Hz, CH_2), 3.99 (d, 1H, J = 15.9 Hz, CH_2), 2.38 (s, 3H, CH_3 at C_6-pyridin-2-yl), 2.32 (s, 3H, CH_3 at C_4-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2 (C=O), 155.3 (pyridin-2-yl-C6), 149.4, 149.7 (pyridin-2-yl-C4 & Ph-C2), 135.7 (pyridin-2-yl-C2), 132.5, 128.8, 122.4, 119.9 (Ph-C), 114.5 (pyridin-2-yl-C3 & C5), 57.0 (C2). 35.7 (C5) 23.3, 23.7 (CH_3 at $C_{4,6}$-pyridin-2-yl); MS (ESI): *m/z* 442.0 (M^+). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{13}BrClN_3O_3S+H^+$ *m/z* 441.9628, Found 441.9994.

4.2.19. 3-(5-bromo-4,6-dimethylpyridin-2-yl)-2-(2,6-difluorophenyl)thiazolidin-4-one (19)

White solid, yield: 80.5%. Mp: 118-119 °C; IR (KBr): ν_{max} C=O 1699 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.03 (s, 1H, H_3-pyridin-2-yl), 7.23-7.13 (m, 2H, $H_{3,5}$-PhH), 6.86 (t, 2H, J = 8.7 Hz, H_4-PhH & CH), 4.26 (d, 1H, J = 15.8 Hz, CH_2), 3.87 (d, 1H, J = 15.8 Hz, CH_2), 2.46 (s, 3H, CH_3 at C_6-pyridin-2-yl), 2.39 (s, 3H, CH_3 at C_4-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0 (C=O), 162.3, 162.2 (Ph-C2 & C6), 159.0, 155.1, 149.2 (pyridin-2-yl-C6, C4 & C2), 129.5 (Ph-C4), 119.6, 118.5 (pyridin-2-yl-C5 & C3), 114.4, 111.8, 111.5 (Ph-C1, C3 & C5) 53.2 (C2), 35.0 (C5), 24.6, 23.7 (CH_3 at $C_{4,6}$-pyridin-2-yl); MS (ESI): *m/z* 399.1 (M^+). HRMS (ESI): $(M+H)^+$, calcd. for $C_{16}H_{13}BrF_2N_2OS+H^+$ *m/z* 398.9978, Found 398.9401.

4.2.20. 3-(5-bromo-4,6-dimethylpyridin-2-yl)-2-(2,6-dimethoxyphenyl)thiazolidin-4-one (20)

Dark yellow solid, yield: 65.5%. Mp: 115-117 °C; IR (KBr): ν_{max} C=O 1702 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.90 (s, 1H, H_3-pyridin-2-yl), 7.44 (s, 1H, CH), 7.15 (t, 1H, J = 8.3 Hz, H_4-PhH), 6.50 (d, 2H, J = 8.2 Hz, $H_{3,5}$-PhH), 4.17 (dd, 2H, J = 15.3 Hz, 1.5 Hz, CH_2), 3.91-3.73 (m, 6H, di-OCH_3), 2.42 (s, 3H, CH_3 at C_6-pyridin-2-yl), 2.36 (s, 3H, CH_3 at C_4-pyridin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.3 (C=O), 159.2 (Ph-C2 & C6), 158.9 (pyridin-2-yl-C6), 151.2 (pyridin-2-yl-C4), 143.6 (pyridin-2-yl-C2), 128.7 (Ph-C4), 116.9 (pyridin-2-yl-C3 & C5), 110.1 (Ph-C1), 105.5 (Ph-C3 & C5), 61.1 (C2), 54.7 (OCH_3 at $C_{2,6}$-Ph), 32.5 (C5), 24.7, 23.8 (CH_3 at $C_{4,6}$-pyridin-2-yl); MS (ESI): *m/z* 423 (M^+). Anal. Calcd for $C_{18}H_{19}BrN_2O_3S$: C, 51.07; H, 4.52; N, 6.62. Found: C, 51.15; H, 4.50; N, 6.59.

4.2.21. 2-(2,6-dimethoxyphenyl)-3-(4,6-dimethoxypyrimidin-2-yl)thiazolidin-4-one (21)

White solid, yield: 72.6%. Mp: 125-126 °C; IR (KBr): ν_{max} C=O 1731cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.18-7.12 (m, 2H, $H_{3,5}$-PhH), 6.53 (d, 2H, J = 8.3 Hz, C_4-pyrimidin-2-yl & H_4-PhH), 5.71 (s, 1H, CH), 4.14 (dd, 2H, J = 15.4 Hz, 0.9 Hz, CH_2), 3.86-3.75 (m, 12H,

OCH_3 at $C_{2,6}$-PhH & $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4 (C=O), 170.6 (pyrimidin-2-yl-C4 & C6), 158.6 (Ph-C2 & C6), 155.1 (pyrimidin-2-yl-C2), 128.1 (Ph-C4), 108.1 (Ph-C1), 104.5 (Ph-C3 & C5), 78.1 (pyrimidin-2-yl-C5), 58.9 (C2), 56.1 (OCH_3 at $C_{2,6}$-Ph), 54.8 (OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 31.2 (C5); MS (ESI): *m/z* 423 (M^+). MS (ESI): *m/z* 378.1 $(M+H)^+$. HRMS (ESI): $(M+H)^+$, calcd. for $C_{17}H_{19}N_3O_5S+H^+$ *m/z* 378.1124, Found 378.1052.

4.2.22. 2-(2,6-dimethoxyphenyl)-3-(5-methylisoxazol-3-yl)thiazolidin-4-one (22)

White solid, yield: 86.7%. Mp: 101-102 °C; IR (KBr): ν_{max} C=O 1705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.21 (t, 1H, J = 8.3 Hz, H_4-PhH), 6.97 (s, 1H, H_4-isoxazol-3-yl-H), 6.78 (s, 1H, CH), 7.59-6.49 (m, 2H, $H_{3,5}$-PhH), 4.06 (dd, 2H, J = 15.5 Hz, 1.3 Hz, CH_2), 3.92, 3.76 (s, 6H, OCH_3 at $C_{2,6}$-Ph), 2.33 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2 (C=O), 169.3 (isoxazol-3-yl-C5), 158.1 (isoxazol-3-yl-C3), 157.8, 129.5, 116.5, 104.3 (Ph-C), 95.4 (isoxazol-3-yl-C4), 56.4, 55.8 (OCH_3 at $C_{2,6}$-Ph), 53.7 (C2), 34.4 (C5), 12.5 (CH_3 at C_5-isoxazol-3-yl); MS (ESI): *m/z* 320.9 (M^+). HRMS (ESI): $(M+H^+)$, calcd. for $C_{15}H_{16}N_2O_4S+H^+$ *m/z* 321.0909, Found 321.0919.

4.2.23. 2-(2,6-dichlorophenyl)-3-(9H-purin-6-yl)thiazolidin-4-one (23)

White solid, yield: 65.4 %. Mp: 156-158 °C; IR (KBr): ν_{max} C=O 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO); δ 12.72 (br, 1H, NH, purin-6-yl), 8.59 (d, 2H, J = 6.8 Hz, $H_{2,8}$-purin-6-yl), 7.56 (s, 1H, CH), 7.48-7.45 (m, 1H, H_4-PhH), 7.28-7.21 (m, 2H, $H_{3,5}$-PhH), 4.19 (s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO): δ 171.5 (C=O), 150.8 (purin-6-yl-C2 & C4), 147.3 (purin-6-yl-C6), 135.1 (purin-6-yl-C8 & Ph-C1), 133.6 (Ph-C2 & C6), 133.0 (Ph-C4), 131.2, 131.0 (Ph-

C3 & C5), 129.3 (purin-6-yl-C5), 58.3 (C2), 34.4 (C5); MS (ESI): *m/z* 367.2 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{14}H_9Cl_2N_5OS+H^+$ *m/z* 365.9983, Found 365.9975.

