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Synthesis, characterization and antiviral 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-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) were synthesized and structurally confirmed by elemental, IR, ^{1}H NMR and MS spectral analysis. Further, the antiviral screening of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) against a broad panel of DNA and RNA viral strains indicated that 2-(4-chlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4a**) showed some antiviral activity against herpes simplex virus-1 (KOS) (EC_{50} = 47 µM), herpes simplex virus-2 (G) (EC_{50} = 42 µM), herpes simplex virus-1 TK KOS ACV^r (EC_{50} = 47 µM), vaccinia virus (EC_{50} = 45 µM) and vesicular stomatitis virus (EC_{50} = 51 µM) tested in human embryonic lung (HEL) cell cultures. 5-methyl-2-(4-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (**4g**) and 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) showed some antiviral activity against cytomegalovirus [EC_{50} = 15 and 100 µM (AD-169) and EC_{50} = 10 and 100 µM (Davis strain), respectively] tested in HEL cell cultures. 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) and 5-methyl-2-(2-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (**4e**) showed antiviral activity against feline corona virus (FIPV) (EC_{50} = 18 and 41 µM, respectively) tested in Crandell-Rees feline kidney (CRFK) cell cultures and can be considered as lead compounds for the development of novel antiviral agents.*

Keywords: Phenylhydrazine, 1,3-thiazolidin-4-one, 2-sulfanylpropanoic acid, antiviral activity, cytotoxicity, MTT assay.

INTRODUCTION

DNA and RNA viruses are incriminated in the causation of many diseases in human as well as in animal hosts. Amongst DNA-containing viruses, the herpes group of viruses [1], especially herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), cytomegalovirus (CMV) and varicella-zoster virus (VZV) are more important in that they cause a variety of illnesses that are very common in humans. Herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2), cause recurrent infections affecting the skin, the mouth (gingivostomatitis), the lips (herpes labialis), the eyes (herpes keratitis), hands (herpes whitlow), encephalitis, meningitis, orofacial and genital lesions [2], cytomegalovirus (CMV) gives rise to pneumonitis, retinitis and is associated with certain vascular diseases [3], varicella-zoster virus (VZV) is an ethiological agent of chickenpox and shingles [4]. Vaccinia virus (VV) associated diseases are a serious problem for human health as well as veterinary medicine [5]. Feline herpes virus 1 (FHV-1) is one of the most common viruses among cats [6], associated with respiratory disorders and ocular disease, including keratitis, conjunctivitis, corneal sequestration, keratoconjuctivitis and ultimately, loss of sight [7].

Reovirus-1, vesicular stomatitis virus (VSV), alphavirus (e.g. sindbis virus, parainfluenza-3 virus (PI-3V), respiratory syncytial virus (RSV), influenza viruses (INF), coxsackievirus B4 (CV B4), puntatoro virus (PTV) and feline coronavirus (FCoV) are few examples of RNA viruses, which are enveloped single-stranded RNA-containing viruses except reovirus-1 which contains a double stranded RNA. Coxsackieviruses are the most common cause of viral myocarditis, associated with the development of pleurodynia, pancreatitis, meningitis, hepatitis and encephalitis [8]. Punta Toro Virus (PTV) is transmitted by sandflies and cause an acute febrile illness [9]. Influenza viruses, parainfluenza-3 virus and respiratory syncytial virus (RSV) are an important cause of respiratory tract infections, including acute pulmonary diseases (pneumonia) [10], bronchitis, bronchiolitis [11], and chronic lung disease (chronic wheezing, asthma) [12] in people of all ages.

Vesicular stomatitis virus causes an economically important disease in cattle and horses [13]. Feline coronavirus (FCoV) affects both wild and domestic cats [14], can cause multiple illnesses ranging from a mild symptomless enteric infection, especially in kittens, to a lethal, systemic disease known as feline infectious peritonitis (FIP) [15].

