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# Synthesis, Characterization and Anti-HIV Evaluation of Some Novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones

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**Abstract**: A series of novel 2-(substituted phenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) were synthesized, structurally confirmed by elemental, IR, $^1$H NMR and MS spectral analysis. Further evaluated 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 in comparison with nevirapine (NVP), azidothymidine (AZT), dideoxycytidine (DDC) and dideoxyinosine (DDI), which were used as reference drugs.
**Keywords**: Phenylhydrazine, 1,3-thiazolidin-4-one, 2-sulfanylpropanoic acid, anti-HIV activity, cytotoxicity, MTT assay.

## 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)-5-methyl-3-(phenylamino)-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.

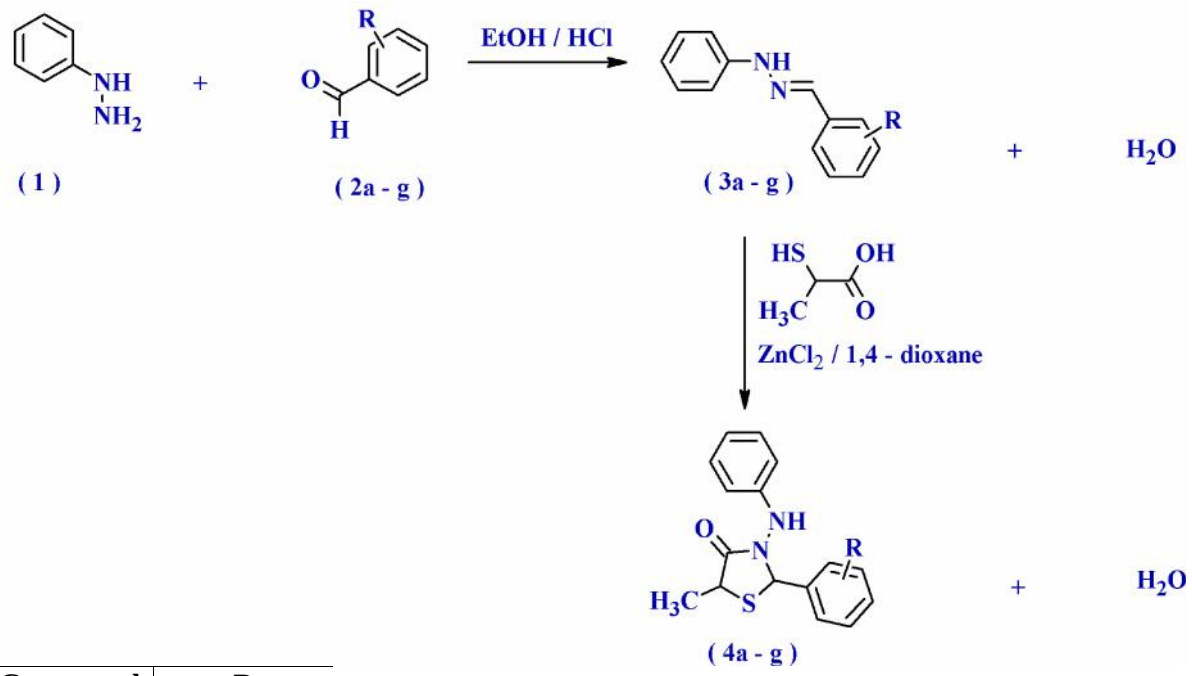
## Materials And Methods

### Experimental

Phenylhydrazine, 4-chlorobenzaldehyde, 2,3-dichlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 4-bromobenzaldehyde, 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde and 2-sulfanylpropanoic acid were commercially obtained from Aldrich (Milwaukee, WI). Dry 1,4-dioxane, anhydrous zinc chloride, chloroform, concentrated hydrochloric acid, sodium hydroxide, sodium bicarbonate, 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 Toluene: Hexane (1:4 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). 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 ±0.4% of the theoretical values. Mass spectra (MS) were recorded on a Q-TOF micromass spectrometer by using electronspray ionization (ESI) technique. 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)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (4a-g)**




| Compound | R |
|---|---|
| **4a** | 4-Cl |
| **4b** | 2,3-$(Cl)_2$ |
| **4c** | 2,4-$(Cl)_2$ |
| **4d** | 4-Br |
| **4e** | 2-$NO_2$ |
| **4f** | 3-$NO_2$ |
| **4g** | 4-$NO_2$ |

