# Synthesis, Characterization and Anticancer Evaluation of Some Novel 2-(Substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones

**Dr. G. Nagalakshmi**
Associate Professor, Department of Pharmaceutical Chemistry, The Oxford College of Pharmacy, Bengaluru, Karnataka, India

**Abstract:** A series of novel 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones (**4a-g**) were synthesized and structurally confirmed by elemental analysis, IR, $^1$H NMR and MS spectral data. All the 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 lymphoma ascites (DLA) cancer cell line by trypan blue exclusion method, in comparison with standard drug doxorubicin hydrochloride. Out of these seven compounds, two compounds 2-(2,4-dichlorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4b**) and 2-(4-bromophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4g**) inhibited 100% and 78% DLA tumor cells at 100 mcg/ml concentration, whereas standard drug doxorubicin exhibit 100% DLA inhibition at a concentration of 100 mcg/ml. From the above study, compound **4b** and compound **4g** which showed better results (> 60% inhibition) at lowest concentration were further selected for screening *in vivo* anticancer activity against Dalton's lymphoma ascites (DLA) cancer cell line at the dose of 50 mg/kg body weight/i.p. in comparison with 5-fluorouracil (20 mg/kg body weight/i.p.) by determining different parameters like body weight analysis, packed cell volume, viable tumor cell count, increase in life span (%), followed by hematological profiles [red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb) and platelet count] and serum biochemical parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC) and triglycerides (TG)] of DLA bearing mice. In the *in vivo* anticancer evaluation, among two compounds screened, compound **4b** emerged as more potent inhibitor of DLA with an increase in life span (ILS) of 88.11%, whereas standard drug 5-fluorouracil exhibit ILS of 92.20%. The *in vivo* anticancer experimental results indicated that, compound **4b** and 5-fluorouracil showed significant ($p < 0.01$) decrease in body weight gain, packed cell volume, viable tumor cell count and increased the life span of DLA tumor bearing mice, followed by hematological and serum biochemical profiles were significantly restored to normal levels in compound **4b** and 5-Fluorouracil ($p < 0.01$) treated groups as compared to DLA control mice.

**Keywords:** Pyridin-4-amine, 2-sulfanylpropanoic acid, 1,3-thiazolidin-4-one, anticancer activity, DLA cells, trypan blue exclusion method.

## Introduction

Cancer is believed to result from unlimited growth of a given cell, due to inability of cells to undergo differentiation and/ or apoptosis (Zhou et al., 2008). Two major concerns with currently available anticancer drugs are their inability to discriminate between normal and tumor cells and hence unpleasant drug toxicities and development of resistance due to expression of drug transporters. Hence, targeting of proliferative pathways resulting in cell death via apoptosis or prevention of cell division via cell cycle arrest, are considered effective strategies for fighting this disease. Hence the discovery and development of new therapeutic agents without side effects is the need of the hour. Therefore, a more reasonable approach would be to synthesize novel compounds which are effective against cancer while at the same time exhibiting minimal toxicity to normal cellular functions.

1,3-thiazolidin-4-one derivatives have been found to exhibit diverse biological activities such as analgesic (Vigorita et al., 2001), anti-inflammatory (Geronikaki et al., 2008), antiangiogenic (Chandrappa et al., 2010), anti-HIV (Balzarini et al., 2009), in vitro anti-Toxoplasma gondii (de Aquino et al., 2008), antimicrobial (de Aquino et al., 2008), antimycobacterial (Babaoglu et al., 2003), antimalarial (Singh et al., 2004), trypanocidal (Smith et al., 2009), antischistosomal (Ottana et al., 2007), anticonvulsant (Ulusoy et al., 1996)], antihistaminic (Diurno et al., 1999), antidiabetic (Shingalapur et al., 2010), antiarrhythmic (Jackson et al., 2007) and antihypertensive properties (Bhandari et al., 2009).

To search for more specific and novel 1,3-thiazolidin-4-one analogues with a wide therapeutic window for the cytoselective anticancer activity, we synthesized some novel 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones and evaluated them for their in vitro and in vivo antitumor activity against Dalton's lymphoma ascites (DLA) cells by trypan blue exclusion method.

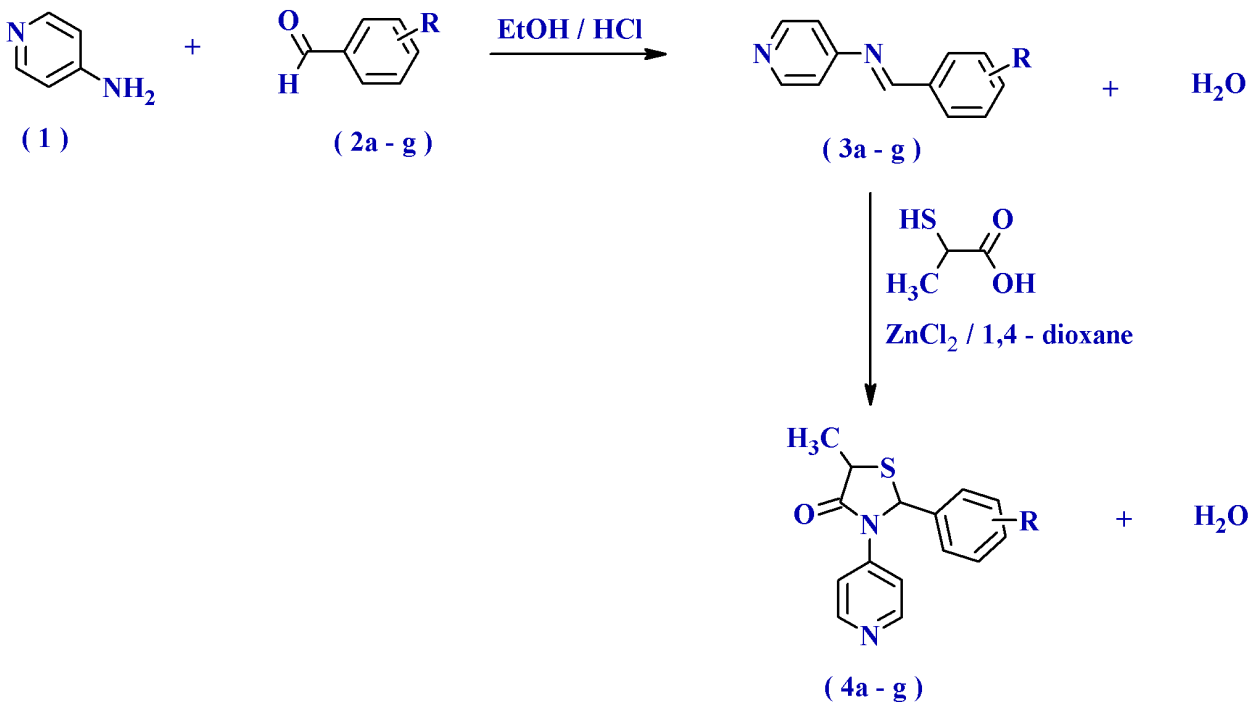
## Materials and Methods

### Experimental

Pyridin-4-amine, 4-chlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 2-fluorobenzaldehyde, 4-fluorobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde and 4-bromobenzaldehyde and 2-sulfanylpropanoic acid, were commercially obtained from Aldrich (Milwaukee, WI) and dry 1,4-dioxane, anhydrous zinc chloride, dimethylformamide, chloroform, concentrated hydrochloric acid 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 Chloroform: Methanol: Formic acid (10:2:0.2 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 $cm^{-1}$. $^1H$ 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). Mass spectra (MS) were recorded on a Q-TOF micromass spectrometer by using electronspray ionization (ESI) technique. 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-(pyridin-4-yl)-1,3-thiazolidin-4-ones (4a-g)**




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

| | |
|---|---|
| **4g** | 4-Br |

**Synthesis of N-[(Z)-(substitutedphenyl)methylidene]pyridin-4-amine (3a-g):**
A mixture of pyridin-4-amine (**1**) (0.01 mol) and different aromatic aldehydes (**2a-g**) (0.01 mol) (4-chlorobenzaldehyde (**2a**), 2,4-dichlorobenzaldehyde (**2b**), 2-fluorobenzaldehyde (**2c**), 4-fluorobenzaldehyde (**2d**), 3-nitrobenzaldehyde (**2e**), 4-nitrobenzaldehyde (**2f**) and 4-bromobenzaldehyde (**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 Chloroform: Methanol: Formic acid (10:2:0.2 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 schiff's bases (**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-(pyridin-4-yl)-1,3-thiazolidin-4-one (4a-g):**
A mixture of N-[(Z)-(substitutedphenyl)methylidene]pyridin-4-amine (**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 10-12 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 DMF. 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 N-[(Z)-(substitutedphenyl)methylidene]pyridin-4-amine (3a-g) and 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones (4a-g)**

| Compound | Mol. Formula/ Mol. weight | Yield (%) | M.p. (°C) | [a]Rf |
|---|---|---|---|---|
| **3a** | $C_{12}H_9ClN_2$/216.67 | 72.7 (1.58 g) | 81.8 - 83.8 | 0.50 |
| **3b** | $C_{12}H_8Cl_2N_2$/251.11 | 84.1 (2.11 g) | 79.2 - 80.9 | 0.56 |
| **3c** | $C_{12}H_9FN_2$/200.21 | 62.5 (1.25 g) | 73.9 - 75.9 | 0.52 |
| **3d** | $C_{12}H_9FN_2$/200.21 | 69.4 (1.39 g) | 82.2 - 83.9 | 0.49 |
| **3e** | $C_{12}H_9N_3O_2$/227.22 | 75.7 (1.72 g) | 67.4 - 68.9 | 0.44 |
| **3f** | $C_{12}H_9N_3O_2$/227.22 | 86.4 (1.96 g) | 96.2 - 97.5 | 0.63 |
| **3g** | $C_{12}H_9BrN_2$/261.12 | 81.2 (2.12 g) | 94.2 - 95.2 | 0.65 |
| **4a** | $C_{15}H_{13}ClN_2OS$/304.79 | 64.5 (0.49 g) | 122.1 - 124.2 | 0.84 |
| **4b** | $C_{15}H_{12}Cl_2N_2OS$/339.24 | 70.2 (0.60 g) | 132 - 134 | 0.82 |
| **4c** | $C_{15}H_{13}FN_2OS$/288.34 | 54.1 (0.39 g) | 114 - 116 | 0.79 |
| **4d** | $C_{15}H_{13}FN_2OS$/288.34 | 60.3 (0.44 g) | 127.3 - 129.1 | 0.75 |
| **4e** | $C_{15}H_{13}N_3O_3S$/315.35 | 63.6 (0.50 g) | 109.2 - 111.4 | 0.63 |
| **4f** | $C_{15}H_{13}N_3O_3S$/315.35 | 74.5 (0.59 g) | 146 - 148 | 0.85 |
| **4g** | $C_{15}H_{13}BrN_2OS$/349.25 | 71.0 (0.62 g) | 138 - 140 | 0.89 |

