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Synthesis, characterization and antiviral evaluation of some novel N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides

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ABSTRACT

*A series of novel N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (**4a-g**) were synthesized and structurally confirmed by elemental analysis, IR, ^{1}H NMR and MS spectral data. Further, the antiviral screening of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (**4a-g**) against a broad panel of DNA and RNA viral strains indicated that N-[2-(4-chlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (**4a**) and N-[5-methyl-2-(2-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzamide (**4e**) showed some antiviral activity against feline corona virus (FIPV) (EC_{50} = 70 ± 15 and 74 ± 31 µM, respectively) and may be selected as lead compound for the development of novel antiviral agents.*

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

INTRODUCTION

Many diseases are caused by different members of DNA- and RNA-containing viruses. Amongst the DNA-containing viruses, the herpes group of viruses [1], especially herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2) commonly 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) giving rise to pneumonitis, retinitis and is associated with certain vascular diseases [3]; varicella-zoster virus (VZV) is the etiological agent of chickenpox and shingles [4]. Vaccinia virus (VV) associated diseases are a serious problem for human health as well as in 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]. Except reovirus-1 (dsRNA), 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), punta toro virus (PTV) and feline coronavirus (FCoV) are examples of enveloped single-stranded RNA-containing viruses. Vesicular stomatitis virus causes an economically important disease in cattle and horses [8]. Coxsackieviruses are the most common cause of viral myocarditis, associated with the development of pleurodynia, pancreatitis, meningitis, hepatitis and encephalitis [9]. Punta Toro Virus (PTV) is transmitted by sandflies and causes an acute febrile illness [10]. Feline coronavirus (FCoV) affects both wild and domestic cats [11], can cause a mild symptomless enteric infection, especially in kittens, and is also associated with a lethal, systemic disease known as feline infectious peritonitis (FIP) [12]. Influenza viruses, parainfluenza-3 virus and respiratory syncytial virus (RSV) are an important

cause of respiratory tract infections, including acute pulmonary diseases (pneumonia) [13], bronchitis, bronchiolitis [14], and chronic lung disease (chronic wheezing, asthma) [15] in people of all ages.

The treatment of viral infectious diseases still remains an important challenge because of the emergence of drug-resistant virus strains due to the rapid mutability of the virus [16]. Many existing therapies are also hampered by significant drug-associated toxicities that limit their duration of use. As a consequence, there is an increasing need for less toxic, orally bioavailable, low potential for *in vivo* nephrotoxicity, sufficient metabolic stability, high inhibitory activity against drug-resistant virus mutants and broad spectrum agents to address virus infections in the growing immunocompromised population, particularly in AIDS patients and organ transplant recipients.

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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (**4a-g**) and tested for its *in vitro* cytotoxicity and antiviral activity, against a broad panel of DNA and RNA viruses.