**Synthesis of (1Z)-1-(substitutedbenzylidene)-2-phenylhydrazine (3a-g)**

A mixture of phenylhydrazine (**1**) (0.01 mol) and different aromatic aldehydes (**2a-g**) (0.01 mol) (4-chlorobenzaldehyde (**2a**), 2,3-dichlorobenzaldehyde (**2b**), 2,4-dichlorobenzaldehyde (**2c**), 4-bromobenzaldehyde (**2d**), 2-nitrobenzaldehyde (**2e**), 3-nitrobenzaldehyde (**2f**) and 4-nitrobenzaldehyde (**2g**)) dissolved in absolute ethanol (20 ml) in presence of catalytic amount of conc. hydrochloric acid (0.5 ml) was refluxed for 5-6 h. The progress of the reaction was monitored by TLC using Toluene: Hexane (1:4 v/v) as eluents. After the completion of the reaction, the reaction mixture was cooled, concentrated under rotary vacuum. Then the resulting residue was poured into crushed ice and the product separated was filtered, washed with cold water, dried and crystallized from chloroform. Adopting the above procedure seven different phenylhydrazones (**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)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (4a-g)** A mixture of (1Z)-1-(substitutedbenzylidene)-2-phenylhydrazine (**3a-g**) (0.01 mol), 2-sulfanylpropanoic acid (0.015 mol) and anhydrous zinc chloride (0.5 g) in dry 1,4-dioxane (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, 1,4-dioxane was removed under reduced pressure. The final residue obtained was poured into crushed ice and the separated solid was neutralized by adding 10% sodium bicarbonate solution, for the removal of unreacted 2-sulfanylpropanoic acid. The neutralized solid product was filtered, washed with cold water, dried and crystallized from 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 $CCID_{50}$ (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.

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

## Results And Discussion

### Chemistry

In the present study, a series of novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) were synthesized according to scheme 1. Phenylhydrazine (**1**) on condensation with different aromatic aldehydes (**2a-g**) in presence of catalytic amount of concentrated hydrochloric acid in absolute ethanol resulted in the formation of (1Z)-1-(substitutedbenzylidene)-2-phenylhydrazine (**3a-g**) with 85.8 - 94.9% 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 Toluene: Hexane (1:4 v/v) and Benzene: Chloroform (1:1 v/v) as a solvent system and the spots being visualized under iodine vapours. The structures of the synthesized compounds (**3a-g**) were confirmed on the basis of elemental analysis, FT-IR and $^1$H NMR spectral data (Results and discussion part).

The FT-IR spectra of synthesized compounds (**3a-g**) showed absorbtion bands ranging from 1694.16 - 1587.13 $cm^{-1}$ for azomethine (>C=N) formation and 1598.7 - 1405.85 $cm^{-1}$ for C=C ring stretch of phenyl ring, 3056.62 - 3018.05 $cm^{-1}$ for aromatic C-H and 3393.14 -3300.57 $cm^{-1}$ for N-H, secondary amine. The IR spectra of compound (**3a-g**) displayed bands at about 1378.85 - 1295.93 $cm^{-1}$ and 837.919 - 636.394 $cm^{-1}$ associated with C-N stretch, secondary aromatic amine and C-Cl functions. In the IR spectra of compound (**3a-g**), some significant stretching bands due to C-Br, asymmetric $ArNO_2$, symmetric $ArNO_2$ and C-N, $ArNO_2$, were observed at 641.25 - 506.223 $cm^{-1}$, 1569.77 - 1529.27 $cm^{-1}$, 1348 -1324.86 $cm^{-1}$ and 896.737 - 851.418 $cm^{-1}$, respectively. In the $^1$H NMR spectra of compound (**3e**), aromatic (9H) protons appeared as a multiplet (9H) at δ 6.931 - 7.601 ppm, NH proton appeared as a broad singlet (1H) at δ 8.113 ppm and N=CH proton appeared as a singlet (1H) at δ 8.308 ppm, which proved the formation of azomethine.

Compounds (**3a-g**), which on cyclisation with 2-sulfanylpropanoic acid in dry 1,4-dioxane in presence of anhydrous zinc chloride offered the corresponding 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-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, $^1$H NMR and mass spectral data (experimental part).

