

Synthesis, Characterization and Anti-HIV Evaluation of Some Novel 2-[(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones

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Abstract: A series of novel 2-[(substituted phenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (**4a-g**) were synthesized, structurally confirmed by elemental analysis, IR, ¹H NMR, MS spectral analysis and further evaluated for their anti-HIV activity and cytotoxicity in MT-4 cell cultures infected with wild-type HIV-1 strain III_B and HIV-2 strain ROD in comparison with azidothymidine (AZT) and dideoxyinosine (DDI), which were used as reference drugs.

Keywords: Isothiocyanatobenzene, 1,3-thiazolidin-4-one, chloroacetic acid, anti-HIV activity, cytotoxicity.

Introduction

HIV-1 (human immunodeficiency virus type 1), a retrovirus of the lentivirus family, is the etiological agent of AIDS [1], an infection characterized by loss of helper T lymphocytes and heavy damage of lymphatic tissue. Global estimates of WHO/UNAIDS showed that 34 million people had been infected with HIV/AIDS at the end of 2010, with 2.7 million getting newly infected with the virus and 1.8 million reported deaths because of AIDS [2]. An estimated 4.0 million people are living with HIV in South-East Asia Region.

The current therapy against AIDS is based on seven classes of anti-HIV drugs: the nucleoside and nucleotide reverse transcriptase inhibitors (indicated as NRTIs and NtRTIs, respectively), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the protease inhibitors (PIs), the integrase inhibitors (INI), the chemokine (C-C motif) receptor 5 (CCR5) inhibitor and the fusion inhibitor (FI) [3]. NRTIs, NtRTIs, NNRTIs and PIs are combined in the highly active antiretroviral therapy (HAART), which dramatically reduces the incidence of AIDS infection and death.

Despite the fact that HAART combination regimens have significantly decreased the morbidity and mortality among patients with HIV infections, by bringing the viral replication to very low levels, they are still unable to eradicate the virus [4]. So, the continued suppression of the virus by long-term use of the anti-retroviral drugs induces the emergence of drug-resistant viral mutants and the undesirable metabolic side effects. Moreover, when individuals develop resistance to one antiretroviral agent within a class, there is often, but not always, development of cross-resistance to other agents of the same class.

In addition to the facts that millions of people still need HAART treatment, the utility of antiretroviral drugs is further limited by viral resistance and toxicity issues [5]. Unfortunately still there exists no safe, effective vaccine for prevention of HIV either upon pre-exposure or post-exposure prophylaxis. Hence the

current need is availability of more potent, less toxic, easily available, cost-effective therapies not only to treat HIV, but also to prevent its transmission. This is particularly critical in regions of the world such as sub-Saharan Africa, where 67% of the world's HIV infected individuals reside [6].

Reverse Transcriptase (RT) is a key enzyme which plays an essential and multifunctional role in the replication of the human immunodeficiency virus (HIV) [7] and thus represents an attractive target for the development of new drugs useful in AIDS therapy. RT is necessary for the catalytic transformation of single-stranded viral RNA into the double-stranded linear DNA which is integrated into host cell chromosomes [7]. Drug targeted at HIV-RT can be divided into two categories: (i) nucleoside and nucleotide RT inhibitors, and (ii) non-nucleoside RT inhibitors (NNRTIs) [8]. However, in view of the increasing incidence of resistance to current drug regimens and the frequency of adverse events, the development of novel, selective, potent, safe, inexpensive antiviral agents, that are also effective against mutant HIV strains, remains a high priority for medical research.

Antiviral research in the past has primarily focused on the development of nucleoside analogues but of late, non-nucleoside derivatives [9] have also received considerable attention as an alternative therapy. Among the non-nucleoside analogues, 1,3-thiazolidin-4-one is an interesting molecule, which has been found to exhibit diverse biological activities.

The modeling studies carried out on 1*H*, 3*H*-thiazolo[3,4-*a*]benzimidazole (TBZ) analogues (Figure 1) [10], a class of NNRTIs, highlighted the importance of 2,6-dihalo substitution on the phenyl ring at C1 of the nucleus for the activity and also their ability to take "butterfly-like" shape on binding to the receptor site [11]. In this background TBZ analogues were modified by opening imidazole ring of TBZ (Figure 1) to generate 2,3-diaryl-1,3-thiazolidin-4-ones [12] as a new NNRTI scaffold to inhibit HIV-1 RT.



Figure 1.: 1-Aryl-1*H*, 3*H*-thiazolo[3,4-*a*]benzimidazole (TBZ) analogues

1,3-thiazolidin-4-one derivatives have been found to exhibit diverse biological activities such as analgesic [13], anti-inflammatory [14], antiangiogenic [15], anti-HIV [16], *in vitro* anti-*Toxoplasma gondii* [17], antimicrobial [17], antimycobacterial [18], antimalarial [19], trypanocidal [20], antischistosomal [21], anticonvulsant [22], antihistaminic [23], antidiabetic [24], antiarrhythmic [25] and antihypertensive [26] properties.