[a]Chloroform: Methanol: Formic acid (10:2:0.2 v/v) for compound (**3a-g**) and Benzene: Chloroform (1:1 v/v) for compound (**4a-g**)

**N-[(Z)-(4-chlorophenyl)methylidene]pyridin-4-amine (3a):** IR (KBr, $cm^{-1}$): 3141.47 (aromatic C-H), 1602.56 (C=N), 815.742, 711.604 (C-Cl), 1602.56, 1521.56 (C=N, C=C ring stretch), 1315.21, 1213.01, 1078.98 (In-plane ring C-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 7.286-7.593 (m, 8H, Ar-H, PyH), 8.079 (s, 1H, N=CH-Ar). Anal. calcd. for $C_{12}H_9ClN_2$: C, 66.52; H, 4.19; N, 12.93. Found: C, 66.56; H, 4.25; N, 12.91.

**N-[(Z)-(2,4-dichlorophenyl)methylidene]pyridin-4-amine (3b)**: IR (KBr, $cm^{-1}$): 3147.26, 3081.69, 3023.84 (aromatic C-H), 1686.44 (C=N), 854.311, 820.563, 754.031 (C-Cl), 1686.44, 1583.27, 1462.74 (C=N, C=C ring stretch), 1375.96, 1247.72, 1195.65, 1131.05, 1096.33, 1051.01 (In-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 7.270-7.633 (m, 7H, Ar-H, PyH), 8.263 (s, 1H, N=CH-Ar). Anal. calcd. for $C_{12}H_8Cl_2N_2$: C, 57.40; H, 3.21; N, 11.16. Found: C, 57.42; H, 3.25; N, 11.18.

**N-[(Z)-(2-fluorophenyl)methylidene]pyridin-4-amine (3c)**: IR (KBr, $cm^{-1}$): 3144.37, 3027.69 (aromatic C-H), 1603.52 (C=N), 1313.29, 1220.72, 1153.22, 1059.69 (C-F), 1603.52, 1522.52 (C=N, C=C ring stretch), 900.594, 809.956, 755.959 (out-of-plane ring C-H bend);

$^1$H NMR (DMSO-$d_6$, δ ppm): 6.707 (br s, 2H, Ar-H), 7.200-7.415 (m, 4H, Ar-H, PyH), 7.590-7.634 (m, 1H, PyH), 8.097 (br s, 2H, N=CH-Ar, PyH). Anal. calcd. for $C_{12}H_9FN_2$: C, 71.99; H, 4.53; N, 13.99. Found: C, 72.04; H, 4.62; N, 14.02.

**N-[(Z)-(4-fluorophenyl)methylidene]pyridin-4-amine** (**3d**): IR (KBr, $cm^{-1}$): 3144.37, 3027.69 (aromatic C-H), 1603.52 (C=N), 1313.29, 1220.72, 1154.19, 1059.69 (C-F), 1603.52, 1522.52 (C=N, C=C ring stretch), 899.63, 809.956, 755.959 (out-of-plane ring C-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.704-6.723 (d, 2H, Ar-H), 7.192-7.453 (m, 4H, Ar-H, PyH), 7.587-7.633 (m, 1H, PyH), 8.090-8.108 (d, 2H, N=CH-Ar, PyH). Anal. calcd. for $C_{12}H_9FN_2$: C, 71.99; H, 4.53; N, 13.99. Found: C, 72.02; H, 4.58; N, 13.96.

**N-[(Z)-(3-nitrophenyl)methylidene]pyridin-4-amine** (**3e**): IR (KBr, $cm^{-1}$): 3068.02 (aromatic C-H), 1615.90 (C=N), 1582.56, 1534.23 (asymmetric ($ArNO_2$) $(N=O)_2$), 1352.37, 1275.33 (symmetric ($ArNO_2$) $(N=O)_2$), 811.24 (C-N, $ArNO_2$), 1615.90, 1582.56, 1534.23, 1471.71, 1446.15, 1399.03 (C=N, C=C ring stretch), 933.21, 917.82, 811.24, 729.42, 677.58 (out-of-plane ring C-H bend), 1202.58, 1101.74, 1076.51, 1008.18 (In-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 6.831-6.862 (m, 2H, Ar-H), 7.260-7.544 (m, 5H, Ar-H, PyH), 8.280 (s, 2H, N=CH-Ar, PyH). Anal. calcd. for $C_{12}H_9N_3O_2$: C, 63.43; H, 3.99; N, 18.49. Found: C, 63.51; H, 4.08; N, 18.52.

**N-[(Z)-(4-nitrophenyl)methylidene]pyridin-4-amine** (**3f**): IR (KBr, $cm^{-1}$): 1518.67 (asymmetric ($ArNO_2$) $(N=O)_2$), 1372.1, 1341.25, 1273.75 (symmetric ($ArNO_2$) $(N=O)_2$), 860.096 (C-N, $ArNO_2$), 2981.41 (aromatic C-H), 1597.73 (C=N), 1597.73, 1518.67, 1460.81, 1405.85 (C=N, C=C ring stretch), 913.129, 860.096, 771.387, 693.284 (out-of-plane ring C-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 8.078-8.240 (m, 7H, Ar-H, PyH), 8.390 (s, 1H, PyH), 8.416 (s, 1H, N=CH-Ar).

**N-[(Z)-(4-bromophenyl)methylidene]pyridin-4-amine** (**3g**): IR (KBr, $cm^{-1}$): 3136.65 (aromatic C-H), 1601.59 (C=N), 529.364 (C-Br), 1601.59, 1520.6 (C=N, C=C ring stretch), 897.701, 812.849 (out-of-plane ring C-H bend), 1314.25, 1212.04, 1071.26 (In-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 6.442-6.589 (m, 3H, Ar-H), 7.360-7.387 (m, 2H, Ar-H, PyH), 7.535-7.590 (m, 2H, PyH), 8.262 (s, 2H, N=CH-Ar, PyH). Anal. calcd. for $C_{12}H_9BrN_2$: C, 55.20; H, 3.47; N, 10.73. Found: C, 55.24; H, 3.52; N, 10.76.

**2-(4-chlorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4a**): IR (KBr, $cm^{-1}$): 2921.63 (methyl C-H, γas $CH_3$), 2853.17 (methyl C-H, γs $CH_3$), 1701.87 (C=O, thiazolidin-4-one), 1402 (C-N, tertiary aromatic amine), 2921.63 (aromatic C-H), 1627.63, 1488.78, 1402 (C=N, C=C ring stretch), 1264.11, 1091.51, 1015.34 (In-plane ring C-H bend), 828.277 (C-Cl);

$^1$H NMR ($CDCl_3$, δ ppm): 7.021-7.422 (m, 8H, Ar-H, PyH), 3.577 (s, 1H, N-CH-Ar), 3.970-4.045 (q, 1H, CH-$CH_3$), 1.628-1.731 (d, 3H, CH-$CH_3$); ESI-MS: m/z 306 $[M + 1]^+$. Anal. calcd. for $C_{15}H_{13}ClN_2OS$: C, 59.11; H, 4.30; N, 9.19. Found: C, 59.16; H, 4.36; N, 9.17.

**2-(2,4-dichlorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4b**): IR (KBr, $cm^{-1}$): 1702.84 (C=O, thiazolidin-4-one), 1401.03 (C-N, tertiary aromatic amine), 2921.63 (methyl C-H, γas $CH_3$), 2853.17 (methyl C-H, γs $CH_3$), 2921.63 (aromatic C-H), 1637.27, 1488.78, 1401.03 (C=N, C=C ring stretch), 1266.04, 1091.51, 1010.52 (In-plane ring C-H bend), 827.312 (C-Cl); $^1$H NMR ($CDCl_3$, δ ppm): 7.020-7.422 (m, 7H, Ar-H, PyH), 3.577 (s, 1H, N-CH-Ar), 3.968-4.044 (q, 1H, CH-$CH_3$), 1.597-1.724 (d, 3H, CH-$CH_3$); Anal. calcd. for $C_{15}H_{12}Cl_2N_2OS$: C, 53.11; H, 3.57; N, 8.26. Found: C, 53.17; H, 3.61; N, 8.28.

**2-(2-fluorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4c**): IR (KBr, $cm^{-1}$): 2921.63 (methyl C-H, γas $CH_3$), 2853.17 (methyl C-H, γs $CH_3$), 1702.84 (C=O, thiazolidin-4-one), 1401.03 (C-N, tertiary aromatic amine), 2921.63 (aromatic C-H), 1613.16, 1489.74, 1401.03 (C=N, C=C ring stretch), 1401.03, 1267, 1090.55, 1015.34 (C-F), 827.312 (out-of-plane ring C-H bend); $^1$H NMR ($CDCl_3$, δ ppm): 6.975-7.423 (m, 8H, Ar-H, PyH), 3.577 (s, 1H, N-CH-Ar), 3.970-4.045 (q, 1H, CH-$CH_3$), 1.254-1.730 (d, 3H, CH-$CH_3$); Anal. calcd. for $C_{15}H_{13}FN_2OS$: C, 62.48; H, 4.54; N, 9.72. Found: C, 62.56; H, 4.63; N, 9.75.