The FT-IR spectrum of compound (**4a-g**) showed strong absorbtion band at 1779.97 - 1714.41 $cm^{-1}$ for C=O of 1,3-thiazolidin-4-one, while the band at 2974.66 - 2925.48 $cm^{-1}$, 2858.95 $cm^{-1}$, 1384.64 - 1323.89 $cm^{-1}$, 782.958 - 692.32 $cm^{-1}$, 3082.65 - 3067.23 $cm^{-1}$ and 3297.68 $cm^{-1}$, respectively confirms the presence of methyl C-H asymmetric, methyl C-H symmetric, C-N stretch of tertiary aromatic amine, C-S stretch, aromatic C-H and N-H stretch of secondary amine. 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 823.455 - 748.245 $cm^{-1}$ and 559.255 $cm^{-1}$ associated with C-Cl and C-Br functions. The IR spectrum of compound (**4a-g**) showed asymmetric $ArNO_2$ stretching bands at 1553.38 $cm^{-1}$, symmetric $ArNO_2$ at 1324.86 $cm^{-1}$ and C-N, $ArNO_2$ at 862.025 $cm^{-1}$, in addition to stretching band at 1646.91 - 1465.63 $cm^{-1}$ attributed to C=C of aromatic ring.

In the $^1$H NMR spectra of compound (**4c**), aromatic (8H) protons appeared as a multiplet (8H) at 7.318-7.409 ppm, N-H proton appeared as a singlet (1H) at 8.568 ppm, C-2 of 1,3-thiazolidin-4-one, N-CH-Ar proton appeared as a singlet (1H) at 6.302 ppm, $\underline{CH}$-$CH_3$ protons appeared as a quartet (1H) at 3.998-4.067 ppm and CH-$\underline{CH_3}$ protons appeared as a doublet (3H) at 1.733-1.757 ppm, which proved the closure of 1,3-thiazolidin-4-one ring. The results of elemental analyses were within ±0.4% of the theoretical values.

**Table 1: Physical data of (1Z)-1-(substitutedbenzylidene)-2-phenylhydrazine (3a-g) and 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (4a-g)**

| Compound | Mol. Formula/ Mol. Weight | Yield (%) | mp (°C) | [a]Rf |
|---|---|---|---|---|
| 3a | $C_{13}H_{11}ClN_2$/230.69 | 94.9 (2.19 g) | 111.6-113.4 | 0.73 |
| 3b | $C_{13}H_{10}Cl_2N_2$/265.14 | 89.4 (2.37 g) | 117.4-119.3 | 0.84 |
| 3c | $C_{13}H_{10}Cl_2N_2$/265.14 | 89.8 (2.38 g) | 133.4-135.3 | 0.88 |
| 3d | $C_{13}H_{11}BrN_2$/275.14 | 85.8 (2.36 g) | 102.8-104.2 | 0.72 |
| 3e | $C_{13}H_{11}N_3O_2$/241.25 | 88.7 (2.14 g) | 151.2-152.5 | 0.59 |
| 3f | $C_{13}H_{11}N_3O_2$/241.25 | 90.8 (2.19 g) | 116.5-117.9 | 0.35 |
| 3g | $C_{13}H_{11}N_3O_2$/241.25 | 88.4 (2.13 g) | 150.4-152.2 | 0.26 |
| 4a | $C_{16}H_{15}ClN_2OS$/318.82 | 73.1 (2.33 g) | 161.4-163.2 | 0.47 |
| 4b | $C_{16}H_{14}Cl_2N_2OS$/353.27 | 77.8 (2.75 g) | 177.2-179.3 | 0.62 |
| 4c | $C_{16}H_{14}Cl_2N_2OS$/353.27 | 76.7 (2.71 g) | 195.8-197.4 | 0.66 |
| 4d | $C_{16}H_{15}BrN_2OS$/363.27 | 65.8 (2.39 g) | 168.2-170.4 | 0.58 |
| 4e | $C_{16}H_{15}N_3O_3S$/329.37 | 77.4 (2.55 g) | 213.4-215.2 | 0.79 |
| 4f | $C_{16}H_{15}N_3O_3S$/329.37 | 75.9 (2.50 g) | 178.2-180.3 | 0.82 |
| 4g | $C_{16}H_{15}N_3O_3S$/329.37 | 78.3 (2.58 g) | 220.2-221.9 | 0.89 |

