SYNTHESIS, CHARACTERIZATION AND ANTIPROLIFERATIVE ACTIVITY OF SOME NOVEL 2-(SUBSTITUTEDPHENYL)-5-METHYL-3-(PHENYLAMINO)-1,3-THIAZOLIDIN-4-ONES

G. Nagalakshmi*, T. K. Maity and B. C. Maiti

Division of Medicinal and Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, West Bengal, India

Summary

1,3-Thiazolidin-4-one is a versatile lead molecule for designing potential bioactive agents. In the present study, phenylhydrazine (**1**) on condensation with different aromatic aldehydes (**2a-g**) in presence of catalytic amount of concentrated hydrochloric acid in absolute ethanol yield (1Z)-1-(substitutedbenzylidene)-2-phenylhydrazine (**3a-g**), which on cyclisation with 2-sulfanylpropanoic acid in dry 1,4-dioxane in presence of anhydrous zinc chloride afford the corresponding 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**). The structure of the newly synthesized compounds (**3a-g**) and (**4a-g**) were confirmed by IR and ^{1}H NMR spectral data. All the newly synthesized 1,3-thiazolidin-4-one analogues (**4a-g**) at various concentrations (10, 20, 50, 100 and 200 mcg/ml) have been evaluated for *in vitro* cytotoxicity against Dalton's ascites lymphoma (DAL) cancer cell line by trypan blue exclusion method. Compound 2-(2,4-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4c**), 2-(2,3-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4b**) and 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) inhibited 70%, 68% and 61% DAL tumor cells at 100 mcg/ml concentration, whereas standard drug doxorubicin exhibit 100% DAL inhibition at a concentration of 100 mcg/ml. From the above study, compounds **4b**, **4c** and **4d** which showed better results (> 50% inhibition) at lowest concentration were selected for their *in vitro* antiproliferative activity against L929 lung fibroblast cell line by using MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. In the antiproliferative assay, among three compounds screened, compound **4d** emerged as more potent inhibitor (11.3% at 5 mcg/ml conc) of L929 with an IC_{50} of 20.7 µg/ml.

Keywords: Phenylhydrazine, 1,3-thiazolidin-4-one, 2-sulfanylpropanoic acid, antitumor activity, antiproliferative activity, DAL cells, MTT assay.

***Author for correspondence**: nagalakshmipharma@gmail.com

Introduction

Cancer is the second leading cause of mortality in developed countries and developing countries as well [1]. Lung cancer is the foremost and leading cause among cancer related deaths reported worldwide and in united states (~ 1,60,000 deaths annually) [1]. Despite advances in cancer research, the overall five year survival of lung cancer patients remains a dismal 15% in contrast to most other solid organ tumors according to the American Association for Cancer Research. Non-small cell lung cancer (NSCLC) constitutes around 80% of all bronchogenic malignancies. Despite continuous research and progress in tumor therapy, chemotherapy as a complete is still a dream in majority of solid tumors. Half of all cancer patients fail to respond to chemotherapy or relapse after an initial response and ultimately succumb to the metastatic disease. The major concern with currently available anticancer drugs is that eventhough they are potent there is an inability to discriminate between normal and tumor cells and hence unpleasant drug toxicities. Another major concern is the development of resistance due to expression of drug transporters. As a result, targeting of proliferative pathways resulting in cell death via apoptosis or prevention of cell division via cell arrest, are considered effective strategies for fighting this disease. Therefore, a more promising approach lies in the synthesis of novel compounds which are effective against cancer cells while at the same time exhibiting minimal toxicity with no or minimal alteration in normal cellular functions.

1,3-thiazolidin-4-one derivatives have been found to exhibit diverse biological activities such as analgesic [2], anti-inflammatory [2,3], antiproliferative [4], antiangiogenic [5], anti-HIV [6], *in vitro* anti-*Toxoplasma gondii* activity [7], antimicrobial [7,8], antimycobacterial [9], antimalarial [10], trypanocidal [11], antischistosomal [12], anticonvulsant [13], antihistaminic [14], anti-cyclooxygenases (COX-1 and COX-2) [15], antidiabetic [16], antiarrhythmic [17] and antihypertensive agents [18].