To search for more specific and novel 1,3-thiazolidin-4-one analogues with a wide therapeutic window and anti-HIV activity, we synthesized some novel 2-[(substituted phenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones and evaluated them for their anti-HIV activity and cytotoxicity in MT-4 cells infected with wild-type HIV-1 strain III_B and HIV-2 strain ROD by MTT assay method.

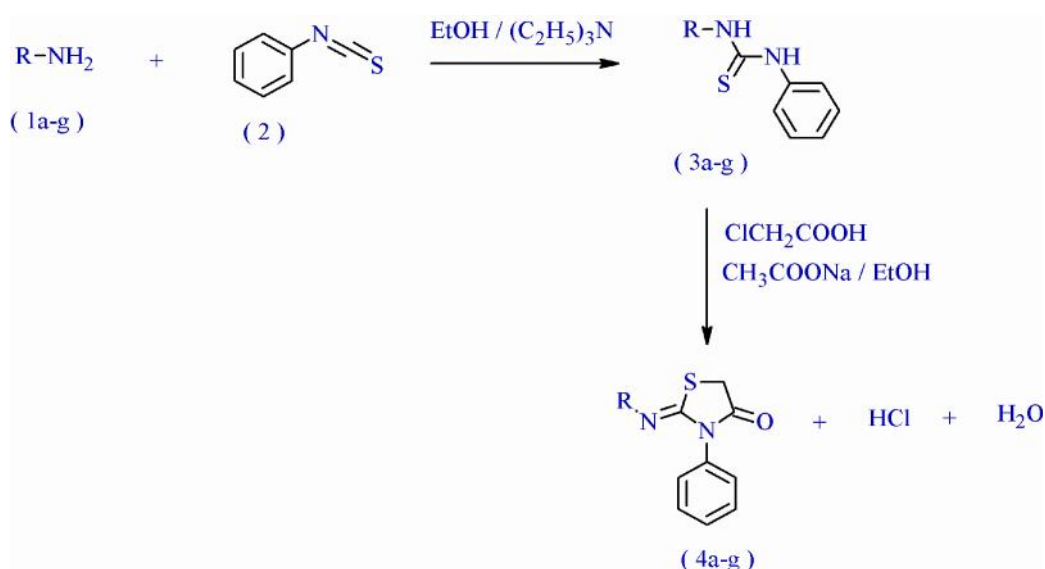
Materials And Methods

Experimental

3,4-dimethylaniline, pyrimidin-2-amine, 1,3-thiazol-2-amine, pyridin-3-amine, 4-methylpyridin-2-amine, 3,5-dimethylaniline, 2,6-dimethylaniline, isothiocyanatobenzene and chloroacetic acid were commercially obtained from Aldrich (Milwaukee, WI). Triethylamine, anhydrous sodium acetate, diethyl ether, hexane, chloroform, ethylacetate, dimethyl sulphoxide and silica gel-G were purchased from Merck, Mumbai, India. Melting points were determined in open capillary tubes using Veego melting point apparatus (Model: VMP-DS) and are uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel-G plates of 0.5 mm thickness using Hexane: Ethylacetate (4.5:0.5 v/v) and Benzene: Chloroform

(1:1 v/v) as a solvent system and the spots being visualized under iodine vapours. Concentration of the solution after the reaction completion involved the use of a rotary evaporator (Eyela, Japan) operating under reduced pressure. Infrared (IR) spectra were recorded on a Jasco FTIR-4100 spectrophotometer (Jasco Ltd, Tokyo, Japan) using KBr pellet disc technique in the range of 4000-400 cm^{-1} . ^1H NMR spectra were recorded on a Bruker DPX 300 (operating at 300 MHz) and Bruker DPX 600 (operating at 600 MHz) NMR spectrometer using CDCl_3 as solvent and TMS as internal standard (chemical shifts in δ , ppm). Spin multiplets are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra (MS) were recorded on a Q-TOF micromass spectrometer by using electrospray ionization (ESI) technique. The elemental analyses (C, H, N) were performed using a Perkin-Elmer 2400 CHN analyzer. Analyses indicated by the symbols of the element were within $\pm 0.4\%$ of the theoretical values. 1,3-thiazolidin-4-one derivatives (**4a-g**) were synthesized as per the reactions outlined in the Scheme 1. The respective physico-chemical characteristics of all the synthesized compounds have been presented in Table 1.

