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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and biological evaluation of thiazolidine-2,4-dione derivatives

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Abstract As a part of our continuation studies in developing new derivatives as dual antimicrobial/antitumor agents we describe the synthesis of new (*Z*)-2-(5-arylidene-2,4-dioxothiazolidin-3-yl) acetic acid derivatives (**3a**–**m**). The chemical structures of the compound were elucidated by FTIR, ^{1}H NMR, ^{13}C NMR, and elemental analysis data. The antimicrobial activity of all products was examined. All newly synthesized compounds were tested for their in vitro anticancer activity against four cancer cell lines. Among the synthesized compounds, **3a** exhibited notable activity against HeLa, HT29, A549, and MCF-7 cell lines with IC_{50} values of 55, 40, 38, and 50 μM, respectively. In order to predict the drug likeliness of the synthesized compounds on the guidelines of Lipinski rule of five studies was carried out using Pallas software.

Keywords Antimicrobial activity · Cytotoxic activity · Thiazolidine-2,4-dione · Drug likeliness

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Introduction

The development of novel antimicrobial and anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. Despite major breakthroughs in many areas of modern medicine, significant rise in multi-drug resistant microbial infections and cancer has become an economic as well as a serious health care problem. In some microbial infections, strains of microbes have become resistant to all available drugs. There is an increasing need for new medicinal organic agent because a dose of anticancer drug sufficient to kill cancer cells is often lethal to the normal tissue and leads to many side effects, which in turn, limits its treatment efficacy (Aydemir and Bilaloglu, 2003). The thiazolidine-2,4-dione (TZDs) is an imperative scaffold that is not only synthetically important but also possesses a wide range of promising biological activities. The TZDs moiety is extensively utilized as a carboxylic acid mimetic to improve the metabolic stability and therapeutic profile of bioactive agents (Boyd, 2007; McIntyre *et al*., 2007; Elte and Blickle, 2007). During recent years there has been a large investigation on different classes of TZDs compounds, many of these are known to possess antibacterial and antifungal (Ayhan-Kilcigil and Altanlar, 2000; Heerding *et al*., 2003; Bozdag-Dundar *et al*., 2007; Tuncbilek and Altanlar, 2006) properties. In addition, this class of compounds has several other potentially beneficial effects including on lipid profile, blood pressure lowering, and anti-inflammatory effects (Kalaitzidis *et al*., 2009). TZDs target vascular cells (Kurebayashi *et al*., 2005) and monocytes/macrophages (Jiang *et al*., 1998; Ricote *et al*., 1998) to inhibit the production of pro-inflammatory cytokines as well as the expression of inducible nitric oxide synthase and cell adhesion molecules. These drugs may also be beneficial in multiple sclerosis and neurodegenerative diseases, including Alzheimer's and

Parkinson's, at least partially due to their anti-inflammatory activity (Heneka and Landreth, 2007). These observations have encouraged us to synthesize some new compounds with potential biological activity. Hence, herein, we report the synthesis, characterization, and investigation of antimicrobial and cytotoxic properties of (*Z*)-2-(5-benzylidene-2,4-dioxothiazolidin-3yl)acetic acid derivatives.