Hydrazine derivatives are widely used compounds in the pharmaceutical, agrochemical, polymer and dye industries and also as precursors in organic synthesis [19]. Many hydrazine derivatives show significant biological activity and several compounds with hydrazine moiety were shown to be effective for treatment of tuberculosis, parkinson's disease and hypertension [20] and used as antidepressant drugs [21]. Hydrazine based peptidomimetics (azapeptides) were found to be potent agents against hepatitis [22], AIDS [23] and SARS viruses [24]. Some hydrazine derivatives such as phthalazin-1-yl-hydrazine are widely used as general antihypertensive and vasodilator agents and are considered at present as a first-line drug in the management of pregnancy-induced hypertension [25].

Hydrazones and their derivatives constitute an important class of compounds in organic chemistry. These compounds have interesting biological properties, such as antimicrobial [26], anticancer [27], antiviral [28], antimalarial [29], antitubercular [30], antiplatelet [31], antipyretic [32], analgesic [33], anti-inflammatory [34], anticonvulsant [35] and potent inhibitors of macrophage migration inhibitory factor (MIF) proinflammatory activity and survival improving agents in sepsis [36].

To search for more selective and novel 1,3-thiazolidin-4-one analogues with a wide therapeutic window for the cytoselective anticancer activity, we synthesized 2-(4-

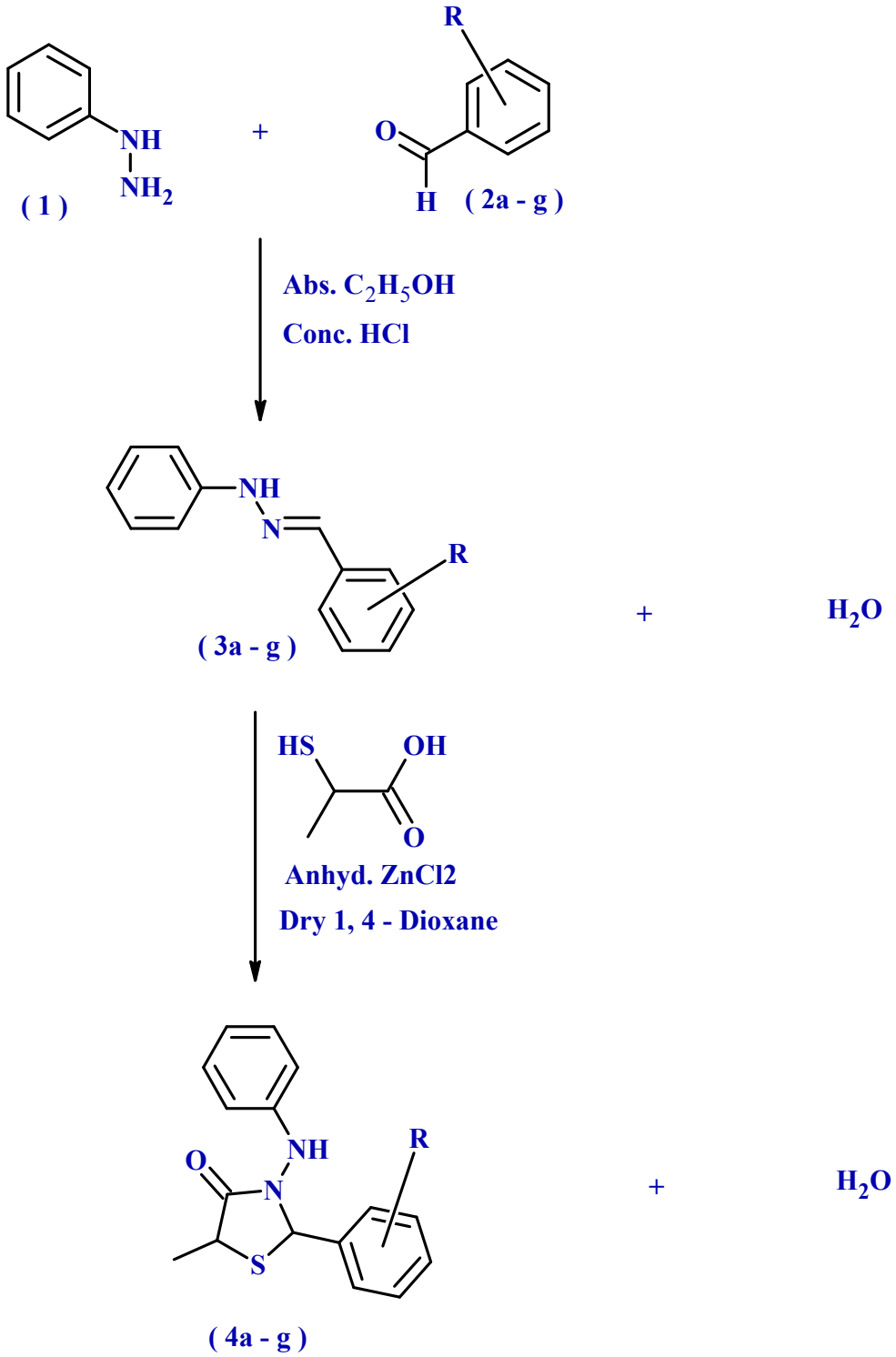
substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones and evaluated them for their *in vitro* antitumor activity against Dalton's ascites lymphoma (DAL) cells by trypan blue exclusion method and antiproliferative activity against L929 lung fibroblast cell line by MTT assay method.