MATERIALS AND METHODS

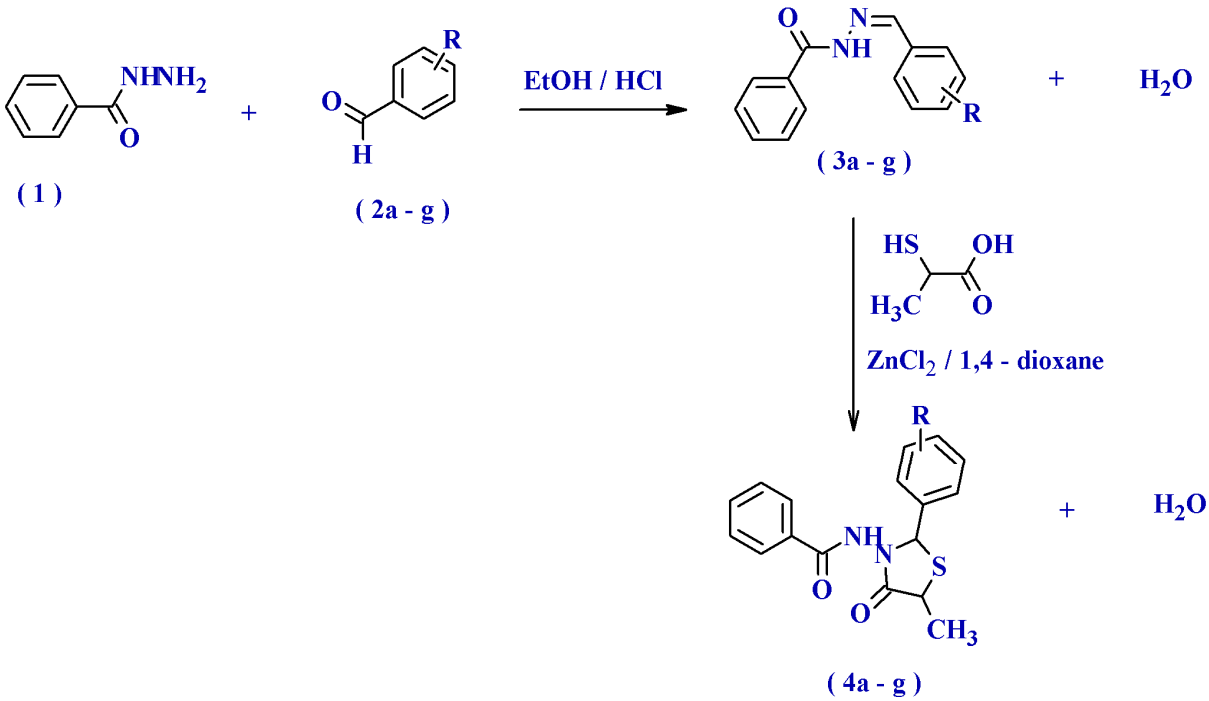
Experimental

Benzohydrazide, 4-chlorobenzaldehyde, 2,3-dichlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 4-bromo benzaldehyde, 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, ethylacetate hexane, benzene, sodium bicarbonate 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 Ethylacetate: Hexane (1:2 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}. ^{1}H NMR spectra were recorded on a Bruker DPX 300 (operating at 300 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 electronspray 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 ±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.

Synthesis of N'-[(E)-(substitutedphenyl)methylidene]benzohydrazide (3a-g)

A mixture of benzohydrazide (**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**)) were dissolved in absolute ethanol (20 ml) in presence of catalytic amount of conc. hydrochloric acid (0.5 ml) was refluxed for 4-5 h. The progress of the reaction was monitored by TLC using Ethylacetate: Hexane (1:2 v/v) as eluents. After the completion of the reaction, the crystalline product that separated out was filtered, washed with cold water, dried and crystallized from chloroform. Adopting the above procedure seven different schiff's base (**3a-g**) was synthesized. Percentage yield, melting point and Rf value of the synthesized compound (**3a-g**) were determined and presented in Table 1.

Scheme 1: Synthetic route for the preparation of novel N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4a-g)

A mixture of N'-[(E)-(substitutedphenyl)methylidene]benzohydrazide (**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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (**4a-g**) were synthesized according to scheme 1. The target compounds (**4a-g**) were prepared from benzohydrazide (**1**) on condensation with different aromatic aldehydes (**2a-g**) in presence of catalytic amount of concentrated hydrochloric acid in absolute ethanol yield N'-[(E)-(substitutedphenyl)methylidene]benzohydrazide (**3a-g**) in 59.8 - 92.03% 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 Ethylacetate: Hexane (1:2 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 compound (**3a-g**) was 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 1653.66 - 1644.98 cm^{-1} for azomethine (>C=N) formation and 1648.84 - 1450.21 cm^{-1} for C=C ring stretch of phenyl ring, 3084.58 - 3017.09 cm^{-1} for aromatic C-H and 3389.28 - 3175.22 cm^{-1} for N-H, secondary amide. The IR spectra of compound (**3a-g**) displayed bands at about 1648.84 - 1623.77 cm^{-1}, 1588.09 - 1524.45 cm^{-1} and 821.527 - 706.783 cm^{-1} associated with C=O stretch, amide I band, N-H bend, secondary acyclic amide, amide II band 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 696.177 cm^{-1}, 1567.84 - 1520.6 cm^{-1}, 1353.78 - 1344.14 cm^{-1} and 892.88 - 809.956 cm^{-1}, respectively. In the ^{1}H NMR spectra of compound (**3c**), aromatic (5H) protons appeared as a multiplet (5H) at δ 7.468 - 7.516 ppm, CONH proton appeared as a singlet (1H) at δ 9.423 ppm, aromatic (3H) protons appeared as a multiplet (3H) at δ 7.881 - 7.903 ppm and N=CH proton appeared as a singlet (1H) at δ 9.004 ppm, which proved the formation of azomethine.

Compound (**3a-g**), which on cyclization with 2-sulfanylpropanoic acid in dry 1,4-dioxane in presence of anhydrous zinc chloride afford the corresponding N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide

(**4a-g**) in 57.5 - 80.29% 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 (Results and discussion part).

