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ORIGINAL ARTICLE

Synthesis of thiazolidine-2,4-dione derivatives: anticancer, antimicrobial and DNA cleavage studies

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Abstract In the search of efficient anticancer agents, here, new 5-(4-alkylbenzyledene)thiazolidine-2,4-dione derivatives (**5a–g**) have been successfully synthesized and characterized and are evaluated for anticancer and antimicrobial activities using DNA cleavage studies. In vitro studies on anticancer activity of compound **5d** (NSC: 768619/1) was done against the full panel of 60 human tumor cell lines. The five-level dose activity results revealed that, the compound **5d** was active against all the cell lines, it has shown potential activity against leukemia SR (GI_{50}: 2.04 μM), non-small cell lung cancer NCI-H522 (GI_{50}: 1.36 μM), colon cancer COLO 205 (GI_{50}: 1.64 μM), CNS cancer SF-539 (GI_{50}: 1.87 μM), melanoma SK-MEL-2 (GI_{50}: 1.64 μM), ovarian cancer OVCAR-3 (GI_{50}: 1.87 μM), renal cancer RXF 393 (GI_{50}: 1.15 μM), prostate cancer PC-3 (GI_{50}: 1.90 μM), and breast cancer MDA-MB-468(GI_{50}: 1.11 μM). DNA cleavage studies revealed that at 50 μg/mL concentration, partial DNA digestion was observed and when the concentration is increasing to threefold (150 μg/mL), complete linear DNA digestion and partial supercoiled DNA digestion was observed. Further antimicrobial studies indicate that all the synthesized compounds except compound **5a** possess prominent activity against all the screened microbial species. This study throws a ray of light in the field of anticancer drugs.

Keywords Anticancer activity · Antimicrobial activity · DNA cleavages studies · 4-hydroxybenzyledenethiazolidine-2 · 4-dione · Cancer

Introduction

Cancer is one of the world's most serious illnesses; every ten in a hundred people are suffering from cancer [1]. Clinically, many chemotherapeutic drugs provide a satisfactory response when they are first exposed to the tumors, but they cause a variety of side effects to the patients. Therefore, there is an urgent need for potential, selective anticancer drugs in modern oncology [2]. On the other hand, typhoid, cholera, and pneumonia are common worldwide bacterial diseases caused by Gram-negative bacteria. When comparing Gram-positive and Gram-negative bacteria, many species of Gram-negative bacteria are pathogenic. This pathogenic capability is usually associated with certain components of Gram-negative cell walls, in particular the lipopolysaccharide (also known as LPS or endotoxin) layer [3]. If the endotoxin enters the circulatory system, it causes a toxic reaction; thus, outer membrane protects the bacteria from several antibiotics, dyes, and detergents that would normally damage the inner membrane or cell wall (peptidoglycan). The outer membrane also provides these bacteria with resistance to lysozyme and penicillin; therefore, drugs which possess a lipophilic nature can damage lipopolysaccharide layer. Larger alkyl groups when introduced into the drug will increase hydrophobicity as well as biological activity [4–6]. Drug binding causes structural and

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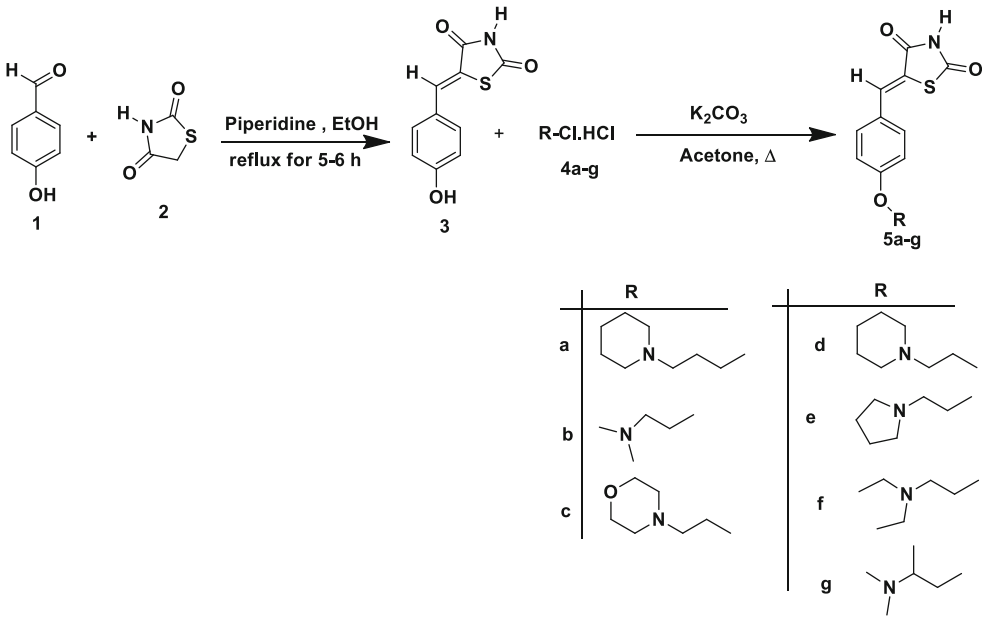
conformational changes in the DNA such as DNA bending and winding double or single strand breaks resulting in DNA damage, which inhibits DNA transcription and replication [7, 8]. In order to treat diseases like those which are mentioned above, many potential drugs are designed to target DNA [9]. 2,4-Thiazolidinedione is one of the important pharmacophores in many in vivo studies on thiazolidinedione derivatives proved they have the capacity to reduce the plasma glucose levels. Besides their antidiabetic potency, 2,4-thiazolidinediones suppress the growth of several cancer cell lines including the colon, breast, and prostate in vivo and in vitro [10, 11]. Romeo Romagnoli et al. reported anticancer activity of 5-benzylidene thiazolidine-2,4-dione derivatives (0.19 to 3.2 μM) against murine leukemia (L1210), murine mammary carcinoma (FM3A), human T lymphocyte (CEM), and human cervix carcinoma (HeLa) cells [12]. In another report, a series of 5-acridin-9-ylmethylene-3-benzyl-thiazolidine-2,4-dione analogs with general structure 2, with a moderate antiproliferative activity (IC50: 4.1–58 μM) against a wide panel of cancer cell lines [13]. On the other hand, huge number of literature reports are available on antimicrobial activity of 2,4-thiazolidinedione derivatives [14, 15]. Recent patent literature discloses (Z)-5-decylidenethiazolidine-2,4-dione as a good antifungal against *Candida albicans* [16]. Very recently, Singanan Ponnuchamy et al. identified the antimycobacterial activity of novel hybrid arylidene thiazolidine-2,4-diones [17].

