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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vivo anticancer and antiangiogenic effects of novel thioxothiazolidin-4-one derivatives against transplantable mouse tumor

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Abstract A series of novel thioxothiazolidin-4-one derivatives **5(a–g)** were synthesized by the coupling of different amines containing aliphatic, substituted aromatic, and heterocyclic moieties, such as oxadiazol, pyrazole, isoxazole, and piperazine with 2-(5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid. All compounds were characterized by ^{1}H NMR, LCMS, FTIR and elemental analysis. In this study, we investigated the possibility that these novel thioxothiazolidin-4-one derivatives **5(a–g)** inhibits tumor growth and tumor induced angiogenesis using mouse Ehrlich Ascites Tumor (EAT) as a model system. Our results demonstrated that the compounds significantly reduced ascites tumor volume, cell number, and increased the life span of EAT-bearing mice. In addition, the compounds manifested strong antiangiogenic effects and suppressed tumor induced endothelial proliferation in the mice peritoneum. From our findings, it is noted that the derivatives **5(a–e)** may be possible candidates for anticancer therapy with the ability to inhibit tumor angiogenesis and tumor cell proliferation.

Keywords Thioxothiazolidin-4-one · EAT cells · Lifespan · Anticancer · Antiangiogenesis

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Introduction

Cancer is a disease of worldwide importance. Its incidence in the developed countries is increasing, and its mortality occupies the second rank in the order of death causes. Similar tendency can be observed in the developing world: the gradual improvement in the life expectancy is associated with an elevated cancer incidence and mortality. Accordingly, we might assume that malignancy will be soon a global problem with its consecutive burden. Therefore, it is easy to understand that cancer therapy is in the focus of common interest. For now, the treatment of any malignancy is based on surgery, radiotherapy, and drug therapy.

The growth and metastasis of cancer cells are dependent on angiogenesis. Angiogenesis has been functionally defined as the sprouting of new vessels from preexisting blood vessels and is an essential process in wound healing. Furthermore, angiogenesis is a critical process in tumor cells invasion (Zetter, 1998). It is deemed that only those cells that have completed neovascularization are capable of growing violently in size and volume, especially when the tumor size is beyond 2–3 mm. The process of angiogenesis induced by tumor cells consists of several distinct stages, including slow growth of tumor cells without blood vessels and then releasing specific angiogenic growth factors (Fontanini *et al*., 1999).

In the rapid developing field of small molecule combinatorial chemistry, it particularly appeals easy way for the synthesis of small heterocyclic compound libraries (Gordon *et al*., 1994; Gordon and Steele, 1995; Green, 1995; Murphy *et al*., 1995; Ruhland *et al*., 1996). An important application of small molecule libraries is the preparation of a directed or focused combinatorial library for assay against a specific biological target. As part of our search for biologically active compounds with sulphur- and nitrogen-containing heterocycles, 4-thiazolidinones substituted in the 2 position were proven to be biologically very potent and selective (McLamore *et al*., 1952; Sobin, 1952; Tanabe *et al*., 2006). A wide spectrum of pharmacological activities has been reported for these compounds. Some of these therapeutic areas include antimicrobial (Franzen, 2000), anticancer (Lakhan and Rai, 1987; Hour *et al*., 2000), antiviral (Hamel *et al*., 1996), anticonvulsant (Corbett *et al*., 2000; Archana *et al*., 2003), antifungal (Samir *et al*., 2007; Bartroli *et al*., 1998), anti-inflammatory (Goel *et al*., 1999), analgesic (Smith and Dewitt, 1996), and antiproliferative activities (Herschman, 1996; Veeresa *et al*., 2004). Thiazolidinones derivatives possess broad pharmacological action on the central nervous system, especially anti-HIV agents (Barreca *et al*., 2001) and cyclooxygenase (COX) inhibitors (Chen *et al*., 2004).

In this context, the synthesis of libraries centered on a known lead compound are valuable for the evaluation of the tumor growth inhibitory activities of novel synthetic thiazolidinone analogs on Ehrlich ascites tumor cells in vivo. The observations suggest that the synthetic thiazolidinone analogs most likely target tumor proliferation and tumor progression.