[a]Hexane: Toluene (4:1 v/v) for compound (**3a-g**) and Benzene: Chloroform (1:1 v/v) for compound (**4a-g**)

**(1Z)-1-(4-chlorobenzylidene)-2-phenylhydrazine** (**3a**)

IR (KBr, $cm^{-1}$): 3309.25 (N-H, secondary amine), 3050.83 (aromatic C-H), 1595.81, 1516.74, 1485.88 (C=C aromatic ring), 1352.82, 1301.72 (C-N, secondary aromatic amine), 1595.81 (C=N), 826.348, 748.245, 692.32, 644.108 (C-Cl), 1516.74 (N-H bending, secondary amine); $^1$H NMR ($CDCl_3$, δ ppm): 7.131-7.549 (m, 5H, Ar-H), 7.957-8.124 (m, 4H, Ar-H), 7.695 (s, 1H, N=CH), 8.437 (s, 1H, NH). Anal. calcd. for $C_{13}H_{11}ClN_2$: C, 67.68; H, 4.81; N, 12.14. Found: C, 67.72; H, 4.86; N, 12.10.

**(1Z)-1-(2,3-dichlorobenzylidene)-2-phenylhydrazine** (**3b**)

IR (KBr, $cm^{-1}$): 3300.57 (N-H, secondary amine), 3056.62, 3018.05 (aromatic C-H), 1596.77, 1570.74, 1514.81, 1488.78, 1446.35, 1405.85 (C=C aromatic ring), 1348.96, 1295.93 (C-N, secondary aromatic amine), 1596.77 (C=N), 837.919, 781.993, 754.031, 698.105, 636.394 (C-Cl), 1514.81 (N-H bending, secondary amine); $^1$H NMR ($CDCl_3$, δ ppm): 7.241-7.375 (m, 3H, Ar-H), 6.881-7.213 (m, 5H, Ar-H), 7.884 (br s, 1H, NH), 8.055 (s, 1H, N=CH). Anal. calcd. for $C_{13}H_{10}Cl_2N_2$: C, 58.89; H, 3.80; N, 10.57. Found: C, 58.98; H, 3.89; N, 10.6.

**(1Z)-1-(2,4-dichlorobenzylidene)-2-phenylhydrazine** (**3c**)

IR (KBr, $cm^{-1}$): 3296.71 (N-H, secondary amine), 3056.62 (aromatic C-H), 1594.84, 1581.34, 1516.74, 1489.74, 1478.17, 1443.46 (C=C aromatic ring), 1378.85, 1256.4 (C-N, secondary aromatic amine), 1594.84 (C=N), 815.742, 752.102, 691.355, 639.287 (C-Cl), 1516.74 (N-H bending, secondary amine); $^1$H NMR ($CDCl_3$, δ ppm): 6.880-7.115 (m, 3H, Ar-H), 7.221-7.470 (m, 5H, Ar-H), 7.979 (s, 1H, NH), 8.008 (s, 1H, N=CH). Anal. calcd. for $C_{13}H_{10}Cl_2N_2$: C, 58.89; H, 3.80; N, 10.57. Found: C, 58.94; H, 3.85; N, 10.59.

**(1Z)-1-(4-bromobenzylidene)-2-phenylhydrazine (3d)**

IR (KBr, $cm^{-1}$): 3305.39 (N-H, secondary amine), 3048.91 (aromatic C-H), 1694.16, 1592.91, 1514.81, 1485.88 (C=C aromatic ring), 1348.96 (C-N, secondary aromatic amine), 1694.16, 1592.91 (C=N), 641.25,

506.223 (C-Br), 1514.81 (N-H bending, secondary amine), 906.379, 818.634, 750.174, 692.32 (out-of-plane ring C-H bend). $^1$H NMR ($CDCl_3$, δ ppm): 6.903-7.620 (m, 9H, Ar-H), 8.275 (s, 1H, N=CH), 8.114 (br s, 1H, NH). Anal. calcd. for $C_{13}H_{11}BrN_2$: C, 56.75; H, 4.03; N, 10.18. Found: C, 56.81; H, 4.09; N, 10.2.