Inspired by the wide range of useful activities of the 2,4-thiazolidinedione derivatives, [18–20] efforts are made to explore the potential biological activities of various heterocyclic compounds We have synthesized and studied their anticancer, antimicrobial, and DNA cleavage activities.

Results and discussion

Chemistry

The preparation of 5-(4-alkylbenzyledene) thiazolidine-2,4-dione derivatives is outlined in Scheme 1. The compound 4-hydroxybenzyledenethiazolidines-2,4-dione (**3**) was obtained by the knoevenagel condensation of 4-hydroxybenzaldehyde with 2,4-thiazolidinedione as described in earlier reports [21]; formation of the intermediate was confirmed by the ^{1}H NMR spectral data, 5-methylidene proton signal was displayed in the range 7.7–7.8 ppm as singlet, and NH proton was observed at 12.48 as a broad singlet; these observations were in full agreement with the previous literature reports [22–24]. Further, the reaction of tertiary alkyl amino chlorohydrochlorides (**4a**–**g**) with 4-hydroxybenzyledenethiazolidines-2,4-dione (**3**) in acetone and backed K_2CO_3 and under reflux conditions produced 5-(4-alkylbenzyledene)thiazolidine-2,4-dione derivatives in good yields. The assignment of structure for compounds (**5a**–**g**) was supported by IR, mass, and NMR spectral studies. Melting points were determined in open capillaries using Stuart SMP30 apparatus and are uncorrected. The progress of the reactions as well as purity of the compounds was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets using hexane/ethyl acetate (7/3) as eluent. IR spectra were recorded on Perkin-Elmer 100S spectrophotometer using KBr pellet. NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Elemental analyses were performed on a Carlo Erba modal EA1108 and mass spectra were recorded on a Jeol JMSD-300 spectrometer.



Scheme 1 Synthesis of 5-(4-alkylbenzyledene)thiazolidine-2,4-dione derivatives (**5a**–**g**)

Table 1 In vitro antibacterial activity of thiazolidine2,4 dione derivatives (**5a**–**g**)

Zone of inhibition in mm							
S. no	Compound	V.c.	K.p	S.a.	C.a.	S.t.	E.c.
1	5a	–	–	–	17	21	22
2	5b	16	11	14	18	16	12
3	5c	20	21	21	21	22	27
4	5d	19	–	11	20	14	23
5	5e	13	14	18	19	20	22
6	5f	15	–	10	16	13	15
7	5g	22	21	19	22	12	28
8	Am	–	–	38	–	–	–
9	Ka	39	37	–		40	15
10	Kt	–	–	–	28	–	–

Ampicillin (10 μg/disk), kanamycin (30 μg/disk), and ketoconazole (25 μg/disk) were used as positive references. Compounds 5a–g (100 μg/disk)

Am ampicillin, *Ka* kanamycin, *Kt* ketoconazole, *V.c. Vibrio cholera*, *K.p. Klebsiella pneumoniae*, *S.a. Staphylococcus aureus*, *C.a. Candida albicans*, *S.t. Salmonella typhi*, *E.c. Escherichia coli*

Biological evaluation

Antimicrobial activities

The in vitro antimicrobial activity was performed using the disk diffusion method, (Supplementary File) against Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli*, *Vibrio cholera*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Candida albicans* as fungus. Ampicillin and kanamycin were used as positive controls for bacteria and ketoconazole for fungi. The screening was performed according to the standard procedure [25, 26]. In view of the highly pathogenic nature of Gram-negative bacteria, we evaluate the antimicrobial activity on more number of Gram-negative bacteria than Gram-positive bacteria. Zone of inhibition values of the compounds (**5a**–**g**) and the standards are presented in Table 1. From the antimicrobial data, we observed that except **5a**, all the compounds (**5b**–**g**) possess activity at 100 μg/disk on both bacterial and fungal species. Meltem ceylan unlusoy et al. reported the synthesis and antimicrobial activity of 2,4-thiazolidione derivatives at 3000 μg/mL [27]. Our newly synthesized compounds antimicrobial activity is satisfactory than their results.