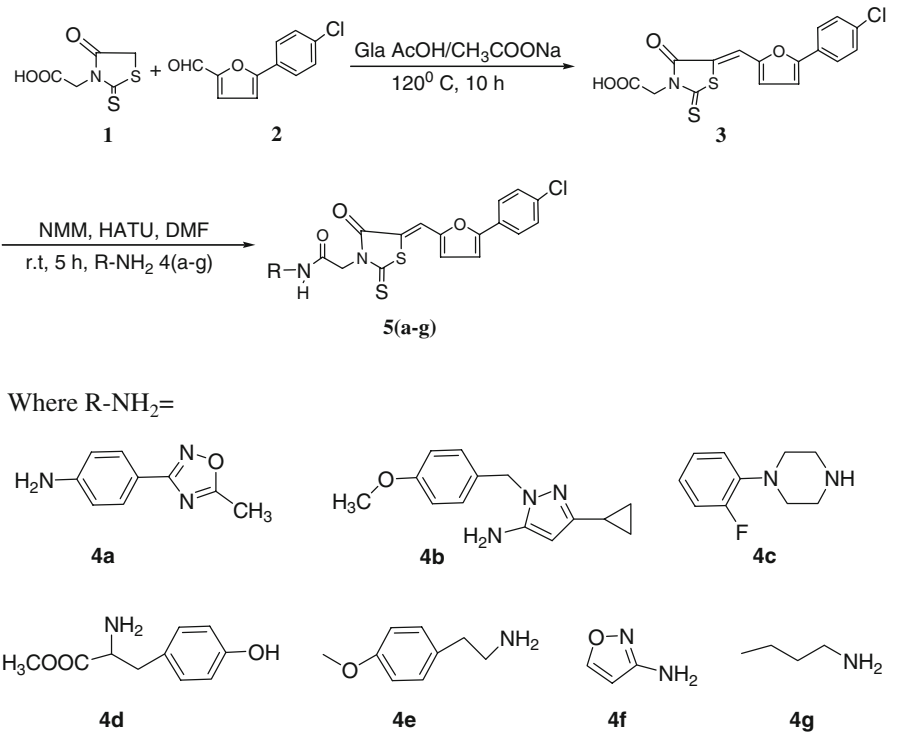
Chemistry

Thiazolidinone derivatives **5(a–g)** were prepared by the method summarized in Scheme 1. Initially the compound **3** was synthesized by the condensation reaction with rhodhanine-3-acetic acid **1** with phenyl furfural **2** by using sodium acetate and acetic acid. The obtained yield was found to be 80%. Finally, the obtained intermediate **3** was treated with substituted aliphatic, aromatic and heterocyclic amines in presence of *N*-methyl morpholine, HATU, and *N,N*-dimethyl formamide as a solvent at room temperature to give the target molecules with a good yield ranging from 76–86% with high purity. All the synthesized molecules **5(a–g)** were characterized by ^{1}H NMR, LCMS, IR, and elemental analysis.

Results and discussion

Chemistry

The coupling reaction of thiazolidinone moiety with different substituted amines was confirmed by the ^{1}H NMR and IR data. The disappearance of $-NH_2$ peak and



Scheme 1 Synthesis of thiazolidinone derivatives **5(a-g)**

–COOH peaks confirms the formation of our target molecules. From IR data, appearance of HN-C=O symmetric stretching frequency ranging from 3478–3385 cm^{-1} and appearance of –C=O in thiazolidinone at 1637–1603 cm^{-1} confirms the formation of products. All the synthesized molecules **5(a–g)** were evaluated for their anticancer activity.

Biology

The peritoneum of the mice were cut open and the inner lining of the peritoneal cavities were examined for angiogenesis in both control and compound **5(a–g)** treated tumor bearing mice and photographed. All data were analyzed by using one-way ANOVA and observed for significance at $P < 0.05$ level.