The treatments of viral infections still remain an important challenge to medical fraternity because of the emergence of multiple drug-resistant strains due to the rapid mutability of the viruses [16]. Many existing therapies are also hampered by significant drug-associated toxicities that limit their use. As a consequence, there is an increasing need for drugs which are less toxic, orally bioavailable, with a low potential for *in vivo* nephrotoxicity, with sufficient metabolic stability, with a high inhibitory activity against drug-resistant mutants and with a broad spectrum activity to counter multiple viral infections in the growing immunocompromised host population, including organ transplant recipients and AIDS patients.

Antiviral research in the past has primarily focused on the development of nucleoside analogues but of late, non-nucleoside derivatives [17] 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 such as analgesic [18], anti-inflammatory [19], antiangiogenic [20], anti-HIV [21], *in vitro* anti-*Toxoplasma gondii* [22], antimicrobial [23], antimycobacterial [24], antimalarial [25], trypanocidal [26], antischistosomal [27], anticonvulsant [28], antihistaminic [29], antidiabetic [30], antiarrhythmic [31] and antihypertensive properties [32]. Review of literature has shown that a large number of 1,3-thiazolidin-4-ones have been synthesized and evaluated for various activities in the past, however their potential antiviral properties have not been investigated in depth.

Guided by the information discussed above, we have synthesized some novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) and tested for its *in vitro* cytotoxicity and antiviral activity, against a broad panel of DNA and RNA viruses.

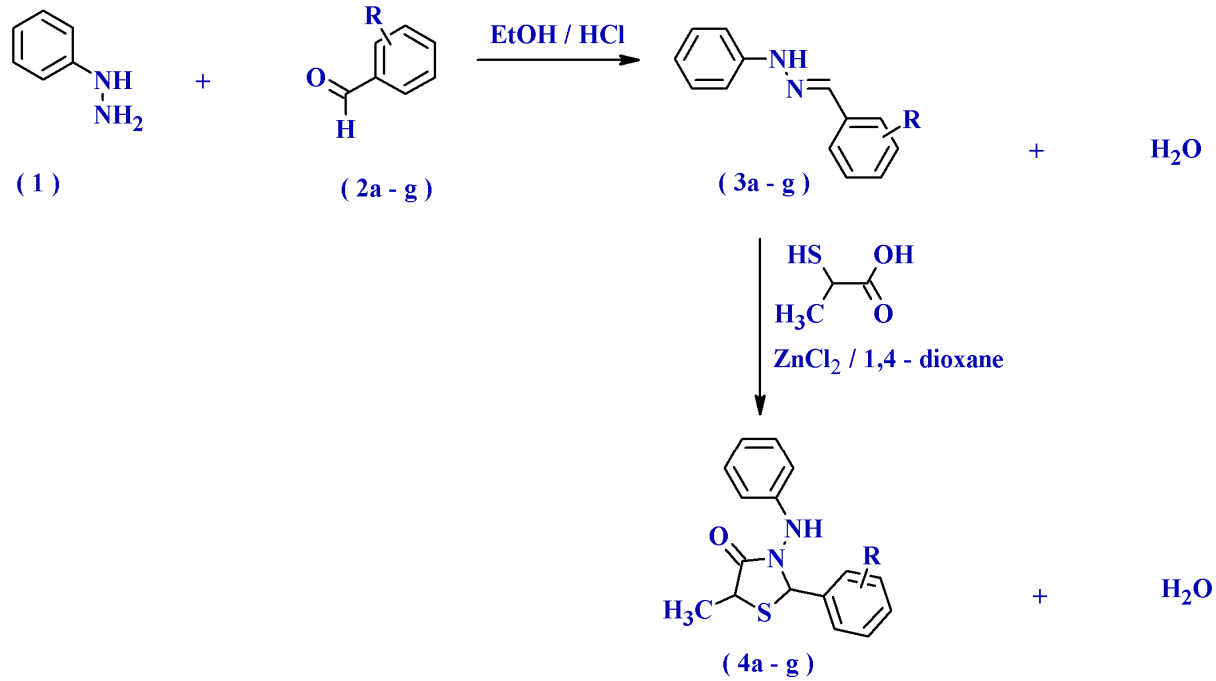
MATERIALS AND METHODS

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, benzene, toluene, hexane 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.