Scheme 1: Synthetic route for the preparation of novel 2-[(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (4a-g)



Compound	a	b	c	d	e	f	g
R							

Synthesis of 1-(substitutedphenyl)-3-phenylthiourea/1-phenyl-3-(heteroaryl)thiourea (3a-g)

A mixture of different aromatic/heteroaromatic amines (**1a-g**) (3,4-dimethylaniline (**1a**), pyrimidin-2-amine (**1b**), 1,3-thiazol-2-amine (**1c**), pyridin-3-amine (**1d**), 4-methylpyridin-2-amine (**1e**), 3,5-dimethylaniline (**1f**) and 2,6-dimethylaniline (**1g**)) (0.01 mol) and isothiocyanatobenzene (**2**) (0.01 mol) dissolved in absolute ethanol (20 ml) in presence of catalytic amount of triethylamine (0.5 ml) was refluxed for 6-8 h. The progress of the reaction was monitored by TLC using Hexane: Ethylacetate (4.5:0.5 v/v) as eluents. After the completion of the reaction, the reaction mixture was concentrated under rotary vacuum, cooled and kept overnight in the refrigerator. The solid thus separated out was filtered, washed with diethyl ether (3 \times 5 ml), dried and crystallized from chloroform. Adopting the above procedure seven different thioureas (**3a-g**) was synthesized. Percentage yield, melting point and Rf value of the synthesized compound (**3a-g**) were determined and presented in Table 1.

Synthesis of 2-[(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (4a-g)

A mixture of 1-(substitutedphenyl)-3-phenylthiourea/1-phenyl-3-(heteroaryl)thiourea (**3a-g**) (0.01 mol), chloroacetic acid (0.01 mol) and anhydrous sodium acetate (0.01 mol) in absolute ethanol (30 ml) was refluxed for 8-10 h. The progress of the reaction was monitored by TLC using Benzene: Chloroform (1:1 v/v) as eluents. After the completion of TLC, absolute ethanol was removed under reduced pressure. The final residue obtained was poured into crushed ice and the separated solid was filtered, washed with cold water, dried and crystallized from absolute chloroform. Adopting the above procedure seven different 1,3-thiazolidin-4-one analogues (**4a-g**) was synthesized. Percentage yield, melting point and Rf value of the synthesized compound (**4a-g**) were determined and presented in Table 1.

Anti-HIV Activity

Cells:

MT-4 cells were grown and maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 0.1% sodium bicarbonate and 20 µg gentamicin per mL [27].

Evaluation of the antiviral activity of the compounds against HIV-1 strain (III_B) and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [28]. Stock solutions (10 × final concentrations) of test compounds were added in 25 µL volumes of two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1 (III_B) [29] or HIV-2 strain (ROD) [30] stock (50 µL) at 100-300 CCID₅₀ (50% cell culture infectious dose) was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the cytotoxicity of the test compound. Exponentially growing MT-4 cells [31] was centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL and 50 µL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow-colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Agros Organics, Geel, Belgium) by the enzyme mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically [32]. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland) at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells.

EC₅₀ was defined as the concentration of the drug required for 50% inhibition of virus-induced cytopathicity. CC₅₀ was defined as the concentration of the drug required for reducing the viability of mock-infected cells by 50%. CC₅₀, EC₅₀, and the selectivity index (SI = CC₅₀/ EC₅₀) were then calculated and results analysed (Table 2).

Results And Discussion

Chemistry

In the present study, a series of novel 2-[(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (**4a-g**) were synthesized according to scheme 1. Aromatic/heteroaromatic amines (**1a-g**) on condensation with isothiocyanatobenzene (**2**) in presence of catalytic amount of triethylamine in absolute ethanol resulted in the formation of 1-(substitutedphenyl)-3-phenylthiourea/1-phenyl-3-(heteroaryl)thiourea (**3a-g**) with 54.7 - 74.5% yields (scheme 1). The physical data of the synthesized compounds (**3a-g**) and (**4a-g**) are presented in Table 1. The purity of the compounds was checked by thin layer chromatography (TLC) showed disappearance of reactant spot on silica gel-G plates of 0.5 mm thickness using Hexane: Ethylacetate (4.5:0.5 v/v) and Benzene: Chloroform (1:1 v/v) as a solvent system and the spots being visualized under iodine vapours. The structure of the synthesized compounds (**3a-g**) was confirmed on the basis of elemental analysis, FT-IR and ¹H

