Med Chem Res (2012) 21:3214–3223
DOI 10.1007/s00044-011-9876-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

New thiazolidine-2,4-diones as antimicrobial and cytotoxic agent

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Received: 6 May 2011 / Accepted: 4 November 2011 / Published online: 13 November 2011

Abstract New (*Z*)-5-substituted-2,4-thiazolidinediones (**3a**–**m**) were easily prepared by the condensation of thiazolidine-2,4-dione (**1**) with suitable aldehydes (**2a**–**m**) via microwave irradiation technique. The reaction between (*Z*)-5-substituted-2,4-thiazolidinediones and 4-(bromomethyl) benzoic acid, using potassium carbonate as base in refluxing acetone, followed by a workup in acidic medium provided 4-(((*Z*)-5-substituted-2,4-dioxothiazolidin-3-yl)methyl) benzoic acid derivatives (**4a**–**m**). The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectral studies, and elemental analysis. All compounds were evaluated for their in vitro antimicrobial and cytotoxic activities. Antibacterial and antifungal results revealed that most of the compounds showed significant activity where as compounds **4c** and **4g** are found to be broad spectrum antibacterial and antifungal properties, the MIC values were observed in the range of 2–4 and 2–8 μg/ml, respectively. In MTT cytotoxicity studies, the compound **4g** was found most potent. In HeLa, HT29, A549, and MCF-7 cells, the IC_{50} values were observed in the range of 30–36 μM.

Keywords Thiazolidinedione · Antibacterial activity · Antifungal activity · Cytotoxic activity · Drug likeliness

Introduction

The development of new antimicrobial and anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. Cytotoxicity and genotoxicity of anticancer drugs to the normal cells are major problems in cancer therapy and engender the risk of inducing secondary malignancy (Aydemir and Bilaloglu, 2003). A dose of anticancer drug sufficient to kill tumor cells is often toxic to the normal tissue and leads to many side effects, which in turn, limits its treatment efficacy. In recent years, there has been a concerned search for the discovery and development of novel selective antimicrobial and anti-tumor agents, devoid of many of the unpleasant side effects of conventional antimicrobial and antitumor agents. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which are ligand-activated transcription factors. So for, three PPAR isotopes have been reported: PPARα, PPARβ, and PPARγ. Originally, PPAR activity was thought to be limited to lipid metabolism and glucose homeostasis. Later studies showed that PPAR activation regulates inflammatory responses, cell proliferation and differentiation, as well as apoptosis (Houseknecht *et al*., 2002; Sung *et al*., 2004).

Thiazolidine-2,4-dione (TZDs) are a class of insulin sensitizing drugs which include ciglitazone, pioglitazone, and rosiglitazone. Apart from their known antidiabetic activity, the ability of TZDs to contribute to cancer therapy has been evidenced by numerous in vitro and in vivo studies (Takashima *et al*., 2001; Galli *et al*., 2004; Yoshizumi *et al*., 2004; Betz *et al*., 2005; Turturro *et al*., 2004; Shiau *et al*., 2005; Han and Roman 2006; Kaminskyy *et al*., 2009; Li *et al*., 2010; Patil *et al*., 2010), antimicrobial and anticancer (Mohsen *et al*., 1985), antibacterial and antifungal

Electronic supplementary material The online version of this article (doi:10.1007/s00044-011-9876-x) contains supplementary material, which is available to authorized users.

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(Ayhan-Kilcigil and Altanlar, 2000; Heerding *et al*., 2003; Tuncbilek and Altanlar, 2006; Bozdag-Dundar *et al*., 2007; Mentese *et al*., 2009; Alegaon and Alagawadi, 2011) activity. While TZDs are known to stimulate PPAR-γ receptor, they also have multiple PPAR-γ independent effects and the specific role of PPAR-γ activation in the anticancer effects of TZDs is still under investigation. It has been reported that there exists of a 3 order-of-magnitude discrepancy between the concentration required to mediate antitumor effects and that for PPAR-γ activation (Wei *et al*., 2009). Thus, the dose required for anticancer activity of thiazolidine-2,4-diones would be significantly lower than that required to bring hypoglycemic activity. We report herein the synthesis, characterization and investigation of antimicrobial and cytotoxic properties of 4-((-5-benzylidene-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives.