The IR spectrum of compound (**4a-g**) showed strong absorbtion band at 1777.08 - 1690.3 cm^{-1} for C=O of 1,3-thiazolidin-4-one, while the band at 2928.38, 2858.95 - 2851.24 cm^{-1}, 1383.68 - 1346.07 cm^{-1}, 785.85 - 696.177 cm^{-1}, 3074.94 - 3021.91 cm^{-1} and 3386.39 - 3167.51 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 amide. 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 1694.8 - 1640.16 cm^{-1}, 1583.27 - 1525.42 cm^{-1}, 883.238 - 696.177 cm^{-1} and 559.255 cm^{-1} associated with C=O, amide I band, N-H bend, secondary acyclic amide, amide II band, C-Cl and C-Br functions. The IR spectrum of compound (**4a-g**) showed asymmetric $ArNO_2$ stretching bands at 1589.06 - 1525.42 cm^{-1}, symmetric $ArNO_2$ at 1397.17 - 1300.75 cm^{-1}, C-N, $ArNO_2$ at 859.132 - 855.275 cm^{-1}, in addition to stretching band at 1684.52 -1465.63 cm^{-1} attributed to C=C of aromatic ring. In the ^{1}H NMR spectra of compound (**4c**), aromatic (5H) protons appeared as a multiplet (5H) at δ 7.459 - 7.564 ppm, CONH proton appeared as a singlet (1H) at δ 9.681 ppm, C-2 of 1,3-thiazolidin-4-one, N-CH-Ar proton appeared as a singlet (1H) at δ 6.30 ppm, aromatic (3H) protons appeared as a multiplet (3H) at δ 7.884 - 7.958 ppm, $\underline{CH}$-CH_3 protons appeared as a quartet (1H) at δ 3.952 - 4.042 ppm and CH-$\underline{CH_3}$ protons appeared as a doublet (3H) at δ 1.647 - 1.656 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 N'-[(E)-(substitutedphenyl)methylidene]benzohydrazide (3a-g) and N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4a-g)

Compound	Mol. Formula/Mol. Weight	Yield (%)	M.p. (˚C)	[a]Rf
3a	$C_{14}H_{11}ClN_2O$/258.70	89.7 (2.32 g)	192.6 - 193.7	0.81
3b	$C_{14}H_{10}Cl_2N_2O$/293.15	88.6 (2.6 g)	198.3 - 199.9	0.89
3c	$C_{14}H_{10}Cl_2N_2O$/293.15	92.03 (2.7 g)	201.2 - 202.1	0.91
3d	$C_{14}H_{11}BrN_2O$/303.15	82.14 (3.25 g)	209.6 - 211.7	0.85
3e	$C_{14}H_{11}N_3O_3$/269.26	69.82 (1.88 g)	184.5 - 186.2	0.63
3f	$C_{14}H_{11}N_3O_3$/269.26	59.8 (1.61 g)	196.8 - 198.2	0.70
3g	$C_{14}H_{11}N_3O_3$/269.26	76.2 (2.05 g)	235.5 - 236.7	0.65
4a	$C_{17}H_{15}ClN_2O_2S$/346.83	69.4 (2.41 g)	207.3 - 209.4	0.53
4b	$C_{17}H_{14}Cl_2N_2O_2S$/381.28	57.5 (2.19 g)	226.1 - 228.4	0.66
4c	$C_{17}H_{14}Cl_2N_2O_2S$/381.28	64.2 (2.45 g)	241.3 - 243.1	0.84
4d	$C_{17}H_{15}BrN_2O_2S$/391.28	74.32 (2.91 g)	250 - 252.2	0.44
4e	$C_{17}H_{15}N_3O_4S$/357.38	80.29 (2.87 g)	214 - 216.4	0.41
4f	$C_{17}H_{15}N_3O_4S$/357.38	62.71 (2.24 g)	235.3 - 237.4	0.58
4g	$C_{17}H_{15}N_3O_4S$/357.38	67.1 (2.40 g)	262.4 - 264.2	0.83

*[a]Ethylacetate: Hexane (1:2 v/v) for compound (**3a-g**) and Benzene: chloroform (1:1 v/v) for compound (**4a-g**)*

N'-[(E)-(4-chlorophenyl)methylidene]benzohydrazide (3a)

IR (KBr, cm^{-1}): 3051.8 (aromatic C-H), 1632.45, 1588.09, 1485.88 (C=C aromatic ring), 3178.11 (N-H, secondary amide), 1632.45 (C=O, amide I band), 1653.66, 1632.45 (C=N), 1588.09 (N-H bend, secondary acyclic amide, amide II band), 820.563, 697.141 (C-Cl), 1299.79, 1168.65, 1085.73, 1008.59 (In-plane ring C-H bend); ^{1}H NMR ($CDCl_3$, δ ppm): 7.414-7.441 (m, 5H, Ar-H), 7.763-7.790 (m, 4H, Ar-H), 8.604 (s, 1H, N=CH), 9.909 (br s, 1H, CONH). Anal. calcd. for $C_{14}H_{11}ClN_2O$: C, 65.00; H, 4.29; N, 10.83. Found: C, 65.12; H, 4.35; N, 10.86.