4.2.24. 2-(2,6-difluorophenyl)-3-(9H-purin-6-yl)thiazolidin-4-one (24)

White solid, yield: 70.2 %. Mp: 147-149 °C; IR (KBr): ν_{max} C=O 1635 cm^{-1}; ^1H NMR (300 MHz, DMSO); δ 12.70 (br, 1H, NH, purin-6-yl), 8.61 (d, 2H, J = 10.17 Hz, $H_{2,8}$-purin-6-yl), 7.39-7.29 (m, 1H, H_4-PhH), 7.11 (s, 1H, CH), 7.06 (t, 2H, J = 9.1 Hz, $H_{3,5}$-PhH), 4.21 (d, 1H, J = 16.0 Hz, CH_2), 4.13 (d, 1H, J = 16.7 Hz, CH_2); ^{13}C NMR (75 MHz, DMSO): δ 171.7 (C=O), 161.9 (Ph-C2 & C6), 158.6, 151.0 (purin-6-yl-C2 & C4), 147.6 (purin-6-yl-C6), 131.4 (purin-6-yl-C8), 131.3 (Ph-C4), 116.8 (purin-6-yl-C5), 115.8 (Ph-C1), 112.8, 112.5 (Ph-C3 & C5), 58.0 (C2), 33.7 (C5); MS (ESI): *m/z* 334.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{14}H_9F_2N_5OS+H^+$ *m/z* 334.0574, Found 334.0581.

4.2.25. 2-(2,6-dichlorophenyl)-3-(4,6-dimethoxypyrimidin-2-yl)thiazolidin-4-one (25)

White solid, yield: 80.8%. Mp: 122-123 °C; IR (KBr): ν_{max} C=O 1741cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.31-7.25 (m, 3H, $H_{3,4,5}$-PhH), 7.16 (t, 1H, J = 8.0 Hz, H_5-pyrimidin-2-yl), 5.78 (s, 1H, CH), 4.19 (dd, 1H, J = 15.8 Hz, 1.3 Hz, CH_2), 3.92 (d, 1H, J = 15.8 Hz, CH_2), 3.79 (s, 6H, di-OCH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4 (C=O), 170.3 (pyrimidin-2-yl-C4 & C6), 155.3 (pyrimidin-2-yl-C2), 135.7, 134.4, 133.1, 130.7, 129.0, 128.4 (Ph-C), 86.7 (pyrimidin-2-yl-C5), 59.2 (OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 54.1 (C2). 35.3 (C5); MS (ESI): *m/z* 386.2 (M^+). HRMS (ESI): (M^+), calcd. for $C_{15}H_{13}Cl_2N_3O_3S^+$ *m/z* 385.0055, Found 385.0096.

4.2.26. 2-(2-chloro-6-fluorophenyl)-3-(4,6-dimethoxypyrimidin-2-yl)thiazolidin-4-one (26)

White solid, yield: 84.8%. Mp: 124-125 °C; IR (KBr): ν_{max} C=O 1737cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.29-7.19 (m, 2H, $H_{3,5}$-PhH), 7.01-6.94 (m, 2H, H_5-pyrimidin-2-yl & H_4-PhH), 5.78 (s, 1H, CH), 4.18 (d, 1H, J = 15.9 Hz, CH_2), 3.91 (d, 1H, J = 15.8 Hz, CH_2), 3.80 (s, 6H, di-OCH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4 (C=O), 170.1 (pyrimidin-2-yl-C4 & C6), 162.3 (Ph-C2), 159.0 (pyrimidin-2-yl-C2), 155.3, 133.0, 129.2, 125.5 (Ph-C), 86.5 (pyrimidin-2-yl-C5), 58.0 (OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 54.1 (C2). 30.9 (C5); MS (ESI): *m/z* 370.2 (M+H$^+$). HRMS (ESI): (M+H$^+$), calcd. for $C_{15}H_{13}ClFN_3O_3S$+H$^+$ *m/z* 370.0428, Found 370.0423.

4.2.27. 2-(2,6-difluorophenyl)-3-(4,6-dimethoxypyrimidin-2-yl)thiazolidin-4-one (27)

White solid, yield: 90.2%. Mp: 117-118 °C; IR (KBr): ν_{max} C=O 1737cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.26-7.19 (m, 1H, H_5-pyrimidin-2-yl), 6.91 (t, 3H, J = 8.6 Hz, $H_{3,4,5}$-PhH), 5.78 (s, 1H, CH), 4.23 (d, 1H, J = 15.2 Hz, CH_2), 4.16 (d, 1H, J = 14.2 Hz, CH_2), 3.84 (s, 6H, di-OCH_3 at $C_{4,6}$-pyrimidin-2-yl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4 (C=O), 169.9 (pyrimidin-2-yl-C4 & C6), 162.0, 161.9 (Ph-C2 & C6), 158.6 (pyrimidin-2-yl-C2), 129.4, 112.0, 111.9, 111.6 (Ph-C), 86.5 (pyrimidin-2-yl-C5), 54.0 (OCH_3 at $C_{4,6}$-pyrimidin-2-yl), 53.2 (C2). 34.6 (C5); MS (ESI): *m/z* 354.2 (M+H$^+$). HRMS (ESI): (M+H$^+$), calcd. for $C_{15}H_{13}F_2N_3O_3S$+H$^+$ *m/z* 354.0724, Found 354.0718.

4.3. General procedure for the preparation of compounds (28-33) from pyridyl-2-yl/4-cyanophenyl hydrazine

An equimolar mixture of 2,6-dihalo-substituted benzaldehyde (1.0 mmol) and aryl/heteroaryl hydrazine (1.0 mmol) was refluxed in toluene using a Dean–Stark trap for 5 hours. The solvent was evaporated under reduced pressure by using rotary evaporator. The solid hydrazone intermediates were added to the excess mercaptoacetic acid (5 mmol), and then

heated at 60 °C for 12 hours until reaction was completed, as shown by TLC. Ethyl acetate (5 ml) was added, the organic layer was washed with saturated sodium bicarbonate (3 X 20 ml) and water (1 X 10 ml), dried with sodium sulphate, and concentrated to give an oil. The oil was purified by column chromatography on silica gel using hexane/ethyl acetate as eluent.

4.3.1. 2-(2,6-dichlorophenyl)-3-(pyridin-2-ylamino)thiazolidin-4-one (28)

White solid, yield: 75.9%. Mp: 118-119 °C; IR (KBr): ν_{max} -NH- 3350, C=O 1699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.21 (d, 1H, J = 4.4 Hz, H_6-pyridin-2-yl), 7.59-7.54 (m, 1H, H_4-pyridin-2-yl), 7.40 (d, 1H, J = 7.7 Hz, H_4-PhH), 7.32-7.20 (m, 2H, $H_{3,5}$-PhH), 7.03 (s, 1H, CH), 6.87 (t, 1H, J = 5.0 Hz, H_5-pyridin-2-yl), 6.67 (d, 1H, J = 8.2 Hz, H_3-pyridin-2-yl), 6.43 (br, 1H, NH), 3.85 (s, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (C=O), 157.1, 148.3, 138.1, 136.4, 134.4, 132.1, 130.7, 130.2, 128.9, 117.1, 107.4 (aromatic-C), 57.9 (C2), 30.8 (C5); MS (ESI): *m/z* 340.4 (M^+). HRMS (ESI): ($M+H^+$), calcd. for $C_{14}H_{11}Cl_2N_3OS+H^+$ *m/z* 340.0078, Found 340.0081.