NMR spectral data (Results and discussion part). The FT-IR spectra of the synthesized compounds (**3a-g**) showed absorption bands ranging from 3337.21 - 3208.97 cm^{-1} for N-H, secondary amine and 1256.4 - 1018.23 cm^{-1} for C=S stretch, 3141.47 - 3023.84 cm^{-1} for aromatic C-H and 1667.16 - 1405.85 cm^{-1} for C=N & C=C ring stretch of phenyl ring. The IR spectra of compound (**3a-g**) displayed bands at about 1352.82 - 1322.93 cm^{-1} and 742.46 - 603.61 cm^{-1} associated with C-N stretch, secondary aromatic amine and C-S functions. In the IR spectra of compound (**3a-g**), some significant stretching bands due to methyl C-H asymmetric and methyl C-H symmetric, were observed at 2965.02 - 2917.77 cm^{-1} and 2882.09 - 2858.95 cm^{-1} , respectively. In the ^1H NMR spectra of compound (**3a**), aromatic (5H) protons appeared as a multiplet (5H) at 7.090 - 7.183 ppm, NH proton appeared as a broad singlet (2H) at 7.8 ppm, aromatic (3H) protons appeared as a multiplet (3H) at 7.386 - 7.399 ppm and methyl protons appeared as a singlet (6H) at 2.249 ppm, which proved the formation of 1-(substitutedphenyl)-3-phenylthiourea.

Compounds (**3a-g**), which on cyclisation with chloroacetic acid in absolute ethanol in presence of anhydrous sodium acetate offered the corresponding 2-[(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-one (**4a-g**) in 65.8 - 78.3% yields (scheme 1). The structure of the synthesized compound (**4a-g**) was established on the basis of elemental analysis, FT-IR and ^1H NMR and mass spectral data (Results and discussion part).

The FT-IR spectrum of compound (**4a-g**) showed strong absorption band at 1725.98 cm^{-1} for C=O of 1,3-thiazolidin-4-one, while the band at 2936.09 - 2918.73 cm^{-1} , 2866.67 - 2804.96 cm^{-1} , 1374.03 - 1329.68 cm^{-1} , 754.031 - 601.682 cm^{-1} and 3116.4 - 3013.23 cm^{-1} , respectively confirms the presence of methylene C-H asymmetric, methylene C-H symmetric, C-N stretch of tertiary aromatic amine, C-S and aromatic C-H stretch. This is considered to be a strong confirmation for the 1,3-thiazolidin-4-one nucleus formation. The IR spectrum of compound (**4a-g**) displayed bands at about 2981.41 cm^{-1} , 2866.67 cm^{-1} and 1672.95 - 1407.78 cm^{-1} associated with methyl C-H asymmetric, methyl C-H symmetric, C=N and C=C of aromatic ring functions. In the ^1H NMR spectra of compound (**4a**), aromatic (5H) protons appeared as a multiplet (5H) at 7.238 - 7.544 ppm, methyl (6H) protons appeared as a singlet (6H) at 2.279 ppm, aromatic (3H) protons appeared as a multiplet (3H) at 6.830 - 7.172 ppm and methylene (2H) protons of 1,3-thiazolidin-4-one C₅-H appeared as a singlet (2H) at 3.924 ppm, which proved the closure of 1,3-thiazolidin-4-one ring. The results of elemental analyses were within $\pm 0.4\%$ of the theoretical values.

Table 1: Physical data of 1-(substitutedphenyl)-3-phenylthiourea/1-phenyl-3-(heteroaryl)thiourea (3a-g**) and 2-(substitutedphenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (**4a-g**)**

Compound	Mol. Formula/ Mol. Weight	Yield (%)	M.p. ($^{\circ}\text{C}$)	^a Rf
3a	C ₁₅ H ₁₆ N ₂ S/256.37	71.8 (1.84 g)	142.6-144.4	0.43
3b	C ₁₁ H ₁₀ N ₄ S/230.29	54.7 (1.26 g)	188-190	0.74
3c	C ₁₀ H ₉ N ₃ S ₂ /235.33	68.4 (1.61 g)	134-136	0.71
3d	C ₁₂ H ₁₁ N ₃ S/229.3	59.3 (1.36 g)	155-157	0.69
3e	C ₁₃ H ₁₃ N ₃ S/243.33	64.9 (1.58 g)	160-161	0.57
3f	C ₁₅ H ₁₆ N ₂ S/256.37	69.8 (1.79 g)	173-175	0.51
3g	C ₁₅ H ₁₆ N ₂ S/256.37	74.5 (1.91 g)	203-205	0.46
4a	C ₁₇ H ₁₆ N ₂ OS/296.39	64.1 (1.90 g)	188.2-190.4	0.77
4b	C ₁₃ H ₁₀ N ₄ OS/270.31	50.3 (1.36 g)	232.4-234.2	0.55
4c	C ₁₂ H ₉ N ₃ OS ₂ /275.35	61.4 (1.69 g)	182.2-184.4	0.52
4d	C ₁₄ H ₁₁ N ₃ OS/269.32	52.9 (1.43 g)	193.5-194.2	0.48
4e	C ₁₅ H ₁₃ N ₃ OS/283.35	58.6 (1.66 g)	207.2-208.9	0.73
4f	C ₁₇ H ₁₆ N ₂ OS/296.39	56.7 (1.68 g)	218.4-219.9	0.84
4g	C ₁₇ H ₁₆ N ₂ OS/296.39	65.5 (1.94 g)	249.5-250.8	0.80