Antiviral Assays

The antiviral assays [33], other than the anti-HIV assays, were based on inhibition of virus-induced cytopathogenicity or plaque formation in respective cell cultures. The viruses included in the panel are herpes simplex virus type 1 (HSV-1) (KOS), herpes simplex virus type 2 (HSV-2) (G), thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to acyclovir (ACV^r), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV) [AD-169 and Davis strain] and varicella-zoster virus (VZV) [TK^+ (OKA strain) and TK^- (07/1 strain)], parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, Punta Toro virus, respiratory syncytial virus, Influenza A [H1N1 subtype (A/PR/8/34) and H3N2 subtype (A/HK/7/87)], Influenza B (B/HK/5/72), feline corona virus (FIPV) and feline herpes virus. Specific cell cultures were utilized for antiviral assays. Human embryonic lung cell (HEL) cultures was utilized for herpes simplex virus type 1 (KOS), herpes simplex virus type 2 (G), thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to acyclovir (ACV^r), vaccinia virus, vesicular stomatitis virus, cytomegalovirus and varicella-zoster virus. Simian Kidney cell (Vero) cultures was utilized for parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4 and Punta toro viruses, human cervix carcinoma cell (HeLa) cultures for vesicular stomatitis virus, coxsackie virus B4 and respiratory syncytial virus, Madin Darby canine kidney cell (MDCK) cultures for Influenza A [H1N1 subtype (A/PR/8/34) and H3N2 subtype (A/HK/7/87)]] and influenza B (B/HK/5/72) viruses and Crandell-Rees feline kidney cells (CRFK) for feline corona virus (FIPV) and feline herpes viruses (Table 2-Table 8).

Human embryonic lung (HEL) [34], simian kidney (Vero), human cervix carcinoma (HeLa) cells and Crandell-Rees feline kidney (CRFK) [35] cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and 0.075% sodium bicarbonate.

Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1$CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8 µM) of the tested compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

Cytotoxicity Assays

Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96 well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37°C, the cell number was determined with a coulter counter. The 50% cytostatic concentration (for MDCK and CRFK cells) [36] (CC_{50}) was calculated as the compound concentration required to reduce cell growth by 50% relative to the number of the cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity (for HEL, Vero and HeLa cells) [36] was also expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology (Table 2-Table 8).

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 ^1H NMR spectral data (experimental 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 ^1H 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, ^1H 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 ^1H 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); ^1H 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-<u>CH</u>-Ar), 4.024-4.041 (q, 1H, <u>CH</u>-CH_3), 1.739-1.751 (d, 3H, CH-<u>CH_3</u>). 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.

Antiviral Evaluation
The results of antiviral screening at the 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) against a broad panel of DNA and RNA viruses, including human cytomegalovirus (CMV) [AD-169 and Davis] (Table 2), herpes simplex virus-1 (KOS) (HSV-1 KOS), herpes simplex virus-2 (G) (HSV-2 G), vaccinia virus (VV), vesicular stomatitis virus (VSV), thymidine kinase-deficient (TK^-) KOS strain of HSV-1 resistant to acyclovir (ACV^r) (HSV-1 TK^- KOS ACV^r) and varicella-zoster virus (VZV) [thymidine kinase positive (TK^+) VZV (OKA strain) and thymidine kinase deficient (TK^-) VZV (07/1 strain)] in human embryonic lung (HEL) cell cultures (Table 3 and Table 8), vesicular stomatitis virus (VSV), coxsackie virus B4 (CV-B4) and respiratory syncytial virus (RSV) in HeLa cell cutures (Table 4), parainfluenza-3 virus (PI-3V), reovirus-1 (RV-1), sindbis virus (SV), coxsackie virus B4 (CV-B4) and punta toro virus (PTV) in Vero cell cultures (Table 5), influenza A [H1N1 subtype (A/PR/8/34)