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 available and obtained from Aldrich (Milwaukee, WI) and dry 1,4-dioxane, anhydrous zinc chloride, chloroform, concentrated hydrochloric acid and silica gel-G were purchased from Merck (Mumbai) and were used without further purification. 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) NMR spectrometer using $CDCl_3$ and DMSO-d_6 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).

Scheme 1: Synthetic route for the preparation of novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (4a-g)



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

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 (%)	M.p. (˚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

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), 1352.82, 1301.72, 1256.4, 1133.94, 1087.66, 1005.7 (In-plane ring C-H bend); ^{1}H NMR ($CDCl_3$, δ ppm): 6.906-7.339 (m, 5H, Ar-H), 7.695 (s, 1H, N=CH), 8.437 (s, 1H, NH), 7.862-8.124 (m, 4H, Ar-H).

(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), 1348.96, 1295.93, 1246.75, 1185.04, 1148.4, 1039.44 (In-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 6.881-7.213 (m, 5H, Ar-H), 8.055 (s, 1H, N=CH), 7.884 (br s, 1H, NH), 7.241-7.375 (m, 3H, Ar-H).

(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), 1378.85, 1256.4, 1150.33, 1094.4, 1044.26 (In-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 6.880-7.470 (m, 8H, Ar-H), 7.979 (s, 1H, N=CH), 8.008 (s, 1H, NH).

(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), 1348.96, 1255.43, 1136.83, 1067.41, 1001.84 (In-plane ring C-H bend), 906.379, 818.634, 750.174, 692.32 (out-of-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 6.903-7.620 (m, 7H, Ar-H), 7.970 (s, 1H, N=CH), 8.114 (s, 1H, NH), 8.245-8.275 (m, 2H, Ar-H).

(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), 896.737, 745.352, 691.355 (out-of-plane ring C-H bend), 1335.46, 1254.47, 1161.9, 1129.12 (In-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 6.931-7.306 (m, 5H, Ar-H), 8.308 (s, 1H, N=CH), 8.113 (br s, 1H, NH), 7.365-8.002 (m, 4H, Ar-H).

(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), 1348.0, 1259.29, 1144.55 (In-plane ring C-H bend); ^1H NMR ($CDCl_3$, δ ppm): 6.903-7.152 (m, 4H, Ar-H), 7.679 (s, 1H, N=CH), 8.427 (br s, 1H, NH), 7.254-7.540 (m, 5H, Ar-H).