Results and discussion

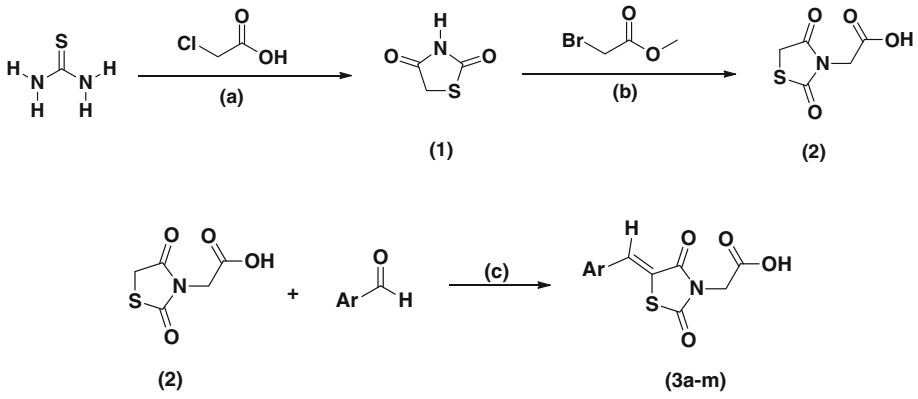
Chemistry

The synthesis of (*Z*)-2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids (**3a**–**m**) was achieved through the versatile and efficient synthetic route outlined in Scheme 1. The starting material TZDs (**1**) was prepared according to earlier reported method (Prashanth Kumar *et al*., 2006) with minor modification. The condensation of compound (**1**) with methylbromoacetate in the presence of potassium carbonate as a base, followed by acidic hydrolysis provided compound (2,4-dioxothiazolidin-3-yl)acetic acid (**2**) (Maccari *et al*., 2005). Further, the condensation of compound (**2**) with the appropriate substituted aldehydes, using piperidine as base in refluxing ethanol for 24 h, provided compounds (**3a**–**m**). Structure of the synthesized compounds was established on the basis of physicochemical, elemental analysis, and spectral data. The (*Z*)-4-(5-arylidene-2,4-dioxothizolidin-3-yl) methyl benzoic acid derivatives were prepared and characterized previously (Maccari *et al*., 2005; Bruno *et al*., 2002; Rakowitz *et al*., 2006). The title compounds (*Z*)-4-(5-arylidene-2,4-dioxothizolidin-3-yl) methyl benzoic acids (**3a**–**m**) were obtained only as *Z* configuration, as expected from the literature and confirmed by ^{1}H NMR, ^{13}C NMR spectra, and X-ray crystallographic data (Tan *et al*., 1986; Albuquerque *et al*., 1995; Vogeli *et al*., 1978; Bruno *et al*., 2002). The ^{1}H NMR spectra of the title compounds (**3a**–**m**) showed only one signal attributable to the resonance of the 5-methylidene proton in the range δ 7.50–8.03 ppm. In ^{13}C NMR spectra, the 5-methylidene carbon and C-5 of the thiazolidinedione ring resonated at δ 113.46 and 128.02 ppm, respectively. In the ^{1}H NMR spectra of compounds (**3a**–**m**), a signal attributable to N–CH_2 resonance was diagnostic: in particular ^{1}H NMR spectra showed a singlet at δ 4.61–4.74 ppm for acetic acid derivatives. In ^{13}C NMR spectra, besides two signals attributable to the resonances of 2- and 4-carbonylic groups of the TZDs ring at 160.31–169.16 ppm, another signal due to the resonances of the carboxylic carbon appeared in the same range.

Antimicrobial activity

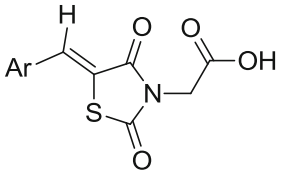
The newly synthesized compounds were evaluated for their in vitro antibacterial activity against two Gram-positive bacteria namely, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 35550); two Gram-negative bacteria namely, *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 25619); and four fungal strains of *Candida albicans* (ATCC 2091), *Aspergillus flavus* (NCIM No. 524), *Aspergillus niger* (ATCC 6275), and *Cryptococcus neoformans* (Clinical isolate) fungi. The MIC values were determined by using the *twofold serial* dilution technique in Mueller–Hinton broth and Sabouraud dextrose agar for the antibacterial and antifungal assays, respectively (National Committee for Clinical Laboratory Standards, 2000). Ciprofloxacin was used as the reference standard for antibacterial activity while ketoconazole was used as the reference standard for antifungal activity. Minimum Inhibitory Concentration (MIC) was recorded as the lowest concentration of a compound that inhibits the growth of tested microorganisms.