Results and discussion

Chemistry

The synthesis of (*Z*)-4-(5-arylidene-2,4-dioxothizolidin-3-yl) methyl benzoic acids **4a–m** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. The starting material thiazolidine-2,4-dione **1** was prepared according to earlier reported method (Prashanth Kumar *et al*., 2006) with minor modification. Further, the condensation of compound **1** with the appropriate substituted aldehydes, using piperidine as base in refluxing toluene for 15 h, provided compounds **3a–m.** The reaction time was significantly reduced (10 min) by using microwave (MW) irradiation (200 psi, 700 W maximum powers) at 140°C. The introduction of 5-aryl or 5-heteroarylidene moieties provided only *Z* isomers, as already demonstrated by X-ray diffraction studies (Bruno *et al*., 2002; Ottana *et al*., 2005).

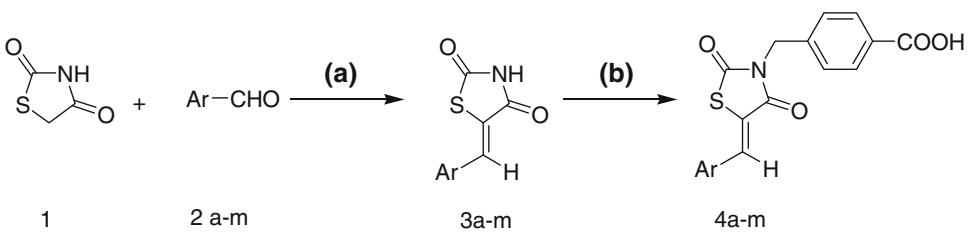
The reaction between (*Z*)-5-arylidene-2,4-thiazolidinediones **3a–m** and 4-(bromomethyl) benzoic acid, using potassium carbonate as base, provided (*Z*)-4-(5-arylidene-2,4-dioxothizolidin-3-yl) methyl benzoic acids **4a–m**. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectral studies, and elemental analysis. The IR spectra of 4-((-5-benzylidene-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives **4a–m** exhibited a very broad absorption band in the region of 3,380–2,570 cm^{-1} attributable to the stretching vibration of carboxylic OH. The ^{1}H NMR spectra revealed that two singlets at the region of δ 4.61–5.02 ppm and δ 7.67–8.12 ppm were assigned to the methylene protons of N-CH_2 and 5-methylidene protons in all title compounds, respectively. The ^{13}C NMR spectral analyses were consistent with the assigned structures. No large differences were found in ^{13}C chemical shift for methylene carbon of N-*C*H$_2$ (δ 43.31–47.38 ppm) and 5-methylidene carbon (δ 130.20–137.77 ppm) in all title compounds. In addition, in ^{13}C NMR spectra, besides two signals due to the resonances of 2-and 4-carbonylic groups of the thiazolidine-2,4-dione ring at δ 164.5–169.8 ppm, another singlet attributable to the resonance of the carboxylic carbon was present in the same range.

Pharmacology

Antibacterial and antifungal activity

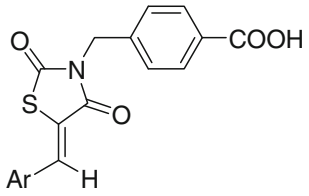
The newly synthesized compounds were evaluated for their in vitro antibacterial activity against *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 25619), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 35550), antifungal activity against *Candida albicans* (ATCC 2091), *Aspergillus flavus* (NCIM No. 524), *Aspergillus niger* (ATCC 6275), and *Cryptococcus neoformans (Clinical isolate)* by using the twofold serial dilution technique (National Committee for Clinical Laboratory Standards, 2006) and results are summarized in Table 1. Ampicillin and ciprofloxacin were used as the reference standard for antibacterial activity while ketoconazole was used as the reference standard for antifungal activity.

In the light, interesting antimicrobial activities of (*Z*)-4-(5-arylidene-2,4-dioxothizolidin-3-yl) methyl benzoic acid derivatives inhibited the growth of bacteria with MIC values ranging between 2 and 128 μg/ml and showed



Scheme 1 Preparation of 4-(((*Z*)-5-substituted-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives **4a–m**: reagents: *a* piperidine, acetic acid, toluene, 110°C for 15 h or MW irradiation 700 W, 10 min, 75–83%; *b* $BrCH_2C_6H_4COOH$, K_2CO_3, acetone, reflux, 48–72 h, 50–62%

Table 1 Results of antibacterial and antifungal activities of compounds **4a–m** [minimum inhibitory concentration (MIC) values in μg/ml]