Anticancer activity

In vitro anticancer activity was carried out at National Cancer Institute, Bethesda, USA [28]. Among all the compounds, **5a**, **5c**, **5d**, and **5f** were selected and initially screened at a single high dose of 10^{-5} M concentration. The entire 60 human cancer cell lines were organized into nine sub-panels derived from

Table 2 Growth percent and growth inhibition (GI %) in single dose assay (10^{-5} M) for compound **5a**

Panel/cell line	Growth percent	Growth inhibition (GI %)
Leukemia		
CCRF-CEM	90.38	9.62
HL-60 (TB)	91.76	8.24
K-562 0.99	84.80	15.2
MOLT-4	97.97	2.03
RPMI-8226	96.32	3.68
SR	83.79	16.21
Non-small cell lung cancer		
A549/ATCC	101.37	−1.37
HOP-62	94.81	5.19
HOP-92	75.24	24.76
NCI-H226	94.34	5.66
NCI-H23	98.96	1.04
NCI-H322M	96.54	3.46
NCI-H460	97.69	2.31
NCI-H522	103.10	−3.1
Colon cancer		
COLO 205	104.40	−4.4
HCC-2998	106.34	−6.34
HCT-116	91.74	8.26
HCT-15	93.21	6.79
HT29	99.97	0.03
KM12	102.68	−2.68
SW-620	98.44	1.56
CNS Cancer		
SF-268	95.09	4.91
SF-295	96.60	3.4
SF-539	99.44	0.56
SNB-19	95.40	4.6
SNB-75	86.07	13.93
U251	101.27	−1.27
Melanoma		
LOX IMVI	83.72	16.28
MALME-3M	93.97	6.03
M14	87.81	12.19
MDA-MB-435	98.19	1.81
SK-MEL-2	107.85	−7.85
SK-MEL-28	100.86	−0.86
SK-MEL-5	100.35	−0.35
UACC-257	92.69	7.31
UACC-62	92.49	7.51
Ovarian cancer		
IGROV1	100.47	−0.47
OVCAR-3	98.33	1.67
OVCAR-4	96.51	3.49
OVCAR-5	92.07	7.93
OVCAR-8	103.09	−3.09

Table 2 (continued)

Panel/cell line	Growth percent	Growth inhibition (GI %)
NCI/ADR-RES	107.07	−7.07
SK-OV-3	84.50	15.5
Renal cancer		
786-0	89.50	10.5
A498	65.18	34.82
ACHN	87.17	12.83
CAKI-1	97.99	2.01
RXF 393	108.84	−8.84
SN12C	93.20	6.8
UO-31	82.72	17.28
Prostate cancer		
PC-3	90.28	9.72
DU-145	113.92	−13.92
Breast cancer		
MCF7	108.29	−8.29
MDA-MB-231/ATCC	89.17	10.83
BT-549	90.00	10.00
T-47D	80.32	19.68
MDA-MB-468	105.27	−5.27

nine different human cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Output from the single-dose screen is reported as a graph of mean growth percent of the treated cells (Supplementary file). From the graph, both growth inhibition values (between 0 and 100) and cytotoxicity values (less than 0) can be detected. The results were analyzed by COMPARE program [29]. The percentage growth inhibition (GI %) of the treated cells at 10^{-5} M concentration of compounds **5a**, **5f**, **5d**, and **5c** are presented in **(**Tables 2, 3, 4, and 5).

Among the four compounds selected for the first dose, compound **5d** has shown significant growth inhibition against a variety of cell lines at a single dose of 10^{-5} M concentration and it has been further evaluated for five dose screening at five different minimal concentrations against 60 full cell lines. Dose-response curves of compound **5d** were created by plotting cytotoxic effect against the $\log_{10}$ of the drug concentration for each cell line (Fig. 1**;** Supplementary data). Cytotoxic effects of each compound were determined as GI_{50}, TGI, and LC_{50} values, which represent the molar drug concentration required to cause 50 % growth inhibition, concentration required to cause total growth inhibition, and the concentration that kills 50 % of the cells, respectively. The compound **5d** has exhibited broad spectrum of growth inhibition activity against nine tumor cell lines with average GI_{50} values (MGMID) 1.18–2.44 μM namely, leukemia SR (GI_{50}: 2.04 μM), non-small cell lung cancer NCI-H522 (GI_{50}: 1.36 μM), colon cancer COLO 205 (GI_{50}: 1.64 μM), CNS cancer SF-539 (GI_{50}:

Table 3 Growth percent and growth inhibition (GI %) in single dose assay (10^{-5} M) for compound **5f** (NSC: 768618/1)

Panel/cell line	Growth percent	Growth inhibition (GI %)
Leukemia		
CCRF-CEM	92.91	7.09
HL-60 (TB)	100.43	−0.43
K-562 0.99	86.27	13.73
MOLT-4	97.05	2.95
RPMI-8226	98.02	1.98
SR	96.99	3.01
Non-small cell lung cancer		
A549/ATCC	101.06	−1.06
HOP-62	85.16	14.84
HOP-92	85.37	14.63
NCI-H226	110.35	−10.35
NCI-H23	108.79	−8.79
NCI-H322M	93.69	6.31
NCI-H460	99.75	0.25
NCI-H522	106.49	−6.49
Colon cancer		
COLO 205	104.69	−4.69
HCC-2998	103.42	−3.42
HCT-116	102.17	−2.17
HCT-15	97.61	2.39
HT29	99.92	0.08
KM12	103.18	−3.18
SW-620	102.06	−2.06
CNS cancer		
SF-268	97.33	2.67
SF-295	90.77	9.23
SF-539	98.86	1.14
SNB-19	98.19	1.81
SNB-75	85.90	14.1
U251	103.08	−3.08
Melanoma		
LOX IMVI	92.59	7.41
MALME-3M	101.09	−1.09
M14	103.05	−3.05
MDA-MB-435	94.31	5.69
SK-MEL-2	101.81	−1.81
SK-MEL-28	101.85	−1.85
SK-MEL-5	104.79	−4.79
UACC-257	100.41	−0.41
UACC-62	99.69	0.31
Ovarian cancer		
IGROV1	103.17	−3.17
OVCAR-3	95.04	4.96
OVCAR-4	102.79	−2.79
OVCAR-5	110.45	−10.45
OVCAR-8	109.10	−9.1

