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Research Letter

Design, Synthesis, and Evaluation of Thiazolidinone Derivatives as Antimicrobial and Anti-viral Agents

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A series of 1,3-thiazolidin-4-one derivatives were prepared by the reaction of respective aromatic amine, aromatic aldehyde, and thioglycolic acid in dry benzene/toluene. The newly synthesized compounds were characterized on the basis of elemental analysis, IR, ^{1}HNMR, and mass spectra. The newly synthesized final compounds were evaluated for their *in vitro* antibacterial, antifungal, and anti-viral activities. Preliminary results indicated that some of the compounds demonstrated antibacterial activity in the range of 7–13 μg/mL, antifungal activity in the range of 13–17 μg/mL, comparable with the standard drugs, ciprofloxacin and fluconazole. Structure–activity relationship studies revealed that the nature of the substituents at the 2 and 3 positions of the thiazolidinone nucleus had a significant impact on the *in vitro* antimicrobial and anti-viral activity of these classes of agents.

Key words: antibacterial, antifungal, anti-viral, Schiff's reaction, thiazolidinones

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Viral infections caused by the rapid emergence of anti-viral drug-resistant strains have become a serious globe threat and this has fostered the search for new anti-viral agents directed against unexplored drug targets. Various nucleoside analogs (currently used as anti-viral agents) often inhibit viral polymerases enzyme. However, only few non-nucleoside anti-viral agents are currently used in the market.

Thiazolidin-4-ones have been reported to posses a wide range of biological activities including antibacterial (1), antitubercular (2), antitumor (3), antihistaminic (4), anti-inflammatory (5), and anticonvulsant activity (6). Several 2,3-diaryl-1,3-thiazolidin-4-ones have proved to be particularly effective against non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) (7). These compounds may be considered as an 'open compound model' (8) of previously described 1H,3H-thiazolo[3,4-a]benzimidazoles (TBZs) (9) because they contain necessary pharmacophoric elements of those HIV-1 NNRTIs, namely a benzene-fused ring, an aryl group at C-1 and the nitrogen atom of the thiazole nucleus. Structure activity relationship (SAR) studies have shown that the anti-HIV activity strongly depends on the nature of substituents at C-2 and N-3 of the thiazolidinone ring. It has been demonstrated that a high anti-viral activity was associated with the presence of a 2,6-dihalo-substituted phenyl ring at C-2 and pyridin-2-yl or pyrimidin-2-yl rings at N-3 (8,10).

In 2002, the synthesis of diastereoisomers of trans- and cis-5-methyl-2,3-diaryl-1,3-thizolidin-4-ones from 2,6-dihalobenzaldehyde, heteroaromatic amine, and racemic 2-mercaptopropionic acid was reported and the author concluded that stereochemistry has not influenced the anti-HIV activity of the compounds. Surprisingly, the stereoselectivity of thiazolidin-4-ones has not yet been profoundly studied (11). Rao *et al.* (12) synthesized and evaluated anti-HIV activity of new 2,3-diaryl-1,3-thiazolidin-4-ones and their results of the *in vitro* tests showed that some of them were highly effective inhibitors of HIV-1 replication at 10–40 nM concentrations with minimal cytotoxicity.

In 2007, the anti-HIV activity of 2-aryl-3-heteroaryl-1,3-thiazolidin-4-ones was studied and concluded when one of their synthesized compound was found to be the most promising compound and active against HIV-1 replication exhibiting EC_{50} at 0.26 μM with minimal toxicity in MT-4 cells as compared to 0.35 μM for thiazobenzimidazole (TBZ) (13). In 2007 and 2009, synthesis of a series of novel thiazolidin-4-ones bearing a lipophilic adamantyl substituent at position 2 or 3 was performed by Balzarini *et al.* (14,15) and reported that majority of the compounds showed a modest anti-HIV-1 activity, whereas 2-adamantan-1-yl-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one was endowed with a remarkable anti-viral potency (EC_{50} = 0.67 μM).

Three 3-substituted-2-adamantyl-4-thiazolidinones were screened against twelve bacterial strains, and the minimal inhibitory concen-

trations (MIC, 125 mg/mL) indicated that these compounds were poor antibacterial agents (16). Antibacterial activity of some new 2,3-diaryl-1,3-thiazolidin-4-ones were reported in 2006 (17). Prompted by the earlier observations, we designed and synthesized a series of novel thiazolidin-4-one derivatives (**1**–**10**) bearing the different aryl, heteroaryl substituent at position 2, and several substituents on the nitrogen atom in the thiazolidine ring.