4.3.2. 2-(2-chloro-6-fluorophenyl)-3-(pyridin-2-ylamino)thiazolidin-4-one (29)

White solid, yield: 95.8%. Mp: 135-136 °C; IR (KBr): ν_{max} -NH- 3401, C=O 1707 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.21 (d, 1H, J = 4.4 Hz, H_6-pyridin-2-yl), 7.59 (t, 1H, J = 7.3 Hz, H_4-pyridin-2-yl), 7.20 (d, 1H, J = 7.9 Hz, H_5-PhH), 7.10 (t, 1H, J = 10.6 Hz, H_3-PhH), 6.87 (t, 1H, J = 5.3 Hz, H_4-PhH), 6.73-6.63 (m, 3H, $H_{3,5}$-pyridin-2-yl & CH-thiazolidin-4-one), 6.50 (br, 1H, NH), 3.91 (d, 1H, J = 15.6 Hz, CH_2), 3.77 (d, 1H, J = 15.6 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (C=O), 163.9, 157.3, 148.3, 138.2, 134.7, 130.6, 127.5, 125.9, 124.8, 115.8, 107.2 (aromatic-C), 56.7 (C2), 29.7 (C5); MS (ESI): *m/z* 324.0 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{14}H_{11}ClFN_3OS+H^+$ *m/z* 324.0374, Found 324.0906.

4.3.3. 2-(2,6-difluorophenyl)-3-(pyridin-2-ylamino)thiazolidin-4-one (30)

White solid, yield: 93.6%. Mp: 128-129 °C; IR (KBr): ν_{max} -NH- 3345, C=O 1699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 8.20 (d, 1H, J = 4.6 Hz, H_6-pyridin-2-yl), 7.59 (t, 1H, J = 7.4 Hz, H_4-pyridin-2-yl), 7.36-7.31 (m, 1H, H_4-PhH), 6.96-6.83 (m, 3H, $H_{3,5}$-PhH & H_5-pyridin-2-yl), 6.68 (d, 1H, J = 8.2 Hz, H_3-pyridin-2-yl), 6.58 (br, 1H, NH), 7.41 (s, 1H, CH), 3.94 (d, 1H, J = 15.6 Hz, CH_2), 3.77 (d, 1H, J = 15.5 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8 (C=O), 163.6, 163.5, 158.7, 157.3, 148.2, 138.1, 117.0, 112.3, 111.2, 111.8, 107.1 (aromatic-C), 52.5 (C2), 29.8 (C5); MS (ESI): *m/z* 308.2 ($M+H^+$). HRMS (ESI): ($M+H^+$), calcd. for $C_{14}H_{11}F_2N_3OS+H^+$ *m/z* 308.0669, Found 308.0616.

4.3.4. 4-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-ylamino)benzonitrile (31)

White solid, yield: 55.2 %. Mp: °C; IR (KBr): ν_{max} -NH- 3421, C=O 1699, CN 2220 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.57 (d, 2H, J = 8.1 Hz, $H_{2,6}$-Ph-4-yl), 7.43-7.24 (m, 2H, $H_{3,5}$-Ph-1-yl), 6.84 (d, 3H, J = 8.2 Hz, H_4-Ph-1-yl & $H_{3,5}$-Ph-4-yl), 6.09 (s, 1H, CH), 3.91 (d, 1H, J = 16.0 Hz, CH_2), 3.85 (d, 1H, J = 15.8 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2 (C=O), 148.1, 135.3, 132.8, 132.7, 130.6, 129.7, 129.4, 128.1, (aromatic-C), 118.1 (CN), 112.2, 103.2 (aromatic-C), 56.3 (C2), 29.6 (C5); MS (ESI): *m/z* 364.0 (M^+). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{11}Cl_2N_3OS+H^+$ *m/z* 364.0078, Found 364.0049.

4.3.5. 4-(2-(2-chloro-6-fluorophenyl)-4-oxothiazolidin-3-ylamino)benzonitrile (32)

White solid, yield: 75.8 %. Mp: °C; IR (KBr): ν_{max} -NH- 3421, C=O 1699, CN 2223 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.51 (d, 2H, J = 8.5 Hz, $H_{2,6}$-Ph-4-yl), 7.34-7.20 (m, 2H, $H_{3,5}$-Ph-1-yl), 7.10, (t, 1H, J = 10.5 Hz, H_4-Ph-1-yl), 6.79 (d, 2H, J = 8.5 Hz, $H_{3,5}$-Ph-4-yl), 6.62 (s, 1H, NH), 6.42 (s, 1H, CH), 3.91 (d, 1H, J = 15.7 Hz, CH_2), 3.74 (d, 1H, J = 15.8 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (C=O), 159.5, 149.3, 133.8, 130.9, 130.7, 126.1, 124.3,

124.1, (aromatic-C), 119.2 (CN), 115.7, 115.4, 113.1, 104.1 (aromatic-C), 56.3 (C2), 29.6 (C5); MS (ESI): *m/z* 347.0 (M^+). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{11}ClFN_3OS$ $+H^+$ *m/z* 348.0374, Found 348.0381.

4.3.6. 4-(2-(2,6-difluorophenyl)-4-oxothiazolidin-3-ylamino)benzonitrile (33)

White solid, yield: 91.2 %. Mp: °C; IR (KBr): ν_{max} -NH- 3398, C=O 1705, CN 2221 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$); δ 7.56 (d, 2H, J = 8.4 Hz, $H_{2,6}$-Ph-4-yl), 7.39-7.31 (m, 1H, H_3-Ph-1-yl), 6.99 (t, 2H, J = 8.9 Hz, $H_{4,5}$-Ph-1-yl), 6.83 (d, 2H, J = 8.4 Hz, $H_{3,5}$-Ph-4-yl), 6.31 (s, 1H, NH), 6.21 (s, 1H, CH), 3.95 (d, 1H, J = 15.3 Hz, CH_2), 3.75 (d, 1H, J = 15.7 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2 (C=O), 162.6, 162.5, 159.3, 149.3, 133.7, 131.1, 131.0 (aromatic-C), 119.1 (CN), 115.2, 114.8, 113.1, 112.4, 104.2 (aromatic-C), 52.2 (C2), 29.6 (C5); MS (ESI): *m/z* 331.0 (M^+). HRMS (ESI): ($M+H^+$), calcd. for $C_{16}H_{11}F_2N_3OS+H^+$ *m/z* 332.0669, Found 332.0667.

4.4. *In vitro* HIV-RT kit assay

The enzymatic HIV-RT inhibition assay was performed by using an RT assay kit following manufacturer's instructions (Roche Diagnostics GmbH, Germany).[10,18] Briefly, the reaction mixture containing template/primer complex, 2'-deoxy-nucleotide-5'-triphosphates (dNTPs) and reverse transcriptase (RT) enzyme in the lysis buffer was incubated for 1 hour at 37 °C and subsequently, the reaction mixture was transferred to streptavidin-coated microtitre plate (MTP). The biotin labeled dNTPs that are incorporated in the template due to the activity of RT bind to streptavidin. The unbound dNTPs were washed using wash buffer and anti-digoxigenin-peroxidase (anti-DIG-POD) was added onto MTP. The DIG-labeled dNTPs incorporated in the template was 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 catalyses by a peroxide enzyme. The absorbance of the sample was determined at OD_{405} nm using microtiter plate ELISA reader. The resulting color intensity is directly proportional to the actual RT activity. The percentage inhibitory activity of RT inhibitors was calculated by comparing with a sample that does not contain an inhibitor. The percentage inhibition was calculated by the formula as given;

$$\% \text{ Inhibition } = 100 - \left[\frac{\text{O.D. 405nm with inhibitor}}{\text{O.D. 405nm without inhibitor}} X\,100 \right]$$

4.5. *In vitro* Anti-HIV assay

All the thiazolidin-4-one compounds discussed here were evaluated for in vitro antiviral activity against HIV-1_{IIIB}, HIV-1_{ADA5}, HIV-1_{UG070} and HIV-1_{VB59} in TZM-bl cells as per previously reported procedure.[10,19]

Cell line and viral Stocks

Cell Line: TZM-bl (JC53BL-13) cells were obtained from the NIH AIDS Research and Reference Reagent Programme (ARRRP), NIH, USA. These are genetically modified HeLa cells, which express CD4, CXCR4, and CCR5 receptors. In addition, the cells also contain Tat-responsive reporter genes encoding firefly luciferase (Luc) and *Escherichia coli* β-galactosidase under the regulatory control of an HIV-1 long terminal repeat. The cells were maintained in Dulbecco's modified Eagle's medium DMEM (Gibco, USA) containing 10% heat inactivated fetal bovine serum (Morgate, Australia), HEPES (Gibco, USA), penicillin and

streptomycin (Gibco, USA) and gentamicin (Sigma, USA). Cultures were incubated at 37 ^{o}C in a humidified 5% CO_2 atmosphere and used when 80% confluency was achieved.