(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), 1324.86, 1264.11, 1159.01, 1102.12 (In-plane ring C-H bend); ^1H 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).

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), 1384.64, 1279.54, 1210.11, 1137.8, 1099.23, 1049.09 (In-plane ring C-H bend). ^1H NMR ($CDCl_3$, δ ppm): 7.214-7.297 (m, 5H, Ar-H), 8.572 (s, 1H, NH), 6.301 (s, 1H, N-CH-Ar), 7.308-7.415 (m, 4H, Ar-H), 4.013-4.053 (q, 1H, CH-CH_3), 1.618-1.677 (d, 3H, CH-CH_3).

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), 1383.68, 1277.61, 1141.65, 1099.23, 1048.12 (In-plane ring C-H bend). ^1H 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), 1.640-1.669 (d, 3H, CH-CH_3), 7.385-7.457 (m, 3H, Ar-H).

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), 1383.68, 1278.57, 1211.08, 1143.58, 1099.23, 1048.12 (In-plane ring C-H bend). ^1H NMR ($CDCl_3$, δ ppm): 6.981-7.150 (m, 5H, Ar-H), 8.568 (s, 1H, NH), 6.302 (s, 1H, N-CH-Ar), 3.998-4.067 (q, 1H, CH-CH_3), 1.618-1.665 (d, 3H, CH-CH_3), 8.149-8.207 (m, 3H, Ar-H).

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), 1383.68, 1278.57, 1209.15, 1137.8, 1099.23, 1049.09 (In-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), 1.614-1.683 (d, 3H, CH-CH_3).

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), 1324.86, 1272.79, 1145.51, 1101.15, 1049.09 (In-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), 4.009-4.051 (q, 1H, CH-CH_3), 1.605-1.677 (d, 3H, CH-CH_3).

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), 1323.89, 1276.65, 1212.04, 1144.55, 1100.19, 1049.09 (In-plane ring C-H bend). ^{1}H NMR ($CDCl_3$, δ ppm): 7.219-7.260 (m, 5H, Ar-H), 8.448 (s, 1H, NH), 6.301 (s, 1H, N-CH-Ar), 4.011-4.052 (q, 1H, CH-CH_3), 1.640-1.678 (d, 3H, CH-CH_3), 7.389-7.432 (m, 4H, Ar-H).

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), 948.806, 862.025, 823.455, 782.958, 695.212 (out-of-plane ring C-H bend), 1324.86, 1275.68, 1144.55, 1101.15, 1050.05 (In-plane ring C-H bend). ^{1}H NMR ($CDCl_3$, δ ppm): 7.219-7.261 (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), 1.641-1.672 (d, 3H, CH-CH_3), 7.389-7.433 (m, 4H, Ar-H).

***In vitro* Evaluation of Antitumor Activity:**

The newly synthesized 1,3-thiazolidin-4-one analogues (**4a-g**) were studied for short term *in vitro* cytotoxicity using Dalton's ascites lymphoma (DAL) cells. The DAL cells were maintained in Swiss albino mice by intraperitoneal transplantation of 1×10^6 cells/animal. The tumor (DAL) cells were aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with normal saline (0.9% NaCl w/v) and checked for viability using trypan blue dye exclusion method [37].

The DAL suspension was added to tubes containing 5 different concentrations of the test compounds and the volume was made up to 1ml. Control tube contained only cell suspension. Doxorubicin hydrochloride was used as standard. These assay mixtures were incubated for 3 h at 37˚ C. After incubation, 0.4% trypan blue was added to each tube and mixed gently. The no of dead cells and the percentage of these cells were determined by using a hemocytometer. The antitumor screening results have been presented in Table 2.