**2-(4-fluorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4d**): IR (KBr, $cm^{-1}$): 2979.56 (methyl C-H, γas $CH_3$), 1714.35 (C=O, thiazolidin-4-one), 1366.58, 1342.65 (C-N, tertiary aromatic amine), 2979.56 (aromatic C-H), 1644, 1601.71, 1525.42, 1461.32, 1410.52 (C=N, C=C ring stretch), 1461.32, 1410.52, 1366.58, 1342.65, 1273.58, 1172.36, 1101.08, 1012.31 (C-F), 771.99, 720.25, 690.30 (C-S); $^1$H NMR ($CDCl_3$, δ ppm): 7.018-7.412 (m, 8H, Ar-H, PyH), 2.968 (s, 1H, N-CH-Ar), 3.491-3.722 (q, 1H, CH-$CH_3$), 1.254-1.412 (d, 3H, CH-$CH_3$).

**5-methyl-2-(3-nitrophenyl)-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4e**): IR (KBr, $cm^{-1}$): 2980.45 (methyl C-H, γas $CH_3$), 2920.66 (methyl C-H, γs $CH_3$), 1715.37 (C=O, thiazolidin-4-one), 1405.85 (C-N, tertiary aromatic amine), 2980.45 (aromatic C-H), 1599.66, 1460.81, 1405.85 (C=N, C=C ring stretch), 773.315, 694.248 (C-S), 1599.66 (asymmetric ($ArNO_2$) $(N=O)_2$), 1273.75 (symmetric ($ArNO_2$) $(N=O)_2$), 862.025 (C-N, $ArNO_2$); $^1$H NMR ($CDCl_3$, δ ppm): 8.183-8.403 (m, 8H, Ar-H, PyH), 3.922 (m, 2H, N-CH-Ar, CH-$CH_3$), 1.402-1.462 (d, 3H, CH-$CH_3$); Anal. calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 57.21; H, 4.24; N, 13.36.

**5-methyl-2-(4-nitrophenyl)-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4f**): IR (KBr, $cm^{-1}$): 2982.37 (methyl C-H, γas $CH_3$), 1714.41 (C=O, thiazolidin-4-one), 1404.89 (C-N, tertiary

aromatic amine), 2924.52 (methyl C-H, γs $CH_3$), 2982.37 (aromatic C-H), 1600.63, 1519.63, 1461.78, 1404.89 (C=N, C=C ring stretch), 1273.75, 1168.65, 1105.01, 1017.27 (In-plane ring C-H bend), 772.351, 693.284 (C-S), 1519.63 (asymmetric ($ArNO_2$) $(N=O)_2$), 1273.75 (symmetric ($ArNO_2$) $(N=O)_2$), 860.096 (C-N, $ArNO_2$); $^1H$ NMR ($CDCl_3$, δ ppm): 8.149-8.403 (m, 8H, Ar-H, PyH), 4.378-4.474 (m, 2H, N-CH-Ar, CH-$CH_3$), 1.401-1.462 (d, 3H, CH-$CH_3$); Anal. calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 57.19; H, 4.20; N, 13.34.

**2-(4-bromophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one** (**4g**): IR (KBr, $cm^{-1}$): 3140.68 (aromatic C-H), 2974.25 (methyl C-H, γas $CH_3$), 1702.06 (C=O, thiazolidin-4-one), 1335.63, 1316.96 (C-N, tertiary aromatic amine), 1601.68, 1526.06, 1485.96, 1429.94 (C=N, C=C ring stretch), 665.41, 631.11, 614.47, 585.28, 526.77 (C-Br), 716.30, 684.14, 665.41, 631.11, 614.47 (C-S); $^1H$ NMR ($CDCl_3$, δ ppm): 8.181-8.402 (m, 8H, Ar-H, PyH), 4.378-4.473 (m, 2H, N-CH-Ar, CH-$CH_3$), 1.401-1.461 (d, 3H, CH-$CH_3$); ESI-MS: m/z 350 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{13}BrN_2OS$: C, 51.59; H, 3.75; N, 8.02. Found: C, 51.63; H, 3.81; N, 8.05.

**In vitro Evaluation of Antitumor Activity**

**Cell lines**

Dalton's lymphoma ascites (DLA) cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation (0.2 ml of 1 × $10^6$ cells/ml). DLA cells (9 days old) were aspirated from the peritoneal cavity in mice, washed with saline and given intraperitoneally to develop ascites tumor.

All the synthesized 1,3-thiazolidin-4-one analogues (**4a-g**) were studied for short term in vitro cytotoxicity using Dalton's lymphoma ascites (DLA) cells. The DLA cells were maintained in Swiss albino mice by intraperitoneal transplantation of 1×$10^6$ cells/mice. The tumor (DLA) 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 (Richardson et al., 1995).

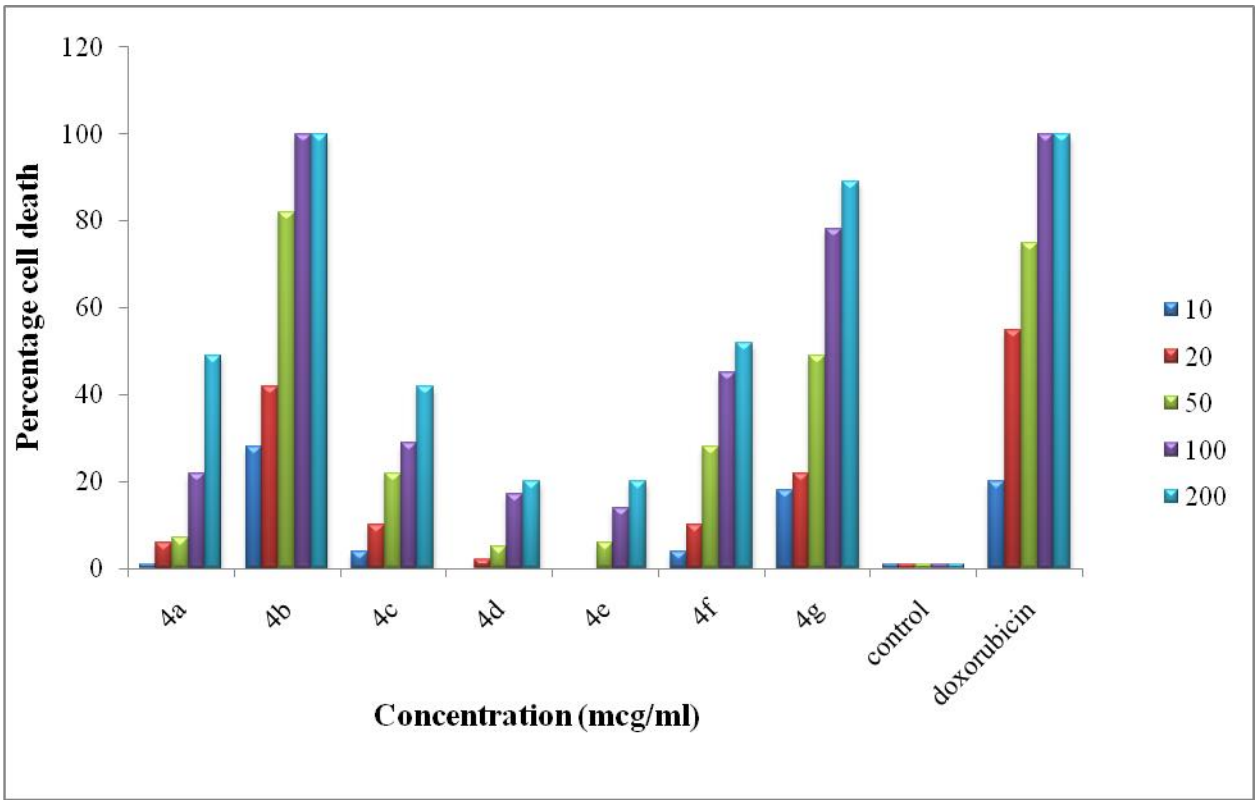
The DLA suspension (1×$10^6$ cells in 0.1 ml) was added to tubes containing 5 different concentrations (10, 20, 50, 100 and 200 mcg/ml) of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. Doxorubicin hydrochloride was used as standard. These assay mixtures were incubated for 3 h at 37° C and percentage of dead cells were evaluated by Trypan blue exclusion method. The antitumor screening results were presented in Table 2 and Figure 1.

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

| Compound | Percentage cell death, concentration in µg/ml | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 50 | 100 | 200 |
| 4a | 01 | 06 | 07 | 22 | 49 |
| 4b | 28 | 42 | 82 | **100** | **100** |
| 4c | 04 | 10 | 22 | 29 | 42 |
| 4d | 0 | 02 | 05 | 17 | 20 |
| 4e | 0 | 0 | 06 | 14 | 20 |
| 4f | 04 | 10 | 28 | 45 | 52 |
| 4g | 18 | 22 | 49 | **78** | **89** |
| Doxorubicin | 20 | 55 | 75 | 100 | 100 |

Control tube contains only 1 dead cell.

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




**Acute toxicity study of the synthesized compounds**

**Animals**

Swiss albino mice of 8-10 weeks old (20 ± 5 g body weight) of either sex was acclimatized to the laboratory conditions for 2 weeks before performing the experiments. The animals were housed in sterile polypropylene cages and maintained under controlled room temperature (23 ± 2° C) and relative humidity (55 ± 5%) with 12:12 h light and dark cycle. All the animals were provided with commercially available standard mice food pellets (Hindustan Lever Ltd., Bangalore, India) and water ad libitum. The guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA, Reg. No. 367) were followed and the study was approved by the University Animal Ethics Committee of Jadavpur University, Kolkata, India.

**Acute toxicity study**

The $LD_{50}$ value of synthesized 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) in Swiss albino mice was determined (Litchfield et al., 1949) and it was found to be 500 mg/kg body weight/i.p. The biological evaluation was carried out at $1/10^{th}$ of maximum tolerated dose, i.e., 50 mg/kg body weight/i.p.