Viral Stocks: Lab adapted HIV-1_{IIIB} (X4, subtype B), HIV-1_{ADA5} (R5, subtype B) and the primary isolates HIV-1_{UG070} (X4, subtype D) were obtained from NIH ARRRP, whereas R5 tropic isolate HIV-1_{VB59} (subtype C) is an indian isolate from the National AIDS Research Institute, Pune. The viruses were grown in PHA-P (5µg/ml) (Sigma Aldrich, USA) activated peripheral blood mononuclear cells (PBMC) derived from healthy donors. Virus production was quantified in cell culture supernatants by HIV p24 antigen detection kit (Advanced Bioscience Laboratories Inc. Kit, USA). Aliquots of cell-free viral culture supernatants were centrifuged, filtered and stored at -70 ^{o}C. The $TCID_{50}$ (50% Tissue culture infective dose) of each virus stock was determined in the TZM-bl cells using Spearman Karber formula.

4.5.1. Cytotoxicity assay in TZM-bl cell line

The TZM-bl cells (1×10^4 cells/well) were seeded in a 96-well plate and incubated overnight at 37 ^{o}C with 5% CO_2 atmosphere. Next day, the medium was replaced with 100 µl of two fold dilution series of each compound in quadruplicate and the plate was incubated further. After 48 hours, cell viability was determined by measuring the mitochondrial-dependent conversion of the MTT salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma Aldrich, USA) to a coloured formazan product and the CC_{50} (concentration showing 50% toxicity) was calculated. In this assay, the measured absorbance is proportional to the viable cell number and inversely to the degree of cytotoxicity. For each compound the results are represented as mean of three independent assays.

4.5.2. Cell associated Anti-HIV-1 assay

The TZM-bl cells (1×10^4/well) were seeded in a 96-well plate and incubated overnight at 37 ^oC with 5% CO_2 atmosphere. On the subsequent day, the cells were infected with 400 $TCID_{50}$ HIV-1 stock and incubated for 2 hours at 37 ^oC with 5% CO_2 atmosphere. Serial dilutions of each compound showing more than 50% viability were then added in duplicates onto the cells. Anti-HIV-1 activity was determined after 48 hours of incubation by measuring the relative luminescence units (RLU) using the Britelite Plus substrate (Perkin Elmer). In this assay, RLU is directly proportional to the number of virus particles and inversely to the percent of inhibition. The concentration showing 50% inhibition (IC_{50}) was determined using Luc software. The results were interpreted as mean of two independent assays. Nevirapine was used as a positive control.

4.6. Docking studies

The Autodock 4.0 program was used to locate the appropriate binding orientations and conformations of thiazolidin-4-ones of HIV-1 RT inhibitors.[16] Autodock is an extensively used automated procedure for predicting the interactions of small molecules, such as peptides, enzyme inhibitors, and drugs, to macromolecules, such as proteins, enzymes, antibodies, DNA, and RNA. The structure of the non-nucleoside binding site (NNBS) of RT was taken from the crystal structure of HIV-1 RT in complex with its inhibitor TMC278 filed in the Brookhaven Protein Data Bank with the entry code 2ZD1.[17] The molecular structures of thiazoldin-4-one derivatives were built using the SKETCH option in Sybyl 7.3 version. Geometry optimizations of all chemical structures were carried out using the Tripos force field with a distance-dependent dielectric and the Powell conjugate gradient algorithm. Gasteiger-Hückel charges were used. Prior to docking studies, all water molecules, TMC278 (ligand)

and magnesium ion were removed from the original Protein Data Bank files. Polar hydrogen atoms were added and gasteiger charges, atomic solvation parameters and fragmental volumes were allocated to the protein. All torsions in thiazolidin-4-ones were treated as flexible by allowing them to rotate freely. The grid map calculation was performed using Autogrid 4.0 program, with a dimension of 60 X 60 X 60 points, a grid-point spacing of 0.375 Å and the maps were centered on the ligand binding site. The Lamarckian genetic algorithm (LGA) in Autodock 4.0 was used to explore the energy landscape. The hybrid search technique consists of a global optimizer modified from a genetic algorithm with 2-point crossover, random mutation, and a local optimizer with a Solis and Wets algorithm. A docking box with a dimension of 60 X 60 X 60 points with a grid spacing of 0.375 Å was defined in the calculations. Random conditions were used in the settings of seed, initial quaternion, coordinates, and torsions. A 0.2 Å step was used for translation and a 25-degree was used for quaternion and torsion. The maximum number of energy evaluation with 250 000 and the maximum number of generations with 27 000 was applied. The rate of gene mutation and crossover was set to 0.02 and 0.8, respectively. The number of cycles was set to 100. As a result, a total of 100 docking configurations were obtained in each docking calculation. A "preferable" docking configuration was chosen based on the lowest empirical binding free energy and the most frequent cluster.

Acknowledgements

This work is supported by ICMR project GAP 0044. We thank CSIR, New Delhi for supporting Dr. V. Murugesan for Senior Research fellowship. The authors thank the Director, CDRI for support and the SAIF for the spectral data. CDRI manuscript number: 18/2014/SBK

References and notes

1. De Béthune, M. P. *Antiviral Res.* **2010**, 85, 75.

2. Ammaranond, P.; Sanguansittianan, S. *Fundam Clin Pharmacol*. **2012**, 26, 146.

3. Adams, J.; Patel, N.; Mankaryous, N.; Tadros, M.; Miller, C. D. *Ann Pharmacother*. **2010**, 44, 157.

4. Hawkins, T. *Antiviral Res*. **2010**, 85, 201.

5. Menéndez-Arias, L. *Antiviral Res*. **2010**, 85, 210.

6. Deeks, E. D.; Keating, G. M. *Drugs*. **2008**, 68, 2357.

7. Das, K.; Bauman, J. D.; Clark, A. D. Jr.; Frenkel, Y. V.; Lewi, P. J.; Shatkin, A. J.; Hughes, S. H.; Arnold, E. *Proc Natl Acad Sci U S A*. **2008**, 105, 1466.

8. Barreca, M. L.; Balzarini, J.; Chimirri, A.; Clercq, E. D.; Luca, L. D.; Holtje, H. D.; Holtje, M.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Rao, A.; Zappala, M. *J Med Chem*. **2002**, 45, 5410.

9. Rao, A.; Balzarini, J.; Carbone, A.; Chimirri, A.; Clercq, E. D.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Zappalà, M. *Antivir Res*. 2004, 63, 79.

10. Murugesan, V.; Tiwari, V. S.; Saxena, R.; Tripathi, R.; Paranjape, R.; Kulkarni, S.; Makwana, N.; Suryawanshi, R.; Katti, S. B. *Bioorg Med Chem*. **2011**, 19, 6919.

11. Janssen, P. A.; Lewi, P. J.; Arnold, E.; Daeyaert, F.; De Jonge, M.; Heeres, J.; Koymans, L.; Vinkers, M.; Guillemont, J.; Pasquier, E.; Kukla, M.; Ludovici, D.; Andries, K.; De

Béthune M. P.; Pauwels, R.; Das, K.; Clark, A. D. Jr.; Frenkel, Y. V.; Hughes, S. H.; Medaer, B.; De Knaep, F.; Bohets, H.; De Clerck, F.; Lampo, A.; Williams, P.; Stoffels, P. *J Med Chem*. **2005**, 48, 1901.

12. Kelly, T. A.; Proudfoot, J. R.; McNeil, D. W.; Patel, U. R.; David, E.; Hargrave, K. D.; Grob, P. M.; Cardozo, M.; Agarwal, A.; Adams, J. *J Med Chem*. **1995**, 38, 4839.