Measurement of potential cytotoxicity by MTT assay:

The cytotoxicity was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [37-40], which is based on the reduction of yellow tetrazolium salt by mitochondrial dehydrogenase of metabolically active viable cells to a blue-purple formazan that can be measured spectrophotometrically. Hence, the intensity of the colour in the solution is directly proportional to cell viability. In order to evaluate the effects of the newly synthesized 1,3-thiazolidin-4-one analogues (**4a-g**) on cell proliferation, L929 lung fibroblast cells were seeded into 96-well flat bottom titre plates containing 200 µl minimum essential medium (MEM) with 10% fetal calf serum (FCS) and incubated for 24 h at 37˚ C for the attachment of cells. All test compounds were dissolved in dimethyl sulfoxide (DMSO), prior to dilution. After incubation, various concentrations of the test compound (0.2, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 µg/ml) were added to the wells in triplicates and the incubation was continued for 48 h. Control groups included treatment with 0.1% DMSO.

20 µl of MTT was added to each well before 4 h of the completion of incubation. After the incubation period, the plates were centrifuged, the supernatant was removed and 100 µl of DMSO was added to each well, to dissolve the formazan crystal produced by the MTT. The plate was then incubated at room temperature for 15 min and the absorbance at optical density (OD) was measured in an enzyme linked immunosorbent assay (ELISA) reader (Auto reader 4011, Awareness Technologies Inc., USA) at 570 nm with reference of 690 nm. Results were evaluated by comparing the absorbance of the wells containing compound treated cells with the absorbance of wells containing 0.1% DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100% in the solvent control. The results were expressed as IC_{50}, concentration causing 50% growth inhibition. IC_{50} values for each compound were calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give percentage of growth (PG) values and the results are given in Table 3. The relationship between cell viability and drug concentrations was plotted to obtain the survival curve of lung tumor cell line of the specified compound are illustrated in Figure 2-4.

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). The structures of the synthesized compounds (3a-g) were confirmed on the basis of melting point, IR and ^{1}H NMR spectral data (experimental part).

The 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 (5H) protons appeared as a multiplet (5H) at δ 6.931 - 7.306 ppm, NH proton appeared as a broad singlet (1H) at δ 8.113 ppm, aromatic (4H) protons appeared as a multiplet (4H) at δ 7.365 - 8.002 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 IR and ^{1}H NMR spectral data (experimental part).

The 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 (5H) protons appeared as a multiplet (5H) at 6.981-7.15 ppm, N-H proton appeared asa 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, aromatic (3H) proton appeared as a multiplet (3H) at 8.149-8.207 ppm, $CH\text{-}CH_3$ protons appeared as a quartet (1H) at 3.998-4.067 ppm and $CH\text{-}CH_3$ protons appeared as a doublet (3H) at 1.618-1.665 ppm, which proved the closure of 1,3-thiazolidin-4-one ring.

Antitumor evaluation:

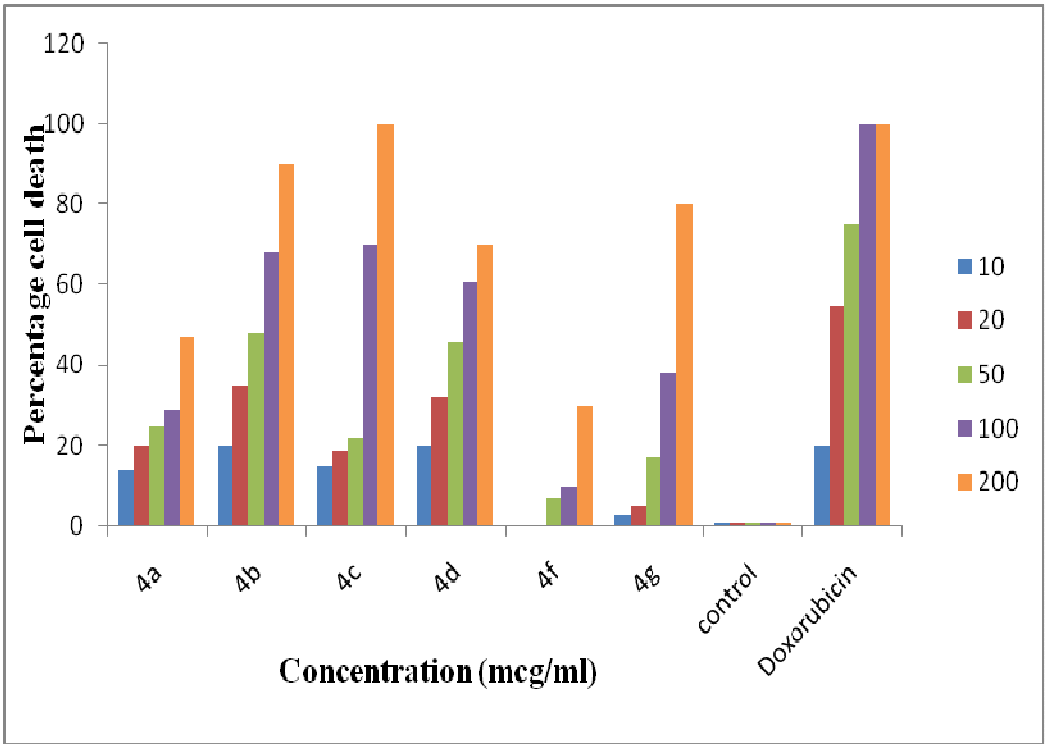
Chemotherapy is the primary therapeutic modality of treatment for the both localized and metastatic cancers. The newly synthesized 1,3-thiazolidin-4-one analogues (**4a-g**) at different concentration (10, 20, 50, 100 and 200 mcg/ml) were evaluated for *in vitro* cytotoxicity against DAL cancer cells by trypan blue exclusion method. The *in vitro* screening results are summarized in Table 2 and Figure 1. Screening results of *in vitro* antitumor activity (Table 2 and Figure 1) reveal that compound 2-(2,4-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4c**), 2-(2,3-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4b**), and 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) inhibited 70%, 68% and 61% DAL tumor cells at 100 mcg/ml concentration, showing marked antitumor activity, which is comparable to the standard drug doxorubicin which exhibit 100% DAL inhibition at a concentration of 100 mcg/ml. At 100 mcg/ml concentration, compound **4a** and **4g** inhibited 29% and 38% DAL tumor cells, exhibiting moderate antitumor activity, whereas compound **4f** inhibited 10% DAL tumor cells displaying a mild antitumor activity. 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (**4a-g**) exhibited dose-dependent significant increase in cytotoxicity against DAL cells. From the above study, compounds **4b**, **4c** and **4d** which showed better results (> 50% inhibition) at standard drug concentration (100 mcg/ml) were selected for their *in vitro* antiproliferative activity against L929 lung fibroblast cell line by using MTT assay method.

Table 2: *In vitro* cytotoxicity of some novel 2-(substitutedphenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-ones (4a-g) against Dalton's ascites lymphoma (DAL) cells

Compound	Percentage cell death, concentration in µg/ml				
	10	**20**	**50**	**100**	**200**
4a	14	20	25	29	47
4b	20	35	48	**68**	**90**
4c	15	19	22	**70**	**100**
4d	20	32	46	**61**	**70**
4f	0	0	07	10	30
4g	03	05	17	38	80
Doxorubicin	20	55	75	**100**	**100**

Control tube contains only 1 dead cell. Compound **4e** - sparingly soluble in DMSO.

Figure 1: Antitumor activity of synthesized 1,3-thiazolidin-4-one analogues (4a-g) against Dalton's ascites lymphoma cells



Evaluation of antiproliferative activity:

Induction of cell death or inhibition of cell proliferation is an important property for chemotherapeutic agents. The antiproliferative effect of compound **4b**, **4c** and **4d** was evaluated by measuring the level of cell proliferation after incubation of the cells with the test samples using MTT colorimetric assay, which evaluates the capacity of the mitochondrial enzyme succinate dehydrogenase of viable cells to reduce MTT to formazan crystals. The results, expressed as percentage of cell proliferation compared with cells control (cells treated with vehicle, DMSO 0.1%) are depicted in Figure 2-4.