In continuation of our research on anticancer and angioinhibitory activities, we report the growth inhibitory and anti-angiogenic effects of the novel synthetic thiazolidinone analogs **5(a–g)** on the Ehrlich's ascites tumor cells grown in the peritoneal cavity of Swiss albino mice (Chandru and Sharada, 2007a, b; Chandru *et al*., 2007a, b; Ananda Kumar *et al*., 2008). The compounds **5(a–e)** inhibited the tumor growth resulting in increase in the Medial Survival Time (MST) and % Increase in Life Span (ILS) of tumor bearing mice. The tumor growth delay by the compounds was further supported with the decrease in cell number and ascites volume. These findings suggest that the effect of compounds is on the proliferating tumor cells. Our reports agree with the earlier studies on in vitro antiproliferative activity against human colon cancer cell lines of thiazolidinone (Roman *et al*., 2006). Few thiazolidinone possess in vitro antiproliferative activity by acting as inhibitors of translation initiation process. Various thiazolidinone (Veeresa *et al*., 2004; Rosaria *et al*., 2005) have been reported for antitumor activities (Chimirri *et al*., 1986). Over expression of COX has been shown to be associated with a wide variety of cancers and the role of COX in tumor cell proliferation has been demonstrated (Williams *et al*., 1999). Thiazolidinones derivatives are found to be cyclooxygenase (COX) inhibitors (Chen *et al*., 2004).

The results on anti-angiogenic studies of **5(a–g)** compounds demonstrated that, in addition to being good anti-tumor agents, the compounds can also act as effective angiogenic inhibitors. These compounds prevented the proliferation of endothelial cells in tumor bearing mouse peritoneum. Tumor angiogenesis is a complex process of proliferation and migration of endothelial cells towards the cancer mass that secretes a variety of growth factors (Kato *et al*., 1994). Thiazolidinone have been demonstrated as potential antitumor agents on human colorectal carcinoma (Vigorita *et al*., 1997; Vigorita *et al*., 2001; Ottana *et al*., 2002), and in this study, the angioinhibitory effects may be due to inhibition of growth factors which requires further investigations.

The coupling of different amines containing aliphatic, substituted aromatic and heterocyclic moieties such as oxadiazol, pyrazole, isoxazole, piperazine with 2-(5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid leads to the production of novel compounds **5(a–g)**. Among the derivatives, compound **5b** exhibited maximum tumor growth inhibition compared to **5a** due to the presence of pyrazole with cyclopropyl ring, where as **5a** substituted with a

oxadiazol moiety in the phenyl ring exhibited lower tumor growth inhibitory effects. Compound **5e** contains a phenyl ring with good electron donating methoxy group and compound **5c** contains phenyl ring with electron withdrawing fluorine group and **5d** has phenolic group and all these compounds exhibited comparable antitumor response. However, the compounds **5f** with isoxazole moiety and **5g** with butyl chain did not show effective antitumor response. The additional modification and diversification of functional groups in order to improve the anti-cancer activity is currently in progress.

Conclusion

Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives gave good yield with purity. The results of the present findings are encouraging, and the compounds **5(a–e)** have shown good antitumor and anti-angiogenic effects against transplantable mouse Ehrlich ascites tumor. Further studies on the thiazolidinone derivatives are of great importance because the compounds may lead to potential therapeutic agents for treatment of cancer.

Experimental

Melting points were determined using SELACO-650 hot stage melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as internal standard (chemical shift in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analyses were obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck made TLC plates.

Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **(3)**

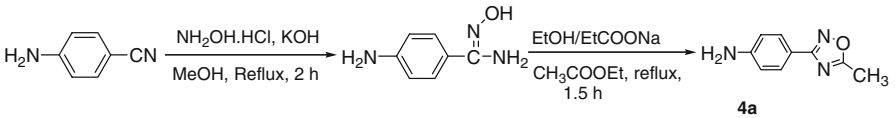
A mixture of 3-rhodanine-3-acetic acid **1** (1.0 g, 0.01 mmol), 5-(3-chlorophenyl)-furfural **2** (1.08 g, 0.01 mmol) and anhydrous sodium acetate (1.3 g, 0.03 mmol) were taken in 10 ml glacial acetic acid. The reaction mixture was heated to 120 °C in an oil bath for 10 h. Then the reaction mixture was cooled, filtered, and washed with ether. A reddish solid compound was obtained (1.75 g, 75%). The schematic representation of the synthesized compound is shown in Scheme 1. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.5 (s, 1H, –COOH), 7.9 (d, 1H, Ar–H), 7.6 (t, 1H, Ar–H), 7.5 (d, 1H, Ar–H), 7.4 (d, 2H, Ar–H), 7.1 (d, 1H, Ar–H), 4.5 (s, 2H, –CH_2). MS: 379.97.