Materials and Methods

Chemistry

Melting points were determined using an open-ended capillary method and are uncorrected. The reaction was monitored by TLC. UV/VIS spectra were taken in a Shimadzu UV/VIS 1700 spectrophotometer (Kyoto, Japan). FT-IR was recorded on a Jasco FT-IR spectrophotometer (Tokyo, Japan), and ^{1}HNMR spectra were recorded at 300 MHz on a Bruker FT-NMR spectrometer (Ettlingen, Germany) and mass spectra on a Varian Atlas CH-7 mass spectrometer (Tokyo, Japan) at 70 eV. The elemental analysis was obtained on a Vario-EL instrument.

General procedure for synthesis of 1,3-thiazolidin-4-ones

We followed the method reported by Rawal *et al.* (13) with slight modification to synthesize 1,3-thiazolidin-4-one derivatives. The appropriate (hetero) aromatic amine (sulphanilamide, *para* chloro aniline, *ortho* amino pyridine, 5-chloro-2-amino pyridine, 2-amino-6-methyl pyridine, 2-amino-4,6-dimethyl pyridine, 2-amino-6-methyl-pyridine, phenyl ethyl amine) (1.0 mmol) and appropriate (hetero) aromatic aldehyde (*para* methoxy benzaldehyde, *para* nitro benzaldehyde, 2,5-dimethoxy benzaldehyde, pyridine-2-aldehyde, 2,6-dichloro benzaldehyde, 2,6-dimethoxy benzaldehyde) (1.0 mmol) was stirred under reflux for 0.5–2.5 h in dry benzene/toluene (10 mL). Then, mercaptoacetic acid (0.2 mL, 2 mmol) was added, and the mixture was refluxed for further 3–45 h. The reaction was monitored by TLC (Mobile phase: Cyclohexane and ethyl acetate 8:2, spots were visualized by exposing them to iodine vapor). After completion of the reaction, 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% sodium hydrogen carbonate, and then finally with brine. The organic layer was dried over sodium sulfate and filtered, and solvent was removed under reduced pressure to obtain crude (solid or oily) product. The oily residue was made solid by treatment with a mixture of ethanol and diethyl ether. All the compounds were recrystallized from ethanol (Table 1). Each of the synthesized compounds was highly soluble in DMSO, soluble in DMF, $CHCl_3$, ethanol and methanol, and sparingly soluble in water. The structures of synthesized compounds were elucidated by the means of FTIR, ^{1}HNMR, FAB-MS, and elemental analyses. The results of elemental analyses for C, H, and N of all the synthesized compounds were within ±0.4% of the theoretical values.

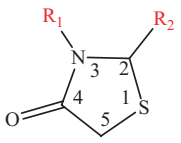
2-(4-methoxyphenyl)-3-(4-sulphonamidophenyl) thiazolidin-4-one [1]

Yield: 0.73 g (40.22%); IR (KBr, in cm^{-1}): 3422 (N-H str. for -NH_2), 1686.30 (C=O str.), 1490.32, 1358, 1275, 1152.26 (S=O str. for SO_2); ^{1}HNMR ($CDCl_3$, δ in ppm): 3.72 (s, 3H, OCH_3), 3.89 (d, 1H, J = 15.7 Hz, 5-H_A), 4.15 (dd, 1H, J = 15.7 and 1.9 Hz, 5-H_B), 5.94 (s, 1H, H-2), 6.93–8.01 (m, 8H, ArH), 10.02 (br s, 2H, SO_2NH_2); Mass: 365 (M+H)$^+$; Anal. Cal. C, 52.73; H, 4.43; N, 7.69; Found C, 52.78; H, 4.39; N, 7.66.

3-(4-chlorophenyl)-2-(4-nitrophenyl) thiazolidin-4-one [2]

Yield: 0.68 g (38.47%); IR (KBr, in cm^{-1}): 1688.56, 1432.80, 1373, 1348.78 (N=O str. for $ArNO_2$), 872; ^{1}HNMR (DMSO d6, δ in ppm): 4.01 (d, 1H, J = 15.9 Hz, 5-H_A), 4.25 (dd, 1H, J = 15.9 and 1.6 Hz, 5-H_B), 6.08 (s, 1H, H-2), 7.04–8.13 (m, 8H, ArH); Mass: 335 (M+H)$^+$; Anal. Cal. C, 53.81; H, 3.31; N, 8.37; Found C, 53.95; H, 3.30; N, 8.40.