Table 3 (continued)

Panel/cell line	Growth percent	Growth inhibition (GI %)
NCI/ADR-RES	84.60	15.4
SK-OV-3		
Renal cancer		
786-0	99.55	0.45
A498	75.79	24.21
ACHN	102.38	−2.38
CAKI-1	97.43	2.57
RXF 393	113.31	−13.31
SN12C	99.13	0.87
UO-31	88.54	11.46
Prostate cancer		
PC-3	101.27	−1.27
DU-145	103.86	−3.86
Breast cancer		
MCF7	100.07	−0.07
MDA-MB-231/ATCC	107.50	−7.50
BT-549	98.58	1.42
T-47D	93.68	6.32
MDA-MB-468	115.87	−15.87

1.87 μM), melanoma SK-MEL-2 (GI_{50}: 1.64 μM), ovarian cancer OVCAR-3 (GI_{50}: 1.87 μM), renal cancer RXF 393 (GI_{50}: 1.15 μM), prostate cancer PC-3 (GI_{50}: 1.90 μM), and breast cancer MDA-MB-468 (GI_{50}: 1.11 μM) cell lines **(**Table 6**)**. Out of these nine different cell lines, compound **5d** was highly active on breast cancer MDA-MB-468 (GI_{50}: 1.11 μM) cell lines. These findings may have an impact on further investigations in this field in search of potent anticancer agents.

DNA cleavage studies

DNA cleavage studies were analyzed by agarose gel electrophoresis method [30]. Test samples (1 mg/mL) were prepared in DMF. The samples (25 μg) were added to the isolated pUC-19 plasmid. The samples were incubated for 2 h at 37 °C and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for 2 h. Removed the gel and stained with 10 μg/mL ethidium bromide for 10–15 min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard DNA marker.

Table 4 Growth percent and growth inhibition (GI %) in single dose assay (10^{-5} M) for compound **5d** (NSC: 768619/1)

Panel/cell line	Growth percent	Growth inhibition (GI %)
Leukemia		
CCRF-CEM	−12.19	Cytotoxic
HL-60 (TB)	8.72	91.28
K-562 0.99	5.25	94.75
MOLT-4	4.20	95.80
RPMI-8226	19.74	80.26
SR	1.70	98.30
Non-small cell lung cancer		
A549/ATCC	3.44	96.56
HOP-62	11.22	88.78
HOP-92	−13.48	Cytotoxic
NCI-H226	16.17	83.83
NCI-H23	6.57	93.43
NCI-H322M	41.47	58.53
NCI-H460	29.71	70.29
NCI-H522	−23.00	Cytotoxic
Colon cancer		
COLO 205	−97.93	Cytotoxic
HCC-2998	41.62	58.38
HCT-116	6.47	93.53
HCT-15	11.35	88.65
HT29	15.01	84.99
KM12	17.59	82.41
SW-620	8.71	91.29
CNS cancer		
SF-268	53.95	46.05
SF-295	21.96	78.04
SF-539	45.27	54.73
SNB-19	53.63	46.37
SNB-75	40.50	59.50
U251	10.79	89.21
Melanoma		
LOX IMVI	−51.42	Cytotoxic
MALME-3M	−2.39	Cytotoxic
M14	28.77	71.23
MDA-MB-435	20.79	79.21
SK-MEL-2	5.71	94.29
SK-MEL-28	50.13	49.87
SK-MEL-5	49.54	50.46
UACC-257	36.39	63.61
UACC-62	28.96	71.04
Ovarian cancer		
IGROV1	35.88	64.12
OVCAR-3	12.68	87.32
OVCAR-4	0.26	99.74
OVCAR-5	76.42	23.58
OVCAR-8	−2.72	Cytotoxic
NCI/ADR-RES	32.37	67.63
SK-OV-3	71.48	28.52
Renal cancer		
786-0	22.97	77.03
A498	−10.26	Cytotoxic
ACHN	23.81	76.19
CAKI-1	0.59	99.41
RXF 393	28.62	71.38
SN12C	45.54	54.46
UO-31	−36.14	Cytotoxic
Prostate cancer		
PC-3	−6.12	Cytotoxic
DU-145	27.90	72.10

Table 4 (continued)

Panel/cell line	Growth percent	Growth inhibition (GI %)
Breast cancer		
MCF7	16.17	83.83
MDA-MB-231/ATCC	26.77	73.23
BT-549	32.26	67.74
T-47D	20.44	79.56
MDA-MB-468	29.54	70.46

The DNA cleavage activities of the compounds **5a**–**g** are presented in (Figs. 2 and 3). It was observed that control DNA is having three forms of DNA (form I, II, and III) in the presence of 5 mM $FeSO_4$ the complete DNA cleavage was observed; however, compounds **5a**–**g** partially cleaved the DNA. The observations made in DNA binding study of synthesized compounds interacting with *E. coli* DNA reveal the significant intercalative mode of interaction of the compounds was observed; concentration and integrity of control are much better than screened compounds. At 50 μg/mL concentration, compounds **5a** and **5f** possess less DNA cleavage, partial cleavage was observed for other series of compounds, with the increasing the concentration to three-fold (150 μg/mL) complete linear DNA (form III) cleavage and partially cleavage was observed on supercoiled DNA (form I).