N'-[(E)-(2,3-dichlorophenyl)methylidene]benzohydrazide (3b)

IR (KBr, cm^{-1}): 3026.73 (aromatic C-H), 1647.88, 1553.38, 1450.21, 1409.71 (C=C aromatic ring), 3178.11 (N-H, secondary amide), 1647.88 (C=N, C=O, amide I band), 1553.38 (N-H bend, secondary acyclic amide, amide II band), 784.886, 706.783 (C-Cl), 1292.07, 1185.04, 1154.19, 1045.23 (In-plane ring C-H bend); ^{1}H NMR ($CDCl_3$, δ ppm): 7.566-7.591 (m, 5H, Ar-H), 7.899-7.923 (m, 3H, Ar-H), 9.686 (br s, 1H, CONH), 9.082 (s, 1H, N=CH). Anal. calcd. for $C_{14}H_{10}Cl_2N_2O$: C, 57.36; H, 3.44; N, 9.56. Found: C, 57.44; H, 3.53; N, 9.6.

N'-[(E)-(2,4-dichlorophenyl)methylidene]benzohydrazide (3c)

IR (KBr, cm^{-1}): 3070.12 (aromatic C-H), 1644.98, 1583.27, 1551.45, 1467.56 (C=C aromatic ring), 3234.04 (N-H, secondary amide), 1644.98 (C=N, C=O, amide I band), 1551.45 (N-H bend, secondary acyclic amide, amide II band), 821.527, 693.284 (C-Cl), 1281.47, 1140.69, 1101.15, 1051.98 (In-plane ring C-H bend); ^{1}H NMR ($CDCl_3$, δ

ppm): 7.468-7.516 (m, 5H, Ar-H), 7.881-7.903 (m, 3H, Ar-H), 9.423 (br s, 1H, CONH), 9.004 (s, 1H, N=CH). Anal. calcd. for $C_{14}H_{10}Cl_2N_2O$: C, 57.36; H, 3.44; N, 9.56. Found: C, 57.4; H, 3.49; N, 9.58.

N'-[(E)-(4-bromophenyl)methylidene]benzohydrazide (3d)
IR (KBr, cm^{-1}): 3069.16 (aromatic C-H), 1644.98, 1584.24, 1551.45, 1465.23 (C=C aromatic ring), 3235.97 (N-H, secondary amide), 1644.98 (C=N, C=O, amide I band), 1551.45 (N-H bend, secondary acyclic amide, amide II band), 696.177 (C-Br), 1281.47, 1140.69, 1100.19, 1051.98 (In-plane ring C-H bend), 946.877, 862.025, 823.455 (out-of-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 7.465-7.595 (m, 5H, Ar-H), 7.887-8.180 (m, 4H, Ar-H), 9.591 (br s, 1H, CONH), 9.006 (s, 1H, N=CH). Anal. calcd. for $C_{14}H_{11}BrN_2O$: C, 55.47; H, 3.66; N, 9.24. Found: C, 55.54; H, 3.75; N, 9.28.

N'-[(E)-(2-nitrophenyl)methylidene]benzohydrazide (3e)
IR (KBr, cm^{-1}): 3084.58 (aromatic C-H), 1623.77, 1524.45, 1436.71 (C=C aromatic ring), 3389.28 (N-H, secondary amide), 1524.45 (N-H bend, secondary acyclic amide, amide II band), 1623.77 (C=N, C=O, amide I band), 942.056, 809.956, 732.817, 699.069 (out-of-plane ring C-H bend), 1524.45 (asymmetric ($ArNO_2$) $(N=O)_2$), 1350.89 (symmetric ($ArNO_2$) $(N=O)_2$), 809.956 (C-N, $ArNO_2$); ^1H NMR ($CDCl_3$, δ ppm): 7.484-7.702 (m, 9H, Ar-H), 8.737 (s, 1H, N=CH), 9.404 (br s, 1H, CONH). Anal. calcd. for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.51; H, 4.18; N, 15.66.