The MIC results of the in vitro antibacterial and antifungal activity screening of a novel series of (*Z*)-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids (**3a**–**m**) are summarized in Table 1. All the reported compounds exhibited moderate to substantial in vitro anti-microbial activity against the tested bacterial strains. At the outset it can be stated that the series of (*Z*)-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids have displayed more promising and interesting antibacterial and antifungal activities. Among the compounds



Scheme 1 Preparation of 2-(5-arylidene-2,4-dioxothiazolidin-3yl)acetic acid derivatives. Reagents and conditions: (*a*) (I) H_2O, RT 3 h, (II) HCl, 110 °C for 15 h, (*b*) (I) K_2CO_3, acetone, reflux, 24 h, (II) AcOH, HCl, reflux, 2 h (*c*) Piperidine, acetic acid, ethanol reflux, 24 h

Table 1 In vitro antimicrobial activity of (*Z*)-2-(5-arylidene-2,4-dioxothiazolidin-3yl)acetic acid derivatives **3a–m** expressed as Minimum inhibitory concentration (MIC) in μg/ml



Compounds	Ar	*E.c*	*P.a*	*S.a*	*E.f*	*C.a*	*C.n*	*A.f*	*A.n*
3a	2-ClC_6H_4	>128	>128	64	32	64	16	32	64
3b	2,3-di-Cl C_6H_3	>128	64	16	16	16	32	16	16
3c	2-Cl-5-NO_2 C_6H_3	>128	64	64	64	128	128	16	16
3d	4-Cl-3-NO_2 C_6H_3	>128	128	64	64	>128	>128	128	128
3e	2,3-di-OH C_6H_3	>128	>128	64	64	>128	64	64	>128
3f	2-OH-5-NO_2 C_6H_3	>128	>128	64	64	64	>128	>128	>128
3g	3,5-di-Br-4-OH C_6H_2	>128	>128	64	32	32	64	32	32
3h	4-Br-2-F C_6H_3	>128	64	32	32	32	16	32	32
3i	4-Br-3-F C_6H_3	>128	64	16	32	16	16	32	16
3j	2,4-di-NO_2 C_6H_3	>128	>128	>128	>128	64	>128	>128	>128
3k	2-Furyl	64	32	16	16	16	64	32	16
3l	2-Thienyl	32	16	16	16	16	16	32	32
3m	1-Methylpyrrol	64	32	64	16	16	32	32	16
Cipro.		2	2	2	2	NT	NT	NT	NT
Keto.		NT	NT	NT	NT	2	1	2	1

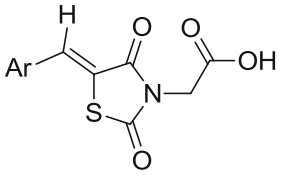
NT not tested, *E.c Escherichia coli*, *P.a Pseudomonas aeruginosa*, *S.a Staphylococcus aureus*, *E.f Enterococcus faecalis*, *C.a Candida albicans*, *C.n Cryptococcus neoformans*, *A.f Aspergillus flavus*, *A.n Aspergillus niger*, *Cipro Ciprofloxacin*, *Keto Ketoconazole*

tested, compounds **3b**, **3k**, and **3l** exhibited excellent antibacterial activity against Gram-positive bacteria like *S. aureus* and *E. faecalis* with a MIC value of 16 μg/ml. Compounds **3i**, **3l**, and **3m** exhibited significant inhibitory activity against *S. aureus, P. aeruginosa,* and *E. faecalis* with a MIC value of 16 μg/ml, respectively. However, all other compounds in the series were found to have least or poor activity against both Gram-positive and Gram-negative bacteria as compared to standard. From the antifungal data through MIC studies, it is evident that most of the tested compounds exhibited excellent antifungal activity, compounds **3b**, **3i**, **3k**, **3l**, and **3m** displayed good antifungal activity against *C. albicans*, and compounds **3b** and **3c** demonstrated significant inhibitory activity against *A. flavus*, *A. niger* with a MIC value of 16 μg/ml, also compounds **3i**, **3k**, and **3m** showed good activity against *A. niger* with a MIC value of 16 μg/ml.