Procedure for the synthesis of 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzeneamine **(4a)**

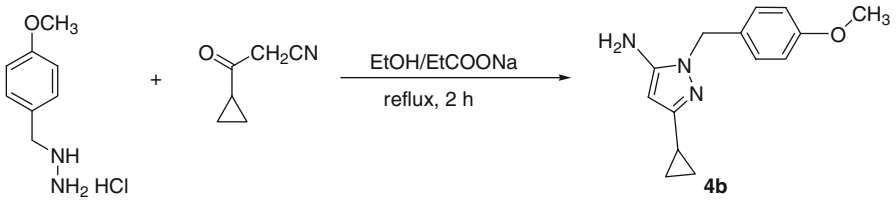
A mixture of 4-amino benzonitrile (1.0 g, 8.4 mmol), hydroxyl amine hydrochloride (0.64 g, 9.2 mmol), and KOH (1.4 g, 25 mmol) were taken in methanol (10 ml). The reaction mixture was refluxed for 2 h. After completion of the reaction, the solvent was removed under reduced pressure. The resulting residue was dissolved in water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained oxime was cyclized using sodium ethoxide (1.09 g, 16.0 mmol) in presence of ethyl acetate (3 volume) to get 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzeneamine as off white solid. The schematic representation is shown in Scheme 2. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.6 (dd, 2H, Ar–H), 6.6 (dd, 2H, Ar–H), 5.7 (bs, 2H, –NH_2), 2.5 (s, 3H, –CH_3).

Procedure for the synthesis of 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(4b)**

1-(4-methoxybenzyl)-2-methylhydrazine salt (1.0 g, 5.36 mmol) and 3-cyclopropyl-3-oxopropanenitrile (0.85 g, 5.36 mmol) were taken in ethanol, and then sodium ethoxide (1.09 g, 16.0 mmol) was added. The reaction mixture was refluxed for 2 h. After completion of the reaction, the solvent was removed under reduced pressure. The workup procedure was same as discussed earlier. The obtained product was white solid. The schematic representation is shown in Scheme 3. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.13 (d, 2H, Ar–H), 6.9 (d, 2H, Ar–H), 5.2 (s, 1H, Ar–H), 5.1 (s, 2H, –CH_2), 3.8 (s, 3H, –OCH_3), 3.3 (br s, 2H, –NH_2), 1.9 (m, 1H, –CH), 0.9 (m, 2H, –CH_2), 0.7 (m, 2H, –CH_2).



Scheme 2 Synthesis of 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzeneamine **(4a)**



Scheme 3 Synthesis of 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(4b)**

General procedure for the synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives **5(a–g)**

A solution of 2-(5-((5-(4-chlorophenyl) furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (1.0 eq) and appropriate amines (1.0 eq) in dry DMF was taken. *N*-methyl morpholine (NMM) (3.0 eq) and 10% of the HATU catalyst were added to the reaction mixture. The reaction mixture was stirred for 5 h at room temperature, and progress of the reaction was monitored by TLC. After completion of the reaction, water was added and the reaction mixture was filtered and finally washed with ether and dried under vacuum.

Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-*N*-(4-(5-methyl-1,2,4-oxadiazol-3-yl)phenyl)acetamide **(5a)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzeneamine **4a** (0.05 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mmol), and 0.01 g HATU. The product obtained was reddish solid (0.12 g, 80%). M.P (°C): 251–253. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.51 (s, 1H, –NH), 7.94 (d, 2H, Ar–H), 7.80 (d, 2H, Ar–H), 7.72 (d, 1H, =CH), 7.46 (d, 2H, Ar–H), 7.3 (d, 1H, Ar–H), 7.01 (d, 1H, Ar–H), 6.68 (d, 1H, Ar–H), 4.53 (s, 2H, –CH_2), 3.1 (s, 3H, –CH_3). MS (−1): 535.70. IR (KBr, cm^{-1}): 3441, 1603, 1364, 1190, 1057. Anal. calcd. for $C_{25}H_{17}ClN_4O_4S_2$ (%): C-55.91, H-3.19, N-10.43, S-11.94. Found C-55.88, H-3.16, N-10.40, S-11.89.