N'-[(E)-(3-nitrophenyl)methylidene]benzohydrazide (3f)
IR (KBr, cm^{-1}): 3024.8 (aromatic C-H), 1610.27, 1552.42, 1449.28, 1407.78 (C=C aromatic ring), 3175.22 (N-H, secondary amide), 1552.42 (N-H bend, secondary acyclic amide, amide II band), 1646.91 (C=N, C=O, amide I band), 943.985, 892.88, 784.886, 749.209, 706.783 (out-of-plane ring C-H bend), 1552.42 (asymmetric ($ArNO_2$) $(N=O)_2$), 1353.78, 1292.07 (symmetric ($ArNO_2$) $(N=O)_2$), 892.88 (C-N, $ArNO_2$); ^1H NMR ($CDCl_3$, δ ppm): 7.414-7.480 (m, 9H, Ar-H), 9.613 (br s, 1H, CONH), 8.605 (s, 1H, N=CH).

N'-[(E)-(4-nitrophenyl)methylidene]benzohydrazide (3g)
IR (KBr, cm^{-1}): 3017.09 (aromatic C-H), 1648.84, 1567.84, 1520.6 (C=C aromatic ring), 3182.93 (N-H, secondary amide), 1567.84, 1520.6 (N-H bend, secondary acyclic amide, amide II band), 1648.84 (C=N, C=O, amide I band), 951.698, 841.776, 695.212 (out-of-plane ring C-H bend), 1567.84, 1520.6 (asymmetric ($ArNO_2$) $(N=O)_2$), 1344.14, 1299.79 (symmetric ($ArNO_2$) $(N=O)_2$), 841.776 (C-N, $ArNO_2$); ^1H NMR ($CDCl_3$, δ ppm): 7.494-7.628 (m, 5H, Ar-H), 9.275 (br s, 1H, CONH), 8.717 (s, 1H, N=CH), 8.173-8.288 (m, 4H, Ar-H). Anal. calcd. for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.49; H, 4.16; N, 15.64.

N-[2-(4-chlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4a)
IR (KBr, cm^{-1}): 3069.16 (aromatic C-H), 1640.16, 1566.88, 1529.27, 1488.78 (C=C aromatic ring), 3386.39, 3217.65 (N-H, secondary amide), 1640.16 (C=O, amide I band), 2928.38 (methyl C-H, γas CH_3), 2858.95 (methyl C-H, γs CH_3), 1349.93 (C-N, tertiary aromatic amine), 823.455, 700.034 (C-Cl), 1566.88, 1529.77 (N-H bend, secondary acyclic amide, amide II band), 700.034 (C-S), 1349.93, 1287.25, 1143.58, 1089.58, 1012.45 (In-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 7.382-7.508 (m, 9H, Ar-H), 9.586 (br s, 1H, CONH), 6.301 (s, 1H, N-<u>CH</u>-Ar), 3.997-4.065 (q, 1H, <u>CH</u>-CH_3), 1.635-1.641 (d, 3H, CH-<u>CH_3</u>). ESI-MS: *m/z* 348 $[M + 1]^+$. Anal. calcd. for $C_{17}H_{15}ClN_2O_2S$: C, 58.87; H, 4.36; N, 8.08. Found: C, 58.91; H, 4.42; N, 8.10.

N-[2-(2,3-dichlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4b)
IR (KBr, cm^{-1}): 3071.08 (aromatic C-H), 1664.27, 1644.02, 1599.66, 1567.84, 1528.31, 1486.85, 1403.92 (C=C aromatic ring), 3386.39, 3218.61 (N-H, secondary amide), 1644.02 (C=O, amide I band), 1772.26 (C=O, thiazolidin-4-one), 1349.93 (C-N, tertiary aromatic amine), 883.238, 821.527, 732.817, 700.998 (C-Cl), 732.817, 700.998 (C-S), 1349.93, 1285.32, 1142.62, 1089.58, 1009.55 (In-plane ring C-H bend), 1567.84, 1528.31 (N-H bend, secondary acyclic amide, amide II band); ^1H NMR ($CDCl_3$, δ ppm): 7.212-7.262 (m, 5H, Ar-H), 9.647 (br s, 1H, CONH), 6.301 (s, 1H, N-<u>CH</u>-Ar), 3.997-4.065 (q, 1H, <u>CH</u>-CH_3), 1.657-1.665 (d, 3H, CH-<u>CH_3</u>), 7.886-7.962 (m, 3H, Ar-H). ESI-MS: *m/z* 381 $[M]^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2S$: C, 53.55; H, 3.70; N, 7.35. Found: C, 53.62; H, 3.78; N, 7.38.