DNA cleavage studies of all the synthesized compounds were correlating with the antimicrobial activity of the compounds, exclusively compound **5a** partially cleave the DNA and it was found that analog **5a** possess less antimicrobial activity, where as compounds **5b**–**5e** possessed marked antimicrobial activity and as well as DNA cleavage activity. It was observed that antimicrobial activity of these compounds may be due to the DNA cleavage.

Materials and methods

All the reagents were procured from Aldrich/Merck and used without further purification. Melting points were determined in open capillaries using Stuart SMP30 apparatus and are uncorrected. The progress of the reactions as well as purity of the compounds was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets using hexane/ethyl acetate (7/3) as eluent. IR spectra were recorded on Perkin-Elmer 100S spectrophotometer using KBr pellet. NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Elemental analyses were performed on a Carlo Erba modal EA1108 and mass spectra were recorded on a Jeol JMSD-300 spectrometer.

Table 5 Growth percent and growth inhibition (GI %) in single dose assay (10^{-5} M) for compound **5c** (NSC: 768620/1)

Panel/cell line	Growth percent	Growth inhibition (GI %)
Leukemia		
CCRF-CEM	95.54	4.46
HL-60 (TB)	93.90	6.10
K-562 0.99	81.87	18.13
MOLT-4	100.53	−0.53
RPMI-8226	100.44	−0.44
SR	95.93	4.07
Non-small cell lung cancer		
A549/ATCC	95.82	4.18
HOP-62	98.47	1.53
HOP-92	75.62	24.38
NCI-H226	101.10	−1.10
NCI-H23	94.48	5.52
NCI-H322M	91.07	8.93
NCI-H460	97.41	2.59
NCI-H522	73.97	26.03
Colon cancer		
COLO 205	102.06	−2.06
HCC-2998	93.76	6.24
HCT-116	93.96	6.04
HCT-15	102.68	−2.68
HT29	101.92	−1.92
KM12	109.03	−9.03
SW-620	102.63	−2.63
CNS cancer		
SF-268	109.99	−9.99
SF-295	92.84	7.16
SF-539	104.02	−4.02
SNB-19	97.78	2.22
SNB-75	76.83	23.17
U251	96.49	3.51
Melanoma		
LOX IMVI	76.23	23.77
MALME-3M	89.06	10.94
M14	88.68	11.32
MDA-MB-435	93.83	6.17
SK-MEL-2	95.44	4.56
SK-MEL-28	99.08	0.92
SK-MEL-5	98.06	1.94
UACC-257	90.81	9.19
UACC-62	99.94	0.06
Ovarian cancer		
IGROV1	107.86	−7.86
OVCAR-3	103.95	−3.95
OVCAR-4	92.91	7.09
OVCAR-5	96.34	3.66
OVCAR-8	104.37	−4.37
NCI/ADR-RES	91.23	8.77
SK-OV-3	83.67	16.33
Renal cancer		
786-0	93.57	6.43
A498	53.05	46.95
ACHN	97.01	2.99
CAKI-1	89.40	10.60
RXF 393	120.96	−20.96
SN12C	99.31	0.69
UO-31	80.26	19.74
Prostate cancer		
PC-3	91.36	8.64
DU-145	110.77	−10.77
Breast cancer		
MCF7	102.21	−2.21
MDA-MB-231/ATCC	101.62	−1.62

Table 5 (continued)

Panel/cell line	Growth percent	Growth inhibition (GI %)
BT-549	89.72	10.28
T-47D	92.58	7.42
sMDA-MB-468	107.26	−7.26

General synthetic procedure for the preparation of compounds (5a–g)

A mixture of 4-hydroxybenzyledenethiazolidines-2,4-dione (**3**) (0.3 g, 1.3 mM) and each of the tertiary alkylamino chloro hydrochloride derivative (1.3 mM) (**4a**–**g**) in acetone (10 mL) containing backed K_2CO_3 (0.54 g, 3.9 mM) were refluxed for 5–6 h. After this time, the mixture was poured onto crushed ice. The precipitate thus obtained was filtered and washed with water and recrystalized from a mixture of ethanol and acetic acid.

Spectral data of compounds **5a**–**g**:

(5-(4-(3-piperidin-1yl)-propoxy)benzylidene)hiazolidine-2,4-dione (**5a**):

White solid, Yield 73 %, M. P 255–260 °C; IR (KBr, υ_{max}, cm^{-1}): 3380, 3054, 2936, 1732, 1696, 1539, 1442, 1299, 1202; ^{1}H NMR (300 MHz, DMSO-d_6 δ ppm): 1.30 (m, 6H), 1.70–1.73 (t, *J* = 4.5 Hz, 2H), 2.24 (m, 4H), 2.51 (m, 2H), 3.69 (m, 2H), 6.92 (d, *J* = 8.4 Hz 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.81 (s, 1H), 10.2 (s, 1H); ^{13}C NMR(75 MHz, DMSO-d_6 δ ppm): 20.71 (CH_2), 23.60(CH_2), 24.03 (CH_2), 25.49 (CH_2), 53.97 (2 x CH_2), 56.16 (CH_2), 59.71(CH_2), 116.3(2 x CH), 123.8 (C), 132.1(C) 132.7(CH), 133.7(2 x CH), 160.1(C) 165.9 (C = O), 167.4 (C = O); MS ESI (M^{+1})*:* 346.8 (30 %). For the M. F $C_{18}H_{22}N_2O_3S$, M. Wt 346.1; Elemental analysis: Anal. Calcd for $C_{18}H_{22}N_2O_3S$: C %, 62.40; H %, 6.40; N %, 8.09. Found C %, 62.49; H %, 6.35; N %, 8.16.