4a-m

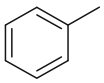
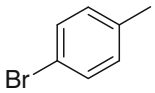
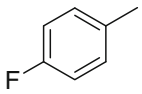
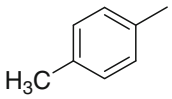
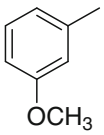
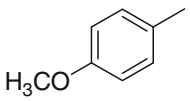
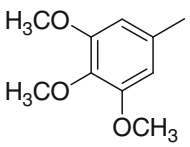
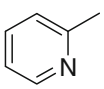
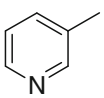
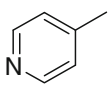
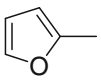
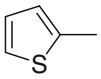
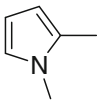
Compound	Ar	*E. c*	*P. a*	*S. a*	*E. f*	*C. a*	*C. n*	*A. f*	*A. n*
4a		4	8	128	64	16	16	32	16
4b		4	4	128	128	16	16	16	32
4c		4	4	2	4	2	4	4	4
4d		16	16	32	32	64	64	32	64
4e		8	8	16	16	32	16	32	32
4f		32	32	16	16	16	16	32	32
4g		8	8	8	8	2	4	4	4
4h		16	16	64	64	32	16	16	32
4i		32	32	128	128	32	32	16	16
4j		16	16	32	16	16	16	32	32

Table 1 continued

Compound	Ar	*E. c*	*P. a*	*S. a*	*E. f*	*C. a*	*C. n*	*A. f*	*A. n*
4k		16	16	32	64	64	64	32	32
4l		8	16	16	32	32	32	8	8
4m		16	16	64	64	32	32	16	16
Amp.		NT	NT	2	2	NT	NT	NT	NT
Cip.		2	2	NT	NT	NT	NT	NT	NT
Ket.		NT	NT	NT	NT	2	2	1	2

E. c Escherichia coli; *P. a Pseudomonas aeruginosa*; *S. a Staphylococcus aureus*; *E. f Enterococcus faecalis*; *C. a Candida albicans*; *C. n Cryptococcus neoformans*; *A. f Aspergillus flavus*; *A. g Aspergillus niger*; *NT* not tested; *Amp* ampicillin; *Cip* ciprofloxacin; *Ket* ketoconazole

antifungal activity with MICs between 2 and 64 μg/ml. According to the antimicrobial studies, most of the compounds showed such activity, albeit lower than their antifungal efficacy. This difference may be due to the differences between the cell structure of bacteria and yeast. While the cell wall of fungi contains chitin, the cell wall of bacteria contains murein (Eweis *et al*., 2006). In addition, fungi contain ergosterol in their cell membranes instead of the cholesterol found in the cell membranes of animals (Kitamura *et al*., 1999). According to antibacterial studies, the efficacy against Gram-negative is higher than Gram-positive bacteria. Compounds **4a**, **4b**, **4c**, **4e**, **4g**, and **4l** showed high activity against *E. coli* and **4a**, **4b**, **4c**, **4e**, and **4g** showed good activity against *P. aeruginosa* as compared to other compounds. Out of 13, 11 compounds showed moderate activity against Gram-positive bacteria. In addition, **4c** and **4g** exhibited broad spectrum activity against all bacterial strains, with MIC values 2–8 μg/ml. Antifungal results indicated that compounds **4c** and **4g** have showed good activity against *C. albicans*, *C. neoformans*, *A. flavus*, and *A. niger*. Compound **4l** showed significant activity against *A. flavus* and *A. niger* while other compounds exhibit moderate activity against all tested fungal strains.

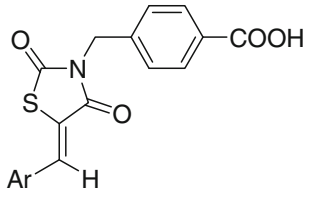
Cytotoxic activity

The MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay (Mosmann, 1983) was used to evaluate cytotoxic activity of the synthesized compounds against four human cancer cell lines including HeLa (cervical carcinoma), HT29 (colorectal cancer), A549 (lung cancer), MCF-7 (breast adenocarcinoma) cell lines (National Centre for Cell Science NCCS, Pune, India). The inhibition of the cell proliferation was determined 24 h after cells were exposed to the tested compounds. The IC_{50} (the concentration that causes 50% growth inhibition) values were determined and summarized in Table 2. Among the thirteen 4-(((*Z*)-5-substituted-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives, compound **4g**, which was 3,4,5-trimethoxyphenyl, exhibited the good inhibitory activity against HeLa, HT29, A549, and MCF-7 cell lines, with the inhibitory concentration (IC_{50}) values of 30, 32, 36, and 30 μM, respectively. Compounds **4a**, **4d**, **4h**, **4i**, **4j**, **4k**, **4l**, and **4m** showed weak cytotoxic activities against all tested human tumor cell lines with IC_{50} values of 60–100 μM. As shown in Table 2, compounds **4b**, **4c**, **4e**, and **4f** showed moderate cytotoxic activities against all tested human tumor cell lines with IC_{50} values of 30–48 μM. In general, structures with electron-donating substituents showed marginal activity, the position of substituents appears to play an important role in activity too.