**In-vivo Pharmacological Screening**

Based upon the in-vitro cytotoxicity assay results in-vivo pharmacological screening of few selected compounds (compound **4b** and compound **4g**) were further selected for screening in vivo anticancer activity against Dalton's lymphoma ascites (DLA) cancer cell line at the dose of 50 mg/kg body weight/i.p. in comparison with 5-fluorouracil (20 mg/kg body weight/i.p.) by determining different parameters like body weight analysis, packed cell volume, viable tumor cell count, increase in life span (%), followed by hematological profiles [red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb) and platelet count] and serum biochemical parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC) and triglycerides (TG)] of DLA bearing mice (Tables 3-5 and Figures 2-4).

**Anticancer activity**

**Animals**

Swiss albino mice of 8-10 weeks old (20 ± 5 g body weight) of either sex were acclimatized to the laboratory conditions for 2 weeks before performing the experiments. The animals were housed in sterile polypropylene cages and maintained under controlled room temperature (23 ± 2° C) and relative humidity (55 ± 5%) with 12:12 h light and dark cycle. All the animals were provided with commercially available standard mice food pellets (Hindustan Lever Ltd., Bangalore, India) and water ad libitum. The guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals

(CPCSEA, Reg. No. 367) were followed and the study was approved by the University Animal Ethics Committee of Jadavpur University, Kolkata, India.

**Preparation of test solution of compounds**

Synthesized 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) were weighed and dissolved in 0.1% v/v DMSO to obtain the required concentrations and administered intraperitoneally on day 1 to day 10 of tumor inoculation in the volume of 0.1 ml/10 g mice. All the compounds were tested at the dose of 50 mg/kg body weight/i.p. The dose of 5-Fluorouracil (5-FU) selected was 20 mg/kg body weight/i.p (Sreelatha et al., 2011).

**Transplantation of tumor and treatment schedule**

Antitumor activities of synthesized 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) were determined by using Dalton's lymphoma ascites (DLA) tumor model in mice. Swiss albino mice were divided into five groups (n = 12). The Dalton's lymphoma ascites (DLA)-bearing mice (donor) were used for the study, 15 days after tumor transplantation (Sathisha et al., 2010). Tumor viability was determined by trypan blue exclusion test and cells were counted using haemocytometer. Cell viability was always found to be 95% or more. The ascitic fluid was suitably diluted in normal saline to get a concentration of $10^6$ cells/ml of tumor cell suspension (Sathisha et al., 2010).

All the animals were injected with DLA cells (0.2 ml of $1\times10^6$ cells/mouse) intraperitoneally except the normal group, for the development of ascites tumor (Dongre et al., 2008). The mice were weighed on the day of tumor inoculation and then once in two days thereafter. In this instance, tumor cells multiplied relatively freely within the peritoneal cavity. Ascites were developed in the cavity. A day of incubation was allowed to establish the disease in the body before starting the administration of the drug. Group I served as normal and group II served as the tumor (DLA) control. These two groups received 0.2 ml of 0.1% DMSO (Chandrappa et al., 2010). Group III served as a positive control and was treated with 5-fluorouracil (20 mg/kg body weight/i.p.) (Sreelatha et al., 2011). Group IV and Group V were treated with synthesized 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) at 50 mg/kg body weight/i.p., respectively. All these treatments were given 24 h after the tumor inoculation, once daily for 10 days (Jose et al., 2001). After the last dose and 24 h fasting, six mice from each group were sacrificed for the study of antitumor, hematological and biochemical parameters. The rest of the animals were kept to check the average life span and change in the body weight.

**Tumor growth response**

The anticancer effect of 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) was assessed by the determination of body weight gain (g), packed cell volume (%), viable cell count and increase in life span (%).

**Determination of packed cell volume**

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by using graduated centrifuge tube, and packed cell volume was determined by centrifuging at 1000 rpm for 5 min. From the packed cell volume (PCV), the percentage of tumor inhibition was calculated (Christina et al., 2003).

**Estimation of viable and non-viable tumor cell count**

The ascitic fluid was taken in a white blood cell (WBC) pipette and diluted 100 times. Then a drop of the diluted suspension was placed on the Neubauer counting chamber and the cells were then stained with trypan blue (0.4% w/v) dye. The cells that did not take up the dye were viable (non stained) and those took the stain were non-viable. Those viable and non-viable cells were counted.

$$\text{Cell count} = \frac{(\text{number of cells} \times \text{dilution factor})}{(\text{area} \times \text{thickness of liquid film})}$$

**Determination of mean survival time and percentage increase in life span**

The effect of compounds (compound **4b** and compound **4g**) on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (% ILS) was calculated. Median survival time (MST) for each group was noted and anticancer activity of the test compounds was compared with that of control group by measuring increase in life span (Gupta et al., 2000). Total number of days an animal survived from the day of tumor inoculation was counted; subsequently the mean survival time was calculated. The percentage increase in life span (Dacie et al., 1975) was calculated by using the formula:

Mean survival time* = [(day of first death + day of last death)/2]

*Time denoted by number of days.

Increase in life span (%) = [(MST of treated group/ MST of control group)-1] × 100

Increase in life span of 25% or more over that of control was considered an effective antitumor response (Sharada et al., 1996).

**Body weight**

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 2nd day during the treatment period. An average percentage increase in body weight as compared to day zero was determined.

**Hematological parameters**

At the end of the experimental period, the next day after an overnight fasting blood was collected from freely flowing tail vein and used for the estimation of hemoglobin (Hb) content (Dacie et al., 1975), red blood cell (RBC) count (Dacie et al., 1975, D' Armour et al., 1965), white blood cell (WBC) count (Swarup et al., 1981, Wintrobe et al., 1961) and platelet count by standard procedures.

**Serum biochemical parameters**

The blood for serum biochemistry was allowed to clot at room temperature and was centrifuged at 3000 rpm for 10 min for serum separation (Bromberg et al., 2010). The serum thus obtained were used for the estimation of serum biochemical parameters included aspartate aminotransferase (AST) (Reitman et al., 1957), alanine aminotransferase (ALT) (Reitman et al., 1957), alkaline phosphatase (ALP) (Kind et al., 1954, Wright et al., 1972), total cholesterol (TC) (Wybenga et al., 1970) and triglycerides (TG) (Mendez et al., 1975) by standard colorimetric assays.

**Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)**

Aminotransferases (AST and ALT) were determined according to the method of Reitman and Frankel (1957) (Reitman et al., 1957).

**Serum alkaline phosphatase (ALP)**

Serum alkaline phosphatase activity was assayed by the method of Kind and King (1954) [26] as described by Wright et al. (1972) (Kind et al., 1954, Wright et al., 1972).

**Total Cholesterol and Triglycerides**

Total Cholesterol and Triglycerides in serum were estimated according to the method of Wybenga et al. (1970) (Wybenga et al., 1970) and Mendez et al. (1975) (Mendez et al., 1975) , respectively.

**Statistical analysis**

All values were expressed as mean ± standard error of the mean (SEM). Results were analyzed statistically by using one way-analysis of variance (ANOVA) followed by Newman-Keuls multiple range test. Values of $P < 0.01$ were considered significant.

**Table 3: Anticancer activity of 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones in Dalton's lymphoma ascites (DLA) bearing mice**

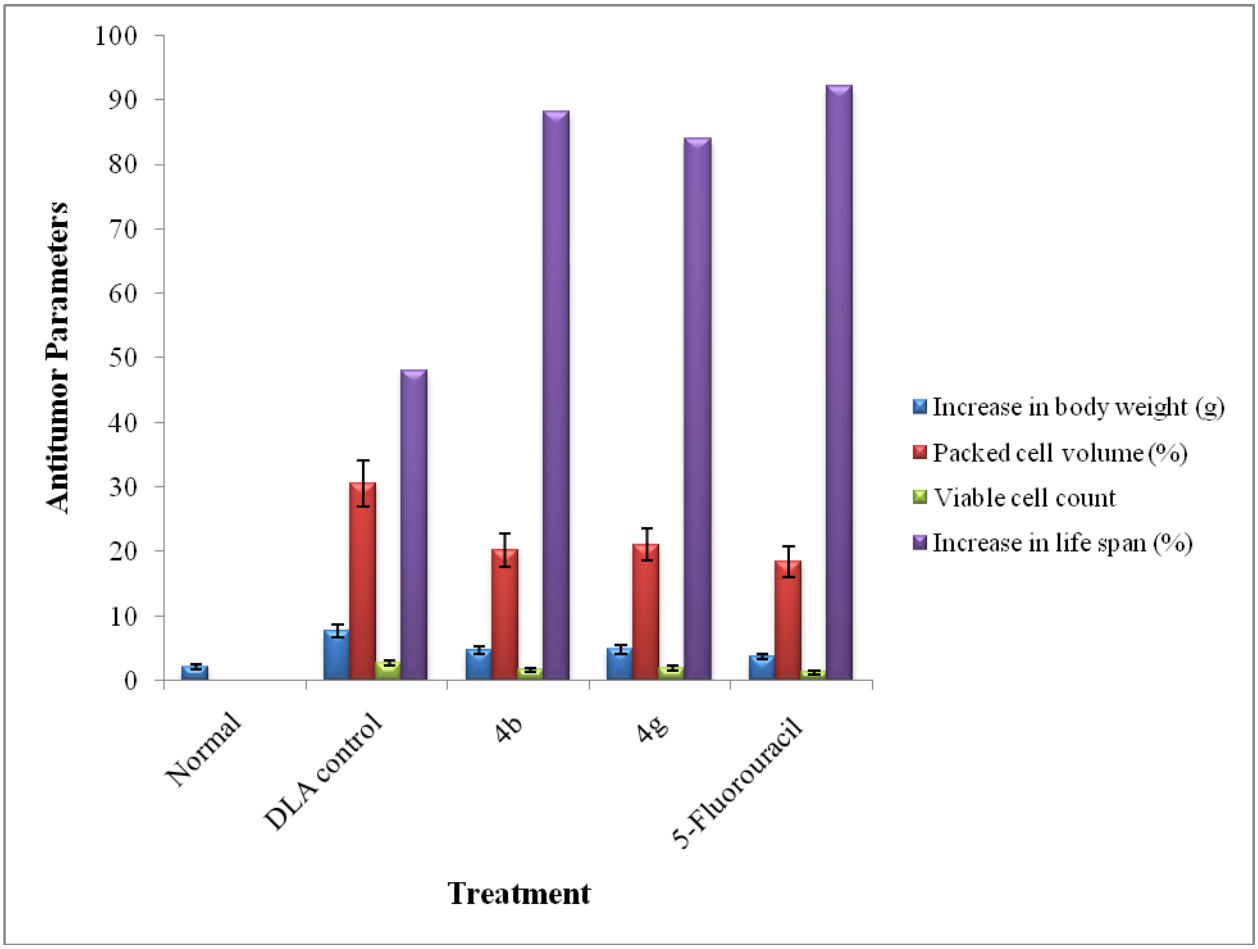
| Groups | Increase in body weight (g) | Packed cell volume (%) | Viable cell count ($\times 10^6$ cells/ml) | Increase in life span (%) |
|---|---|---|---|---|
| Normal (0.1% DMSO) | 2.12 ± 0.44 | - | - | - |
| DLA control ($1\times10^6$ cells/ml per mice) | 7.64 ± 0.95[a] | 30.55 ± 3.55[a] | 2.72 ± 0.33[a] | 48 |
| **4b** (50 mg/kg) + DLA | 4.68 ± 0.60[b] | 20.18 ± 2.62[b] | 1.66 ± 0.30[b] | 88.11 |
| **4g** (50 mg/kg) + DLA | 4.80 ± 0.65[b] | 21.07 ± 2.43[b] | 1.85 ± 0.40[b] | 84.04 |
| 5-Fluorouracil (20 mg/kg) + DLA | 3.73 ± 0.42[b] | 18.40 ± 2.33[b] | 1.25 ± 0.24[b] | 92.20 |

Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using one-way ANOVA followed by Newman-Keuls multiple range test.

[a]P<0.001: between normal and DLA control group.

[b]P<0.01: between compound treated groups and DLA control.

**Figure 2: Effect of Compounds (50 mg/kg) and 5-Fluorouracil (20 mg/kg) on Antitumor Parameters in Dalton's Lymphoma Ascites Bearing Mice**




Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using one-way ANOVA followed by Newman-Keuls multiple range test. [a]P<0.001: between normal and DLA control group. [b]P<0.01: between compound treated groups and DLA control.

**Table 4: Effect of 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones on hematological parameters in Dalton's lymphoma ascites (DLA) bearing mice**

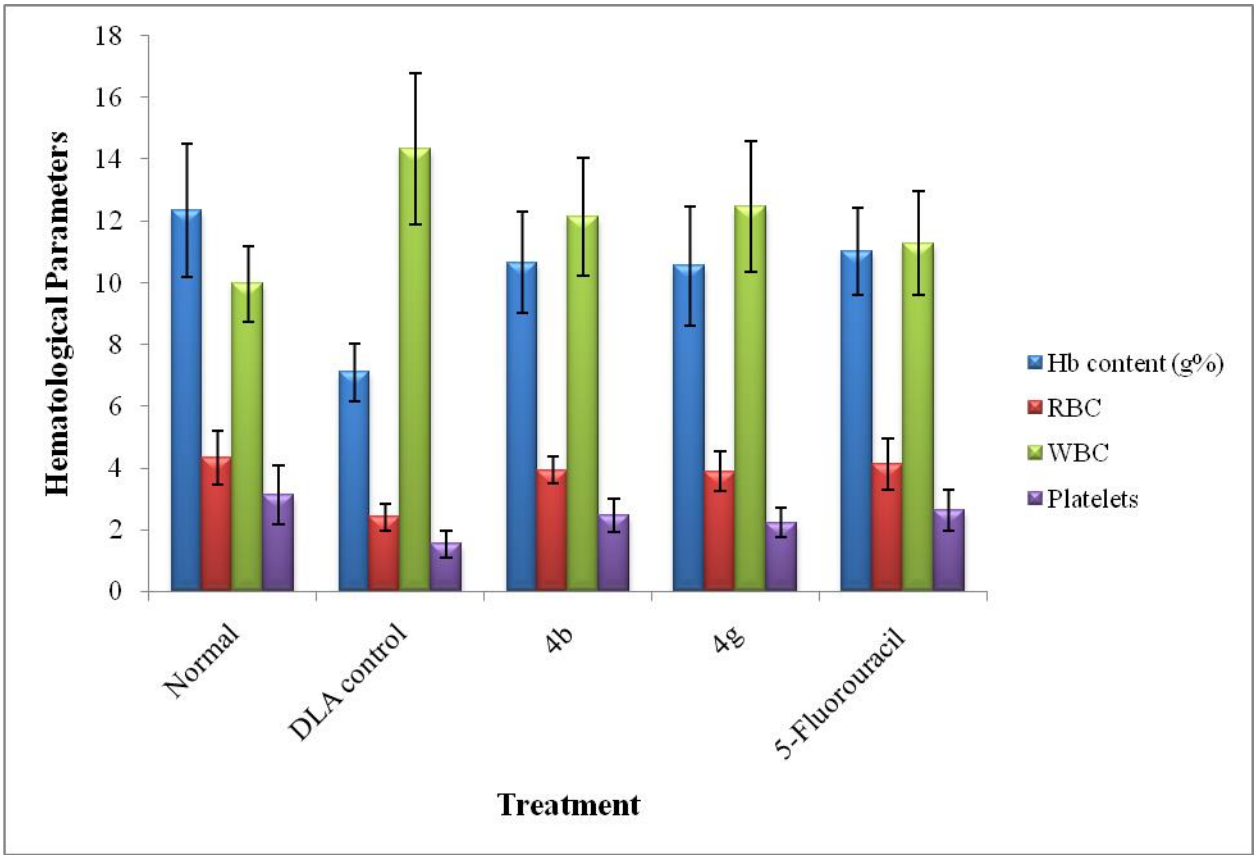
| Groups | Hb content (g%) | RBC ($10^6$ cells/$mm^3$) | WBC ($10^3$ cells/ml) | Platelets ($10^5$ cells/$mm^3$) |
|---|---|---|---|---|
| Normal (0.1% DMSO) | 12.35 ± 2.16 | 4.33 ± 0.87 | 9.96 ± 1.22 | 3.12 ± 0.94 |
| DLA control ($1\times10^6$ cells/ml per mice) | 7.09 ± 0.93[a] | 2.40 ± 0.43[a] | 14.32 ± 2.45[a] | 1.54 ± 0.44[a] |
| **4b** (50 mg/kg) + DLA | 10.65 ± 1.65[b] | 3.93 ± 0.44[b] | 12.12 ± 1.90[b] | 2.46 ± 0.52[b] |
| **4g** (50 mg/kg) + DLA | 10.54 ± 1.94[b] | 3.88 ± 0.64[b] | 12.46 ± 2.10[b] | 2.22 ± 0.48[b] |
| 5-Fluorouracil (20 mg/kg) + DLA | 11.0 ± 1.42[b] | 4.12 ± 0.85[b] | 11.26 ± 1.68[b] | 2.63 ± 0.68[b] |

Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using one-way ANOVA followed by Newman-Keuls multiple range test.

[a]P<0.001: between normal and DLA control group.

[b]P<0.01: between compound treated groups and DLA control.

**Figure 3: Effect of Compounds (50 mg/kg) and 5-Fluorouracil (20 mg/kg) on Hematological Parameters in Dalton's Lymphoma Ascites Bearing**




**Mice**

Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using one-way ANOVA followed by Newman-Keuls multiple range test. [a]P<0.001: between normal and DLA control group. [b]P<0.01: between compound treated groups and DLA control.

**Table 5: Effect of 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones on serum biochemical parameters in Dalton's lymphoma ascites (DLA) bearing mice**

| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | Total cholesterol (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|---|
| Normal (0.1% DMSO) | 36.45 ± 1.17 | 32.27 ± 1.24 | 125.09 ± 2.18 | 99.08 ± 3.50 | 120.81 ± 2.34 |
| DLA control ($1\times10^6$ cells/ml per mice) | 85.0 ± 2.69[a] | 60.18 ± 2.55[a] | 240.18 ± 4.26[a] | 140.86 ± 4.54[a] | 206.14 ± 4.63[a] |
| **4b** (50 mg/kg) + DLA | 62.67 ± 2.19[b] | 43.52 ± 1.49[b] | 180.28 ± 3.31[b] | 118.39 ± 3.53[b] | 160.50 ± 2.48[b] |
| **4g** (50 mg/kg) + DLA | 64.39 ± 1.90[b] | 45.25 ± 1.92[b] | 185.29 ± 2.96[b] | 120.29 ± 2.42[b] | 165.83 ± 2.27[b] |
| 5-Fluorouracil (20 mg/kg) + DLA | 55.22 ± 1.56[b] | 40.40 ± 1.52[b] | 160.26 ± 2.23[b] | 110.44 ± 3.90[b] | 154.40 ± 2.62[b] |