^aHexane: Ethylacetate (4.5: 0.5 v/v) for compound (**3a-g**) and Benzene: Chloroform (1:1 v/v) for compound (**4a-g**)

1-(3,4-dimethylphenyl)-3-phenylthiourea (3a)

IR (KBr, cm^{-1}): 3141.47, 3061.44 (aromatic C-H), 1613.16, 1586.16, 1532.17, 1504.2, 1448.28, 1407.78 (C=C aromatic ring), 3252.36 (N-H, secondary amine), 1328.71 (C-N, secondary aromatic amine), 2917.77 (methyl C-H, as CH_3), 1256.4, 1018.23 (C=S), 875.524, 815.742, 718.354, 686.538 (out-of-plane ring C-H bend), 1532.17 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 7.090-7.183 (m, 5H, Ar-H), 7.386-7.399 (m, 3H, Ar-H), 7.8 (br s, 2H, NH), 2.249 (s, 6H, 2 CH_3). Anal. calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{S}$: C, 70.27; H, 6.29; N, 10.93. Found: C, 70.31; H, 6.33; N, 10.90.

1-phenyl-3-pyrimidin-2-ylthiourea (3b)

IR (KBr, cm^{-1}): 3117.37, 3045.05 (aromatic C-H), 1667.16, 1595.81, 1546.63, 1490.7, 1407.78 (C=N, C=C aromatic ring), 3216.68 (N-H, secondary amine), 1330.64 (C-N, secondary aromatic amine), 1200.47, 1095.37, 1034.62 (C=S), 958.448, 903.487, 838.883, 741.496, 693.284 (out-of-plane ring C-H bend), 1546.63 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 7.168-7.683 (m, 8H, Ar-H, PymH), 8.683 (br s, 2H, NH). Anal. calcd. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{S}$: C, 57.37; H, 4.38; N, 24.33. Found: C, 57.42; H, 4.44; N, 24.35.

1-phenyl-3-(1,3-thiazol-2-yl)thiourea (3c)

IR (KBr, cm^{-1}): 3117.37, 3044.09 (aromatic C-H), 1595.81, 1546.63, 1491.67, 1407.78 (C=N, C=C aromatic ring), 3215.72 (N-H, secondary amine), 1330.64 (C-N, secondary aromatic amine), 1199.51, 1094.4, 1034.62 (C=S), 742.46, 693.284, 603.61 (C-S), 1546.63 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 7.092-7.190 (m, 5H, Ar-H), 7.390-7.404 (d, 2H, thiazole-H), 7.701 (br s, 2H, NH). Anal. calcd. for $\text{C}_{10}\text{H}_9\text{N}_3\text{S}_2$: C, 57.04; H, 3.85; N, 17.86. Found: C, 57.12; H, 3.91; N, 17.88.

1-phenyl-3-pyridin-3-ylthiourea (3d)

IR (KBr, cm^{-1}): 3136.65, 3091.33, 3039.26 (aromatic C-H), 1614.13, 1564.95, 1530.24, 1489.74, 1405.85 (C=N, C=C aromatic ring), 3221.5 (N-H, secondary amine), 1352.82, 1322.93, 1270.86 (C-N, secondary aromatic amine), 1180.22, 1115.62, 1030.77 (C=S), 942.056, 899.63, 863.953, 807.063, 751.138, 689.427 (out-of-plane ring C-H bend), 1530.24 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 6.840-6.848 (d, 1H, Ar-H), 7.239-7.271 (m, 4H, Ar-H), 7.394-7.434 (m, 2H, PyH), 7.679-7.706 (m, 1H, PyH), 8.084-8.093 (d, 1H, PyH), 8.318 (br s, 1H, NH), 13.738 (br s, 1H, NH). Anal. calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{S}$: C, 62.86; H, 4.84; N, 18.33. Found: C, 62.92; H, 4.88; N, 18.35.