Table 1: Structure and physical constant of the synthesized compounds



Comp. code	R_1	R_2	Molecular formula	Mol. weight	Melting point (°C)	Yield (%)
1	4-sulphonamidophenyl	4-methoxyphenyl	$C_{16}H_{16}N_2O_4S_2$	364.4	Gummy	40
2	4-chlorophenyl	4-nitrophenyl	$C_{15}H_{11}ClN_2O_3S$	334.7	Gummy	38
3	pyridin-2-yl	2,5-dimethoxyphenyl	$C_{16}H_{16}N_2O_3S$	316.3	130–132	41
4	5-chloropyridin-2-yl	pyridin-2-yl	$C_{13}H_{10}ClN_3OS$	291.7	155–157	42
5	6-methylpyridin-2-yl	pyridin-2-yl	$C_{14}H_{13}N_3OS$	271.3	163–165	43
6	4-sulphonamidophenyl	2-chlorophenyl	$C_{15}H_{13}ClN_2O_3S_2$	368.8	85–87	40
7	4,6-dimethylpyridin-2-yl	2,6-dichlorophenyl	$C_{16}H_{17}Cl_2N_2OS$	353.2	128–130	38
8	6-methylpyridin-2-yl	2,6-dimethoxyphenyl	$C_{17}H_{18}N_2O_3S$	330.4	146–148	44
9	2-phenylethan-1-yl	2,6-dichlorophenyl	$C_{17}H_{15}Cl_2N_2OS$	352.2	210–212	41
10	2-phenylethan-1-yl	2,5-dimethoxyphenyl	$C_{19}H_{21}NO_3S$	343.4	179–181	40

2-(2,5-dimethoxyphenyl)-3-(pyridin-2-yl) thiazolidin-4-one [3]

Yield: 0.65 g (41.27%); IR (KBr, in cm^{-1}): 1694.13, 1578, 1472.70, 1362.86 (Ar-C-N str.), 1215.33; ^{1}HNMR (DMSOd6, δ in ppm): 3.71 (s, 6H, OCH_3), 3.83 (d, 1H, J = 15.7 Hz, 5-H_A), 4.12 (dd, 1H, J = 15.7 and 1.6 Hz, 5-H_B), 5.95 (s, 1H, H-2), 6.47–6.56 (m, 3H, ArH), 7.18–8.54 (m, 4H, pyridinyl); Mass: 317 $(M+H)^+$; Anal. Cal. C, 60.74; H, 5.10; N, 8.85; Found C, 60.64; H, 5.09; N, 8.84.

3-(5-chloropyridin-2-yl)-2-(pyridine-2-yl) thiazolidin-4-one [4]

Yield: 0.62 g (42.47%); IR (KBr, in cm^{-1}): 1709.59, 1584.24 (C=N str.), 1458.89, 1363.73; ^{1}HNMR ($CDCl_3$, δ in ppm): 3.92 (d, 1H, J = 15.1 Hz, 5-H_A), 4.14 (d, 1H, J = 15.1 Hz, 5-H_B), 6.16 (s, 1H, H-2), 7.29–7.83 (m, 3H, pyridinyl), 8.14–8.78 (m, 4H, pyridinyl); ^{13}CNMR ($CDCl_3$): δ 166.7, 158.6, 151.3, 149.4, 147.2, 138.3, 135.9, 127.7, 123, 120.6, 115.5, 58, 36; Mass: 292 $(M+H)^+$; Anal. Cal. C, 53.52; H, 3.45; N, 14.40; Found C, 53.72; H, 3.45; N, 14.46.

3-(6-methylpyridin-2-yl)-2-(pyridine-2-yl) thiazolidin-4-one [5]

Yield: 0.59 g (43.25%); IR (KBr, in cm^{-1}): 2960, 1694.16, 1586.63, 1490, 1365 (Ar-C-N str.); ^{1}HNMR ($CDCl_3$, δ in ppm): 2.46 (s, 3H, CH_3), 3.90 (d, 1H, J = 15.9 Hz, 5-H_A), 4.14 (d, 1H, J = 15.9 Hz, 5-H_B), 6.46 (s, 1H, H-2), 7.08–7.23 (m, 2H, pyridinyl), 7.58–8.64 (m, 5H, pyridinyl); ^{13}CNMR ($CDCl_3$): δ 168.2, 158.7, 156.3, 152.1, 149.5, 135.7, 123.3, 120.6, 118.6, 111.2, 58.6, 36.1, 20.7; Mass: 272 $(M+H)^+$; Anal. Cal. C, 61.97; H, 4.83; N, 15.49; Found C, 61.85; H, 4.82; N, 15.56.