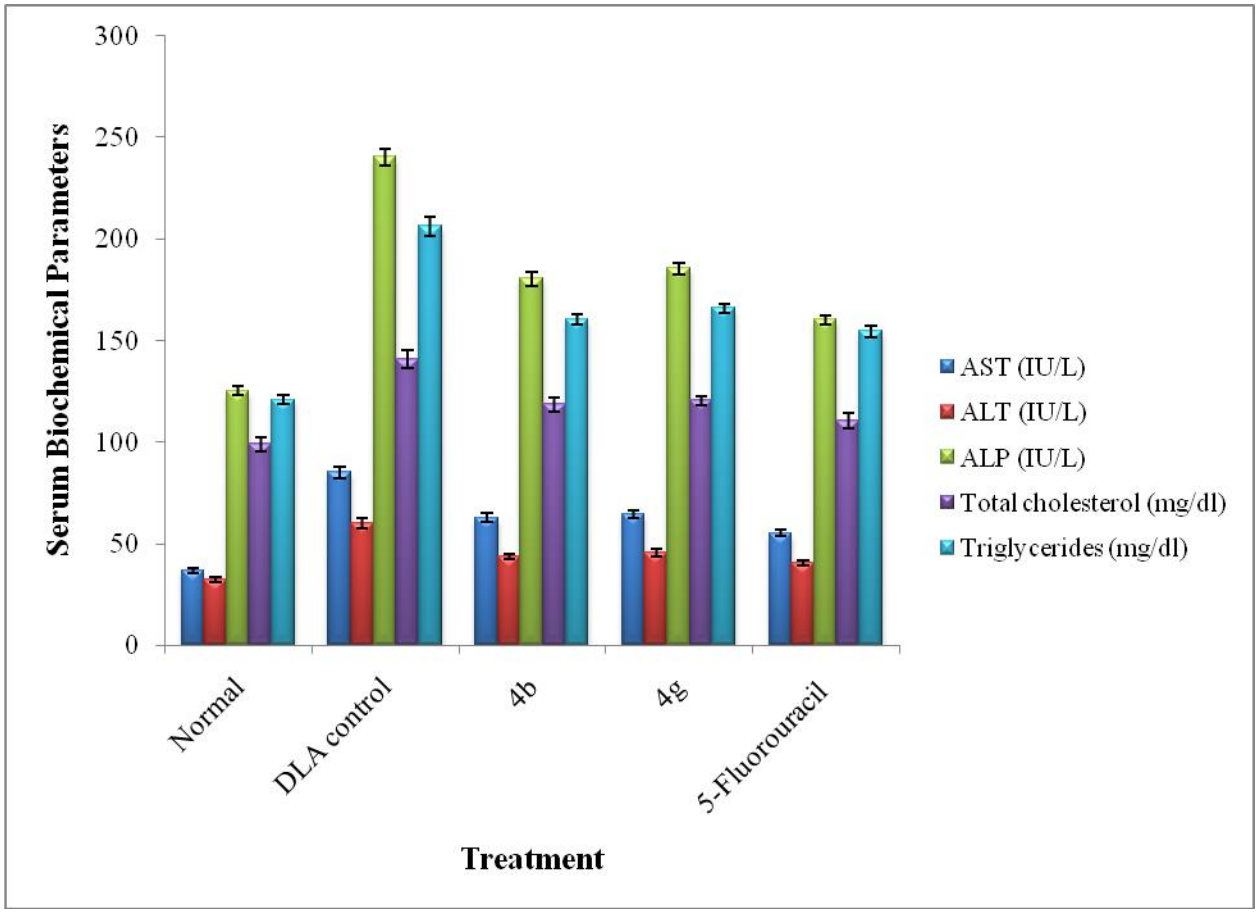
Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using one-way ANOVA followed by Newman-Keuls multiple range test.

[a]P<0.001: between normal and DLA control group.

[b]P<0.01: between compound treated groups and DLA control.

**Figure 4: Effect of Compounds (50 mg/kg) and 5-Fluorouracil (20 mg/kg) on Serum Biochemical Parameters in Dalton's Lymphoma Ascites Bearing Mice**

Values are expressed as mean ± S.E.M., n = 6 mice per group. Data were analyzed by using




one way ANOVA followed by Newman-Keuls multiple range test. [a]P<0.001: between normal and DLA control group. [b]P<0.01: between compound treated groups and DLA control.

## Results and Discussion

**Chemistry:** In the present study, a series of novel 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones (**4a-g**) were synthesized according to scheme 1. The target compounds (**4a-g**) were prepared from pyridin-4-amine (**1**) which on condensation with different aromatic aldehydes (**2a-g**) in presence of catalytic amount of concentrated hydrochloric acid in absolute ethanol yield N-[(Z)-(substitutedphenyl)methylidene]pyridin-4-amine (**3a-g**) in 62.5 - 86.4% 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

Chloroform: Methanol: Formic acid (10:2:0.2 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 (experimental part).

The FT-IR spectra of synthesized compounds (**3a-g**) showed absorbtion bands ranging from 1615.90 - 1597.73 $cm^{-1}$ for imine (>C=N) formation and 1686.44 - 1466.15 $cm^{-1}$ for C=N and C=C ring stretch of substituted phenyl and pyridyl ring. The IR spectra of compound (**3a-g**) displayed bands at about 3147.26 - 3023.84 and 1313.29 - 1059.69 $cm^{-1}$ associated with aromatic C-H and C-F functions. In the IR spectra of compound (**3a-g**), some significant stretching bands due to C-Cl, C-Br, asymmetric $ArNO_2$ and symmetric $ArNO_2$ were observed at 854.311 - 711.604 $cm^{-1}$, 529.364 $cm^{-1}$, 1582.56 - 1518.67 $cm^{-1}$ and 1372.1 - 1341.25 $cm^{-1}$, respectively. $^1$H NMR spectra of compound **3a** showed a sharp, singlet (1H) at δ 8.079 ppm attributed to azomethine (N=CH) and multiplet (8H) observed at δ 7.286 - 7.593 ppm confirmed the presence of four aromatic (phenyl) and four pyridyl protons, respectively. The results of elemental analyses were within ±0.4% of the theoretical values.

Compound (**3a-g**), which on cyclization with 2-sulfanylpropanoic acid in dry 1,4-dioxane in presence of anhydrous zinc chloride offers the corresponding 2-(substitutedphenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-ones (**4a-g**) in 54.1 - 74.5% yields (scheme 1). The structure of the synthesized compound (**4a-g**) was established on the basis of elemental analysis, FT-IR, $^1$H NMR and mass spectral data (experimental part).

The IR spectrum of compound (**4a-g**) showed strong absorbtion band at 1741.41 - 1701.87 $cm^{-1}$ for C=O of 1,3-thiazolidin-4-one, while the band at 2982.37 - 2921.63 $cm^{-1}$, 2853.17 $cm^{-1}$, 1409.89 - 1366.58 $cm^{-1}$ and 772.301 - 690.30 $cm^{-1}$, respectively confirms the presence of methyl C-H asymmetric, methyl C-H symmetric, C-N stretch of tertiary aromatic amine and C-S stretch. This is considered to be a strong confirmation for the 1,3-thiazolidin-4-one nucleus formation. The IR spectrum of compound (**4a-g**) showed strong absorbtions bands in the aromatic C-H region at 3140.68 $cm^{-1}$ and strong C-Cl at 828.277 $cm^{-1}$. The IR spectrum of compound (**4a-g**) showed $ArNO_2$ stretching bands at 1519.63 $cm^{-1}$, 1273.75 $cm^{-1}$, 862.025 $cm^{-1}$, in addition to stretching band at 1461.32 - 1015.34 $cm^{-1}$ attributed to C-F functions. The IR spectra of compound (**4a-g**) displayed bands at about 1644.0 - 1460.81 $cm^{-1}$ associated with C=N and C=C ring stretch of substituted phenyl and pyridyl ring.

In the $^1$H NMR spectra of compound **4a**, aromatic (4H) and pyridyl (4H) protons appeared as a multiplet (8H) at 7.422 - 7.021 ppm, C-2 of 1,3-thiazolidin-4-one, N-CH-Ar proton appeared as a singlet (1H) at 3.577 ppm, CH-$\underline{CH_3}$ protons appeared as a doublet (3H) at 1.731 - 1.628 ppm and $\underline{CH}$-$CH_3$ protons appeared as a quartet (1H) at 4.045 - 3.970

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.

**Antitumor Activity**

Chemotherapy is a major therapeutic approach for the both localized and metastasized 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 DLA 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 the compound 2-(2,4-dichlorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4b**) and 2-(4-bromophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4g**) inhibited 100% and 78% DLA tumor cells at 100 mcg/ml and 100% and 89% at 200 mcg/ml concentration, whereas standard drug doxorubicin exhibit 100% DLA inhibition at a concentration of 100 mcg/ml. At a higher concentration of 200 mcg/ml, compound **4a**, compound **4c** and compound **4f** inhibited 49%, 42% and 52% DLA tumor cells, exhibited moderate antitumor activity, whereas compound **4d** and compound **4e** both inhibited 20% DLA tumor cells displayed mild antitumor activity. 1,3-thiazolidin-4-one analogues (**4a-g**) exhibit dose-dependent significant increase in cytotoxicity when compared to those of standard drug, doxorubicin. From the above study, compound **4b** and compound **4g** which showed better results (> 60% inhibition) at a concentration of 100 mcg/ml were selected for their in vivo anticancer activity against DLA cancer cell line by trypan blue exclusion method.

**Anticancer Activity**

**Antitumor parameters**

Antitumor activity of 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**) against Dalton's lymphoma ascites (DLA) bearing mice was assessed by the parameters such as body weight gain, viable tumor cell count, packed cell volume and increase in life span (%). The results are shown in Table 3 and Figure 2.

The treatment with compound **4b** and compound **4g** at 50 mg/kg body weight significantly ($p < 0.01$) increased the average life span of DLA bearing mice from 48.0% to 88.11 and 84.04%, respectively, when compared with the DLA control group ($p < 0.001$). The standard drug 5-Fluorouracil (20 mg/kg) also significantly ($p < 0.01$) increased the life span to 92.20% (Table 3 and Figure 2). The average weight gain of DLA bearing mice was 7.64 ± 0.95 g, whereas it was reduced to 4.68 ± 0.60 g, 4.80 ± 0.65 g and 3.73 ± 0.42 g for the groups treated with compound **4b**, compound **4g** (50 mg/kg) and 5-fluorouracil (20

mg/kg), respectively. Compound **4b**, compound **4g** and 5-fluorouracil significantly ($p < 0.01$) reduced the body weight gain on day-11 as compared to DLA control (Table 3 and Figure 2). The compound **4b** and compound **4g** treated groups exhibited reduction in body weight is due to decreased tumor burden and the compound **4b** and compound **4g** were effective in suppressing the proliferation of tumor cells.