1-(4-methylpyridin-2-yl)-3-phenylthiourea (3e)

IR (KBr, cm^{-1}): 3136.65, 3091.33, 3039.26 (aromatic C-H), 1614.13, 1530.24, 1488.78, 1405.85 (C=N, C=C aromatic ring), 3220.54 (N-H, secondary amine), 1352.82, 1322.93 (C-N, secondary aromatic amine), 2920.66 (methyl C-H, as CH_3), 2882.09 (methyl C-H, s CH_3), 1179.26, 1115.62, 1030.77 (C=S), 942.056, 863.953, 807.063, 751.138, 689.427 (out-of-plane ring C-H bend), 1530.24 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 6.837-6.845 (d, 1H, Ar-H), 7.240-7.275 (m, 2H, Ar-H), 7.395-7.434 (m, 2H, Ar-H, PyH), 7.691-7.705 (m, 2H, PyH), 8.082-8.091 (d, 1H, PyH), 8.421 (s, 1H, NH), 13.733 (s, 1H, NH), 2.358 (s, 3H, CH_3 at C_4 -Py). Anal. calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_3\text{S}$: C, 64.17; H, 5.39; N, 17.27. Found: C, 64.25; H, 5.45; N, 17.29.

1-(3,5-dimethylphenyl)-3-phenylthiourea (3f)

IR (KBr, cm^{-1}): 3023.84 (aromatic C-H), 1592.91, 1539.88, 1449.24, 1408.75 (C=C aromatic ring), 3208.97 (N-H, secondary amine), 1336.43 (C-N, secondary aromatic amine), 2965.02, 2928.38, (methyl C-H, as CH_3), 2867.63 (methyl C-H, s CH_3), 1193.72, 1130.08, 1047.16 (C=S), 929.521, 835.99, 753.066, 689.427 (out-of-plane ring C-H bend), 1539.88 (N-H bending, secondary aromatic amine); $^1\text{H NMR}$ (CDCl_3 , ppm): 7.102-7.261 (m, 5H, Ar-H), 7.396-7.423 (m, 3H, Ar-H), 8.559 (br s, 2H, NH), 2.354 (s, 6H, 2 CH_3). Anal. calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{S}$: C, 70.27; H, 6.29; N, 10.93. Found: C, 70.36; H, 6.38; N, 10.92.

1-(2,6-dimethylphenyl)-3-phenylthiourea (3g)

IR (KBr, cm^{-1}): 3140.51 (aromatic C-H), 1588.09, 1530.24, 1490.7 (C=C aromatic ring), 3337.21 (N-H, secondary amine), 1343.18 (C-N, secondary aromatic amine), 2960.2 (methyl C-H, as CH_3), 2858.95 (methyl C-H, s CH_3), 1246.75, 1205.29, 1029.8 (C=S), 931.45, 846.597, 778.136, 748.245, 699.069 (out-of-plane ring

C-H bend), 1530.24 (N-H bending, secondary aromatic amine); ^1H NMR (CDCl_3 , ppm): 6.830-6.838 (d, 1H, Ar-H), 7.242-7.266 (m, 2H, Ar-H), 7.399-7.425 (m, 2H, Ar-H), 7.676-7.689 (d, 2H, Ar-H), 8.076-8.085 (d, 1H, Ar-H), 8.660 (br s, 2H, NH), 2.350 (s, 6H, 2 CH_3). Anal. calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{S}$: C, 70.27; H, 6.29; N, 10.93. Found: C, 70.33; H, 6.35; N, 10.95.

(2Z)-2-[(3,4-dimethylphenyl)imino]-3-phenyl-1,3-thiazolidin-4-one (4a)

IR (KBr, cm^{-1}): 3116.4, 3043.12 (aromatic C-H), 1671.98, 1595.81, 1545.67, 1491.67, 1442.49, 1407.78 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 2981.41 (methyl C-H, as CH_3), 2866.67 (methyl C-H, s CH_3), 2928.38 (methylene C-H, as CH_2), 2804.96 (methylene C-H, s CH_2), 1373.07, 1329.68 (C-N, tertiary aromatic amine), 740.531, 690.391, 629.644, 601.682 (C-S); ^1H NMR (CDCl_3 , ppm): 6.830-6.863 (m, 2H, Ar-H), 7.088-7.172 (m, 1H, Ar-H), 7.238-7.544 (m, 5H, Ar-H), 2.279 (s, 6H, 2 CH_3), 3.924 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). ESI-MS: m/z 297 [$\text{M} + 1$] $^+$. Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{OS}$: C, 68.89; H, 5.44; N, 9.45. Found: C, 68.93; H, 5.48; N, 9.48.