13. Herschhorn, A.; Hizi, A. *J Med Chem*. **2008**, 51, 5702.

14. Murugesan, V.; Prabhakar, Y. S.; Katti, S. B. *J Mol Graph Model*. **2009**, 27, 735.

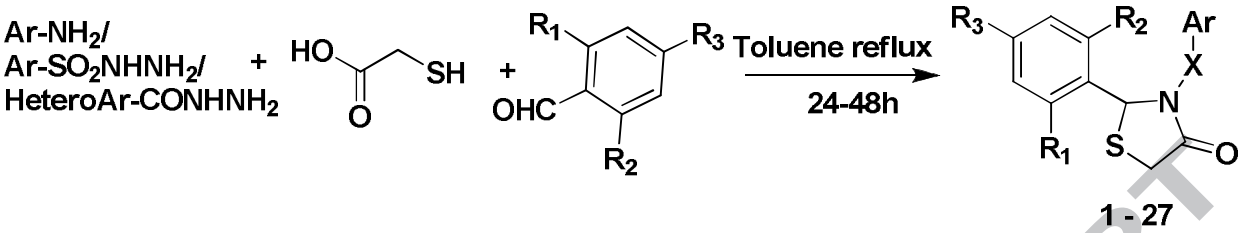
15. Neuenfeldt, P. D.; Drawanz, B. B.; Siqueira, G. M.; Gomes, C. R. B.; Wardell, S. M. S. V.; Flores, A. F. C.; Cunico, W. *Tetrahedron Lett*. **2010**, 51, 3106.

16. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem*. **1998**, 19, 1639.

17. Das, K.; Bauman, J. D.; Clark, A. D. Jr.; Frenkel, Y. V.; Lewi, P. J.; Shatkin, A. J.; Hughes, S. H.; Arnold, E. *Proc. Natl. Acad. Sci. U S A*. **2008**,105, 1466.

18. Reverse Transcriptase Assay, Colorimetric kit, Roche Diagnostics GmbH, Roche Applied Science, Sandhofer Strasse 116, D-68305 Mannheim, Germany.

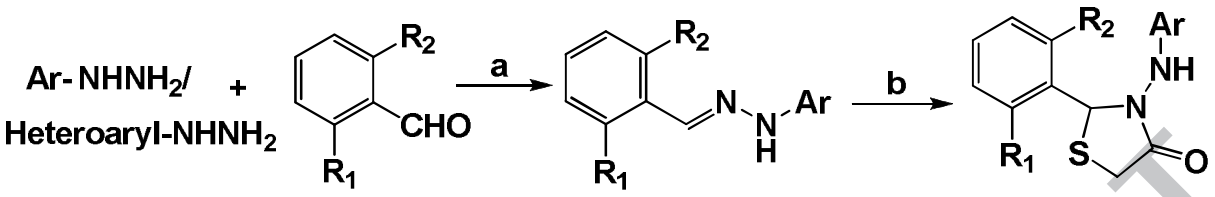
19. Geonnotti, A. R.; Bilska, M.; Yuan, X.; Ochsenbauer, C.; Edmonds, T. G.; Kappes, J. C.; Liao, H. X.; Haynes, B. F.; Montefiori, D. C. *AIDS. Res. Hum. Retroviruses*. **2010**, 26, 279.



1 - 27

Compound No	R_1	R_2	R_3	X	Ar/Heteroaryl
1	H	H	CN	-	5-methylisoxazol-3-yl
2	H	H	CN	-	5-bromo-4,6-dimethylpyridin-2-yl
3	H	H	CN	-	4,6-dimethylpyridin-2-yl
4	H	H	CN	-	4,6-dimethoxypyrimidin-2-yl
5	H	H	CN	-	4,6-dimethylpyrimidin-2-yl
6	H	H	CN	-	4-methylpyrimidin-2-yl
7	H	H	CN	-	4-cyanophenyl
8	H	H	CN	-	3-cyanopyridin-6-yl
9	Cl	Cl	H	-	3-cyanopyridin-6-yl
10	Cl	Cl	H	SO_2NH	4-methylphenyl
11	Cl	F	H	SO_2NH	4-methylphenyl
12	Cl	Cl	H	CONH	pyridin-4-yl
13	Cl	F	H	CONH	pyridin-4-yl
14	H	H	NO_2	CONH	pyridin-4-yl
15	Me	Me	H	CONH	pyridin-4-yl
16	Cl	NO_2	H	-	4,6-dimethylpyridin-2-yl
17	Cl	NO_2	H	-	4,6-dimethoxypyrimidin-2-yl
18	Cl	NO_2	H	-	5-bromo-4,6-dimethylpyridin-2-yl
19	F	F	H	-	5-bromo-4,6-dimethylpyridin-2-yl
20	OMe	OMe	H	-	5-bromo-4,6-dimethylpyridin-2-yl
21	OMe	OMe	H	-	4,6-dimethoxypyrimidin-2-yl
22	OMe	OMe	H	-	5-methylisoxazol-3-yl
23	Cl	Cl	H	-	9H-purin-6-yl
24	F	F	H	-	9H-purin-6-yl
25	Cl	Cl	H	-	4,6-dimethoxypyrimidin-2-yl
26	Cl	F	H	-	4,6-dimethoxypyrimidin-2-yl
27	F	F	H	-	4,6-dimethoxypyrimidin-2-yl

Scheme 1: Synthesis of thiazolidin-4-one compounds (1-27)



Compound No	R_1	R_2	Heteroaryl/Ar
28	Cl	Cl	pyridin-2-yl
29	Cl	F	pyridin-2-yl
30	F	F	pyridin-2-yl
31	Cl	Cl	4-cyanophenyl
32	Cl	F	4-cyanophenyl
33	F	F	4-cyanophenyl

a:Toluene, reflux, 5h
b: Mercaptoacetic acid, 60 °C, 12h

Scheme 2: General synthetic steps for thiazolidin-4-ones (28-33) from pyridin-2-ylhydrazine and para-cyanophenylhydrazine

Table 1: HIV-1 RT inhibitory activity and anti-HIV-1 activity of thaizolidin-4-ones against X4 tropic HIV-1 $_{IIIB}$ and R5 tropic HIV-1 $_{ADA5}$ lab adapted strains

Compound No	Anti-HIV-1 activity[a]					% Inhibition (HIV-RT Kit Assay) 100 µg/ml
	EC_{50} [b](µM)		CC_{50}[c](µM)	SI[d]		
	HIV-1 $_{IIIB}$	HIV-1 $_{ADA5}$	TZM-bl cells	HIV-1 $_{IIIB}$	HIV-1 $_{ADA5}$	
1	88.74	81.10	140.19	1.57	1.72	27.56
2	NI[f]	39.14	43.78		1.11	37.50
3	40.43	26.37	148.36	3.66	5.62	37.92
4	68.02	57.01	86.45	1.27	1.51	12.22
5	115.28	99.30	112.76	0.97	1.13	41.52
6	85.70	54.96	121.12	1.41	2.20	43.52
7	67.82	61.24	74.99	1.10	1.22	ND
8	53.60	74.22	127.63	2.38	1.71	30.36
9	20.52	4.62	59.96	2.92	12.97	42.01
10	29.40	4.55	75.71	2.57	16.63	57.9
11	63.11	64.6	135.70	2.15	2.10	56.3
12	36.66	9.72	170.25	4.64	17.51	18.87
13	NI[f]	NI[f]	104.61			81.65
14	15.68	13.75	58.6	3.73	4.26	23.34
15	29.38	18.90	43.37	1.47	2.29	14.25
16	0.02	0.08	138.80	6940	1735	91.55
17	0.10	0.22	80.64	806.4	366.45	79.43
18	NT[e]	NT[e]				77.75
19	0.35	0.70	67.12	191.77	95.88	81.77
20	2.22	1.51	85.75	38.62	56.78	85.53
21	3.17	4.13	156.32	49.31	37.84	93.81
22	11.33	NI[f]	124.85	11.01		62.18
23	11.82	11.71	13.10	1.10	1.11	50.23
24	36.33	27.84	40.50	1.11	1.45	70.40
25	0.07	0.10	85.43	1220	854.6	76.83
26	0.18	0.16	98.16	545.33	613.5	95.40
27	0.25	0.31	77.54	310.16	250.12	82.94
28	0.99	1.85	149	150.50	80.54	97.42
29	2.10	3.79	152.57	72.65	40.25	97.54
30	17.34	16.21	90.13	5.19	5.56	64.74
31	30.30	5.42	65.33	2.14	12.02	22.24
32	33.55	16.24	76.48	2.27	4.70	26.31
33	42.58	26.58	42.25	1	1.58	34.55
NVP[g]	0.03	0.03	76.13	2057	2057	99.20