In Table 3, the packed cell volume (%) of the DLA control group was 30.55 ± 3.55. When compared to DLA control group, the packed cell volume was reduced significantly ($p < 0.01$) to 20.18 ± 2.62, 21.07 ± 2.43 and 18.40 ± 2.33%, respectively, following treatment with compound **4b**, compound **4g** and 5-fluorouracil. The viable tumor cell count was found to be significantly ($p < 0.001$) increased in DLA control when compared with normal control. Intraperitoneal administration of compound **4b** and compound **4g** at the dose of 50 mg/kg significantly ($p < 0.01$) decreased the viable tumor cell count when compared with DLA control (Table 3 and Figure 2). All these results clearly indicate compound **4b** and compound **4g** have a remarkable capacity to inhibit the growth of solid tumor induced by DLA cell line in experimental animals.

In DLA-bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad et al., 1994). The reliable criteria for judging the value of any anticancer drug are prolongation of life span of the animals (Clarkson et al., 1965) and decrease of WBC from blood (Gupta et al., 2000). Treatment with compound **4b** and compound **4g** caused significant reduction in bodyweight gain, packed cell volume and viable tumor cell count followed by significant increase in the life span of compound treated animals when compared with DLA control, indicating the potent anticancer properties of 1,3-thiazolidin-4-one analogues (compound **4b** and compound **4g**).

Andreani et al. (Andreani et al., 1983) have suggested that an increase in the life span of ascites bearing animals by 25% is considered as an indicative of significant drug activity. Roman et al (Roman et al., 2006)., reported in vitro antiproliferative activity against human colon cancer cell lines of 1,3-thiazolidin-4-one and few 1,3-thiazolidin-4-one possess in vitro antiproliferative activity by acting as inhibitors of translation initiation process. Various 1,3-thiazolidin-4-one (Rosario et al., 2005) have been reported for antitumor activities (Chimirri et al., 1986).

**Hematological parameters**

As shown in Table 4, hemoglobin content, RBC and platelet count in the DLA control was significantly ($p < 0.001$) decreased, compared to normal group. Treatment with compound **4b** and compound **4g** (50 mg/kg) significantly ($p < 0.01$) increased the

hemoglobin content, RBC and platelet count to near-normal levels. The total WBC count was found to be increased significantly in DLA control group when compared with normal group ($p < 0.001$). Administration of compound **4b** and compound **4g** (50 mg/kg) in DLA-bearing mice significantly ($p < 0.01$) reduced the WBC count when compared with DLA control (Figure 3).

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anaemia (Price et al., 1958, Hogland et al., 1982). The anaemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger et al., 1954). Treatment with compound **4b** and compound **4g** brought back the haemoglobin content, RBC, WBC and platelet count more or less to normal levels. This indicates that compound **4b** and compound **4g** possess protective action on the hemopoietic system.

**Serum biochemical parameters**

Alterations in the activities of biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC) and triglycerides (TG) in the serum of DLA-bearing mice is summarized in Table 5 and Figure 4. The levels of serum marker enzymes such as AST, ALT, ALP, TC and TG were found to be significantly ($p < 0.001$) increased in DLA control, when compared with the normal group, whereas treatment with compound **4b**, compound **4g** and 5-fluorouracil significantly ($p < 0.01$) decreased the level of AST, ALT, ALP, total cholesterol and triglycerides in compound **4b**, compound **4g** and 5-fluorouracil treated mice when compared to that of DLA control group as depicted in Table 5 and Figure 4.

Elevated levels of serum enzymes, ALT and AST are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman et al., 1978). Alkaline phosphatase activity on the other hand is related to the functioning of hepatocytes, increase in its ability being due to increased synthesis in the presence of increased biliary pressure (Moss et al). Liver damage induced by tumor cells generally reflects disturbances in liver cell metabolism, which lead to characteristic changes in serum enzyme activities. The increased levels of AST, ALT and ALP in serum may be interpreted as a result of liver damage or as changes in membrane permeability indicating the severity of hepatocellular damage by DLA (Senthilkumar et al., 2008). Treatment with compound **4b** and compound **4g** decreased the serum levels of AST, ALT and ALP towards their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by DLA.

Liver diseases also exhibit changes in blood cholesterol levels. The significant increase in cholesterol noted in serum in this study might have been due to the inability of the diseased liver to remove cholesterol from circulation. Hepatocellular damage also causes a modest hypertriglyceridemia, which is due to biochemical changes affecting transport of triglycerides out of the liver (Ravikumar et al., 2005). It was reported that the presence of tumor in humans or experimental animals is known to affect many functions of the vital organs especially in the liver, even when the site of the tumor does not interfere directly with organ functions (DeWys et al., 1982). The significant restoration of all the above mentioned biochemical parameters towards normal by treatment with compound **4b** and compound **4g** (50 mg/kg) in the present study indicates the protection of vital organs from damage induced by DLA.

The present study clearly demonstrated the tumor inhibitory activity of the 1,3-thiazolidin-4-one derivatives against transplantable tumor cell line (Tables 3-5). In the DLA bearing mice, cells were present in the peritoneal cavity, and the compounds were administered directly into the peritoneum. Thus, tumor inhibition might be due to the direct effect of the compounds on the tumor cells. The standard drug 5-fluorouracil acts cytostatically by interfering with nucleotide metabolism in S phase of the cell cycle (Dash et al., 2011).

In the in vivo anticancer evaluation, among two compounds screened, compound **4b** was the most active emerged as more potent inhibitor of DLA with an increase in life span of 88.11%, whereas compound **4g** exhibited good activity.

From the in vitro and in vivo antitumor and antiproliferative activity data reported in Tables 2-5, 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 **4b** possessing 2,4-dichlorophenyl group substituted at C-2 and pyridin-4-yl ring at N-3 position of 1,3-thiazolidin-4-one nucleus.

## Conclusion

In this study, compound 2-(2,4-dichlorophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4b**) and 2-(4-bromophenyl)-5-methyl-3-(pyridin-4-yl)-1,3-thiazolidin-4-one (**4g**) exhibited significant antitumor activity against DLA cells in vitro. In the in vivo anticancer evaluation, among two compounds screened, compound **4b** was the most active, emerged as more potent inhibitor of DLA with an increase in life span of 88.11%. However, further investigations are needed to understand the mechanism of action of the compounds and to examine the possible utility of the compounds in cancer therapy. These compounds 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.

**Acknowledgements**

The authors are thankful to Jadavpur University, Kolkata for providing the necessary facilities to carry out this research work. The authors express their sincere thanks and acknowledge the financial support from All India Council for Technical Education (AICTE), Quality Improvement Programme, New Delhi, India, for the financial assistance provided to carry out this research work. The authors are also thankful to the Director, Indian Institute of Chemical Biology (IICB), Kolkata for providing spectral data.

**References**

1. Zhou, H., Wu, S., Zhai, S., Liu, A., Sun, Y., Li, R., Zhang, Y., Ekins, S., Swaan, P.W., Fang, B., Zhang, B. and Yan, B (2008). Design, synthesis, cytoselective toxicity, structure-activity relationships and pharmacophore of thiazolidinone derivatives targeting drug-resistant lung cancer cells. Journal of Medicinal Chemistry, 51(5):1242-1251.
2. Vigorita, M.G., Ottana, R., Monforte, F., Maccari, R., Trovato, A., Monforte, M.T. and Taviano, M.F (2001). Synthesis and anti-inflammatory, analgesic activity of 3,3'-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone] chiral compounds. Part 10. Bioorganic & Medicinal Chemistry Letters, 11(21):2791-2794.
3. Geronikaki, A.A., Lagunin, A.A., Hadjipavlou-Litina, D.I., Eleftheriou, P.T., Filimonov, D.A., Poroikov, V.V., Alam, I. and Saxena, A.K (2008). Computer-Aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipooxygenase inhibition. Journal of Medicinal Chemistry, 51(6):1601-1609.
4. Chandrappa, S., Chandru, H., Sharada, A.C., Vinaya, K., Anandakumar, C.S., Thimmegowda, N.R., Nagegowda, P., Karunakumar, M. and Rangappa, K.S (2010). Synthesis and in vivo anticancer and antiangiogenic effects of novel thioxothiazolidin-4-one derivatives against transplantable mouse tumor. Medicinal Chemistry Research, 19:236-249.
5. Balzarini, J., Krzesinska, B.O., Maurin, J.K. and Orzeszko, A (2009). Synthesis and anti-HIV studies of 2- and 3-adamantyl-substituted thiazolidin-4-ones. European Journal of Medicinal Chemistry, 44(1):303-311.
6. de Aquino, T.M., Liesen, A.P., da Silva, R.E.A., Lima, V.T., Carvelho, L.C.S., de Faria, A.R., de Araujo, J.M., de Lima, J.G., Alves, A.J., de Melo, E.J.T. and Goes, A.J.S (2008). Synthesis, anti-Toxoplasma gondii and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-

[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids. Bioorganic & Medicinal Chemistry, 16:446-456.