N-[2-(2,4-dichlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4c)
IR (KBr, cm^{-1}): 3068.19 (aromatic C-H), 1684.52, 1645.95, 1612.2, 1583.27, 1551.45, 1465.63 (C=C aromatic ring), 3232.11 (N-H, secondary amide), 1645.95 (C=O, amide I band), 1777.08, 1710.55 (C=O, thiazolidin-4-one),

1349.93 (C-N, tertiary aromatic amine), 2928.38 (methyl C-H, γas CH_3), 862.025, 822.491, 781.029, 696.177, 621.931 (C-Cl), 696.177, 621.931 (C-S), 1382.71, 1349.93, 1279.54, 1211.08, 1138.76, 1099.23, 1048.12 (In-plane ring C-H bend), 944.949, 862.025, 822.491, 781.029, 696.177, 621.931 (out-of-plane ring C-H bend), 1583.27, 1551.45 (N-H bend, secondary acyclic amide, amide II band), 1465.63 (methyl C-H bend, δas CH_3); ^{1}H NMR ($CDCl_3$, δ ppm): 7.459-7.564 (m, 5H, Ar-H), 9.681 (br s, 1H, CONH), 6.30 (s, 1H, N-CH-Ar), 3.952-4.042 (q, 1H, CH-CH_3), 1.647-1.656 (d, 3H, CH-CH_3), 7.884-7.958 (m, 3H, Ar-H). MS: *m/z* 382 $[M + 1]^+$. Anal. calcd. for $C_{17}H_{14}Cl_2N_2O_2S$: C, 53.55; H, 3.70; N, 7.35. Found: C, 53.59; H, 3.74; N, 7.39.

N-[2-(4-bromophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (4d)
IR (KBr, cm^{-1}): 3074.94 (aromatic C-H), 1646.91, 1583.27, 1466.6 (C=C aromatic ring), 3234.04 (N-H, secondary amide), 1646.91 (C=O, amide I band), 1690.3 (C=O, thiazolidin-4-one), 1383.68 (C-N, tertiary aromatic amine), 2928.38 (methyl C-H, γas CH_3), 559.255 (C-Br), 785.85, 697.141 (C-S), 1383.68, 1279.54, 1209.15, 1139.72, 1100.19, 1051.01 (In-plane ring C-H bend), 947.842, 863.953, 821.527, 785.85, 697.141 (out-of-plane ring C-H bend), 1583.27 (N-H bend, secondary acyclic amide, amide II band), 1466.6 (methyl C-H bend, δas CH_3); ^{1}H NMR ($CDCl_3$, δ ppm): 7.264-7.419 (m, 5H, Ar-H), 9.661 (br s, 1H, CONH), 6.302 (s, 1H, N-CH-Ar), 3.968-4.045 (q, 1H, CH-CH_3), 1.635-1.658 (d, 3H, CH-CH_3), 7.891-7.967 (m, 4H, Ar-H). ESI-MS: *m/z* 392 $[M + 1]^+$. Anal. calcd. for $C_{17}H_{15}BrN_2O_2S$: C, 52.18; H, 3.86; N, 7.16. Found: C, 52.23; H, 3.93; N, 7.14.

N-[5-methyl-2-(2-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzamide (4e)
IR (KBr, cm^{-1}): 3021.91 (aromatic C-H), 1649.8, 1567.84, 1525.42, 1444.42 (C=C aromatic ring), 3167.51 (N-H, secondary amide), 1649.8 (C=O, amide I band), 1715.37 (C=O, thiazolidin-4-one), 1346.07 (C-N, tertiary aromatic amine), 2851.24 (methyl C-H, γs CH_3), 741.496, 699.069 (C-S), 1567.84, 1525.42 (asymmetric ($ArNO_2$) $(N=O)_2$), 1346.07, 1300.75 (symmetric ($ArNO_2$) $(N=O)_2$), 855.275 (C-N, $ArNO_2$), 1444.42 (methyl C-H bend, δas CH_3), 963.269, 855.275, 787.779 (out-of-plane ring C-H bend), 1567.84, 1525.42 (N-H bend, secondary acyclic amide, amide II band); ^{1}H NMR ($CDCl_3$, δ ppm): 7.464-7.595 (m, 5H, Ar-H), 9.800 (br s, 1H, CONH), 6.312 (s, 1H, N-CH-Ar), 3.968-4.044 (q, 1H, CH-CH_3), 1.631-1.638 (d, 3H, CH-CH_3), 7.699-7.820 (m, 4H, Ar-H). ESI-MS: *m/z* 358 $[M + 1]^+$. Anal. calcd. for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.19; H, 4.29; N, 11.79.