2-(2-chlorophenyl)-3-(4-sulphonamidophenyl) thiazolidin-4-one [6]

Yield: 0.73 g (39.57%); IR (KBr, in cm^{-1}): 3416.17 (N-H str. for -NH_2), 1695.12, 1462.74, 1364.19; ^{1}HNMR ($CDCl_3$, δ in ppm): 3.88 (d, 1H, J = 15.7 Hz, 5-H_A), 4.12 (d, 1H, J = 15.7 Hz, 5-H_B), 5.96 (s, 1H, H-2), 6.93–8.02 (m, 8H, ArH), 10.08 (br s, 2H, SO_2NH_2); Mass: 369 $(M+H)^+$; Anal. Cal. C, 48.84; H, 3.55; N, 7.59; Found C, 48.66; H, 3.56; N, 7.62.

2-(2,6-dichlorophenyl)-3-(4,6-dimethylpyridin-2-yl) thiazolidin-4-one [7]

Yield: 0.67 g (37.68%); IR (KBr, in cm^{-1}): 2962, 2873.36, 1697.27, 1589.06, 1436.43, 1362.41 (Ar-C-N str.); ^{1}HNMR ($CDCl_3$, δ in ppm): 2.39 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.89 (d, 1H, J = 15.6 Hz, 5-H_A), 4.12 (dd, 1H, J = 15.6 and 1.4 Hz, 5-H_B), 5.96 (s, 1H, H-2), 6.95 (s, 1H, pyridinyl), 6.98–7.03 (m, 3H, ArH), 8.09 (s, 1H, pyridinyl); ^{13}CNMR ($CDCl_3$): δ 167.9, 156.8, 152.6, 149.3, 147.2, 138.3, 135.9, 127.7, 123, 120.6, 115.5, 58, 36; Mass: 354 $(M+H)^+$; Anal. Cal. C, 54.40; H, 3.99; N, 7.93; Found C, 54.35; H, 4.00; N, 7.96.

2-(2,6-dimethoxyphenyl)-3-(6-methylpyridin-2-yl) thiazolidin-4-one [8]

Yield: 0.72 g (43.64%); IR (KBr, in cm^{-1}): 2870.69, 1694.13, 1585.84, 1497.47, 1358.32, 1215.33; ^{1}HNMR (DMSOd6, δ in ppm): 2.49 (s, 3H, CH_3), 3.72 (s, 6H, OCH_3), 3.89 (d, 1H, J = 15.4 Hz, 5-H_A), 4.12 (d, 1H, J = 15.4 Hz, 5-H_B), 5.92 (s, 1H, H-2), 6.21–6.81 (m, 3H, ArH), 7.13–8.16 (m, 3H, pyridinyl); Mass: 331 $(M+H)^+$; Anal. Cal. C, 61.80; H, 5.49; N, 8.48; Found C, 61.67; H, 5.51; N, 8.46.

2-(2,6-dichlorophenyl)-3-phenethylthiazolidin-4-one [9]

Yield: 0.72 g (40.79%); IR (KBr, in cm^{-1}): 2925, 2852.8, 1685.28, 1432.85, 1244.97 (Al-C-N str.); ^{1}HNMR ($CDCl_3$, δ in ppm): 2.81 (t, 2H, CH_2), 3.43 (t, 2H, CH_2), 3.85 (d, 1H, J = 15.7 Hz, 5-H_A), 4.01 (dd, 1H, J = 15.7 and 1.9 Hz, 5-H_B), 6.10 (s, 1H, H-2), 6.81–7.28 (m, 8H, ArH); Mass: 353 $(M+H)^+$; Anal. Cal. C, 57.96; H, 4.29; N, 3.98; Found C, 58.08; H, 4.40; N, 3.96.