Synthesis of *N*-(1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-yl)-2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetamide **(5b)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **4b** (0.07 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mmol), and 0.01 g HATU. The product obtained was reddish solid (0.14 g, 85%). M.P (°C): 254-256. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.01 (s, 1H, –NH), 7.93 (d, 1H, =CH), 7.61 (m, 2H, Ar–H), 7.52 (d, 2H, Ar–H), 7.44 (d, 1H, Ar–H), 7.2 (d, 2H, Ar–H), 7.23 (d, 2H, Ar–H), 5.24 (s, 1H, Ar–H), 5.13 (s, 2H, –CH_2), 4.9 (s, 2H, –CH_2), 3.83 (s, 3H, –OCH_3), 1.84 (m, 1H, –CH), 0.9 (2H, m, –CH_2), 0.76 (m, 2H, –CH_2). MS (−1): 602.10. IR (KBr, cm^{-1}): 3478, 1609, 1331, 1190, 1057. Anal. calcd. for $C_{30}H_{25}ClN_4O_4S_2$ (%): C-59.54, H-4.16, N-9.26, S-10.60. Found C-50.50, H-4.12, N-9.22, S-10.56.

Synthesis of 5-((5-(4-chlorophenyl)furan-2-yl)methylene)-3-(2-(4-(2-fluorophenyl)piperazin-1-yl)-2-oxoethyl)-2-thioxothiazolidin-4-one **(5c)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)aceticacid **3** (0.1 g, 0.01 mmol), 1-(2-fluorophenyl)piperazine **4c** (0.05 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mmol), and 0.01 g HATU. The product obtained was reddish solid (0.12 g, 77%). M.P (°C): 234–236. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.94 (d, 1H, =CH), 7.82 (d, 2H, Ar–H), 7.62 (m, 1H, Ar–H), 7.5 (m, 2H, Ar–H), 7.42 (d, 1H, Ar–H), 7.1 (m, 4H, Ar–H), 5.04 (s, 2H, –CH_2), 3.6-3.7 (s, 4H, N–CH_2), 2.9-3.0 (s, 4H, N–CH_2). MS (+1): 542.04. IR (KBr, cm^{-1}): 1613, 2925, 1502, 1329, 1208. Anal. calcd. for $C_{26}H_{21}ClFN_3O_3S_2$ (%): C-57.61, H-3.90, N-7.75, S-11.83. Found C-57.57, H-3.86, N-7.71, S-11.79.

Synthesis of methyl 2-(2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetamido)-3-(4-hydroxyphenyl)propanoate **(5d)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), methyl 2-amino-3-(4-hydroxyphenyl)propanoate **4d** (0.06 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mmol), and 0.01 g HATU. The product obtained was reddish solid (0.126 g, 84%). M.P (°C): 228–230. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.43 (s, 1H, –NH), 8.7 (bs, 1H, –OH), 7.9 (d, 2H, Ar–H), 7.8 (d, 3H, Ar–H), 7.73 (d, 1H, =CH), 7.4 (d, 2H, Ar–H), 7.3 (d, 1H, Ar–H), 7.02 (d, 1H, Ar–H), 6.68 (d, 1H, Ar–H), 4.6 (d, 2H, –CH_2), 4.5 (s, 2H, –CH_2), 3.6 (d, 3H, –$COOCH_3$), 4.4 (t, 1H, –CH). MS (+1): 555.10. IR (KBr, cm^{-1}): 3434, 1637, 1316, 1200. Anal. calcd. for $C_{26}H_{21}ClN_2O_6S_2$ (%): C-56.06, H-3.80, N-5.03, S-11.51. Found C-56.01, H-3.76, N-4.98, S-11.47.

Synthesis of *N*-(4-methoxyphenethyl)-2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetamide **(5e)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), 2-(4-methoxyphenyl)ethanamine **4e** (0.04 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mol), and 0.01 g HATU. The product obtained was reddish solid (0.11 g, 80%). M.P (°C): 219–221. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.12 (s, 1H, –NH), 7.9 (d, 1H, =CH), 7.8 (m, 3H, Ar–H), 7.6 (m, 3H, Ar–H), 7.5 (m, 3H, Ar–H), 7.3 (d, 1H, Ar–H), 5.2 (t, 2H, –CH_2), 4.9 (t, 2H, –CH_2), 4.5 (s, 2H, –CH_2), 3.8 (s, 3H, –OCH_3), 4.5 (s, 2H, –CH_2), 3.6 (d, 3H, –$COOCH_3$), 4.4 (t, 1H, –CH). MS (+1): 513. IR (KBr, cm^{-1}): 3461, 1634, 1405, 1325, 1191. Anal. calcd. for $C_{25}H_{21}ClN_2O_4S_2$ (%): C-58.53, H-4.13, N-5.46, S-12.50. Found C-58.49, H-4.09, N-5.42, S-12.45.

Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-*N*-(isoxazol-3-yl)acetamide **(5f)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), isoxazol-3-amine **4f**

(0.02 g, 0.01 mmol), *N*-methyl morpholine (0.04 g, 0.03 mmol), and 0.01 g HATU. The product obtained was reddish solid (0.126 g, 86%). M.P (°C): 249–251. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.6 (s, 1H, –NH), 7.9 (d, 1H, =CH), 7.7 (m, 2H, Ar–H), 7.6 (m, 2H, Ar–H), 7.4 (m, 2H, Ar–H), 7.3 (d, 1H, Ar–H), 4.5 (s, 2H, –CH_2). MS (−1): 445. IR (KBr, cm^{-1}): 3423, 1632, 1330, 1190, 954. Anal. calcd. for $C_{19}H_{12}ClN_3O_4S_2$ (%): C-51.18, H-2.71, N-9.42, S-14.38. Found C-51.15, H-2.68, N-9.38, S-14.34.

Synthesis of *N*-butyl-5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidine-3-carboxamide **(5g)**

It was obtained from 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **3** (0.1 g, 0.01 mmol), butan-1-amine **4g** (0.02 g 0.01 mmol), *N*-methyl morpholine (0.04 g 0.03 mol), and 0.01 g HATU. The product obtained was reddish solid (0.11 g, 80%). M.P (°C): 210-212. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.0 (s, 1H, -NH), 7.86 (d, 1H, =CH), 7.7 (d, 1H, Ar–H), 7.68 (d, 1H, Ar–H), 7.53 (m, 1H, Ar–H), 7.45 (m, 2H, Ar–H), 7.36 (d, 1H, Ar–H), 4.5 (s, 2H, –CH_2), 3.6 (t, 2H, –CH_2), 3.55 (m, 2H, –CH_2), 3.05 (m, 2H, –CH_2), 2.13 (t, 3H, –CH_3). MS (+1): 435.2. IR (KBr, cm^{-1}): 3385, 1637, 1405, 1116. Anal. calcd. for $C_{19}H_{17}ClN_2O_3S_2$ (%): C-54.21, H-4.07, N-6.66, S-15.24. Found C-54.18, H-4.02, N-6.62, S-15.21.

Biology

In vivo anti-cancer activities of synthetic novel thiazolidinones analogs

Animals and tumor

Inbred Swiss albino mice of 6–8 weeks old weighing 25 ± 5 g of either sex were used for the experiments. Ehrlich ascites tumor was grown in adult Swiss albino mice intraperitoneally. Experimental animals were prepared by injecting 5×10^6 viable tumor cells into intraperitoneal cavity of Swiss mice. Tumor growth was followed by recording the animal weights. EAT cells started exponential growth phase from the seventh day after tumor cell injection and the animal succumb to the ascites tumor burden on 14–18 days after injection.

Animal survival

Seven days after tumor cell injection, the animals were divided into groups of five each and were treated as follows: 1) control: 0.2 ml of 0.1% DMSO was given on days 7, 9, and 11 after tumor transplantation; 2) compound-treated groups: the compounds **5(a–g)** were given to seven different groups of tumor bearing mice. Three doses of the compounds **5(a–g)** (100 mg/kg body. wt) were injected ip into the mice on days 7, 9, and 11 after tumor transplantation. All the mice were weighed on the day of tumor inoculation and at weekly intervals. Animal survival was

recorded up to 28 days. The tumor response was assessed on the basis of median survival time (MST) and increase in life span (% ILS) (Sharada *et al*., 1996). MST and % ILS were calculated from the mortality data with in the observation period. Enhancements of life span by 25% are more over that of the control was considered as effective anti-tumor response.