Cytotoxic activity

The MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay 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 (Mosmann, 1983). 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. Results showed that most of the compounds exhibited a considerable effect against A549 (lung cancer) and MCF-7 (breast adenocarcinoma) cell lines. Compounds **3a**, **3b**, **3g**, **3k**, and **3l** showed good cytotoxicity against A549 with IC_{50} values of 38, 37, 45, 36, and 30 μM, respectively, Compounds **3a**, **3c**, **3e**, **3f**, **3g**, **3j**, **3l**, and **3m** displayed significant inhibitory activity with an IC_{50} values ranging from 40–50 μM. In this work we also performed a computational study for prediction of physical–chemical properties of all derivatives and the results are summarized in Table 3. In an effort to study the hydrophobic pattern, we analyzed the theoretical parameters related to the oral bioavailability according to Lipinski "rule of 5." This rule theoretically determines if a chemical compound presents absorption and permeation across the membranes properties that would make it a likely orally active drug in humans. Interestingly our results pointed that most of the compounds obey the Lipinski "rule of 5." We

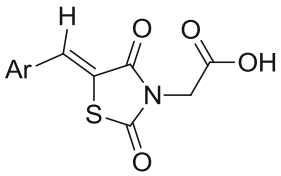
Table 2 In vitro cytotoxicity profile of (*Z*)-2-(5-arylidene-2,4-dioxothiazolidin-3yl)acetic acid derivatives **3a–m** against selected human cancer cell lines IC_{50} (μM)



Compound	Ar	HeLa	HT29	A549	MCF7
3a	2-ClC_6H_4	55	40	38	50
3b	2,3-di-Cl C_6H_3	65	57	37	55
3c	2-Cl-5-NO_2 C_6H_3	77	82	65	48
3d	4-Cl-3-NO_2 C_6H_3	59	70	70	60
3e	2,3-di-OH C_6H_3	>100	>100	50	50
3f	2-OH-5-NO_2 C_6H_3	94	100	54	50
3g	3,5-di-Br-4-OH C_6H_2	75	79	45	50
3h	4-Br-2-F C_6H_3	62	83	60	70
3i	4-Br-3-F C_6H_3	79	81	65	80
3j	2,4-di-NO_2 C_6H_3	65	76	60	40
3k	2-Furyl	50	40	36	54
3l	2-Thienyl	60	50	30	40
3m	1-Methylpyrrol	50	60	60	40
Cisplatin	–	7	4	2	3

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

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



Compound	Ar	MW	Log *P*	HBD	HBA	Rule-5 violation
3a	2-ClC_6H_4	297	1.42	1	5	0
3b	2,3-di-Cl C_6H_3	332	2.05	1	5	0
3c	2-Cl-5-NO_2 C_6H_3	342	1.36	1	8	0
3d	4-Cl-3-NO_2 C_6H_3	342	1.36	1	8	0
3e	2,3-di-OH C_6H_3	295	0.03	3	7	0
3f	2-OH-5-NO_2 C_6H_3	324	0.67	2	9	0
3g	3,5-di-Br-4-OH C_6H_2	437	2.01	2	6	0
3h	4-Br-2-F C_6H_3	360	1.69	1	5	0
3i	4-Br-3-F C_6H_3	360	1.69	1	5	0
3j	2,4-di-NO_2 C_6H_3	353	0.64	1	11	1
3k	2-Furyl	253	0.05	1	6	0
3l	2-Thienyl	269	0.69	1	6	0
3m	1-Methylpyrrol	266	0.18	1	5	0

MW molecular weight, *Log P* calculated using software, *HBD* hydrogen bond donors, *HBA* hydrogen bond acceptor; Lipinski rule of 5: (Molecular weight ≤500, Log *P* ≤5, HBD ≤5 and HBA ≤10) study was carried out using Pallas and molinspiration software

used Molinspiration (Ertl *et al*., 2000) and Pallas (Pallas, 2010) software for calculations of physical–chemical properties.