2-(2,5-dimethoxyphenyl)- 3-phenethylthiazolidin-4-one [10]

Yield: 0.69 g (40.05%); IR (KBr, in cm^{-1}): 2925, 2852.8, 1703.56, 1434.78, 1238.83, 1024.37; ^{1}HNMR (DMSOd6, δ in ppm): 2.81 (t, 2H, CH_2), 3.53 (t, 2H, CH_2), 3.73 (s, 6H, OCH_3), 3.89 (d, 1H, J = 15.7 Hz, 5-H_A), 4.15 (dd, 1H, J = 15.7 and 1.6 Hz, 5-H_B), 5.92 (s, 1H, H-2), 6.46–6.54 (m, 3H, ArH), 7.08–7.48 (m, 5H, ArH); Mass: 344 $(M+H)^+$; Anal. Cal. C, 66.45; H, 6.16; N, 4.08; Found C, 66.57; H, 6.19; N, 4.05.

Table 2: Antibacterial and anti-fungal activity of the compounds (MIC's in μg/mL)

Comp. code	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Penicillium notatum*	*Candida albicans*	*Aspergillus niger*
1	10	11	63	55	–	–	–
2	32	35	71	–	47	–	71
3	16	13	55	48	35	40	65
4	35	28	67	62	48	57	68
5	40	32	78	105	35	–	75
6	07	10	25	35	16	19	17
7	26	22	115	117	28	42	42
8	15	12	27	67	15	17	20
9	13	10	28	38	13	25	35
10	12	16	20	28	63	55	–
Cip	5	5	10	15	–	–	–
Flu	–	–	–	–	15	10	15

MIC, minimum inhibitory concentration; Cip, ciprofloxacin; Flu, fluconazole

Antimicrobial evaluation

In vitro antibacterial activity

Synthesized compounds were evaluated for their *in vitro* antibacterial activity against pathogenic bacteria. The agar dilution method was carried out using Muller–Hinton agar (Hi-Media, Mumbai, India) medium. Suspension of each micro-organism was prepared and applied to plates with serially diluted compounds (DMF, solvent control) to be tested and incubated (approximately 20 h) at 37 °C. The MIC was considered to be the lowest concentration that was completely inhibited growth on agar plates. The bacteria strains *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2065), and *Bacillus subtilis* (NCIM 2063) were used for this study (Table 2).

In vitro antifungal activity

The compounds were evaluated for their *in vitro* anti-fungal activity against pathogenic fungi using agar dilution method with Saburoud's dextrose agar (Hi-Media). Suspension of each fungus was prepared and applied to agar plates with serially diluted compounds to be tested. The plates were incubated at 26 °C for 72 h, and MIC's were determined. The fungus strains *Candida albicans* (NCIM 3102), *Aspergillus niger* and *Penicillium notatum* (NCIM 742) were used for this study (Table 2).

In vitro anti-viral activity

Cytotoxicity and anti-viral activity of all compounds were evaluated against Herpes simplex virus-1 and Herpes simplex virus-2 in HEL cell culture. Cytotoxic concentration was measured, required to cause a microscopically detectable alteration of normal cell morphology. Cytopathogenicity was determined as a concentration required to reduce virus-induced cytopathogenicity by 50%.

Compounds were also evaluated against influenza A H3N2 and influenza B in Madin Darby canine kidney (MDCK) cell cultures and 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. Similarly 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, was also determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay protocol.

The compounds were also tested for anti-HIV activity against replication of HIV-1 (III B) in MT-2 cells at varying concentrations from 100 nM by double dilution technique. The MT-2 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine, UK), supplemented with 10% (v/v) heat-inactivated calf serum and 20-mg/mL gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) was obtained from the culture supernatant of HIV-1-infected MT-2 cell lines, and the virus stocks were stored at −70 °C until used. Anti-HIV assays were carried out in microtitre plates filled with 100 mL of medium and 25 mL volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and mock-infected cells. Fifty microliters of HIV at 100 $CCID_{50}$ medium were added to either the HIV-infected or mock-infected part of the microtitre tray. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT method. The EC_{50} (effective concentration of compound (μM) achieving 50% protection in MT-2 cell lines against the cytopathic effect of HIV-1) and CC_{50} (cytotoxic concentration of compound (μM) required to reduce the viability of mock-infected MT-2 cells by 50%) values (average of two experiments) were calculated and reported.