Molinspiration calculations and "Rule of 5" properties

The structures of thiazolidine-2,4-diones differ in the substitution pattern. Therefore, they may be considered analogous allowing consideration of structure–activity relationships. As can be observed in Table 3, we calculated *c*log*P* and topological polar surface area (TPSA) for all newly synthesized compounds. *c*Log*P* is the partition coefficient between water and octanol as a factor of the lipophilicity of molecules (Jensen *et al*., 2005; Veber *et al*., 2002). PSA is defined as the surface sum over all polar atoms; in particular, TPSA is based on the summation of tabulated surface contributions of polar fragments (Ertl *et al*., 2000). These two properties are commonly used functions for the determination of cell permeability in transport across membranes (Irwin and Shoichet, 2005). We used software of

Table 2 Results of cytotoxic activities of compounds **4a–m** against four different human cancer cell lines (IC_{50}, μM)



4a-m

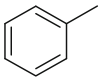
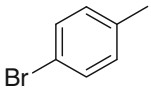
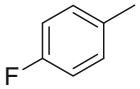
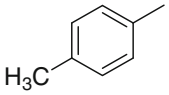
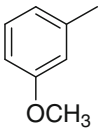
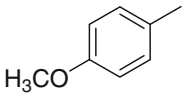
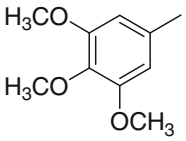
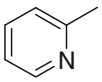
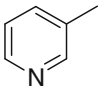
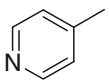
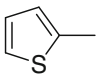
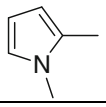
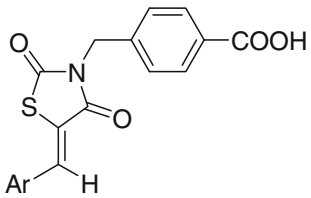
Compound	Ar	HeLa	HT29	A549	MCF-7
4a		72	88	90	100
4b		38	30	42	48
4c		34	32	36	38
4d		68	72	78	100
4e		48	48	55	58
4f		40	44	44	48
4g		30	32	36	30
4h		62	78	80	90
4i		88	90	75	100
4j		60	80	72	84
4k		80	94	76	90

Table 2 continued

Compound	Ar	HeLa	HT29	A549	MCF-7
4l		76	88	80	76
4m		90	88	80	70

Cell lines include cervical carcinoma (HeLa), colorectal cancer (HT29), lung cancer (A549), and breast adenocarcinoma (MCF-7)

Table 3 Molinspiration calculations of the 4-(((*Z*)-5-substituted-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives **4a–m**



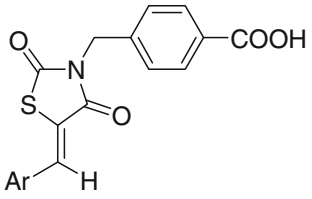
4a-m

Compound	Log*P*	MW	MV	TPSA
4a	3.05	339	284	87.22
4b	3.86	418	302	76.37
4c	3.22	357	289	76.37
4d	3.50	353	301	76.37
4e	3.08	369	310	85.60
4f	3.11	369	310	85.60
4g	2.68	429	361	104.07
4h	1.88	340	280	89.26
4i	1.81	340	280	89.26
4j	1.71	340	280	89.26
4k	2.31	329	266	89.51
4l	2.95	345	275	76.37
4m	2.27	342	286	81.30

Molinspiration for calculations of *c*log*P*, TPSA, and molecular volume (Ertl *et al.*, 2000). In Table 3, *c*log*P* of the compounds **4a–g** are higher than the compounds like **4h–k** and **4m**. Whereas, there is no large difference in TPSA. Therefore, the lipophilicity of **4a–g** is higher than those of **4h–k** and **4m**. The low lipophilicity is known as one of the common cause of poor cell permeability.