Conclusion

In conclusion, we report the synthesis of (*Z*)-(5-arylidene-2,4-dioxothiazolidin-3-yl) acetic acid derivatives and evaluated for their in vitro antimicrobial and cytotoxic activities. The biological activities of (*Z*)-(5-arylidene-2,4-dioxothiazolidin-3-yl) acetic acid depended upon the substituent, compound **3a** exhibited notable in vitro anticancer activity against HeLa, HT29, A549, and MCF-7 cell lines with IC_{50} values of 55, 40, 38, and 50 μM, respectively. Moreover, most of the synthesized compounds obey the Lipinski "rule of 5." Thus the compounds containing 2,4-(dioxothiazolidin-3-yl)acetic acid could constitute an interesting lead for the evaluation of antimicrobial and cytotoxic activity and may be helpful for the design of new therapeutic agents or for further lead optimization.

Experimental protocols

General conditions

All the chemicals used in this study were purchased from Aldrich, Himedia, and SD fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. The progress of the reactions was monitored by TLC F 254 Merck precoated silica gel plates. The IR spectra were recorded on Nicolet Impact 410 FTIR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on AMX-400, Bruker-400 liquid-state NMR spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts were recorded as δ (ppm). Elemental analysis was carried out using a Perkin Elmer 2400-CHN Analyzer. Microwave (MW) experiments were carried out in a domestic oven operating at a frequency of 2450 MHz. Spectra facilities were carried out by Sophisticated Analytical Instruments Facility (SAIF) division of Indian Institute of Science, Bangalore, India.

Chemistry

*General procedure for the preparation of (Z)-2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids (**3a–m**)*

A mixture of TZDs **1** (10 mmol), methylbromoacetate (20 mmol), and potassium carbonate (20 mmol) was refluxed for 24 h; then the solvent was evaporated under reduced pressure. The residue was washed with methanol to provide (2,4-dioxothiazolidin-3-yl)acetic acid methyl ester as oil; glacial acetic acid (17 ml) and 2 N HCl (4.2 ml) were refluxed for 2 h. After evaporation to dryness in vacuo, the crude oil was washed with water and then ethanol to provide pure (2,4-dioxothiazolidin-3-yl)acetic acid as oil (**2**). Piperidine and suitable substituted aldehydes were added to a solution of (2,4-dioxothiazolidin-3-yl)acetic acid in ethanol and the mixture was refluxed for 24 h. The reaction mixture was then poured into water and acidified with acetic acid to give as crude solid (**3a**–**m**), which was recrystallized from appropriate solvents.

*(Z)-2-(5-(2-chlorobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3a**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 215–217 °C. IR (KBr, cm^{-1}) 3364 (OH of COOH), 1710 (C=O), 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 7.95 (s, 1H, –CH=), 7.52–7.68 (m, 4H, Ar–H), 4.72 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 167.06 (C=O, TZD), 165.19 (C=O, $–CH_2C=O$), 164.33 (C=O, TZD), 147.38, 141.20, 137.77, 133.75, 130.24, 119.66, 114 (ArC), 43.31(CH_2). Elemental Anal. Calcd for ($C_{12}H_8ClNO_4S$) C, 48.41; H, 2.71; N, 4.70. Found: C, 48.40; H, 2.69; N, 4.73.

*(Z)-2-(5-(2,3-dichlorobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3b**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 210–213 °C. IR (KBr, cm^{-1}) 3385 (OH of COOH), 1705 (C=O), 1683 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 7.94 (s, 1H, –CH=), 7.80 (d, 1H, Ar–H). 7.51–7.60 (m, 2H, Ar–H), 4.74 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 167.36 (C=O, TZD), 166.90 (C=O, $–CH_2C=O$), 165.50 (C=O, TZD), 141.11, 140.30, 133.95, 127.50, 125.53, 123.95, 113.46 (ArC), 44.46 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7Cl_2NO_4S$) C, 43,39; H, 2.21; N, 4.22. Found: C, 43.36; H, 2.09; N, 4.26.