Results and Discussion

The synthetic approach for the title compounds is shown in Figure 1. Some 1,3-thiazolidin-4-ones were synthesized using the methods reported by Rawal *et al.* (13), with slight modification. The reaction was monitored by TLC. The structures of synthesized intermediates were confirmed by IR spectra, and final compounds were characterized by means of FTIR, ^{1}HNMR, FAB-MS, and elemental analyses.

The investigation of antibacterial, antifungal, and anti-viral screening data revealed that all the tested compounds **1**–**10** showed moderate-to-good inhibition. The compounds **1**, **6**, **8**, **9**, and **10** showed comparatively good activity against all the bacterial strains. The good activity is attributed to the presence of pharmacologically active sulphonamidophenyl group at R_1, 2,6-dichlorophenyl, 2,5-dimethoxyphenyl, and 2,6-dimethoxyphenyl groups attached at R_2 of the 1,3-thiazolidin-4-ones. Introduction of mono or dimethoxyphenyl group and mono or dichlorophenyl at R_2 caused enhancement in activity. While introduction of 6-methylpyridine group at position R_1 caused decrease in activity against most of the strains. The compounds **3** and **4** exhibited moderate activity compared with that of standard against all the bacterial strains.

Compounds **1**, **6**, **9**, and **10** showed good activity against *E. coli*, while compounds **9** and **10** showed good activity against *S. aureus*. The compounds **6**, **7** (Figure 2), **8**, and **9** showed comparatively good activity against all the fungal strains. The structures of these compounds contain 2,6-dichlorophenyl, 2,5-dimethoxyphenyl, and 2,6-dimethoxyphenyl groups attached at R_2. The compounds **3** and **4** exhibited moderate activity against all fungal strains. Results showed that aryl and heterocyclic substitution at R_2 and R_1 gave an enhanced biological effect against all the tested bacterial and fungal strains.

Title compounds were tested against representative members of the virus including Herpes simplex virus-1 (KOS), Herpes simplex virus-2 (G), Influenza A H3N2 subtype, Influenza B (Table 3) and their cytotoxic concentration was evaluated. None of the synthesized compounds are active against Herpes simplex virus-1 (KOS) and Herpes simplex virus-2 (G). The same compound (**7**) showed better anti-viral activity against Influenza A H3N2 subtype and Influenza B at the concentration of 249–263 μM, where as cytotoxicity was found to be >283 μM.

It is evident that the compound containing 2,6-dichlorophenyl group attached at R_2 of the was found to be most active compound among the series. Interestingly, the same 2,6-dichlorophenyl group

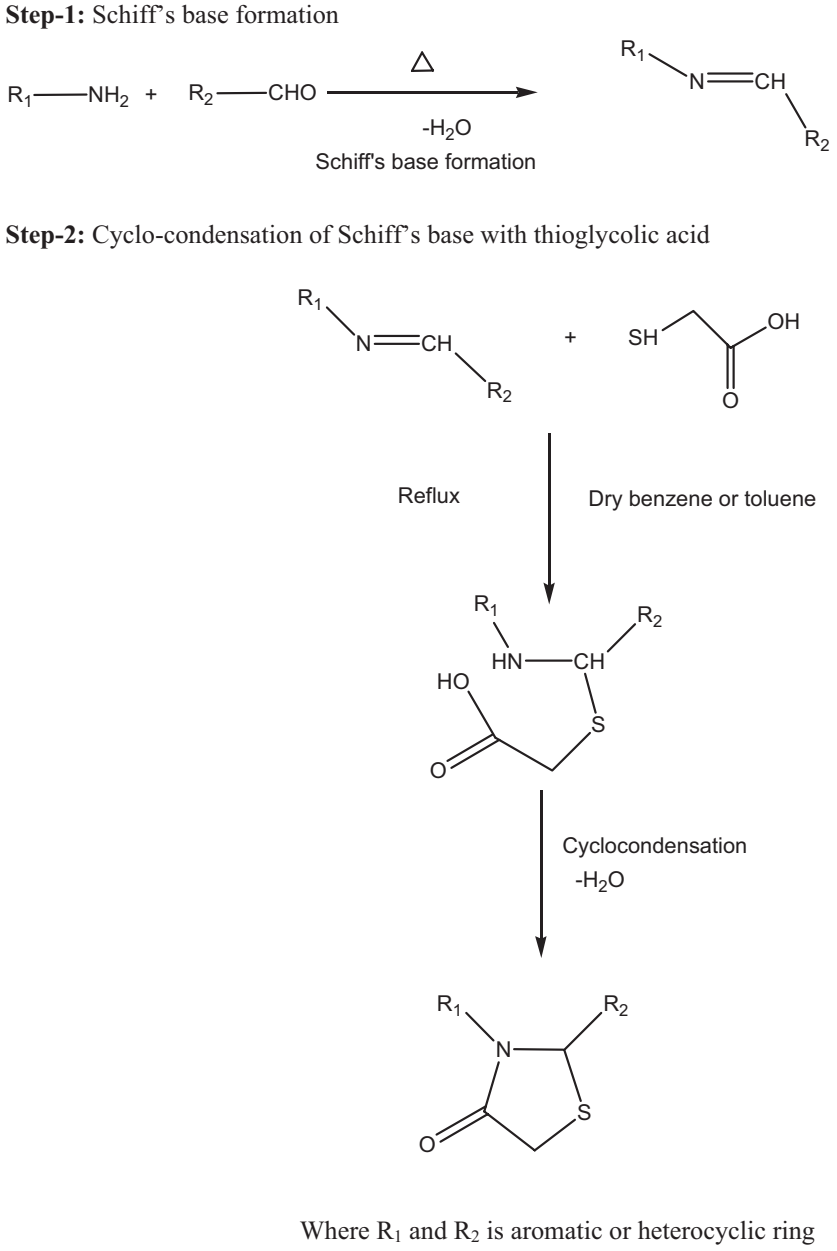
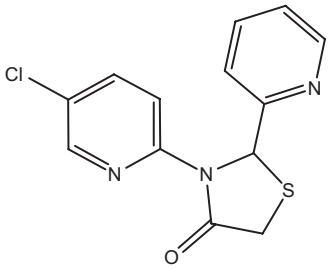