[a] Data represent the mean of two and three independent assays for EC_{50} and $CC_{50,}$ respectively. [b]EC_{50} is the 50% effective concentration required to reduce HIV-1 induced cytopathic effect of HIV-1_{IIIB} and HIV-1_{ADA5} in TZM-bl cell line, [c] The CC_{50} is the 50% cytotoxic concentration for TZM-bl cells, [d] Selectivity index ratio CC_{50}/EC_{50}, [e] Not tested. [f] No inhibition, [g] Nevirapine

Table 2: Anti-HIV-1 activity of thaizolidin-4-ones against X4 tropic HIV-1_{UG070} and R5 tropic HIV-1_{VB59} primary isolates

Compound No	Anti-HIV-1 activity[a]				
	EC_{50} [b](μM)		CC_{50} [c](μM)	SI[d]	
	HIV-1_{UG070}	HIV-1_{VB59}	TZM-bl cells	HIV-1_{UG070}	HIV-1_{VB59}
1	NT[e]	68.72	140.19		2.04
2	NT[e]	NT	43.78		
3	51.06	46.54	148.36	2.90	3.18
4	NI[f]	NI[f]	86.45		
5	NI[f]	NI[f]	112.76		
6	NI[f]	NI[f]	121.12		
7	63.40	55.67	74.99	1.18	1.34
8	105.69	80.07	127.63	1.20	1.59
9	7.13	<7.13	59.96	8.40	<8.40
10	NI[f]	NI[f]	75.71		
11	72.39	81.62	135.70	1.87	1.66
12	NI[f]	26.31	170.25		6.47
13	NT[e]	NT[e]	104.61		
14	10.48	NI[f]	58.6	5.59	
15	38.79	NI[f]	43.37	1.11	
16	0.08	0.08	138.80	1692	1692
17	0.27	0.22	80.64	298	366.54
18	NT[e]	NT[e]			
19	0.50	0.77	67.12	134.24	87.16
20	2.36	2.48	85.75	36.33	34.57
21	8.87	7.28	156.32	17.62	21.47
22	18.44	20.19	124.85	6.77	6.18
23	NT[e]	NT[e]	13.10		
24	56.49	38.55	40.50	0.71	1.05
25	0.20	0.12	85.43	427.15	662.24
26	0.24	0.32	98.16	409	306.75
27	0.53	0.48	77.54	144.39	161.20
28	1.17	1.70	149.00	127.35	87.64
29	4.66	5.31	152.57	32.74	28.73
30	24.37	16.79	90.13	3.69	5.36
31	36.92	25.72	65.33	1.76	2.54
32	49.56	35.02	76.48	1.54	2.18
33	56.86	48.92	42.25	0.74	0.86
NVP[g]	0.03	0.03	76.13	2057	2057

[a] Data represent the mean of two and three independent assays for EC_{50} and $CC_{50,}$ respectively. [b]EC_{50} is the 50% effective concentration required to reduce HIV-1 induced cytopathic effect of HIV-1_{UG070} and HIV-1_{VB59} in TZM-bl cell line, [c] The CC_{50} is the 50% cytotoxic concentration for TZM-bl cells, [d] Selectivity index ratio $CC_{50}/EC_{50,}$, [e] Not tested. [f] No inhibition, [g] Nevirapine

Table 3. Docking score (kcal/mol) values of the five thiazolidin-4-one compound/HIV-1 RT Complexes

Compound no	$\Delta G^{a}_{Binding}$
10	-8.23
12	-7.98
16	-10.08
25	-8.96
28	-9.67

[a]Binding free energy

Figure Captions

Figure 1: The structural fragments of the three selected NNRTIs.

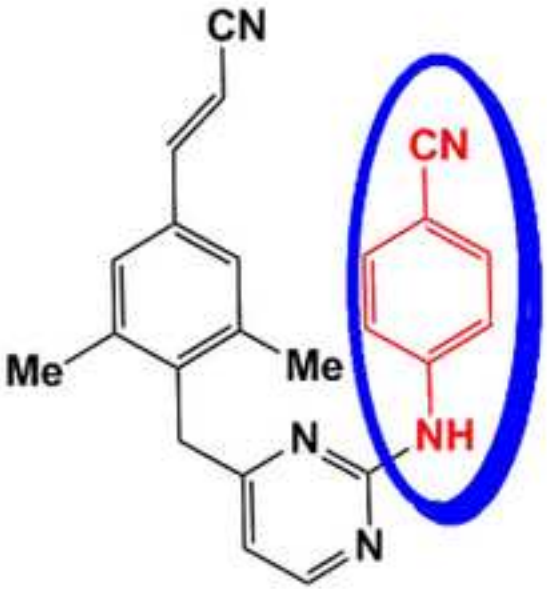
Figure 2: Design of C-2 and N-3 modified thiazolidin-4-ones.

Figure 3: Binding model of compound **16** in the NNRTI-binding pocket residues.

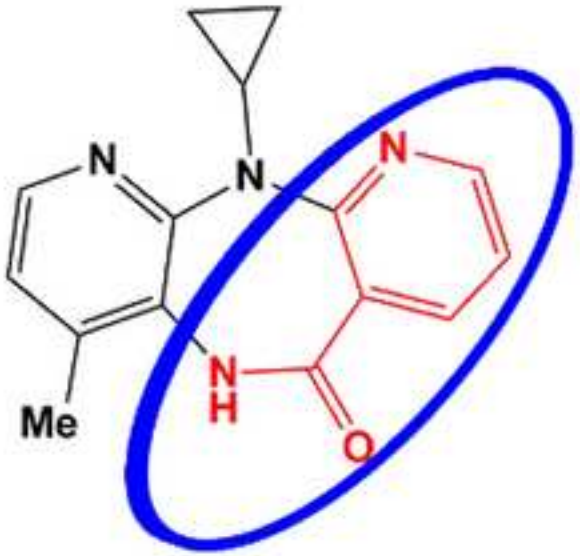
Figure 4: Binding model of compounds **10** (left) and **12** (right) in the NNRTI binding pocket residues.

Figure 5: Binding model of compounds **25** (left) and **28** (right) in the NNRTI binding pocket residues.