**(1Z)-1-(2-nitrobenzylidene)-2-phenylhydrazine (3e)**

IR (KBr, cm$^{-1}$): 3293.82 (N-H, secondary amine), 3051.8 (aromatic C-H), 1598.7, 1569.77, 1536.99, 1490.7 (C=C aromatic ring), 1335.46 (C-N, secondary aromatic amine), 1598.7 (C=N), 1569.77, 1536.99 (asymmetric ($ArNO_2$) $(N=O)_2$), 1335.46 (symmetric ($ArNO_2$) $(N=O)_2$), 896.737 (C-N, $ArNO_2$), 1536.99 (N-H bending, secondary amine); $^1$H NMR ($CDCl_3$, δ ppm): 6.931-7.601 (m, 9H, Ar-H), 8.308 (s, 1H, N=CH), 8.113 (br s, 1H, NH). Anal. calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.8; H, 4.69; N, 17.43.

**(1Z)-1-(3-nitrobenzylidene)-2-phenylhydrazine (3f)**

IR (KBr, cm$^{-1}$): 3318.89 (N-H, secondary amine), 3024.8 (aromatic C-H), 1587.13, 1529.27, 1487.81 (C=C aromatic ring), 1348.0 (C-N, secondary aromatic amine), 1587.13 (C=N), 1529.27 (asymmetric ($ArNO_2$) $(N=O)_2$), 1348.0 (symmetric ($ArNO_2$) $(N=O)_2$), 878.417 (C-N, $ArNO_2$), 1529.27 (N-H bending, secondary amine), 913.129, 878.417, 807.063, 749.209, 696.177 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 6.903-7.152 (m, 4H, Ar-H), 7.679 (s, 1H, N=CH), 8.427 (s, 1H, NH), 7.254-7.540 (m, 5H, Ar-H). Anal. calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.83; H, 4.71; N, 17.40.

**(1Z)-1-(4-nitrobenzylidene)-2-phenylhydrazine (3g)**

IR (KBr, cm$^{-1}$): 3393.14, 3297.68 (N-H, secondary amine), 3044.09 (aromatic C-H), 1597.73, 1556.27, 1531.2, 1492.63, 1405.85 (C=C aromatic ring), 1324.86 (C-N, secondary aromatic amine), 1597.73 (C=N), 1556.27, 1531.2 (asymmetric ($ArNO_2$) $(N=O)_2$), 1324.86 (symmetric ($ArNO_2$) $(N=O)_2$), 851.418 (C-N, $ArNO_2$), 1531.2 (N-H bending, secondary amine), 900.594, 851.418, 746.317, 687.498 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 6.926-7.345 (m, 5H, Ar-H), 7.690 (s, 1H, N=CH), 8.004 (br s, 1H, NH), 7.752-7.781 (m, 2H, Ar-H), 8.205-8.234 (m, 2H, Ar-H). Anal. calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.77; H, 4.66; N, 17.45.

**2-(4-chlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (4a)**

IR (KBr, cm$^{-1}$): 3082.65 (aromatic C-H), 1645.95, 1613.16, 1583.27, 1551.45, 1465.63 (aromatic C=C ring), 1384.64 (C-N, tertiary aromatic amine), 3234.04 (N-H, secondary amine), 2925.48 (methyl C-H, γas $CH_3$), 2858.95 (methyl C-H, γs $CH_3$), 1779.97, 1717.3 (C=O, 1,3-thiazolidin-4-one), 697.141 (C-S), 823.455, 782.958, 697.141 (C-Cl); $^1$H NMR ($CDCl_3$, δ ppm): 7.308-7.393 (m, 4H, Ar-H), 7.457-7.540 (m, 5H, Ar-H), 8.572 (s, 1H, NH), 6.301 (s, 1H, N-CH-Ar), 4.024-4.041 (q, 1H, CH-$CH_3$), 1.739-1.751 (d, 3H, CH-$CH_3$). ESI-MS: *m/z* 320 $[M + 1]^+$. Anal. calcd. for $C_{16}H_{15}ClN_2OS$: C, 60.28; H, 4.74; N, 8.79. Found: C, 60.32; H, 4.79; N, 8.84.