7. Babaoglu, K., Page, M.A., Jones, V.C., Mc Neil, M.R., Dong, C., Naismith, J.H. and Lee, R.E (2003). Novel inhibitors of an emerging target in Mycobacterium tuberculosis; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. Bioorganic & Medicinal Chemistry Letters, 13(19):3227-3230.
8. Singh, B., Mehta, D., Baregama, L.K. and Talesara, G.L (2004). Synthesis and biological evaluation of 7-N-(n-alkoxyphthalimido)-2-hydroxy-4-aryl-6-aryliminothiazolidino[2,3-b]pyrimidines and related compounds. Indian Journal of Chemistry, 43B:1306-1313.
9. Smith, T.K., Young, B.L., Denton, H., Hughes, D.L. and Wagner, G.K (2009). First small molecular inhibitors of T. brucei dolichophosphate mannose synthase (DPMS), a validated drug target in African sleeping sickness. Bioorganic & Medicinal Chemistry Letters, 19(6):1749-1752.
10. Ottana, R., Maccari, R., Ciurleto, R., Vigorita, M.G., Panico, A.M., Cardile, V., Garufi, F. and Ronsivalle, S (2007). Synthesis and in vitro evaluation of 5-arylidene-3-hydroxyalkyl-2-phenylimino-4-thiazolidinones with antigenerative activity on human chondrocyte cultures. Bioorganic & Medicinal Chemistry, 15:7618-7625.
11. Ulusoy, N., Ergenc, N., Ekinci, A.C. and Ozer, H (1996). Synthesis and anticonvulsant activity of some new arylidenehydrazides and 4-thiazolidinones. Monatshefte fur Chemie, 127:1197-1202.
12. Diurno, M.V., Mazzoni, O., Correale, G., Monterrey, I.G., Calignano, A., Rana, G.L. and Bolognese, A (1999). Synthesis and structure-activity relationships of 2-(substitutedphenyl)-3-[3-(N,N-dimethylamino)propyl]-1,3-thiazolidin-4-ones acting as $H_1$-histamine antagonists. IL Farmaco, 54:579-583.
13. Shingalapur, R.V., Hosamani, K.M., Keri, R.S. and Hugar, M.H (2010). Derivatives of benzimidazole pharmacophore: synthesis, anticonvulsant, antidiabetic and DNA cleavage studies. European Journal of Medicinal Chemistry, 45 (5):1753-1759.
14. Jackson, C.M., Blass, B., Coburn, K., Djandjighian, L., Fadayel, G., Fluke, A.J., Hodson, S.J., Janusz, J.M., Murawskej, M., Ridgeway, J.M., White, R.E. and Wu, S (2007). Evaluation of thiazolidine-based blockers of human Kv1.5 for the treatment of atrial arrhythmias. Bioorganic & Medicinal Chemistry Letters, 17:282-284.
15. Bhandari, S.V., Bothara, K.G., Patil, A.A., Chitra, T.S., Sarkate, A.P., Gore, S.T., Dangre, S.C. and Kanchane, C.V (2009). Design, synthesis and pharmacological

screening of novel antihypertensive agents using hybrid approach. Bioorganic & Medicinal Chemistry, 17:390-400.

16. Richardson, D.R., Tran, E.H. and Ponka, P (1995). The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class of effective antiproliferative agents. Blood, 86(11):4295-4306.
17. Litchfield, J.T. and Wilcoxon, F (1949). A simplified method of evaluating dose effect experiments. Journal of Pharmacology and Experimental Therapeutics, 96(2):99-133.
18. Sreelatha, S., Padma, P.R. and Umasankari, E (2011). Evaluation of anticancer activity of ethanol extract of Sesbania grandiflora (Agati sesban) against Ehrlich ascites carcinoma in Swiss albino mice. Journal of Ethnopharmacology, 134(3):984-987.
19. Sathisha, M.P., Budagumpi, S., Kulkarni, N.V., Kurdekar, G.S., Revankar, V.K. and Pai, K.S.R (2010). Synthesis, structure, electrochemistry and spectral characterization of (D-glucopyranose)-4-phenylthiosemicarbazide metal complexes and their antitumor activity against Ehrlich ascites carcinoma in Swiss albino mice. European Journal of Medicinal Chemistry, 45(1):106-113.
20. Dongre, S.H., Badami, S. and Godavarthi, A (2008). Antitumor activity of Hypericum hookerianum against DLA induced tumor in mice and its possible mechanism of action. Phytotherapy Research, 22(1):23-29.
21. Chandrappa, S., Chandru, H., Sharada, A.C., Vinaya, K., Ananda Kumar, C.S., Thimmegowda, N.R., Nagegowda, P., Karuna Kumar, M. and Rangappa, K.S (2010). Synthesis of in vivo anticancer and antiangiogenic effects of novel thioxothiazolidin-4-one derivatives against transplantable mouse tumor. Medicinal Chemistry Research, 19:236-249.
22. Jose, J.K., Kuttan, G. and Kuttan, R (2001). Antitumor activity of Emblica officinalis. Journal of Ethnopharmacology, 75 (2-3):65-69.
23. Christina, A.J.M., Jose, M.A., Robert, S.J.H., Kothai, R., Chidambaranathan, N. and Muthumani, P (2003). Effect of Indigofera aspalathoides against Dalton's ascitic lymphoma. Fitoterapia, 74(3):280-283.
24. Gupta, M., Mazumder, U.K., Rath, N. and Mukhopadhyay, D.K (2000). Antitumor activity of methanol extract of Cassia fistula L. seed against Ehrlich ascites carcinoma. Journal of Ethnopharmacology, 72(1-2):151-156.
25. Dacie, J.V. and Lewis, S.M (1975). Basic hematological techniques. In: Practical hematology. 5th ed. Edinburgh: Churchill Living stone,21-67.

26. Sharada, A.C., Solomon, F.E., Devi, P.U., Udupa, N. and Srinivasan, K (1996). Antitumor and radiosensitizing effects of withaferin A on mouse Ehrlich ascites carcinoma in vivo. Acta Oncologica, 35(1):95-100.
27. D' Armour, F.E., Blood, F.R. and Belden, D.A. The manual for laboratory work in mammalian physiology. 3rd ed. Illinois, Chicago: The University of Chicago Press, 1965:4-6.
28. (a) Swarup, H., Pathak, S.C. and Arrora, S (1981). In: Laboratory techniques in modern biology. New Delhi: Kalyani Publishers,163-186. (b) Wintrobe, M.M., Lee, G.R., Boggs, D.R., Bithel, T.C., Athens, J.W. and Foerester (1961) J. Clinical Hematology. 5th ed. Philadelphia: Les & Febiger,326.
29. Bromberg, N., Dreyfuss, J.L., Regatieri, C.V., Palladino, M.V., Duran, N., Nader, H.B., Harm, M. and Justo, G.Z (2010). Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. Chemico-Biological Interactions; 186(1):43-52.
30. Reitman, S. and Frankel, S.A (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28(1):56-63.
31. (a) Kind, P.R.N. and King, E.J (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrine. Journal of Clinical Pathology, 7(4):322-326. (b) Wright, P.J., Leathwood, P.D. and Plummer, D.T (1972). Enzymes in rat urine: alkaline phosphatase. Enzymologia, 42(4):317-321.
32. Wybenga, D.R., Pleggi, V.J., Dirstine, P.H. and Giorgio, J.D (1970). Direct manual determination of serum total cholesterol with a single stable reagent. Clinical Chemistry, 16(12):980-984.
33. Mendez, J., Franklin, B. and Gahagan, H (1975). Simple manual procedure for determination of serum triglycerides. Clinical Chemistry, 21(6):768-770.
34. Prasad, G.B. and Giri, A (1994). Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. Indian Journal of Experimental Biology, 32(3):155-162.
35. Clarkson, B.D. and Burchenal, J.H (1965). Preliminary screening of antineoplastic drugs. Prog Clin Cancer, 1:625-629.
36. Gupta, M., Mazumder, U.K., Rath, N. and Mukhopadhyay, D.K (2000). Antitumor activity of methanol extract of Cassia fistula L. seed against Ehrlich ascites carcinoma. Journal of Ethnopharmacology, 72:151-156.
37. Andreani, A., Scapini, G., Galatulas, I. and Bossa, R (1983). Potential antitumor agents IX: Synthesis and antitumor activity of two analogues of ketocaine. Journal of Pharmaceutical Sciences, 72(7):814-815.

38. Roman, L., Bory, Z., Dmytro, A., Frank, J., Katarzyna, K. and Andrzej, G (2006). Anticancer thiopyrano[2,3-α][1,3]thiazol-2-ones with norbornane moiety. Synthesis, cytotoxicity, physico-chemical properties, and computational studies. Bioorganic & Medicinal Chemistry, 15: 5230-5240.
39. Rosario, O., Stefania, C., Rosanna, M., Ida, L., Giuseppa, C., Barbara, C., Maria, G.V. and Enrico, M (2005). In vitro antiproliferative activity against human colon cancer cell lines of representative 4-thiazolidinones. Part I. Bioorganic & Medicinal Chemistry Letters, 15(17):3930-3933.
40. Chimirri, A., Grasso, S., Monforte, P., Fenech, G. and Zappala, M (1986). Compounds with potential antitumor activity. V. 2-Substituted 3-[2-(1,3,4-thiadiazolyl)]-4-thiazolidinone. Farmaco, 41:839-851.
41. Price, V.E. and Greenfield, R.E. (1958). Anaemia in cancer. Advances in Cancer Research, 5:199-200.
42. Hogland, H.C (1982). Hematological complications of cancer chemotherapy. Seminars in Oncology, 9(1):95-102.
43. Fenninger, L.D. and Mider, G.B (1954). Energy and nitrogen metabolism in cancer. Advances in Cancer Research, 2:229-253.
44. Drotman, R.B. and Lawhorn, G.T (1978). Serum enzymes are indicators of chemical induced liver damage. Drug and Chemical Toxicology, 1(2):163-171.
45. Moss, D.W. and Butterworth, P.J. Enzymology and Medicine. London: Pitman Medical, 139.
46. Senthilkumar, N., Badami, S., Dongre, S.H. and Bhojraj, S (2008). Antioxidant and hepatoprotective activity of the methanol extract of Careya arborea bark in Ehrlich ascites carcinoma-bearing mice. Journal of Natural Medicines, 62(3):336-339.
47. Ravikumar, V., Shivashangari, K.S. and Devaki, T (2005). Hepatoprotective activity of Tridax procumbens against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology, 101(1-3):55-60.
48. DeWys, W.D (1982). Pathophysiology of cancer cachexia: current understanding and areas for future research. Cancer Research, 42 (2 Suppl):721s-726s.
49. Dash, S., Kumar, B.A., Singh, J., Maiti, B.C. and Maity, T.K (2011). Synthesis of some novel 3,5-disubstituted 1,3,4-oxadiazole derivatives and anticancer activity on EAC animal model. Medicinal Chemistry Research, 20:1206-1213.