To predict the drug likeliness of the synthesized compounds on the guidelines of Lipinski rule of 5 (Molecular weight $\leq$500, log*P* $\leq$5, HBD $\leq$ 5 and HBA $\leq$ 10) study was carried out using Pallas software (Pallas, 2010); the result are given in Table 4. The relevance of the synthesized

Table 4 Drug likeliness of the 4-(((*Z*)-5-substituted-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives **4a–m**



4a-m

Compound	MW	Log*P*	HBD	HBA
4a	339	3.06	1	5
4b	418	3.96	1	5
4c	357	3.19	1	5
4d	353	3.45	1	5
4e	369	3.05	1	6
4f	369	2.92	1	6
4g	429	2.77	1	8
4h	340	2.05	1	6
4i	340	1.88	1	6
4j	340	1.88	1	6
4k	329	2.54	1	6
4l	345	2.90	1	5
4m	342	2.32	1	6

molecules with respect to Lipinski rule of five is as follows. Molecular weight of the compound is important in drug action, if the molecular weight increases beyond a limit, the bulkiness of the compounds also increases, which will affect the drug action (affect the drug receptor/DNA interactions). Molecular weight of compounds lies between 329 and 429 show that these compounds follows Lipinski rule of 5. So the bulkiness of the compounds is in optimum limit for the action. Pharmacokinetic property optimization is a rather complex undertaking that is likely to require changes in those molecular determinants that are responsible for binding affinity and specificity like hydrogen bonds. Hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) groups in the compound optimize the drug receptor interaction. Number of HBA ($\leq$10) and HBD ($\leq$5) in the proposed compounds obeys the Lipinski rule of 5, so it may have some of the compounds good absorption or permeability properties through the biological membrane. Dissolution is highly interdependent influences of aqueous solubility, ionizability (p*K*a) and lipophilicity (log*P*). Furthermore, log*P* is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility. The log*P* values of the synthesized compounds lies in between 2 and 4.

Conclusions

We report the synthesis of 4-((2,4-dioxothiazolidin-3-yl)methyl)benzoic acid derivatives. All compounds were evaluated for their in vitro antimicrobial and cytotoxic activities. Antibacterial and antifungal results revealed that most of the compounds showed significant activity whereas compounds **4c** and **4g** are found to be broad spectrum antibacterial and antifungal properties, the MIC values were observed in the range of 2–4 and 2–8 μg/ml, respectively. In MTT cytotoxicity studies, the compound **4g** was found most potent. In HeLa, HT29, A549, and MCF-7 cells, the IC_{50} values were observed in the range of 30–36 μM. The results indicates that some of functional groups such as 4-bromophenyl, 4-fluorophenyl, and 3,4,5-trimethoxyphenyl group present in these compounds displayed role of biological activity even most of the compounds have similar physicochemical parameters. It can be concluded that a combination of 4-((2,4-dioxothiazolidin-3-yl) methyl) benzoic acid and substituted phenyl has caused an enhanced antimicrobial and cytotoxic effect and hence they are ideally suited for further modification to obtain more efficacious antimicrobial and cytotoxic compounds.

Experimental protocols

General conditions

All the chemicals used in this study were purchased from E. Merck, Fluka and Aldrich. Melting point was determined by electrothermal melting point apparatus and is uncorrected. TLC controls were carried out on precoated silica gel plates (F 254 Merck). The IR spectra were recorded on Nicolet Impact 410 FTIR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on AMX-400, Bruker-400 liquid-state NMR spectrometer using tetramethylsilane (TMS) as the internal standard and DMSO-d_6 as the solvent. Chemical shifts were recorded as δ (ppm). Elemental analysis was carried out using a Perkin Elmer 2400-CHN Analyzer. MW experiments were carried out in a domestic oven (200 psi, 700 W maximum powers). Spectra facilities were carried out by Sophisticated Analytical Instruments Facility (SAIF) division of Indian Institute of Science, Bangalore, India.

Chemistry

General method for the synthesis of 4-(5-arylidene-2,4-dioxothiazolidin-3-yl) methylbenzoic acid (**4a–m**)

A mixture of (*Z*)-5-substituted-2,4-thiazolidinediones (**3a–m**) (10 mmol) and potassium carbonate (20 mmol) in

acetone (100 ml) was refluxed for 45 min. Then 4-(bromomethyl) benzoic acid (20 mmol) was slowly added and the mixture was refluxed for 48–72 h. After evaporation of the solvent under reduced pressure, the crude solid residue was dissolved in methanol; the solution was acidified (pH 3) with HCl and was stirred at room temperature for 30 min. After evaporation to dryness in vacuum, the crude solid was washed with H_2O and recrystallized from methanol/$CHCl_3$ providing pure acid **4a**–**m**.