**2-(2,3-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (4b)**

IR (KBr, cm$^{-1}$): 3067.23 (aromatic C-H), 1645.95, 1582.31, 1465.63 (aromatic C=C ring), 1383.68 (C-N, tertiary aromatic amine), 3235.97 (N-H, secondary amine), 2928.38 (methyl C-H, γas $CH_3$), 1714.41 (C=O, 1,3-thiazolidin-4-one), 697.141 (C-S), 823.455, 781.993, 697.141 (C-Cl); $^1$H NMR ($CDCl_3$, δ ppm): 7.325 - 7.346 (m, 5H, Ar-H), 8.575 (s, 1H, NH), 6.302 (s, 1H, N-CH-Ar), 4.024-4.041 (q, 1H, CH-$CH_3$), 1.666-1.669 (d, 3H, CH-$CH_3$), 7.385-7.396 (m, 3H, Ar-H). Anal. calcd. for $C_{16}H_{14}Cl_2N_2OS$: C, 54.40; H, 3.99; N, 7.93. Found: C, 54.52; H, 4.08; N, 7.96.

**2-(2,4-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (4c)**

IR (KBr, cm$^{-1}$): 3068.19 (aromatic C-H), 1645.95, 1583.27, 1551.45, 1465.63 (aromatic C=C ring), 1383.68 (C-N, tertiary aromatic amine), 3235 (N-H, secondary amine), 2974.66, 2928.38 (methyl C-H, γas $CH_3$), 1715.37 (C=O, 1,3-thiazolidin-4-one), 696.177 (C-S), 822.491, 696.177 (C-Cl), 946.877, 862.025, 822.491, 696.177 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 7.318-7.409 (m, 8H, Ar-H), 8.568 (s, 1H, NH), 6.302 (s, 1H, N-CH-Ar), 3.998-4.067 (q, 1H, CH-$CH_3$), 1.733-1.757 (d, 3H, CH-$CH_3$). ESI-MS: *m/z* 354 $[M + 1]^+$. Anal. calcd. for $C_{16}H_{14}Cl_2N_2OS$: C, 54.40; H, 3.99; N, 7.93. Found: C, 54.45; H, 4.04; N, 7.94.

**2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (4d)**

IR (KBr, $cm^{-1}$): 3081.69 (aromatic C-H), 1645.95, 1613.16, 1583.27, 1551.45, 1465.63 (aromatic C=C ring), 1383.68 (C-N, tertiary aromatic amine), 3234.04 (N-H, secondary amine), 2928.38 (methyl C-H, γas $CH_3$), 1777.08, 1717.3 (C=O, 1,3-thiazolidin-4-one), 697.141 (C-S), 559.255 (C-Br), 946.877, 862.025, 823.455, 781.993, 697.141 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 7.462-7.669 (m, 9H, Ar-H), 8.435 (s, 1H, NH), 6.302 (s, 1H, N-CH-Ar), 3.998-4.067 (q, 1H, CH-$CH_3$), 1.641-1.658 (d, 3H, CH-$CH_3$). ESI-MS: *m/z* 364 $[M + 1]^+$. Anal. calcd. for $C_{16}H_{15}BrN_2OS$: C, 52.90; H, 4.16; N, 7.71. Found: C, 52.93; H, 4.21; N, 7.73.

**5-methyl-2-(2-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (4e)**

IR (KBr, $cm^{-1}$): 3068.19 (aromatic C-H), 1645.95, 1586.16, 1553.38, 1466.6 (aromatic C=C ring), 1324.86 (C-N, tertiary aromatic amine), 3297.68 (N-H, secondary amine), 2928.38 (methyl C-H, γas $CH_3$), 1715.37 (C=O, 1,3-thiazolidin-4-one), 1553.38 (asymmetric ($ArNO_2$) $(N=O)_2$), 1383.68, 1324.86 (symmetric ($ArNO_2$) $(N=O)_2$), 861.06 (C-N, $ArNO_2$), 748.245, 692.32 (C-S), 949.77, 861.06, 824.455, 748.245, 692.32 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 7.313-7.348 (m, 9H, Ar-H), 8.445 (s, 1H, NH), 6.30 (s, 1H, N-CH-Ar), 4.022-4.034 (q, 1H, CH-$CH_3$), 1.742-1.753 (d, 3H, CH-$CH_3$). ESI-MS: *m/z* 330 $[M + 1]^+$. Anal. calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.40; H, 4.65; N, 12.77.