N-[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzamide (4f)
IR (KBr, cm^{-1}): 3024.8 (aromatic C-H), 1649.8, 1604.48, 1567.84, 1525.42, 1444.42 (C=C aromatic ring), 3179.08 (N-H, secondary amide), 1694.8 (C=O, amide I band), 1715.37 (C=O, thiazolidin-4-one), 1346.07, 1301.72 (C-N, tertiary aromatic amine), 2855.1 (methyl C-H, γs CH_3), 740.531, 699.069 (C-S), 1567.84, 1525.42 (asymmetric ($ArNO_2$) $(N=O)_2$), 1346.07, 1301.72 (symmetric ($ArNO_2$) $(N=O)_2$), 855.275 (C-N, $ArNO_2$), 964.233, 855.275, 787.779 (out-of-plane ring C-H bend), 1567.84, 1525.42 (N-H bend, secondary acyclic amide, amide II band), 1444.42 (methyl C-H bend, δas CH_3); ^{1}H NMR ($CDCl_3$, δ ppm): 7.266-7.280 (m, 4H, Ar-H), 7.330-7.598 (m, 5H, Ar-H), 9.93 (br s, 1H, CONH), 6.302 (s, 1H, N-CH-Ar), 3.970-4.045 (q, 1H, CH-CH_3), 1.632-1.639 (d, 3H, CH-CH_3). Anal. calcd. for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.21; H, 4.31; N, 11.75.

N-[5-methyl-2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzamide (4g)
IR (KBr, cm^{-1}): 3073.01 (aromatic C-H), 1646.91, 1583.27, 1466.6 (C=C aromatic ring), 3232.11 (N-H, secondary amide), 1646.91 (C=O, amide I band), 1716.34 (C=O, thiazolidin-4-one), 1383.68 (C-N, tertiary aromatic amine), 2928.38 (methyl C-H, γas CH_3), 697.141 (C-S), 1583.27 (asymmetric ($ArNO_2$) $(N=O)_2$), 1383.68, 1279.54 (symmetric ($ArNO_2$) $(N=O)_2$), 863.953 (C-N, $ArNO_2$), 946.877, 863.953, 821.527 (out-of-plane ring C-H bend), 1583.27 (N-H bend, secondary acyclic amide, amide II band), 1466.6 (methyl C-H bend, δas CH_3); ^{1}H NMR ($CDCl_3$, δ ppm): 7.267-7.281 (m, 4H, Ar-H), 7.320-7.597 (m, 5H, Ar-H), 10.022 (br s, 1H, CONH), 6.310 (s, 1H, N-CH-Ar), 3.970-4.044 (q, 1H, CH-CH_3), 1.630-1.638 (d, 3H, CH-CH_3). ESI-MS: *m/z* 358 $[M + 1]^+$. Anal. calcd. for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.17; H, 4.27; N, 11.80.

Antiviral Evaluation
The results of antiviral screening N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (**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) of HSV-1 resistant to acyclovir (ACV^r) (HSV-1 TK^- ACV^r) and varicella-zoster virus (VZV) [thymidine kinase positive (TK^+) VZV (OKA) and thymidine kinase deficient (TK^-) VZV (07/1)] 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 6) and feline corona virus (FIPV) and feline herpes virus in Crandell-Rees Feline kidney (CRFK) cell cultures (Table 7), were determined by using cytopathicity (CPE) reduction assay [56]. 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). Antiviral activity was expressed as the minimum effective concentration (EC_{50}) required reducing virus-induced cytopathogenicity by 50% and cytotoxicity was expressed as the minimum cytotoxic concentration required causing a microscopically detectable alteration of normal cell morphology.

None of the synthesized compounds displayed antiviral activity against any of the viruses except for compound **4a** and compound **4e** (having a ortho/para substituted electron withdrawing group at C-2 position of 1,3-thiazolidin-4-one ring) that showed some antiviral activity against feline corona virus (FIPV) (EC_{50} = 70 ± 15 and 74 ± 31 µM, respectively) (Table 7). None of the tested 1,3-thiazolidin-4-one analogues showed cytotoxicity in CRFK cells.

Table 2: Cytotoxicity and antiviral activity of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides against cytomegalovirus (CMV) in HEL cell cultures (4a-g)

	Antiviral activity EC_{50} (µM)[a]		Cytotoxicity (µM)	
Compound	**AD-169 strain**	**Davis strain**	**Cell morphology (MCC)[b]**	**Cell growth (CC_{50})[c]**
4a	>20	>100	≥100	100
4b	>20	>100	≥100	>100
4c	>20	>100	≥100	67
4d	>20	>100	≥100	58
4e	>20	>100	≥100	>100
4f	>20	>100	≥100	>100
4g	>20	>20	100	>100
Ganciclovir	6.26	3.1	>350	555
Cidofovir	1.27	0.38	≥300	101

[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%.
HEL cells: Human embryonic lung cells.