*4-[((Z)-5-Benzylidene-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4a**)*

Yield 62%, mp 275–277°C, IR (KBr, cm^{-1}): 3276 (OH), 1720, 1695 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.90 (s, 2H, CH_2), 7.42 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.49–7.56 (m, 3H, Ar–H), 7.62 (d, 2H, Ar–H, $J = 7.4$ Hz), 7.92 (d, 2H, Ar–H, $J = 8$ Hz), 7.97 (s, 1H, –CH=), 12.94 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.37, 121.07, 127.59, 129.34, 129.64, 130.12, 130.21, 130.70, 132.86, 133.51, 140.16, 165.43, 166.90, 167.29; Elemental Anal. Calcd for $C_{18}H_{13}NO_4S$ (339): C, 63.71; H, 3.86; N, 4.13. Found: C, 63.67; H, 3.85; N, 4.11.

*4-[((Z)-5-(4-Bromobenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4b**)*

Yield 52%, mp 262–263°C, IR (KBr, cm^{-1}): 3320 (OH), 1720, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 5.02 (s, 2H, CH_2), 7.47 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.55 (d, 2H, Ar–H, $J = 8.3$ Hz), 7.65 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.81 (d, 2H, Ar–H, $J = 8.3$ Hz), 8.03(s, 1H, –CH=), 13.09 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 46.44, 116.34, 125.37, 126.53, 127.32, 128.02, 129.66, 130.98, 132.34, 149.67, 154.55, 165.76, 167.46, 168.33; Elemental Anal. Calcd for $C_{18}H_{12}BrNO_4S$ (418): C, 51.69; H, 2.89; N, 3.35. Found: C, 51.65; H, 2.86; N, 3.33.

*4-[((Z)-5-(4-Fluorobenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4c**)*

Yield 60%, mp 250–252°C, IR (KBr, cm^{-1}): 3255 (OH), 1725, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.90 (s, 2H, CH_2), 7.36–7.42 (m, 4H, Ar–H), 7.70 (d, 2H, Ar–H, $J = 8.3$ Hz), 7.90 (d, 2H, Ar–H, $J = 6.5$ Hz), 7.98 (s, 1H, –CH=), 12.94 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 47.38, 115.58, 124.36, 125.55, 127.32, 127.98, 128.79, 130.53, 131.88, 148.37, 155.16, 165.37, 166.27, 168.65; Elemental Anal. Calcd for $C_{18}H_{12}FNO_4S$ (357): C, 60.50; H, 3.38; N, 3.92. Found: C, 60.47; H, 3.36; N, 3.90.

*4-[((Z)-5-(4-Methylbenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4d**)*

Yield 58%, mp 262–264°C, IR (KBr, cm^{-1}): 3262 (OH), 1723, 1703 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.36 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 7.25(d, 2H, Ar–H, $J = 7.6$ Hz), 7.36 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.48 (d, 2H, Ar–H, $J = 6.8$ Hz), 7.56 (d, 2H, Ar–H, $J = 6.5$ Hz), 7.95 (s, 1H, –CH=), 12.57 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 21.07, 43.31, 114.00, 119.66, 130.01, 130.24, 133.75, 137.77, 141.20, 157.38, 164.33, 165.19, 167.06; Elemental Anal. Calcd for $C_{19}H_{15}NO_4S$ (353): C, 64.58; H, 4.28; N, 3.96. Found: C, 64.55; H, 4.25; N, 3.97.

*4-[((Z)-5-(3-Methoxybenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4e**)*

Yield 55%, mp 256–258°C, IR (KBr, cm^{-1}): 3330 (OH), 1720, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.97 (s, 3H, OCH_3), 4.89 (s, 2H, CH_2), 7.05–7.19 (m, 2H, Ar–H), 7.40–7.47 (m, 2H, Ar–H), 7.55 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.90 (s, 1H, –CH=),7.93 (d, 2H, Ar–H, $J = 8.3$ Hz), 12.99 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.36, 55.27, 115.44, 116.60, 121.95, 127.56, 129.63, 130.46, 133.48, 134.22, 140.16, 159.62, 165.38, 166.89, 167.24; Elemental Anal. Calcd for $C_{19}H_{15}NO_5S$ (369): C, 61.78; H, 4.09; N, 3.79. Found: C, 61.73; H, 4.04; N, 3.75.