and H3N2 subtype (A/HK/7/87)] and influenza B (B/HK/5/72) in Madin Darby canine kidney (MDCK) cell cultures (Table 7) and feline corona virus (FIPV) and feline herpes virus in Crandell-Rees Feline kidney (CRFK) cell cultures (Table 6), were determined by using cytopathicity (CPE) reduction assays [37]. The antiviral activities were compared with the reference antiviral drugs (ganciclovir, cidofovir, acyclovir, brivudin, DS-5000, (S)-DHPA, ribavirin, zanamivir, amantadine, rimantadine, *Hippeastrum* hybrid agglutinin (HHA) and *Urtica dioica* agglutinin (UDA) (Table 2-Table 8). The antiviral activity was expressed as the minimum effective concentration (EC_{50}) required to reduce virus-induced cytopathogenicity by 50% and cytotoxicity was expressed as the minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology.

Compound **4g** showed antiviral activity against cytomegalovirus (CMV) [EC_{50} = 15 µM (AD-169, Table 2) and EC_{50} = 10 µM (Davis, Table 2)] among the tested (2-substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**), and was comparable to the reference compound, ganciclovir [EC_{50} = 12 µM (AD-169); EC_{50} = 7 µM (Davis)] but less active than cidofovir [EC_{50} = 1.8 µM (AD-169); EC_{50} = 0.97 µM (Davis)]. However, compound **4g** also showed cytostatic activity against HEL cell proliferation at a CC_{50} of 55 µM. Therefore, the antiviral selectivity of **4g** is ~ 3.

It was interesting to note that the position of the different electron withdrawing groups on the phenyl group is of crucial importance for eventual toxicity in the HEL cell cultures. The introduction of 2,3-$(Cl)_2$ and 2,4-$(Cl)_2$ group at *ortho* position attached to the C-2 position of the 1,3-thiazolidin-4-one ring (compound **4b** and compound **4c**) results in relatively high cytotoxicity (CC_{50} = 12 and 16 µM, respectively) (Table 2), whereas introduction of NO_2 at *meta* (compound **4f**) showed a CC_{50} = 51 µM and introduction of a NO_2 and Cl group at the *para* position (compound **4a** and compound **4g**) causes limited toxicity (CC_{50} = 55 and 75 µM, respectively) (Table 2) pointing to the *ortho* substitution of these groups as a prerequisite for cytotoxic activity.

Compound **4a** showed antiviral activity against herpes simplex virus-1 (KOS) (EC_{50} = 47 µM, Table 3), herpes simplex virus-2 (G) (EC_{50} = 42 µM, Table 3), herpes simplex virus-1 TK^- KOS ACV^r (EC_{50} = 47 µM, Table 3), vaccinia virus (VV) (EC_{50} = 45 µM, Table 3) and vesicular stomatitis virus (VSV) (EC_{50} = 51 µM, Table 3) among the tested (2-substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**), but it was less active than the reference compounds brivudin, cidofovir, acyclovir and ganciclovir. In HEL cell cultures (Table 3) compound **4b**, compound **4c**, compound **4e**, compound **4f** and compound **4g** proved inactive against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 TK^- KOS ACV^r, vaccinia virus (VV) and vesicular stomatitis virus (VSV).

In HeLa cell cultures (Table 4) none of the compounds were antivirally active, and compound **4a**, compound **4g** (EC_{50} >4 µM; SI = 5) exhibited moderate cytotoxicity. In Vero cell cultures, Table 5 revealed that none of the compounds were active against parainfluenza-3 virus (PI-3V), reovirus-1 (RV-1), Sindbis virus (SV), Coxsackie virus B4 (CV-B4) and Punta Toro virus (PTV).

Compound **4d** and compound **4e** showed some antiviral activity against feline corona virus (FIPV) tested in CRFK cell cultures with an EC_{50} value of 18 and 41 µM, respectively (Table 6). These compounds did not show toxicity at 100 µM. Table 7 revealed that none of the compounds were inhibitory at subtoxic concentrations against influenza A [H1N1 subtype (A/PR/8/34) and H3N2 subtype (A/HK/7/87)] and influenza B (B/HK/5/72) in MDCK cell cultures. Instead, they were highly cytotoxic in MDCK cells (Table 7).