Figure 1: Scheme for the synthesis of 1,3-thiazolidin-4-one compounds.

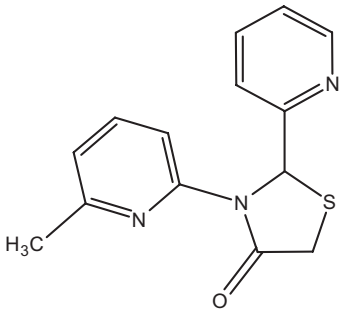
at R_2 and 2-phenylethane group at R_1 (**9**) greatly reduce the spectrum of activity. It may be because of the increase in the distance between the nitrogen atom (3rd position) of thiazolidinone and the substitution ring at this position.

Synthesized compounds were also screened for their anti-HIV activity. The anti-HIV activity and cytotoxicity of synthesized compounds against multiplication of wt HIV-1 (wild-type HIV-1) in acutely infected MT-2 cell line and mock-infected MT-2 cell line, respectively, were screened by MTT methods. The results are given in Table 3. Azidothymidine (AZT), Nevirapine, was considered as reference drugs. All the synthesized 1,3-thiazolidin-4-ones compounds except **1**, **2**, **9**, and **10** exhibited potent inhibitory activities against HIV replication in this assay with EC_{50} values <1.07 μM. The most promising 1,3-thiazolidin-4-one compound was **5** with EC_{50} values of 0.078 and selectivity index (SI) of 205.

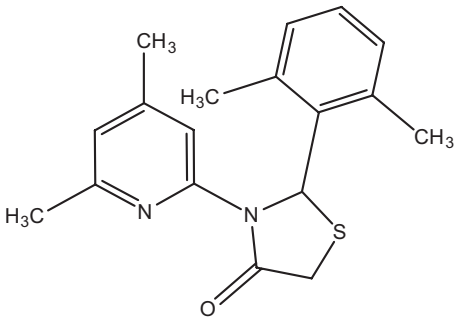
The compounds **4**, **5**, and **7** (Figure 2) (EC_{50} = 0.078 to 0.108 μM) were more potent than nevirapine (EC_{50} = 0.150 μM) in the same assay, but all the thiazolidinone compounds were less potent than AZT (EC_{50} = 0.007 μM). The compounds **1**, **2**, **9**, and **10** were found to be very less potent. 1,3-thiazolidin-4-one compounds substituted with heterocyclic moiety at R_2 and R_1 by 2,6- dichlorphenyl and 2,5-dimethoxyphenyl substituent at R_2 were found to be



Compound **4**



Compound **5**



Compound **7**

Figure 2: Structure of some synthesized 1,3-thiazolidin-4-ones.