5-(4-(2-(dimethylamino)ethoxy)benzylidene)thiazolidine-2,4-dione (**5b**): *White solid,* Yield 77 %, M. P 245–250 °C*;* IR (KBr, υ_{max}, cm^{-1}): 3410, 3027, 2927, 1738, 1689, 1550, 1460, 1270, 1215; ^{1}H NMR (300 MHz, DMSO-d_6 δ ppm): 2.12 (s, 6H), 2.46 (t, 2H), 3.73 (t, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.85 (s, 1H), 10.35 (s, 1H); ^{13}C NMR(75 MHz, DMSO-d_6 δ ppm): 45.0 (CH_3), 45.3 (CH_3), 57.3(CH_2), 65.9(CH_2), 115.3(C), 116.3(2xCH), 123.8(C), 132.2 (2xCH), 132.9(CH), 160.1(C), 165.7(C = O), 167.3(C = O); MS ESI (M^{+1})*:* 293 (80 %) For the M. F $C_{14}H_{16}N_2O_3S$, M. Wt 292; Elemental analysis: Anal. Calcd for $C_{14}H_{16}N_2O_3S$: C %, 57.52; H % , 5.52; N %, 9.58. Found C %, 57.64; H %, 5.41; N %, 9.67.

5-(4-(2-morpholinoethoxy)benzylidene)thiazolidine-2,4-dione (**5c**): White solid, Yield 70 %, M. P 250–255 °C; *^{1}H-NMR (300 MHz, DMSO-d_6 δ ppm):* 2.39 (m, 4H), 2.53 (m, 4H), 3.51 (m, 2H), 3.89 (t, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.89 (s,

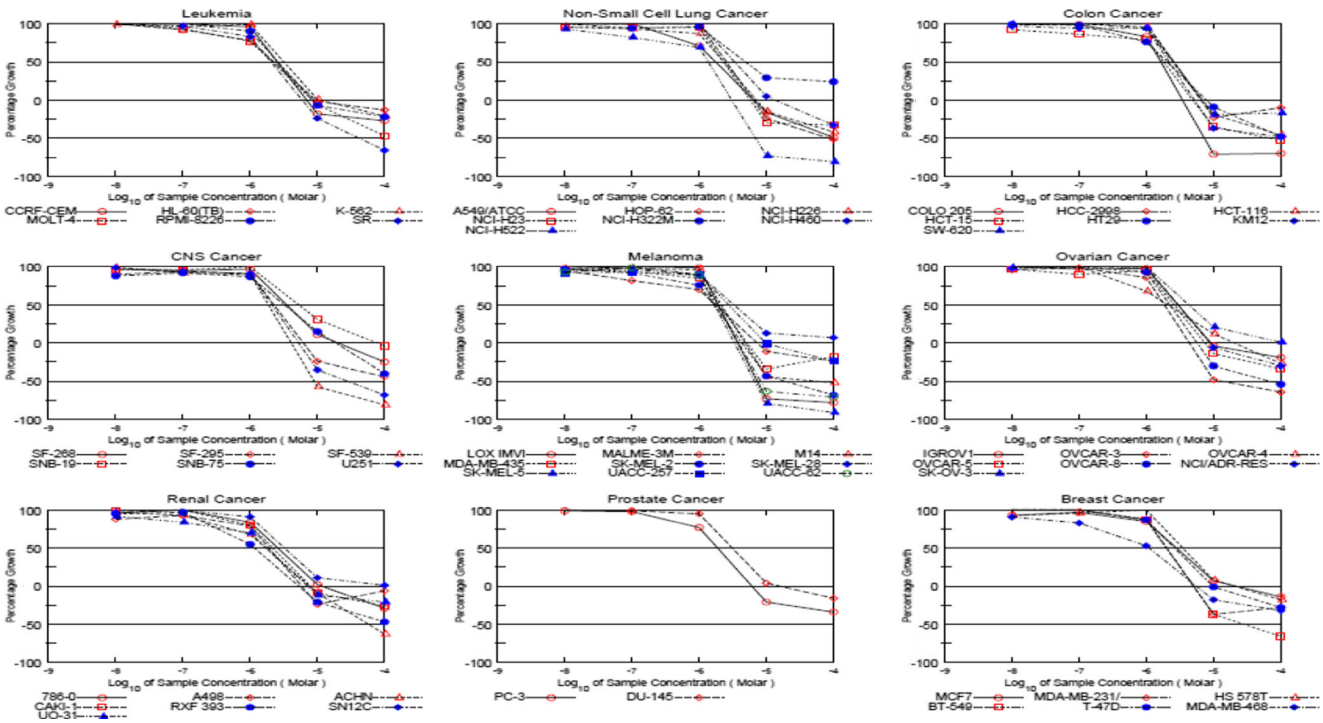


Fig. 1 Five dose-response curves of nine sub-panel cell lines for compound **5d**

Table 6 GI_{50}, TGI, and LC_{50} values of compound **5d** (five-dose level) against 60 human cancer cell lines