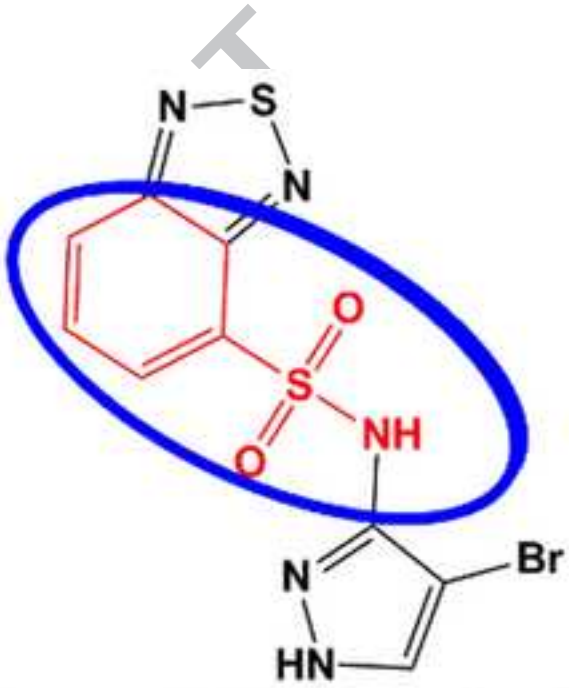
Figure(1)