Table 3: Anti-viral activity of the synthesized compounds

	Herpes simplex virus-1		Herpes simplex virus-2	Influenza A H3N2 subtype		Influenza B	HIV-1(IIIB)	
	(in μM)			(in μM)			(in μM)	
Comp. code	CC_{50}	EC_{50}	EC_{50}	CC_{50}	EC_{50}	EC_{50}	CC_{50}	EC_{50}
1	–	–	–	>274.42	>274.42	>274.42	≥181.65	≥181.65
2	–	–	–	–	–	–	≥154.60	≥154.60
3	≥316.15	>316.15	>316.15	–	–	–	>3	1.064 ± 0.42
4	–	–	–	–	–	–	>10	0.108 ± 0.08
5	–	–	–	–	–	–	>16	0.078 ± 0.03
6	>271.15	>271.15	>271.15	>271.15	>271.15	>271.15	21 ± 8.76	8.153 ± 0.04
7	≥184.03	130.24 ± 4.86	161.38 ± 8.75	>283.13	249.15 ± 8.32	263.31 ± 12.17	>65	0.082 ± 0.05
8	>302.66	296.61 ± 15.55	>302.66	–	–	–	>25	21.10 ± 5.40
9	>283.93	>283.93	>283.93	–	–	–	>228.15	228.15 ± 9.57
10	>218.40	>218.40	>218.40	–	–	–	>302.73	302.73 ± 24.81
Ganciclovir	>391.80	0.196 ± 0.070	0.196 ± 0.058	–	–	–	–	–
Ribavirin	–	–	–	>409.48	32.76 ± 1.40	24.57 ± 0.55	–	–
Azidothymidin	–	–	–	–	–	–	70 ± 3.03	0.007 ± 0.002
Nevirapine	–	–	–	–	–	–	50 ± 2.53	0.150 ± 0.003

EC_{50}, effective concentration of compound (μM) achieving 50% protection in MT-2 cell lines against the cytopathic effect of HIV-1; CC_{50}, cytotoxic concentration of compound (μM) required to reduce the viability of mock-infected MT-2 cells by 50%.

more potent for their anti-HIV activity than the aromatic substitutions at the aforementioned positions. The extrapolation of the distance between the nitrogen atom (3rd position) of thiazolidinone and the substitution ring at this position (compounds **9**) is not favorable for anti-HIV activity. Highly electronegative substituents in this position (compounds **10**) are also not favorable for anti-HIV activity. The results of the present study show that our approach has led to the development of potent anti-HIV agents, more active

than nevirapine. This is in conformity with the earlier observation (13) namely, nature of the substituent at C-2 and N-3 of the thiazolidin-4-one ring and butterfly conformation collectively facilitate better interaction with the allosteric binding pocket residues of HIV-1 RT enzyme. This has a direct bearing on the anti-HIV activity.

In terms of SARs, we can say that the anti-HIV activity was strongly enhanced by introducing a 2-pyridinyl substituent at R_1 of the thiazolidinone ring and in particular by 2-pyridinyl, two chlorine atoms at 2′ and 6′ positions of the phenyl ring at C-2: in fact, derivative **5** was more active than its analogues **4** and **7**, while compound **7** was more active than **9** and **10**. As suggested by molecular modeling studies (18), the introduction of a lipophilic substituent in the hetero-aromatic ring led to a substantial increase in the anti-viral activity. Moreover, we learned that the introduction of a methyl group at the 6 position of the pyridin-2-yl group led to a substantial increase in potency thus confirming that increasing the steric bulk of this aromatic part led to improved anti-viral activity, our findings are consistent with the report of Rao *et al.* (10).

Conclusion

The research study reports the successful synthesis, antibacterial, antifungal, and anti-viral activity of new 1,3-thiazolidin-4-ones carrying biologically active groups at C-2 (position 2) and N (position 3). Their antimicrobial activity study revealed that all of the tested compounds showed moderate-to-good antibacterial and antifungal activities against pathogenic strains. Only few of the synthesized compounds showed better anti-HIV activity than nevirapine. It can be concluded that a combination of two different heterocyclic systems namely 1,3-thiazolidin-4-one and 2-pyridinyl ring (substituted) with an aromatic system namely 2,6-disustituted phenyl or 2-pyridinyl ring has enhanced the antimicrobial and anti-viral effect and hence, they are ideally suited for further modifications to obtain more efficacious antimicrobial compounds.

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