(2Z)-3-phenyl-2-(pyrimidin-2-ylimino)-1,3-thiazolidin-4-one (4b)

IR (KBr, cm^{-1}): 2932.23 (methylene C-H, as CH_2), 2866.67 (methylene C-H, s CH_2), 3116.4, 3043.12 (aromatic C-H), 1671.98, 1595.81, 1545.67, 1490.7, 1441.53, 1407.78 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 1373.07, 1329.68 (C-N, tertiary aromatic amine), 740.531, 689.427, 629.644, 601.682 (C-S); ^1H NMR (CDCl_3 , ppm): 6.997-7.258 (m, 3H, PymH), 7.311-7.679 (m, 5H, Ar-H), 4.628 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). ESI-MS: m/z 271 [$\text{M} + 1$] $^+$. Anal. calcd. for $\text{C}_{13}\text{H}_{10}\text{N}_4\text{OS}$: C, 57.76; H, 3.73; N, 20.73. Found: C, 57.82; H, 3.79; N, 20.71.

(2Z)-3-phenyl-2-(1,3-thiazol-2-ylimino)-1,3-thiazolidin-4-one (4c)

IR (KBr, cm^{-1}): 3098.08 (aromatic C-H), 1633.41, 1568.81, 1530.24, 1460.81, 1400.07 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 2918.73 (methylene C-H, as CH_2), 2820.38 (methylene C-H, s CH_2), 1364.39 (C-N, tertiary aromatic amine), 754.031, 696.177, 625.788 (C-S); ^1H NMR (CDCl_3 , ppm): 7.325-7.551 (m, 5H, Ar-H), 7.690-7.704 (d, 2H, thiazole-H), 3.912 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). ESI-MS: m/z 276 [$\text{M} + 1$] $^+$. Anal. calcd. for $\text{C}_{12}\text{H}_9\text{N}_3\text{OS}_2$: C, 52.34; H, 3.29; N, 15.26. Found: C, 52.42; H, 3.37; N, 15.28.

(2E)-3-phenyl-2-(pyridin-3-ylimino)-1,3-thiazolidin-4-one (4d)

IR (KBr, cm^{-1}): 3098.08, 3050.83, 3013.23 (aromatic C-H), 1641.13, 1568.81, 1531.2, 1460.81, 1400.07 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 1364.39 (C-N, tertiary aromatic amine), 2919.7 (methylene C-H, as CH_2), 2858.95 (methylene C-H, s CH_2), 754.031, 696.177, 625.788 (C-S); ^1H NMR (CDCl_3 , ppm): 6.843-6.850 (d, 1H, Ar-H), 7.239-7.274 (m, 4H, Ar-H), 7.394-7.435 (m, 2H, PyH), 7.679-7.715 (m, 1H, PyH), 8.086-8.095 (d, 1H, PyH), 4.120 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). Anal. calcd. for $\text{C}_{14}\text{H}_{11}\text{N}_3\text{OS}$: C, 62.43; H, 4.12; N, 15.60. Found: C, 62.48; H, 4.18; N, 15.62.

(2E)-2-[(4-methylpyridin-2-yl)imino]-3-phenyl-1,3-thiazolidin-4-one (4e)

IR (KBr, cm^{-1}): 3116.4, 3044.09 (aromatic C-H), 1671.98, 1595.81, 1546.63, 1490.7, 1441.53, 1407.78 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 2981.41 (methyl C-H, as CH_3), 2866.67 (methyl C-H, s CH_3), 2932.23 (methylene C-H, as CH_2), 2812.67 (methylene C-H, s CH_2), 1374.03, 1329.68 (C-N, tertiary aromatic amine), 740.531, 690.391, 629.644, 602.646 (C-S); ^1H NMR (CDCl_3 , ppm): 6.838-6.847 (d, 1H, Ar-H), 7.240-7.275 (m, 2H, Ar-H), 7.395-7.434 (m, 2H, Ar-H), 7.692-7.705 (m, 2H, PyH), 8.083-8.092 (d, 1H, PyH), 2.360 (s, 3H, CH_3 at C_4 -Py), 4.098 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). ESI-MS: m/z 284 [$\text{M} + 1$] $^+$. Anal. calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_3\text{OS}$: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.62; H, 4.66; N, 14.85.

(2E)-2-[(3,5-dimethylphenyl)imino]-3-phenyl-1,3-thiazolidin-4-one (4f)

IR (KBr, cm^{-1}): 3116.4, 3043.12 (aromatic C-H), 1671.98, 1595.81, 1545.67, 1491.67, 1442.49, 1407.78 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 2981.41 (methyl C-H, as CH_3), 2866.67 (methyl C-H, s CH_3), 2936.09 (methylene C-H, as CH_2), 2812.67 (methylene C-H, s CH_2), 1373.07, 1329.68 (C-N, tertiary aromatic amine), 741.496, 690.391, 629.644, 602.646 (C-S); ^1H NMR (CDCl_3 , ppm): 7.109-7.261 (m, 5H, Ar-H), 7.396-7.432 (m, 3H, Ar-H), 2.340 (s, 6H, 2 CH_3), 3.998 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{OS}$: C, 68.89; H, 5.44; N, 9.45. Found: C, 68.98; H, 5.53; N, 9.43.