**5-methyl-2-(3-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (4f)**

IR (KBr, $cm^{-1}$): 3068.19 (aromatic C-H), 1645.95, 1585.2, 1553.38, 1466.6 (aromatic C=C ring), 1323.89 (C-N, tertiary aromatic amine), 3296.71 (N-H, secondary amine), 2928.38 (methyl C-H, γas $CH_3$), 1716.34 (C=O, 1,3-thiazolidin-4-one), 1553.38 (asymmetric ($ArNO_2$) $(N=O)_2$), 1383.68, 1323.89 (symmetric ($ArNO_2$) $(N=O)_2$), 862.025 (C-N, $ArNO_2$), 781.993, 695.212 (C-S), 949.77, 862.025, 823.455, 781.993, 695.212 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 7.315-7.349 (m, 9H, Ar-H), 8.448 (s, 1H, NH), 6.301 (s, 1H, N-CH-Ar), 4.023-4.035 (q, 1H, CH-$CH_3$), 1.743-1.755 (d, 3H, CH-$CH_3$). Anal. calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.44; H, 4.67; N, 12.75.

**5-methyl-2-(4-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (4g)**

IR (KBr, $cm^{-1}$): 3068.19 (aromatic C-H), 1646.91, 1586.16, 1553.38, 1466.6 (aromatic C=C ring), 1324.86 (C-N, tertiary aromatic amine), 3297.68 (N-H, secondary amine), 2927.41 (methyl C-H, γas $CH_3$), 1778.05, 1716.34 (C=O, 1,3-thiazolidin-4-one), 1553.38 (asymmetric ($ArNO_2$) $(N=O)_2$), 1384.64, 1324.86 (symmetric ($ArNO_2$) $(N=O)_2$), 862.025 (C-N, $ArNO_2$), 782.958, 695.212 (C-S); $^1$H NMR ($CDCl_3$, δ ppm): 7.389-7.409 (m, 4H, Ar-H), 7.458-7.468 (m, 5H, Ar-H), 8.447 (s, 1H, NH), 6.302 (s, 1H, N-CH-Ar), 4.023-4.036 (q, 1H, CH-$CH_3$), 1.743-1.755 (d, 3H, CH-$CH_3$),. ESI-MS: *m/z* 330 $[M + 1]^+$. Anal. calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.38; H, 4.63; N, 12.78.

**Anti-HIV Activity**

All the newly synthesized 2-(substituted phenyl)-5-methyl-3-(phenylamino)-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 nevirapine (NVP), azidothymidine (AZT), dideoxycytidine (DDC) 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 $CC_{50}/EC_{50}$ 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)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in MT-4 cells**

| Compound | $EC_{50}$ (µg/ml)[a] | | $CC_{50}$ (µg/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 | >2.51 | >2.51 | 2.51 | 2.51 | <1 | <1 |
| 4b | >2.64 | >2.64 | 2.64 | 2.64 | <1 | <1 |
| 4c | >0.34 | >0.34 | ≥0.34 | ≥0.34 | <orX1 | <orX1 |
| 4d | >3.97 | >3.97 | 3.97 | 3.97 | <1 | <1 |
| 4e | >9.20 | >9.20 | 9.20 | 9.20 | <1 | <1 |
| 4f | >2.37 | >2.37 | 2.37 | 2.37 | <1 | <1 |
| 4g | >6.45 | >6.45 | 6.45 | 6.45 | <1 | <1 |
| Nevirapine | 0.050 | >4.00 | >4.00 | >4.00 | >80 | <1 |
| Azidothymidine (AZT) | 0.0022 | 0.00094 | >25.00 | >25.00 | >11587 | >26731 |
| Dideoxycytidine (DDC) | 0.16 | 0.19 | >20.00 | >20.00 | >127 | >108 |
| Dideoxyinosine (DDI) | 2.09 | 3.78 | >50.00 | >50.00 | >24 | >13 |

[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%.
[c]SI: Selectivity index: ratio $CC_{50}/EC_{50}$. The SI values: ×1 stand for ≥1 or <1.

## Conclusion

In conclusion, we designed and synthesized a series of novel 2-(substituted phenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**), which were structurally confirmed by IR, $^1$H 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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