Rilpivirine

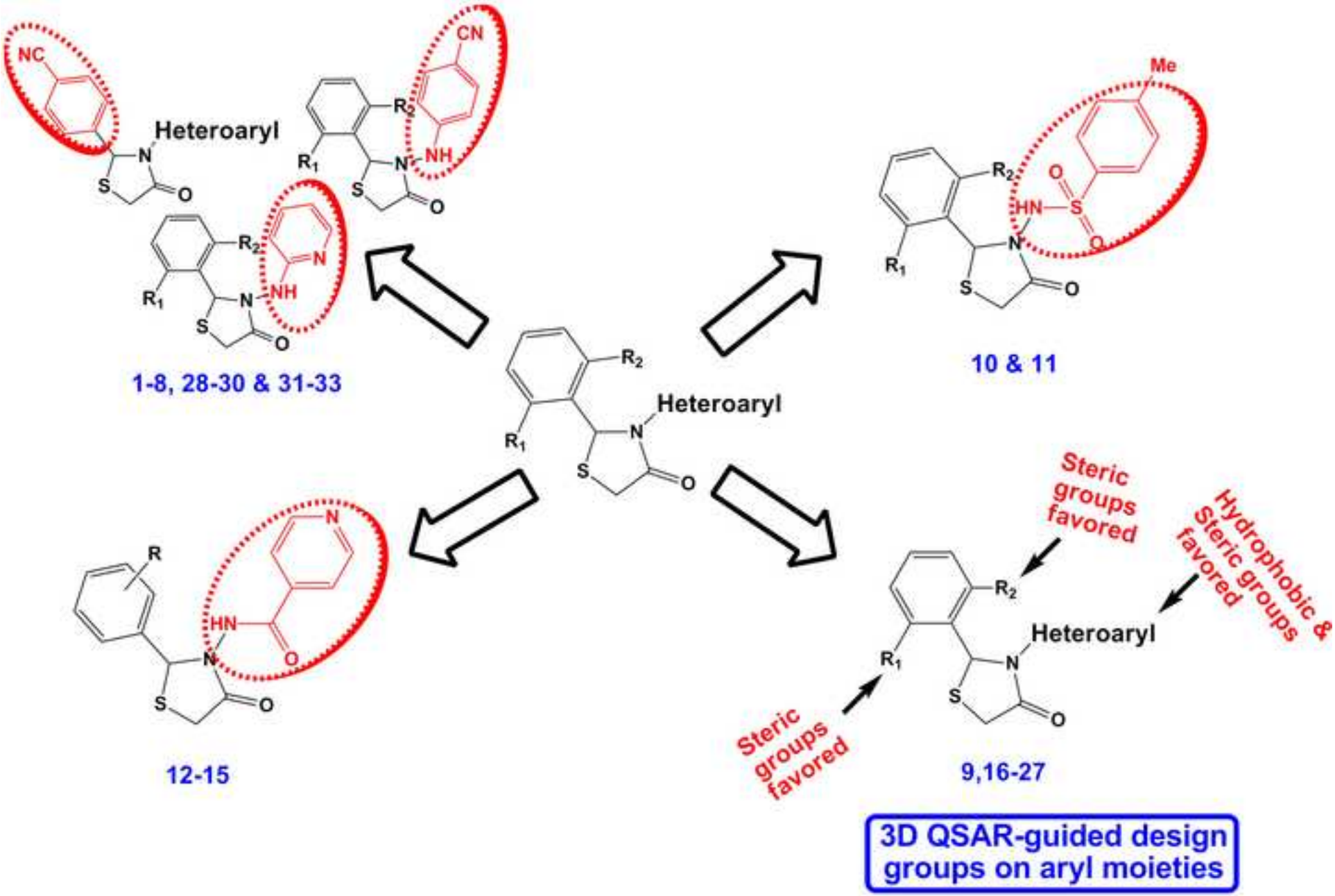


Nevirapine

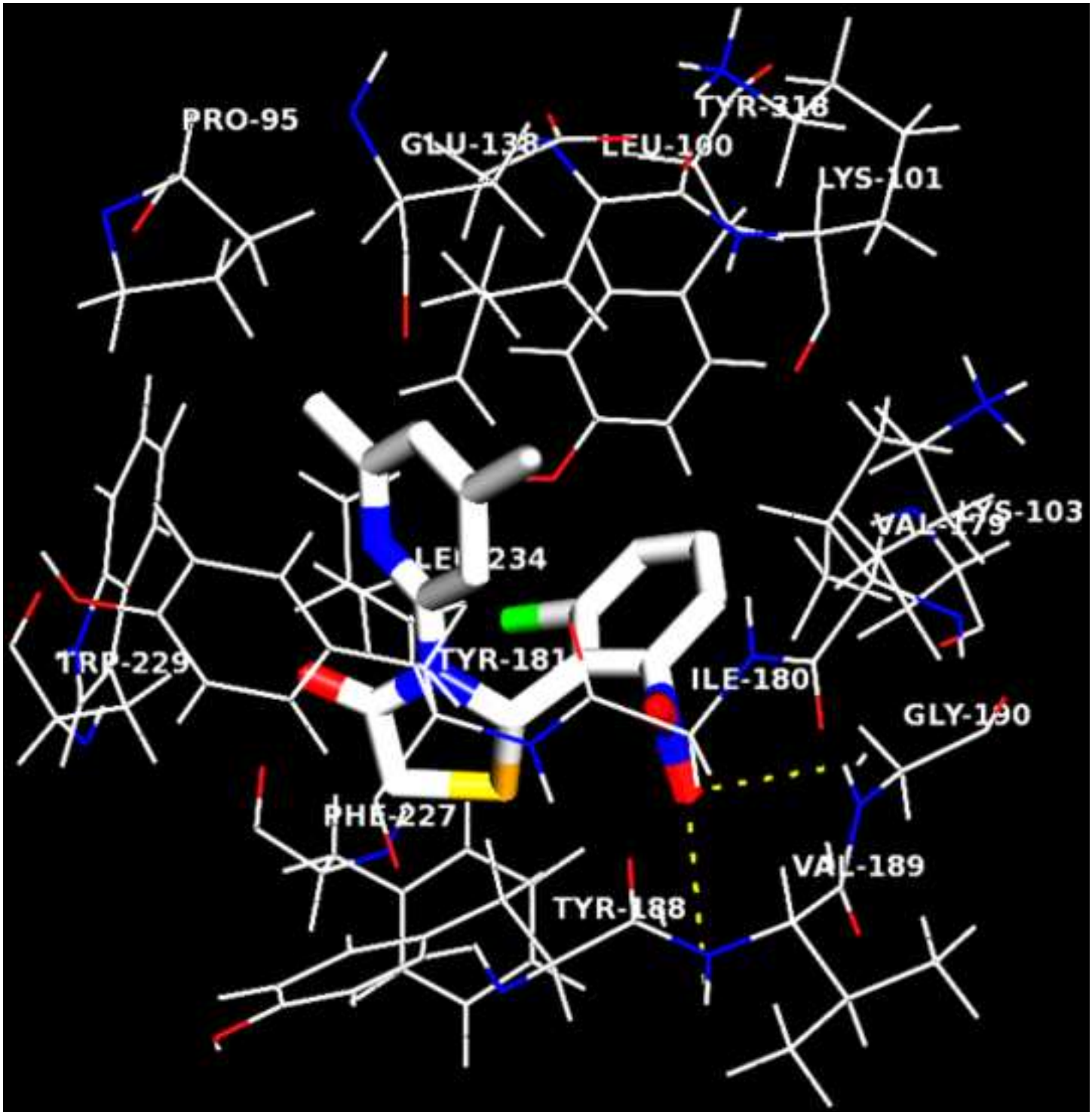


Benzothiadiazole-4-sulfonamide

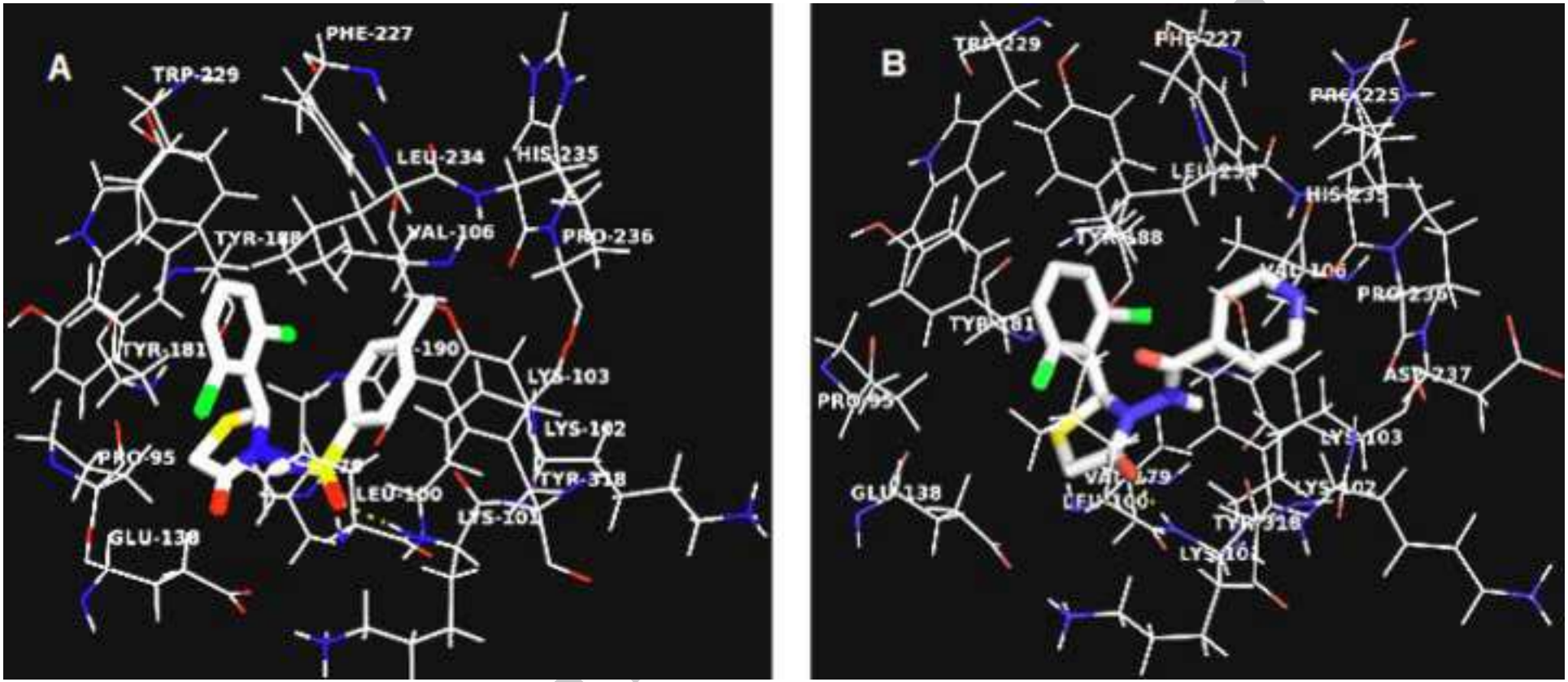
Figure(2)



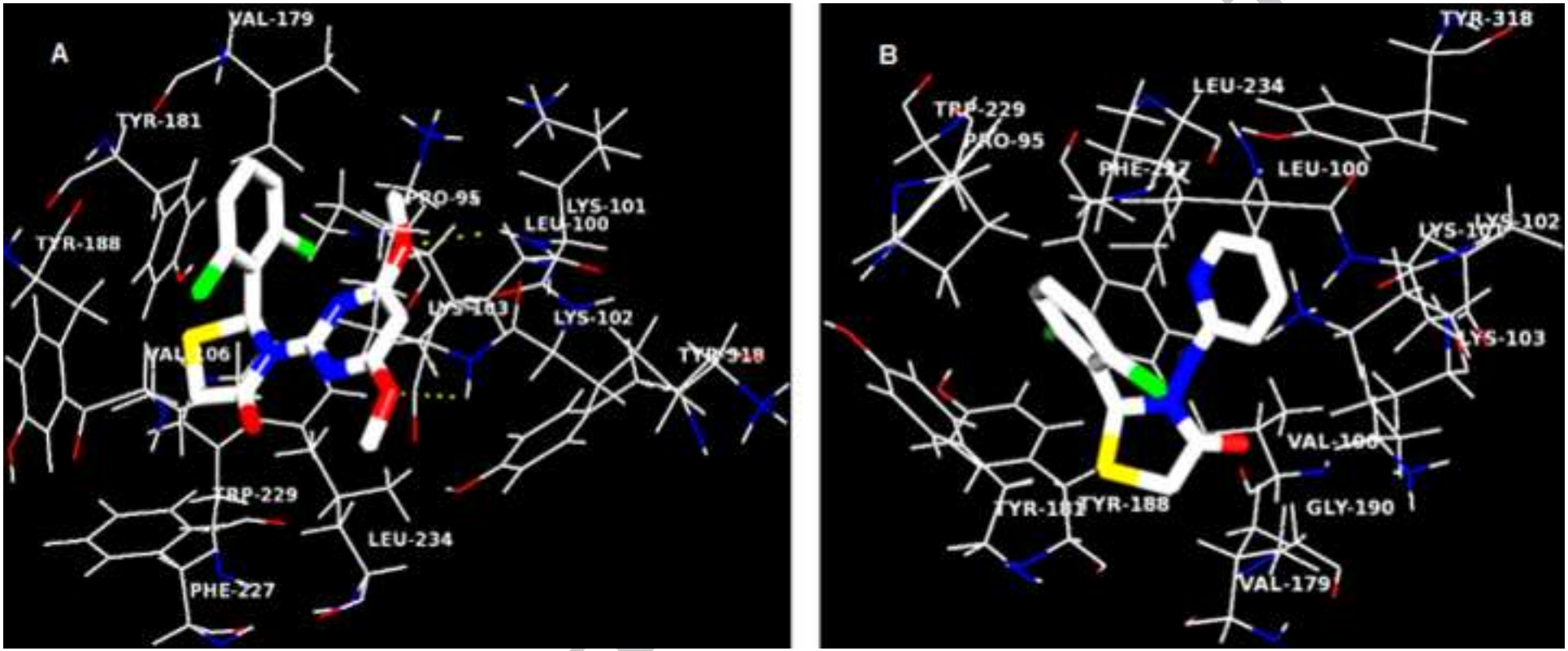
Figure(3)



Figure(4)



Figure(5)



Rational design and synthesis of novel thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors

Vanangamudi Murugesan[a], Nandini Makwana[c], Rahul Suryawanshi[c], Reshu Saxena[b], Rajkamal Tripathi[b], Ramesh Paranjape[c], Smita Kulkarni[c,$], Seturam B. Katti[a,$,*]

[a] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, JankiPuram Extension, Sector-10, Lucknow-226031, Uttar Pradesh, India

[b] Toxicology Division, CSIR-Central Drug Research Institute (CDRI), JankiPuram Extension, Sector-10, Lucknow-226031, Uttar Pradesh, India

[c] Department of Molecular Virology, National AIDS Research Institute (NARI), Pune-411026, Maharashtra, India

[$]Equal contribution

*Corresponding author:

Dr. Seturam B. Katti, CSIR-CDRI, Lucknow, India

sb_katti@cdri.res.in

Tel: 0522-2772454

Fax: 0522-2771941