In vivo tumor growth inhibition

After 7 days of tumor cell injection the animals were divided into groups of ten each; the control group received 0.2 ml of 0.1% DMSO on days 7, 9, and 11 after tumor transplantation. The compounds **5(a–g)** were given to seven different groups of tumor bearing mice as scheduled above. The tumor inhibitory effect of the compounds on EAT cell growth was assessed by counting cell number and ascites volume. On day 12, the control and compounds **5(a–g)-**treated tumor-bearing mice were killed and an incision was made in the abdominal region. EAT cells along with the ascites fluid were harvested in a beaker containing 2 ml of saline and centrifuged at 3000 rpm for 10 minutes at 4 °C. The volume of ascites fluid was obtained by subtracting the volume of saline added from the volume of the supernatant. The harvested EAT cells were resuspended in 0.9% saline and counted using a hemocytometer.

The vehicle-treated control animals developed tumor and died within 14–18 days; median survival time (MST) was 16 days. Three doses of 100 mg/kg body weight of thiazolidinone analogs **5(a–g)** treatments on days 7, 9, and 11 after tumor transplantation increased the MST and % ILS. The compound **5a** showed 23 days of MST with 43.75% ILS, but the compound **5g** was ineffective and produced 12.5%

Table 1 Effect of synthetic novel thiazolidinone analogs **5(a–g)** on survival of mice bearing Ehrlich ascites tumor

Treatment groups	No. of animals	Schedule days	MST (days)[a]	ILS (%)	Av. Wt change[b]
Control (0.1%DMSO)	5	7, 9, 11	16	–	8.52 ± 0.41
5a	5	7, 9, 11	23*	43.75	−3.4 ± 0.5
5b	5	7, 9, 11	25*	56.25	−3.71 ± 0.48
5c	5	7, 9, 11	21*	31.25	−2.6 ± 0.34
5d	5	7, 9, 11	20*	25	−2.22 ± 0.28
5e	5	7, 9, 11	22*	37.5	−2.63 ± 0.31
5f	5	7, 9, 11	19	18.25	−2.41 ± 0.52
5g	5	7, 9, 11	18	12.5	−2.3 ± 0.61

5(a–g) dose in 100 mg/kg body wt

[a] Median survival time (MST) and ILS % was calculated from the mortality data within the observation period

[b] Determined on 12th day of treatment

* Significant difference from control ($P < 0.05$)

ILS, which is less than the effective anti-tumor response (25% ILS). The compound **5e** increased the % ILS, which was doubled to that produced by the compound **5f**. The compound **5c** have shown considerable increase in lifespan of animals. The % ILS reached 50% in the **5d**-treated group. The highest tumor response 56.25% ILS was observed in **5b**-treated group. The decrease in animal body weights in compound-treated groups compared with the control is an indication of inhibition of tumor cell proliferation (Table 1).

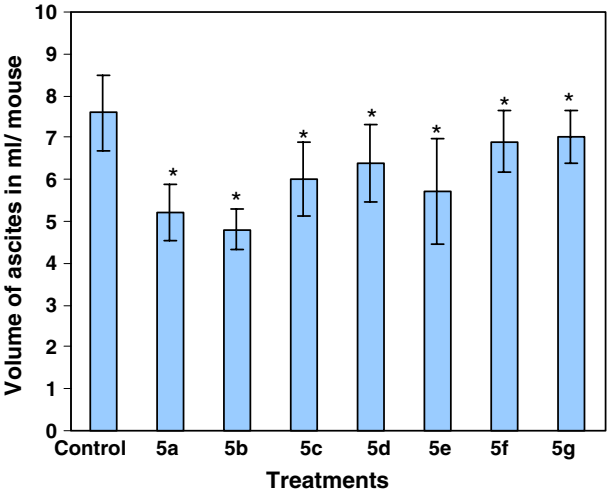


Fig. 1 Effect of the compounds **5(a–g)** on ascites volume of EAT bearing mice. The bar graph represents the effect of the compounds on ascites volume. All treatments showed a significant decrease in ascites volume from that of control ($P < 0.05$). The error bars represent standard deviation of the mean