In HEL cell cultures (Table 8) none of the compounds were active against varicella-zoster virus (VZV) [thymidine kinase positive (TK^+) VZV (OKA strain) and thymidine kinase deficient (TK^-) VZV (07/1 strain)].

Table 2: Cytotoxicity and antiviral activity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones against cytomegalovirus (CMV) in HEL cell cultures (4a-g)

	Antiviral activity EC_{50} (µM)[a]		Cytotoxicity (µM)	
Compound	AD-169	Davis	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
4a	>20	>20	100	75
4b	>20	>20	100	12
4c	>20	>20	100	16
4d	100	100	>100	100
4e	>100	>100	>100	100
4f	>20	>20	100	51
4g	15	10	100	55
Ganciclovir	12	7	>400	N.D.[d]
Cidofovir	1.8	0.97	>300	114

[a]*Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).*
[b]*Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.*
[c]*Cytotoxic concentration required to reduce cell growth by 50%.*
[d]*Not determined*
HEL cells: Human embryonic lung cells.

Table 3: Cytotoxicity and antiviral activity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in HEL cell cultures (4a-g)

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK⁻ KOS ACV^r
4a	≥100	47	42	45	51	47
4b	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100	>100
4d	100	>20	>20	>20	>20	>20
4e	>100	>100	>100	>100	>100	>100
4f	>100	>100	>100	>100	>100	>100
4g	≥100	>100	>100	>100	>100	>100
Brivudin	>250	0.08	146	50	>250	50
Cidofovir	>250	0.9	0.2	4	>250	0.9
Acyclovir	>250	1	0.4	>250	>250	126
Ganciclovir	>100	0.02	0.03	>100	>100	8

[a]*Required to cause a microscopically detectable alteration of normal cell morphology.*
[b]*Required to reduce virus-induced cytopathogenicity by 50%.*
HEL cells: Human embryonic lung cells.

Table 4: Cytotoxicity and antiviral activity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in HeLa cell cultures (4a-g)

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
4a	20	>4	>4	>4
4b	≥100	>100	>100	>100
4c	100	>20	>20	>20
4d	≥20	>20	>20	>20
4e	≥20	>20	>20	>20
4f	100	>20	>20	>20
4g	20	>4	>4	>4
DS-5000	>100	>100	2	2
(µg/ml)	>250	>250	>250	>250
(S)-DHPA	>250	4	22	17
Ribavirin				

[a]*Required to cause a microscopically detectable alteration of normal cell morphology.*
[b]*Required to reduce virus-induced cytopathogenicity by 50%.*
DS-5000: Dextran sulfate-5000; (S)-DHPA: 9-(1,3-dihydroxypropyl)adenine.

Table 5: Cytotoxicity and antiviral activity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in Vero cell cultures (4a-g)

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)				
		Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
4a	20	>4	>4	>4	>4	>4
4b	100	>20	>20	>20	>20	>20
4c	100	>20	>20	>20	>20	>20
4d	100	>20	>20	>20	>20	>20
4e	100	>20	>20	>20	>20	>20
4f	100	>20	>20	>20	>20	>20
4g	≥20	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	20	100	100
(µg/ml)	>250	>250	>250	>250	>250	>250
(S)-DHPA	>250	112	>250	>250	146	112
Ribavirin						

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.
DS-5000: Dextran sulfate-5000; (S)-DHPA: 9-(1,3-dihydroxypropyl)adenine.