*(Z)-2-(5-(2-chloro-5-nitrobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3c**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 221–222 °C. IR (KBr, cm^{-1}) 3360 (OH of COOH), 1705 (C=O), 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.31–8.34 (m, 1H, Ar–H), 7.95–7.97 (m, 2H, Ar–H), 7.89 (s, 1H, –CH=), 4.69 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 167.09 (C=O, TZD), 165.23 (C=O, $–CH_2C=O$), 164.28 (C=O, TZD), 138.43, 137.65, 133.01, 129.12, 128.02, 125.56, 119.18, 107.74 (ArC), 43.29 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7ClN_2O_6S$) C, 42.06; H, 2.06; N, 8.17. Found: C, 42.02; H, 2.02; N, 8.19.

*(Z)-2-(5-(4-chloro-3-nitrobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3d**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 230–231 °C. IR (KBr, cm^{-1}) 3374 (OH of COOH), 1715 (C=O), 1685 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.39 (d, 2H, Ar–H), 7.93 (s, 1H, –CH=), 7.89–7.92(m, 1H, Ar–H), 4.67 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6). 167.01 (C=O, TZD), 165.08 (C=O, –CH_2C=O), 164.33 (C=O, TZD), 139.75, 137.79, 133.93, 128.27, 119.84, 107.74 (ArC), 43.31 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7ClN_2O_6S$) C, 42.06; H, 2.06; N, 8.17. Found: C, 42.03; H, 2.02; N, 8.19.

*(Z)-2-(5-(2-3-dihydroxybenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3e**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 212–213 °C. IR (KBr, cm^{-1}) 3360 (OH of COOH), 1710 (C=O), 1685 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 9.84 (s, 1H, Ar–OH), 9.65 (s, 1H, Ar–OH), 8.03 (s, 1H, –CH=), 6.91–6.93 (m, 1H, Ar–H), 6.75–6.84 (m, 2H, Ar–H), 4.61 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 166.02 (C=O, TZD), 165.41 (C=O, –CH_2C=O), 160.49 (C=O, TZD), 142.21, 141.46, 126.53, 122.72, 121.31, 118.59, 117.65 (ArC), 41.69 (CH_2). Elemental Anal. Calcd for ($C_{12}H_9NO_6S$) C, 48.81; H, 3.07; N, 4.74. Found: C, 48.78; H, 3.05; N, 4.72.

*(Z)-2-(5-(2-hydroxy-5-nitrobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3f**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 205–206 °C. IR (KBr, cm^{-1}) 3370 (OH of COOH), 1705 (C=O) 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.22 (d, 1H, Ar–H), 8.11 (d, 1H, Ar–H), 7.87 (s, 1H, –CH=), 6.87(d, 1H, Ar–H), 4.72 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 167.11 (C=O, TZD), 165.52 (C=O, –CH_2C=O), 164.13 (C=O, TZD), 138.06, 137.16, 133.31, 129.16, 128.14, 124.56, 119.32, 108.48 (ArC), 43.31(CH_2). Elemental Anal. Calcd for ($C_{12}H_8N_2O_7S$) C, 44.45; H, 2.49; N, 8.64. Found: C, 44.40; H, 2.46; N, 8.69.

*(Z)-2-(5-(3,5-dibromo-4-hydroxybenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3g**)* This compound was prepared according to general procedure and it was obtained as a yellow solid, mp 231–233 °C. IR (KBr, cm^{-1}) 3355 (OH of COOH), 1710 (C=O) 1690 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.47 (s, 1H, Ar–OH), 7.74 (s, 2H, Ar–H), 7.68 (s, 1H, –CH=), 4.70 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 166.81(C=O, TZD), 164.34 (C=O, –CH_2C=O), 160.78 (C=O, TZD), 141.02, 133.13, 128.36, 127.69, 124.43, 115.29 (ArC), 42.13(CH_2). Elemental Anal. Calcd for ($C_{12}H_7Br_2NO_5S$) C, 32.98; H, 1.61; N, 3.20. Found: C, 32.93; H, 1.60; N, 3.22.