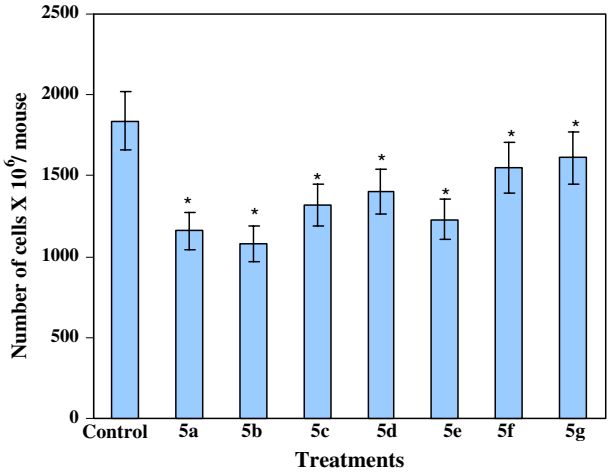


Fig. 2 Effect of the synthetic compounds **5(a–g)** on cell number of EAT-bearing mice. The bar graph represents the effect of the compounds on cell number. All treatments showed a significant decrease in cell number compared with control ($P < 0.05$). The error bars represent standard deviation of the mean

The inhibitory effect of thiazolidinone analogs **5(a–g)** on EAT cells in vivo was evaluated in terms of volume of ascites and total number of cells in mice treated with vehicle or compounds. The mean value of ascites volume and cell number in control animals was found to be 7.6 ± 0.9 ml (Fig. 1) and 1,840 ± 1.62 × 10^6 cells/mouse, respectively (Fig 2). Among the compounds **5(a–g)** treatment, the best response was obtained with the compound **5b**. This compound decreased the mean ascites volume to 4.8 ± 0.49 ml with corresponding reduction of mean cell number to 1,080 ± 0.79 × 10^6 cells/mouse. The compounds **5a** and **5e** showed mean ascites volume of 5.2 ± 0.68 ml and 5.7 ± 1.26 ml, respectively. The compound **5c** and **5d** were equally effective in reducing ascites volume and cell number. Although the compounds **5f** and **5g** were ineffective in prolonging the life span of

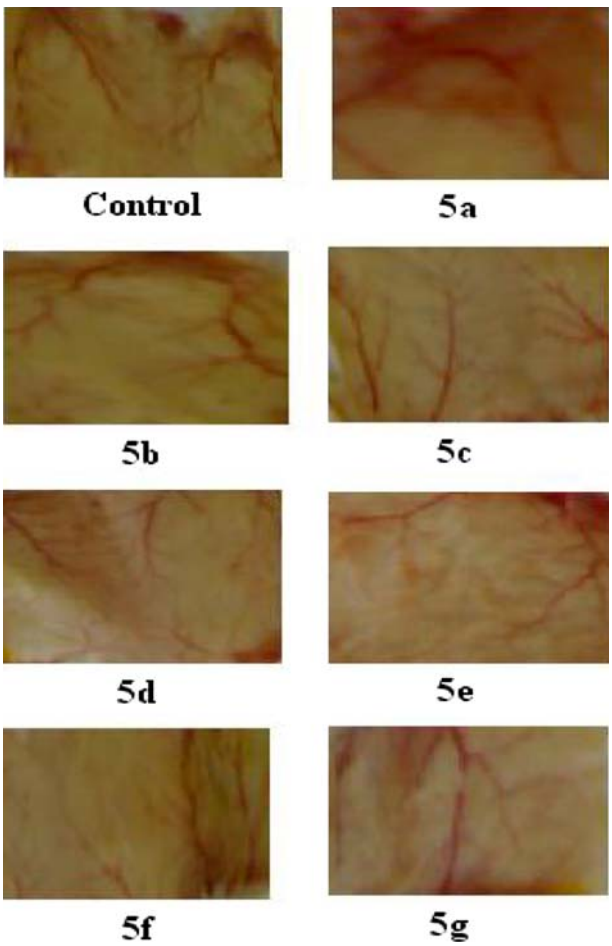


Fig. 3 Suppression of in vivo angiogenesis by synthetic compounds **5(a–g)**. Peritoneal lining of tumor bearing mice treated with vehicle (0.1% DMSO) and thiazolidinone analogs were inspected for anti-angiogenesis effects. Inhibition of angiogenesis was more prominent in **5(a–g)**-treated mice compared with control

EAT-bearing mice, **5f** showed significant decrease in mean value of ascites volume and cell number (Figs. 1 and 2).

Examination of tumor induced peritoneal endothelial proliferation of EAT-bearing mice is a reliable model of in vivo angiogenesis. It is evident from the results that more number of blood vessels were seen in the peritoneum of control EAT-bearing mice compared with **5(a–g)**-treated mice, which demonstrated reduced vasculature (Fig. 3).

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