Panel/cell line	GI_{50} (μM)	MGMID (μM)	TGI (μM)	LC_{50} (μM)
Leukemia				
CCRF-CEM	2.53		6.91	>100
HL-60 (TB)	2.23		9.43	>100
K-562	3.14	2.43	10.8	>100
MOLT-4	2.10		8.53	>100
RPMI-8226	2.57		8.48	>100
SR	2.04		5.94	41.3
Non-small cell lung cancer				
A549/ATCC	1.74		6.55	>100
HOP-62	2.86		6.66	86.2
NCI-H226	2.29		7.11	>100
NCI-H23	2.30	2.68	5.76	>100
NCI-H322M	4.87		>100	>100
NCI-H460	3.38		13.8	>100
NCI-H522	1.36		3.05	6.84
Colon cancer				
COLO 205	1.64		3.45	7.28
HCC-2998	2.89		6.78	>100
HCT-116	2.50		6.85	>100
HCT-15	1.79	2.21	4.90	66.5
HT29	2.03		7.91	>100
KM12	2.16		5.22	>100
SW-620	2.46		6.81	>100
CNS cancer				
SF-268	3.50		20.0	>100
SF-295	2.26		6.17	>100
SF-539	1.87	3.07	4.09	8.96
SNB-19	5.42		76.2	>100
SNB-75	3.27		18.8	>100
U251	2.12		5.31	29.2
Melanoma				
LOX IMVI	1.93		3.77	7.35
MALME-3M	1.77		7.27	>100
M14	2.12		4.81	49.4
MDA-MB-435	1.97		5.21	>100
SK-MEL-2	1.64	2.10	4.32	18.5
SK-MEL-28	3.30		>100	>100
SK-MEL-5	1.70		3.38	6.73
UACC-257	2.72		9.68	>100
UACC-62	1.80		3.85	8.23
Ovarian cancer				
IGROV1	2.94		9.14	>100
OVCAR-3	1.87		4.39	13.4
OVCAR-4	2.08		20.6	>100
OVCAR-5	2.59	2.77	7.39	>100
OVCAR-8	2.22		5.65	70.0
NCI/ADR-RES	3.03		8.74	>100
SK-OV-3	4.70		>100	>100
Renal cancer				
786-0	2.61		11.6	>100
A498	1.91		5.89	>100
ACHN	1.70		7.58	57.7
CAKI-1	2.18	2.08	7.86	>100
RXF 393	1.15		5.27	>100
SN12C	3.26		>100	>100
UO-31	1.76		7.18	>100
Prostate cancer				
PC-3	1.90	2.51	6.09	>100
DU-145	3.12		16.0	>100
Breast cancer				
MCF7	2.83		21.0	>100
MDA-MB-231/ ATCC	2.01		5.04	>100
HS 578T	3.55	2.35	19.9	>100
BT-549	1.99		5.05	28.2
T-47D	2.63		9.74	>100
MDA-MB-468	1.11		5.52	>100

1H), 10.36 (s, 1H); ^{13}C NMR(75 MHz, DMSO-d_6 δ ppm): 53.0(2xCH_2), 54.7(CH_2), 66.1(2xCH_2), 67.5 (CH_2), 116.3(2xCH), 116.5(C), 123.8 (2xCH), 132.5(C), 133.3(CH), 160.0(C), 165.7(C = O), 167.3(C = O); MS ESI (M^{+1}): 335 (100 %). For the M. F $C_{16}H_{18}N_2O_4S$, M. Wt 334; Elemental analysis: Anal. Calcd for $C_{16}H_{18}N_2O_4S$: C %, 57.47; H %, 5.43; N %, 8.38. Found C %, 57.58; H %, 5.37; N %, 8.29.

5-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**5d**): White solid, Yield 80 %, M. P 265–270 °C; IR (KBr, υ_{max}, cm^{-1}): 3380, 3054, 2936, 1732, 1696, 1665, 1539, 1442, 1299, 1202, 933, 749; ^{1}H NMR (300 MHz, DMSO-d_6 δ ppm): 1.42 (m, 6H), 2.32 (m, 4H), 2.46 (t, 2H), 3.73 (t, 2H), 6.92 (d, J = 8.4 Hz 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.82 (s, 1H), 10.40 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6 δ ppm): 23.57 (CH_2), 25.57 (2 x CH_2), 53.87 (2 x CH_2), 54.9 1(CH_2), 60.2 (CH_2), 116.35(2 x CH), 116.53(C), 123.81(C), 132.54(2xCH), 133.27(CH), 160.12(C), 165.71(C = O), 167.24 (C = O); MS ESI (M^{+1}): 333 (100 %). For the M. F $C_{17}H_{20}N_2O_3S$, M. Wt 332; Elemental analysis: Anal. Calcd for $C_{17}H_{20}N_2O_3S$: C %, 61.42; H %, 6.06; N %, 8.43. Found C %, 61.31; H %, 6.18; N %, 8.31.