The selected novel 1,3-thiazolidin-4-one analogues (**4b**, **4c** and **4d**) at different concentrations (0.1, 0.5, 1.0, 2.5 and 5.0 mcg/ml) were tested for their *in vitro* antiproliferative activity against L929 lung fibroblast cell line using MTT assay. The resulting IC_{50} values are summarized in Table 3. The relationship between percent viability and the compound concentration is illustrated in Figure 2-4.

Table 3: IC_{50} values of compound 4b, 4c and 4d against L929 lung fibroblast cell line by MTT assay

Compound	[a]IC_{50} (µg/ml)
	L929
4b	22.98
4c	33.8
4d	20.7

[a]IC_{50} - concentration that causes 50% growth inhibition. [a]IC_{50} value was obtained from a dose-response curve, mean of triplicate wells.

Figure 2: Effect of compound 4b on the viability of L929 lung fibroblast cells

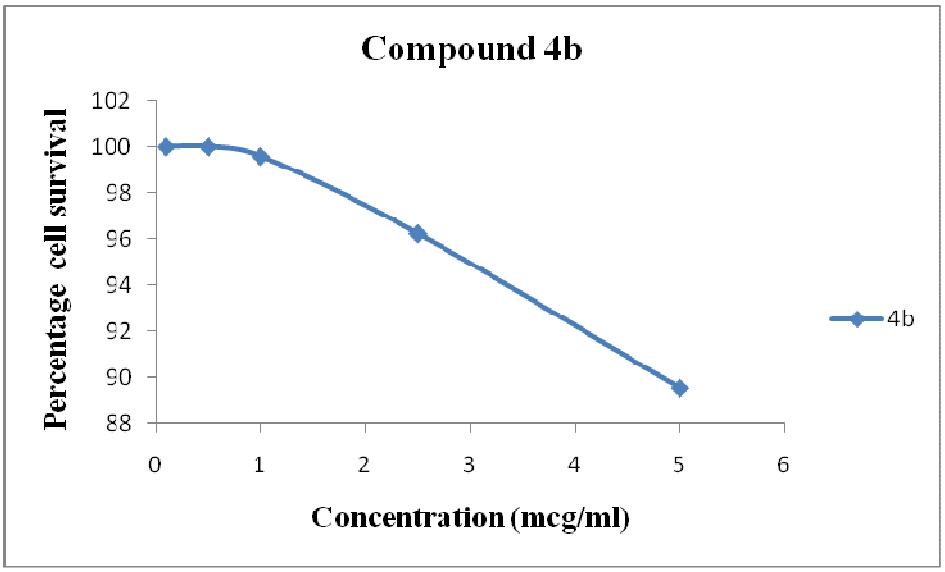


Figure 3: Effect of compound 4c on the viability of L929 lung fibroblast cells

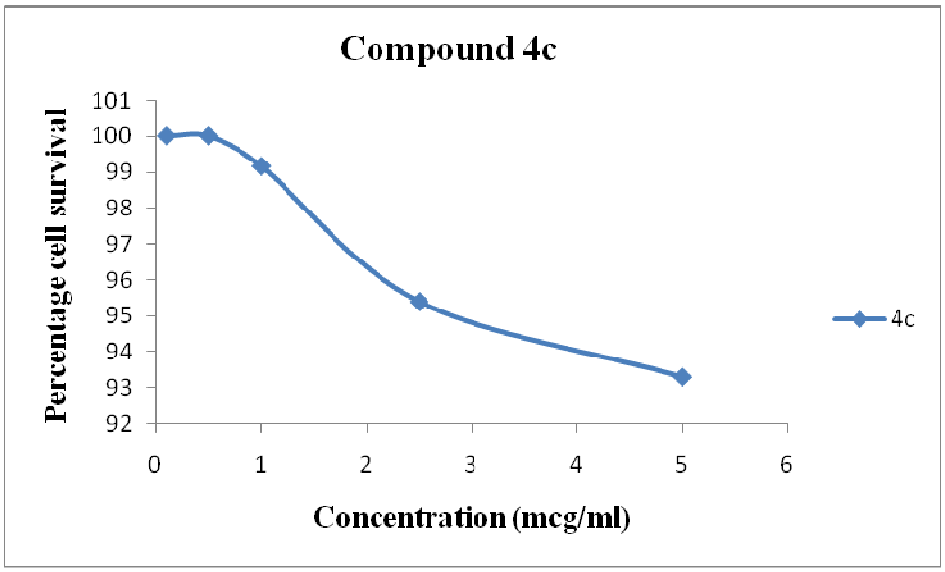
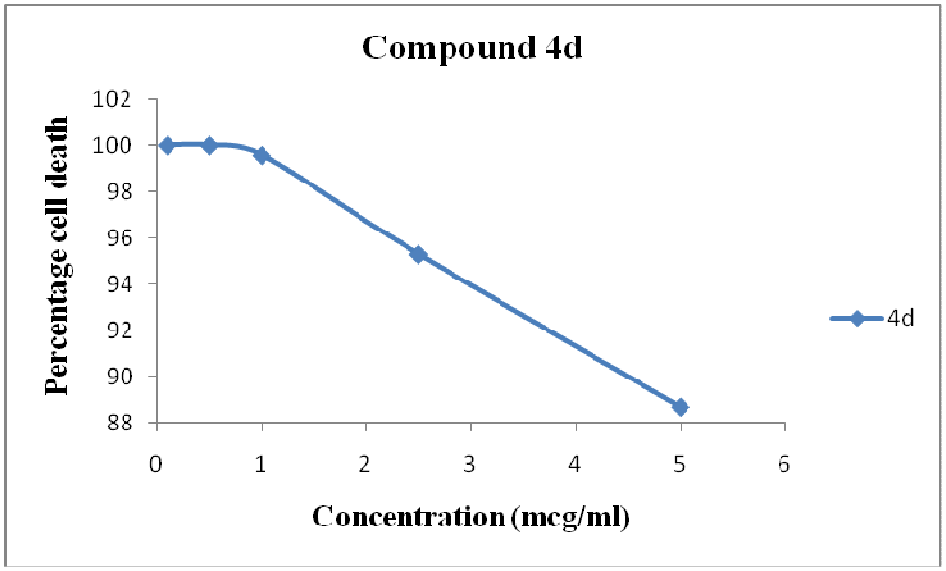


Figure 4: Effect of compound 4d on the viability of L929 lung fibroblast cells



Among three compounds screened, compound 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) showed potent inhibitory activity (11.3% at 5 mcg/ml concentration) with an IC_{50} value of 20.7 mcg/ml. Compound 2-(2,3-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4b**) showed moderate inhibitory activity (10.46% at 5 mcg/ml concentration) with an IC_{50} value of 22.98 mcg/ml and compound 2-(2,4-dichlorophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4c**) showed mild inhibitory (6.69% at 5 mcg/ml concentration) activity against L929 lung fibroblast cell line with an IC_{50} value of 33.8 mcg/ml.

Furthermore, results illustrated that compound (**4d**) exhibited highest antiproliferative activity against L929 cells *in vitro*, and can therefore be candidates for further stages of screening *in vivo*.

From the antitumor and antiproliferative activity data reported in Table 2 and Table 3, it may be inferred that antitumor activity is strongly dependent on the nature of the substituent at C-2 and N-3 of the 1,3-thiazolidin-4-one ring. In a particular, a high activity level was observed for compound (**4d**) possessing a 4-bromophenyl group substituted at C-2 and phenylamino group at N-3 position of the 1,3-thiazolidin-4-one nucleus.

Conclusion

In this study, compound 2-(4-bromophenyl)-5-methyl-3-(phenylamino)-1,3-thiazolidin-4-one (**4d**) exhibited significant antitumor and antiproliferative activity against DAL and L929 cells *in vitro*. This compound could be considered as useful templates or leads for the future development and further structural variation to obtain more potent, selective and less toxic antitumor agents.

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