Table 6: Anti-Feline Corona virus (FIPV) and anti-Feline Herpes virus activity and cytotoxicity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in CRFK cell cultures (4a-g)

Compound	CC_{50}[a] (µM)	EC_{50}[b] (µM)	
		Feline Corona virus (FIPV)	Feline Herpes virus
4a	53	>20	>20
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	18	>100
4e	>100	41	>100
4f	95	>20	>20
4g	76	>20	>20
HHA (µg/ml)	>100	7.5	11
UDA (µg/ml)	21	6.7	2.6
Ganciclovir	>100	>100	3.8

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay (data are the mean of 2 independent experiments).
CRFK cells: Crandell-Rees Feline Kidney cells; HHA: Hippeastrum hybrid agglutinin; UDA: Urtica dioica agglutinin.

Table 7: Anti-influenza virus activity and cytotoxicity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones in MDCK cell cultures (4a-g)

Compound	Concentration unit	Cytotoxicity		Antiviral EC_{50}[c] (µM)					
		CC_{50}[a]	Minimum cytotoxic concentration[b] (µM)	Influenza A H1N1 subtype (A/PR/8/34)		Influenza A H3N2 subtype (A/HK/7/87)		Influenza B (B/HK/5/72)	
				visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
4a	µM	0.8	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
4b	µM	2.2	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
4c	µM	1.0	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
4d	µM	0.6	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
4e	µM	0.5	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
4f	µM	1.6	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
4g	µM	1.7	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
Oseltamivir	µM	>100	>100	1.8	2.3	0.8	0.4	58	67.0
Ribavirin	µM	>100	>100	2.3	3.4	9.0	5.1	9	4.5
Amantadine	µM	>200	>200	4.0	3.8	0.8	0.6	>200	>200
Rimantadine	µM	>200	>200	200	118	0.6	0.3	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
MDCK cells: Madin Darby canine kidney cells; Virus strain: Influenza A/Puerto Rico/8/34 (H1N1), influenza A/Hong Kong/7/87 (H3N2) and influenza B/ Hong Kong/5/72.

Table 8: Cytotoxicity and antiviral activity of 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones against varicella-zoster virus (VZV) in HEL cell cultures (4a-g)

Compound	Antiviral activity EC_{50} (µM)[a]		Cytotoxicity (µM)	
	TK^+ VZV strain	TK^- VZV strain	Cell morphology	Cell growth
	OKA	07-1	(MCC)[b]	(CC_{50})[c]
4a	>100	>100	>100	75.5
4b	>100	>100	>100	12.3
4c	>20	>20	100	16.1
4d	>100	>100	>100	100
4e	>100	>100	>100	100
4f	>20	>20	100	51
4g	>20	>20	100	55.4
Acyclovir	0.93	17.8	>440	840
Brivudin	0.0081	1.08	>300	468

[a]*Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).*
[b]*Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.*
[c]*Cytotoxic concentration required to reduce cell growth by 50%.*
HEL cells: Human embryonic lung cells.

CONCLUSION

In conclusion, we synthesized a series of novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones, which were structurally confirmed by elemental, IR, ^{1}H NMR and MS spectral analysis. Further, the antiviral screening of the 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) against a broad panel of DNA and RNA viral strains indicated that 2-(4-chlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4a**) showed some antiviral activity against herpes simplex virus-1 (KOS) (EC_{50} = 47 µM), herpes simplex virus-2 (G) (EC_{50} = 42 µM), herpes simplex virus-1 TK^- KOS ACV^r (EC_{50} = 47 µM), vaccinia virus (EC_{50} = 45 µM) and vesicular stomatitis virus (EC_{50} = 51 µM) tested in human embryonic lung (HEL) cell cultures. 5-methyl-2-(4-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (**4g**) and 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) showed some antiviral activity against cytomegalovirus [EC_{50} = 15 and 100 µM (AD-169) and EC_{50} = 10 and 100 µM (Davis strain), respectively] tested in HEL cell cultures. 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) and 5-methyl-2-(2-nitrophenyl)-3-(phenylamino)-1,3-thiazolidin-4-one (**4e**) showed antiviral activity against feline corona virus (FIPV) (EC_{50} = 18 and 41 µM, respectively) tested in Crandell-Rees feline kidney (CRFK) cell cultures and could be selected as potential lead compounds for the development of novel antiviral agents.

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