5-(4-(2-(pyrrolidin-1-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**5e**):

Light yellow solid, Yield 73 %, M. P 270–275 °C; ^{1}H NMR (300 MHz, DMSO-d_6 δ ppm): 1.65 (m, 4H), 2.60 (m, 4H), 2.64 (m, 2H), 3.73 (m, 2H), 6.92 (d, J = 8.4 Hz, 2H), 7.48 (s, J = 8.4 Hz, 2H), 7.89 (s, 1H), 10.50 (s, 1H); ^{13}C NMR

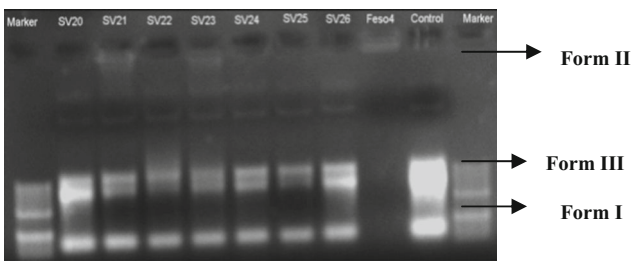


Fig. 2 DNA Cleavages studies of compounds **5a**–**g** at 50 μg/mL concentration. Form I: supercoiled DNA, form II: nicked DNA, form III: linear DNA. Sv20-**5a**, SV2**1-5c**, SV22-**5d**, SV23-**5e**, SV24-**5g**, SV25-**5b**, SV26-**5f**

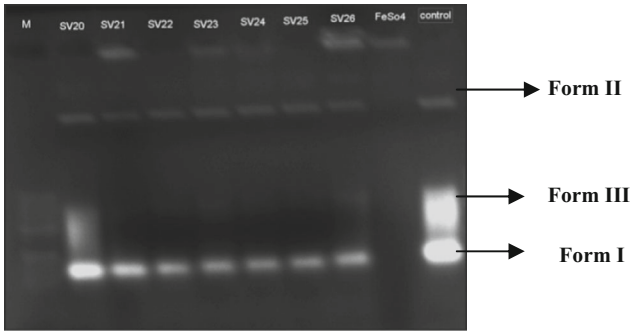


Fig. 3 DNA Cleavages studies of compounds **5a**–**g** at 150 μg/mL concentration. Form I: supercoiled DNA, form II: nicked DNA, form III: linear DNA. Sv20-**5a**, SV21**-5c**, SV22-**5d**, SV23-**5e**, SV24-**5g**, SV25-**5b**, SV26-**5f**

(100 MHz, DMSO-d_6 δ ppm): 23.13(2 x CH_2), 52.37(2 x CH_2), 53.49(CH_2), 60.8 (CH_2), 116.36 (2 x CH), 116.47(C), 123.78(C) 132.56(2 x CH), 133.44(CH), 160.16(C), 165.72, (C = O), 167.30(C = O); MS ESI (M^{+1}): 319 (100 %). For the M. F $C_{16}H_{18}N_2O_3S$, M. Wt 318; Elemental analysis: Anal. Calcd for $C_{16}H_{18}N_2O_3S$: C %, 60.36; H %, 5.70; N %, 8.80. Found C %, 60.25; H %, 5.78; N %, 8.71.

5-(4-(2-(diethylamino)ethoxy)benzylidene)thiazolidine-2,4-dione (**5f**):

White solid, Yield 68 %, M. P 250–255 °C; ^{1}H NMR (300 MHz, DMSO-d_6 δ ppm): 1.23 (m, 6H), 2.34 (m, 4H), 2.48 (t, 2H), 3.83 (t, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.90 (s, 1H), 10.41 (s, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 14.2 (2xCH_3), 49.52, (2 x CH_2), 55.52(CH_2), 69.51(CH_2), 115.72(C), 116.31(2 x CH), 123.82,(C), 132.75 (2 x CH), 133.56 (CH), 160.32(C), 165.82, (C = O), 167.52 (C = O); MS ESI (M^{+1}): 321 (80 %). For the M. F $C_{16}H_{20}N_2O_3S$, M. Wt 320; Elemental analysis: Anal. Calcd for $C_{16}H_{20}N_2O_3S$: C %, 59.98; H %, 6.29; N %, 8.74. Found C %, 59.87; H %, 6.38; N %, 8.81.

5-*(4-(2-(dimethylamino)propoxy)benzylidene)-thiazolidine-2,4-dione* (**5g**):

White solid, Yield 75 %, M. P 225–230 °C; *^{1}H*-NMR (300 MHz, DMSO-d_6 δ ppm): 1.12 (d, 3H), 2.25 (s, 6H), 3.32 (m, 1H), 3.89 (m, 1H), 6.73 (d, *J* = 8.4 Hz, 2H), 7.19 (s, *J* = 8.4 Hz, 2H), 7.84 (s, 2H), 10.30 (s, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 15.2 (CH_3), 49.52, (2 x CH_3), 58.52(CH), 69.51(CH_2), 115.72(C), 16.31(2 x CH), 123.82,(C), 132.75 (2 x CH), 133.56 (CH), 160.32(C), 165.82, (C = O), 167.52 (C = O); MS ESI (M^{+1}): 307 (50 %). For the M. F $C_{15}H_{18}N_2O_3S$, M. Wt 306; Elemental analysis: Anal. Calcd for $C_{15}H_{18}N_2O_3S$: C %, 58.80; H %, 5.92; N %, 9.14. Found C %, 58.89; H %, 5.81; N %, 9.21.

Conclusions

In summary, we have synthesized a new class of 5-(4-alkylbenzyledene)thiazolidine-2,4-dione derivatives (**5a**–**g**) by employing a simple procedure. In our analysis on biological activities, we observed all the compounds displayed marked activity especially analogs **5d** and **5g** has shown potent anticancer activity and antimicrobial activities, respectively. These compounds are better candidates for novel anticancer and antimicrobial agents. We hope this work will contribute to further studies on thiazolidine-2,4-dione derivatives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests. The authors alone hereby stand responsible for the contents of this scientific paper.

Ethical statements This article does not contain any studies with human participants or animals performed by any of the authors.

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