*4-[((Z)-5-(4-Methoxybenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4f**)*

Yield 58%, mp 265–267°C, IR (KBr, cm^{-1}): 3325 (OH), 1725, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.84 (s, 3H, OCH_3), 4.91 (s, 2H, CH_2), 7.11 (d, 2H, Ar–H, $J = 7.3$ Hz), 7.41(d, 2H, Ar–H, $J = 8.1$ Hz), 7.60 (d, 2H, Ar–H, $J = 6.7$ Hz), 7.92 (d, 2H, Ar–H, $J = 6.5$ Hz), 7.94 (s, 1H, –CH=), 12.95 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.26, 55.48, 114.96, 117.69, 125.32, 127.52, 129.62, 132.02, 132.30, 133.57, 140.28, 151.24, 165.53, 166.88, 167.34; Elemental Anal. Calcd for $C_{19}H_{15}NO_5S$ (369): C, 61.78; H, 4.09; N, 3.79. Found: C, 61.75; H, 4.05; N, 3.74.

*4-[((Z)-5-(3,4,5-Trimethoxybenzylidene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4g**)*

Yield 50%, mp 260–262°C, IR (KBr, cm^{-1}): 3360 (OH), 1722, 1704 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.72 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 4.90 (s, 2H, CH_2), 6.95 (s, 2H, Ar–H), 7.42 (d, 2H, Ar–H, $J = 8.3$ Hz), 7.91 (s, 1H, –CH=), 7.92 (d, 2H, Ar–H, $J = 6.7$ Hz), 12.94 (s, 1H,

OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.60, 54.56, 54.98, 115.34, 118.65, 126.48, 127.34, 128.21, 131.62, 132.16, 133.20, 141.32, 152.45, 165.02, 166.43, 168.18; Elemental Anal. Calcd for $C_{21}H_{19}NO_7S$ (429): C, 58.73; H, 4.46; N, 3.26. Found: C, 58.70; H, 4.44; N, 3.23.

*4-[((Z)-2,4-Dioxo-5-((pyridine-2-yl)methylene)thiazolidin-3yl)methyl]benzoic acid (**4h**)*

Yield 58%, mp 275–276°C, IR (KBr, cm^{-1}): 3355 (OH), 1725, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.90 (s, 2H, CH_2), 7.42 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.53–7.67 (m, 4H, pyridine), 7.89 (d, 2H, Ar–H, $J = 6.5$ Hz), 7.97 (s, 1H, –CH=), 12.95 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 45.32, 119.25, 120.24, 124.63, 125.34, 128.46, 129.08, 130.20, 132.12, 140.30, 151.86, 164.36, 166.02, 167.45; Elemental Anal. Calcd for $C_{17}H_{12}N_2O_4S$ (340): C, 59.99; H, 3.55; N, 8.23. Found: C, 59.96; H, 3.52; N, 8.25.

*4-[((Z)-2,4-Dioxo-5-((pyridine-3-yl)methylene)thiazolidin-3yl)methyl]benzoic acid (**4i**)*

Yield 56%, mp 258–260°C, IR (KBr, cm^{-1}): 3370 (OH), 1723, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.89 (s, 2H, CH_2), 7.12 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.21–7.24 (m, 2H, pyridine), 7.39 (d, 2H, pyridine, $J = 5.1$ Hz), 7.90 (d, 2H, Ar–H, $J = 6.5$ Hz), 7.92 (s, 1H, –CH=), 12.93 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 45.54, 118.86, 121.56, 123.54, 125.87, 127.48, 129.77, 130.43, 133.54, 135.86, 145.22, 151.33, 165.40, 166.28, 168.06; Elemental Anal. Calcd for $C_{17}H_{12}N_2O_4S$ (340): C, 59.99; H, 3.55; N, 8.23. Found: C, 59.95; H, 3.53; N, 8.25.

*4-[((Z)-2,4-Dioxo-5-((pyridine-4-yl)methylene)thiazolidin-3yl)methyl]benzoic acid (**4j**)*

Yield 50%, mp 270–272°C, IR (KBr, cm^{-1}): 3325 (OH), 1726, 1704 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.71 (s, 2H, CH_2), 7.20–7.28 (m, 4H, pyridine), 7.50 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.92 (d, 2H, Ar–H, $J = 6.8$ Hz), 8.12 (s, 1H, –CH=), 12.42 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.85, 118.43, 120.98, 122.87, 125.64, 127.55, 129.70, 131.11, 135.80, 149.45 150.01, 165.40, 166.24 167.87; Elemental Anal. Calcd for $C_{17}H_{12}N_2O_4S$ (340): C, 59.99; H, 3.55; N, 8.23. Found: C, 59.95; H, 3.53; N, 8.25.

