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-alkylbenzylidene)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 (GI50: 2.04 μM), non-small cell lung cancer NCI-H522 (GI50: 1.36 μM), colon cancer COLO 205 (GI50: 1.64 μM), CNS cancer SF-539 (GI50: 1.87 μM), melanoma SK-MEL-2 (GI50: 1.64 μM), ovarian cancer OVCAR-3 (GI50: 1.87 μM), renal cancer RXF 393 (GI50: 1.15 μM), prostate cancer PC-3 (GI50: 1.90 μM), and breast cancer MDA-MB-468 (GI50: 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-hydroxybenzylidenethiazolidine-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
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-decyldenedithiazolines-2,4-dione (3) as a good antifungal agent 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-alkylenzylatedene) thiazolidine-2,4-dione derivatives is outlined in Scheme 1. The compound 4-hydroxybenzylabeledethiazolines-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 1H 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-hydroxybenzylatedethiazolines-2,4-dione (3) in acetone and backed K2CO3 and under reflux conditions produced 5-(4-alkylbenzylenatedene)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 F254 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-d6 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-alkylenzylatedene)thiazolidine-2,4-dione derivatives (5a–g)
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*. Ampicillin (10 μg/disk), kanamycin (30 μg/disk), and ketoconazole (25 μg/disk) were used as positive references. Compounds 5a–g (100 μg/disk) 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 1  In vitro antibacterial activity of thiazolidine2,4 dione derivatives (5a–g)

| 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*

### 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          | 84.80          | 15.2                     |
| MOLT-4         | 0.99           | 19.03                    |
| 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                    |
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].

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$_{50}$: 1.18 μM), melanoma LOX IMVI (GI$_{50}$: 2.04 μM), ovarian cancer IGROV1 (GI$_{50}$: 2.44 μM), and breast cancer MCF7 (GI$_{50}$: 3.02 μM).

### 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                    |

### 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                     |
1.87 μM), melanoma SK-MEL-2 (GI50: 1.64 μM), ovarian cancer OVCAR-3 (GI50: 1.87 μM), renal cancer RXF 393 (GI50: 1.15 μM), prostate cancer PC-3 (GI50: 1.90 μM), and breast cancer MDA-MB-468 (GI50: 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 (GI50: 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 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                  |

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                   |
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 F254 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.
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 K2CO3 (0.54 g, 3.9 mM) was 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 recrystallized 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, νmax, cm⁻¹): 3380, 3054, 2936, 1732, 1696, 1539, 1442, 1299, 1202; ¹H NMR (300 MHz, DMSO-d6 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); ¹³C NMR(75 MHz, DMSO-d6 δ ppm): 20.71 (CH₃), 23.60(CH₂), 24.03 (CH₂), 25.49 (CH₂), 53.97 (2 x CH₂), 56.16 (CH₂), 59.71(CH₂), 116.3 (2 x CH), 123.8 (C), 132.11(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 C₁₈H₂₂N₂O₃S, M. Wt 346.1; Elemental analysis: Anal. Calcd for C₁₈H₂₂N₂O₃S: 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)hiazolidine-2,4-dione (5b): White solid, Yield 77 %, M. P 245–250 °C; IR (KBr, νmax, cm⁻¹): 3410, 3027, 2927, 1738, 1689, 1550, 1460, 1270, 1215; ¹H NMR (300 MHz, DMSO-d6 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); ¹³C NMR(75 MHz, DMSO-d6 δ ppm): 45.0 (CH₃), 45.3 (CH₃), 57.3(CH₂), 65.9(CH₂), 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 C₁₄H₁₆N₂O₃S, M. Wt 292; Elemental analysis: Analy. Calcd for C₁₄H₁₆N₂O₃S: C %, 57.52; H % , 5.52; N %, 9.58. Found C %, 57.64; H %, 5.41; N %, 9.67.

5-(4-(2-morpholinoethoxy)benzylidene)hiazolidine-2,4-dione (5c): White solid, Yield 70 %, M. P 250–255 °C; ¹H-NMR (300 MHz, DMSO-d6 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,
Table 6  GL50, TGI, and LC50 values of compound 5d (five-dose level) against 60 human cancer cell lines

| Panel/cell line | GL50 (μM) | MGMTID (μM) | TGI (μM) | LC50 (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-H232       | 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      | 8.76        | >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      | 5.78        | 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     |           |

Table 6 (continued)

| Panel/cell line | GL50 (μM) | MGMTID (μM) | TGI (μM) | LC50 (μM) |
|----------------|-----------|-------------|----------|-----------|
| Non-small cell lung cancer (continued) | | | | |
| 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     |           |

Light yellow solid, Yield 73 %, M. P 270–275 °C; 1H NMR (300 MHz, DMSO-d6 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); 13C 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, SV21-5c, SV22-5d, SV23-5e, SV24-5g, SV25-5b, SV26-5f
Conclusions

In summary, we have synthesized a new class of 5-(4-alkylbenzylidene)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.

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

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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-d6 δ ppm): 23.13 (2 × CH2), 52.37 (2 × CH2), 53.49(CH2), 60.8 (CH2), 116.36 (2 × CH), 116.47(C), 123.78(C) 132.56(2 × 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 C16H18N2O3S, M. Wt 318; Elemental analysis: Anal. Calcd for C16H18N2O3S: 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; 1H NMR (300 MHz, DMSO-d6 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); 13C-NMR (75 MHz, DMSO-d6): δ 14.2 (2 × CH3), 49.52, (2 × CH2), 55.52(CH2), 69.51(CH2), 115.72(C), 116.31(2 × CH), 123.82(C), 132.75 (2 × 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 C16H18N2O3S, M. Wt 320; Elemental analysis: Anal. Calcd for C16H18N2O3S: C %, 58.80; 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; 1H-NMR (300 MHz, DMSO-d6 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); 13C-NMR (75 MHz, DMSO-d6): δ 15.2 (CH3), 49.52, (2 × CH3), 58.52(CH2), 69.51(CH2), 115.72(C), 161.32 (2 × CH), 123.82(2 × CH), 132.75 (2 × 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 C16H18N2O3S, M. Wt 306; Elemental analysis: Anal. Calcd for C16H18N2O3S: C %, 58.80; H %, 5.92; N %, 9.14. Found C %, 58.89; H %, 5.81; N %, 9.21.
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