(2Z)-2-[(2,6-dimethylphenyl)imino]-3-phenyl-1,3-thiazolidin-4-one (4g)

IR (KBr, cm^{-1}): 3116.4, 3044.09 (aromatic C-H), 1672.95, 1595.81, 1545.67, 1490.7, 1407.78 (C=N, C=C aromatic ring), 1725.98 (C=O, 1,3-thiazolidin-4-one), 2981.41 (methyl C-H, as CH_3), 2866.67 (methyl C-H, s CH_3), 2932.23 (methylene C-H, as CH_2), 2812.67 (methylene C-H, s CH_2), 1374.03, 1329.68 (C-N, tertiary aromatic amine), 741.496, 690.391, 602.646 (C-S); $^1\text{H NMR}$ (CDCl_3 , ppm): 6.832-6.839 (d, 1H, Ar-H), 7.242-7.266 (m, 2H, Ar-H), 7.399-7.425 (m, 2H, Ar-H), 7.676-7.689 (d, 2H, Ar-H), 8.088-8.092 (d, 1H, Ar-H), 2.361 (s, 6H, 2 CH_3), 4.186 (s, 2H, 1,3-thiazolidin-4-one C_5 -H). ESI-MS: m/z 297 $[\text{M} + 1]^+$. Anal. Calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{OS}$: C, 68.89; H, 5.44; N, 9.45. Found: C, 68.95; H, 5.50; N, 9.47.

Anti-HIV Activity

All the newly synthesized 2-[(substituted phenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (**4a-g**) were evaluated for their anti-HIV activity and cytotoxicity in MT-4 cell cultures infected with wild-type HIV-1 strain III_B and HIV-2 strain ROD in comparison with azidothymidine (AZT) and dideoxyinosine (DDI), which were used as reference drugs. The results, expressed as EC_{50} (50% effective concentration), CC_{50} (50% cytotoxic concentration) and SI (selectivity index given by the $\text{CC}_{50}/\text{EC}_{50}$ ratio), are summarized in Table 2.

The experimental results indicated that none of the synthesized compounds showed any specific activity against HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cell cultures at subtoxic concentrations.

Based on the experience with this type of molecules, 1,3-thiazolidin-4-one are considered to act on the allosteric site of HIV-RT [33], and a certain degree of flexibility might be required for binding to HIV-1 RT. The absence of anti-HIV potency in most of the compounds was possibly due to their inability to exist in butterfly-like conformation.

Table 2: Anti-HIV activity, cytotoxicity and selectivity index of 2-[(substituted phenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones in MT-4 cells

Compound	EC_{50} ($\mu\text{g/ml}$) ^a		CC_{50} ($\mu\text{g/ml}$) ^b		Selectivity index (SI) ^c	
	HIV-1 (III _B)	HIV-2 (ROD)	HIV-1 (III _B)	HIV-2 (ROD)	HIV-1 (III _B)	HIV-2 (ROD)
4a	>62.18	>62.18	62.18	62.18	<1	<1
4b	>125	>125	>125	>125	X1	X1
4c	>53.45	>53.45	53.45	53.45	<1	<1
4d	>77.28	>77.28	77.28	77.28	<1	<1
4e	>125	>125	>125	>125	X1	X1
4f	>62.00	>62.00	62.00	62.00	<1	<1
4g	>125	>125	>125	>125	X1	X1
Azidothymidine (AZT)	0.002	0.002	>25	>25	>13405	>12077
Dideoxyinosine (DDI)	3.89	7.40	>50	>50	>13	>7

^a EC_{50} : Effective concentration or compound concentration achieving 50% inhibition of HIV-1-induced cytopathicity in MT-4 infected cell cultures.

^b CC_{50} : Cytotoxic concentration or compound concentration that reduces the normal uninfected MT-4 cell viability by 50%.

^cSI: Selectivity index: ratio $\text{CC}_{50}/\text{EC}_{50}$. The SI values: $\times 1$ stand for ≥ 1 or < 1 .

Conclusion

In conclusion, we synthesized a series of novel 2-[(substituted phenyl/heteroaryl)imino]-3-phenyl-1,3-thiazolidin-4-ones (**4a-g**), which were structurally confirmed by IR, ¹H NMR, elemental and MS spectral analysis and evaluated for their inhibition of HIV [HIV-1 (III_B) and HIV-2 (ROD)]-induced cytopathogenicity in MT-4 cell culture. The results indicated that none of the compounds were active against HIV-1 and HIV-2 replication. Although the pharmacological results are not very encouraging, this study provides useful information to further design new anti-HIV agents.

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