*4-[((Z)-5-((Furan-2-yl)methylene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4k**)*

Yield 62%, mp 263–265°C, IR (KBr, cm^{-1}): 3280 (OH), 1720, 1700 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.87 (s, 2H, CH_2), 6.75 (q, 1H, $J = 1.7$, $J = 1.6$ Hz, furan), 7.15 (d, 1H, $J = 3.4$ Hz, furan), 7.40 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.78 (s, 1H, –CH=), 7.91 (d, 2H, Ar–H, $J = 8.2$ Hz), 8.07 (d, 1H, $J = 1.4$ Hz, furan), 12.93 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.50, 120.21, 123.33, 125.42, 127.33, 128.13, 129.69, 131.55, 140.72, 157.64, 162.44, 165.28, 166.23, 168.65; Elemental Anal. Calcd for $C_{16}H_{11}NO_5S$ (329): C, 58.35; H, 3.37; N, 4.25. Found: C, 58.32; H, 3.33; N, 4.26.

*4-[((Z)-2,4-Dioxo-5-((thiophen-2-yl)methylene)thiazolidin-3yl)methyl]benzoic acid (**4l**)*

Yield 62%, mp 258–260°C, IR (KBr, cm^{-1}): 3320 (OH), 1720, 1695 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.90 (s, 2H, CH_2), 7.41–7.43 (d, 1H, $J = 3.7$ Hz, thiophene), 7.60 (d, 1H, $J = 1.6$ Hz, thiophene), 7.76 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.79–7.91 (m, 2H, Ar–H and 1H, thiophene), 7.92 (s, 1H, –CH=), 12.75 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 44.55, 119.65, 123.60, 124.63, 127.63, 128.84, 129.61, 130.29, 139.98, 158.83, 161.38, 165.11, 166.87, 167.25; Elemental Anal. Calcd for $C_{16}H_{11}NO_4S_2$ (345): C, 55.64; H, 3.21; N, 4.06. Found: C, 55.63; H, 3.20; N, 4.02.

*4-[((Z)-5-((1-Methyl-1H-pyrrol-2-yl)methylene)-2,4-dioxothiazolidin-3yl)methyl]benzoic acid (**4m**)*

Yield 55%, mp 255–256°C, IR (KBr, cm^{-1}): 3340 (OH), 1725, 1699 (C=O); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.80 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 6.35 (d, 1H, $J = 3.1$ Hz, pyrrol), 7.28 (m, 2H, Ar–H and 1H, pyrrol), 7.60 (m, 2H, Ar–H and 1H, pyrrol), 7.65 (s, 1H, –CH=), 14.30 (s, 1H, OH); ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 32.76, 44.82, 120.92, 122.76, 125.82, 127.44, 128.02, 129.55, 131.56, 137.53, 157.44, 162.43, 165.43, 167.35, 168.63; Elemental Anal. Calcd for $C_{17}H_{14}N_2O_4S$ (342): C, 59.64; H, 4.12; N, 8.18. Found: C, 59.62; H, 4.10; N, 8.19.

Antimicrobial activity

The cultures were obtained from Mueller–Hinton broth for all the bacterial strains after 24 h of incubation at 37 ± 1°C. Fungi were maintained in Sabouraud dextrose broth after incubation for 24 h at 25 ± 1°C. Testing was carried out in Mueller–Hinton broth and Sabouraud dextrose broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculums size was 10^5 CFU/ml for the antibacterial assay and 10^4 CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37 ± 1°C and after incubation for 48 h at 25 ± 1°C for antifungal assay, the tube with no

growth of microorganism was recorded to represent the MIC expressed in μg/ml. Every experiment in the antibacterial and antifungal assays was performed in triplicate.

Cytotoxic activity

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system. Test compounds were prepared before the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 μM MTT reagent (1 mg/ml) for 5 h at 37°C. The suspension was placed on microvibrator for 10 min and absorbance was recorded by the ELISA reader. The experiment was performed in triplicate. The IC_{50} results are summarized in Table 2.

Acknowledgments Authors are grateful to Dr. A. D. Taranalli, Principal, for providing necessary facilities. Authors are also grateful to NMR Research center, IISC, Bangalore, India, for providing the spectral data.

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