Table 3: Cytotoxicity and antiviral activity of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides in HEL cell cultures (4a-g)

		EC_{50}[b] (µM)				
Compound	**Minimum cytotoxic concentration[a] (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	>20	>20	>20	>20	>20
4b	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100	>100
4d	100	>20	>20	>20	>20	>20
4e	100	>20	>20	>20	>20	>20
4f	100	>20	>20	>20	>20	>20
4g	100	>20	>20	>20	>20	>20
Brivudin	>250	0.01	10	10	>250	>250
Cidofovir	>250	2	2	25	>250	5
Acyclovir	>250	1	1	>250	>250	>250
Ganciclovir	>100	0.02	0.1	>100	>100	100

[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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides 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	100	>20	>20	>20
4b	>100	>100	>100	>100
4c	>100	>100	>100	>100
4d	>100	>100	>100	>100
4e	>100	>100	>100	>100
4f	>100	>100	>100	>100
4g	≥100	>100	>100	>100
DS-5000	>100	>100	9	2
(µg/ml)	>250	125	>250	>250
(S)-DHPA	>250	10	22	6
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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides 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	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100	>100
4e	100	>20	>20	>20	>20	>20
4f	>100	>100	>100	>100	>100	>100
4g	>100	>100	>100	>100	>100	>100
DS-5000	>100	>100	>100	20	>100	>100
(µg/ml)	>250	>250	>250	>250	>250	112
(S)-DHPA	>250	250	>250	>250	>250	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-influenza virus activity and cytotoxicity of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides 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	>100	100	>100	>100	>100	>100	>100	>100
4b	µM	>100	≥100	>100	>100	>100	>100	>100	>100
4c	µM	>100	>100	>100	>100	>100	>100	>100	>100
4d	µM	>100	≥100	>100	>100	>100	>100	>100	>100
4e	µM	>100	≥100	>100	>100	>100	>100	>100	>100
4f	µM	>100	100	>100	>100	>100	>100	>100	>100
4g	µM	>100	100	>100	>100	>100	>100	>100	>100
Zanamivir	µM	>100	>100	0.36	1.1	0.043	0.012	17	53
Ribavirin	µM	23	≥20	8.4	6.7	7.2	6.8	8.9	10
Amantadine	µM	>500	≥500	34	56	0.18	0.07	>500	>500
Rimantadine	µM	41	100	2.7	2.5	0.044	0.042	>500	>500

[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 7: Anti-Feline Corona virus (FIPV) and anti-Feline Herpes virus activity and cytotoxicity of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides in CRFK cell cultures (4a-g)

Compound	CC_{50}[a](µM)	EC_{50}[a](µM)	
		Feline Corona virus (FIPV)	Feline Herpes virus
4a	>100	70 ± 15	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	74 ± 31	>100
4f	>100	>100	>100
4g	>100	>100	>100
HHA (µg/ml)	>100	8.3 ± 0.3	2.7 ± 2.0
UDA (µg/ml)	11 ± 3	2.1 ± 0.1	0.8 ± 0.7
Ganciclovir	>100	>100	1.5 ± 0.3

[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.*
CRFK cells: Crandell-Rees Feline Kidney cells; HHA: Hippeastrum hybrid agglutinin; UDA: Urtica dioica agglutinin.

Table 8: Cytotoxicity and antiviral activity of N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides 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	>20	>20	100	100
4b	>100	>100	>100	>100
4c	>20	>20	100	67
4d	>100	>100	>100	58
4e	>100	>100	>100	>100
4f	>100	>100	>100	>100
4g	>20	>20	100	>100
Acyclovir	0.8	88.9	>400	696
Brivudin	0.0099	28.3	>300	339

[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 N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides, which were structurally confirmed by elemental, IR, ^{1}H NMR and MS spectral analysis. Further, the antiviral screening of the N-[2-(substitutedphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamides (**4a-g**) against a broad panel of DNA and RNA viral strains indicated that N-[2-(4-chlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]benzamide (**4a**) and N-[5-methyl-2-(2-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzamide (**4e**) showed significant antiviral activity against feline corona virus (FIPV) (EC_{50} = 70 ± 15 and 74 ± 31 µM, respectively) in CRFK cell cultures could be selected as potential lead compounds for the development of novel antiviral agents against feline viruses.

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