*(Z)-2-(5-(4-bromo-2-fluorobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3h**)* This compound was prepared according to general procedure and it was obtained as a orange solid, mp 214–216 °C. IR (KBr, cm^{-1}) 3365 (OH of COOH), 1705 (C=O), 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 7.75–7.78 (dd, 1H, Ar–H), 7.50 (s, 1H, –CH=), 7.59–7.61 (dd, 1H, Ar–H), 7.49–7.53 (t, 1H, Ar–H), 4.66 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 169.16 (C=O, TZD), 166.12 (C=O, –CH_2C=O), 162.32 (C=O, TZD), 147.60, 130.66, 128.43, 123.67, 121.07, 119.08, 119.69 (ArC), 39.87 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7BrFNO_4S$) C, 40.02; H, 1.96; N, 3.89. Found: C, 40.01; H, 1.92; N, 3.88.

*(Z)-2-(5-(4-bromo-3-fluorobenzylidene)-2,4-dioxothizolidin-3-yl)acetic acid (**3i**)* This compound was prepared according to general procedure and it was obtained as orange solid, mp 224–226 °C. IR (KBr, cm^{-1}) 3370 (OH of COOH), 1704 (C=O), 1685 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.05–8.07 (dd, 1H, Ar–H), 7.86 (s, 1H, –CH=), 7.67–7.70 (m, 1H, Ar–H), 7.54–7.58 (t, 1H, Ar–H), 4.62 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 165.11 (C=O, TZD), 164.11 (C=O, –CH_2C=O), 160.31(C=O, TZD), 141.08, 134.54, 130.16, 129.09, 128.48, 125.61, 116.04 (ArC), 42.09 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7BrFNO_4S$) C, 40.02; H, 1.96; N, 3.89. Found: C, 40.00; H, 1.95; N, 3.88.

*(Z)-2-(5-(2,4-dinitrobenzylidene)-2,4-dioxothizolidin-3-yl)-acetic acid (**3j**)* This compound was prepared according to general procedure and it was obtained as yellow solid, mp 209–210 °C. IR (KBr, cm^{-1}) 3350 (OH of COOH), 1700 (C=O), 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.16 (s, 1H, –CH=), 8.13–8.14 (dd, 1H, Ar–H), 7.78–7.79 (dd, 1H, Ar–H), 7.31–7.34(m, 1H, Ar–H), 4.68 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 166.95 (C=O, TZD), 165.09 (C=O, –CH_2C=O), 165.23 (C=O, TZD), 138.34, 131.95, 129.12, 124.32, 109.67 (ArC), 41.09 (CH_2). Elemental Anal. Calcd for ($C_{12}H_7N_3O_8S$) C, 40.80; H, 2.00; N, 11.89. Found: C, 40.75; H, 1.98; N, 11.93.

*(Z)-2-(5-(furan-2-ylmethylene)-2,4-dioxothizolidin-3-yl)acetic acid (**3k**)* This compound was prepared according to general procedure and it was obtained as light yellow solid, mp195–196 °C. IR (KBr, cm^{-1}) 3250 (OH of COOH), 1705 (C=O), 1690 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.14 (d, 1H, furan), 7.70 (s, 1H, –CH=), 7.25 (d, 1H, furan), 7.75–7.76 (dd, 1H, furan), 4.63 (s, 2H, CH_2). ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 169.30 (C=O, TZD), 167.27 (C=O, –CH_2C=O), 166.12 (C=O, TZD), 149.47, 148.82, 120.83, 119.36, 118.68, 114.06, 45.43 (CH_2). Elemental Anal. Calcd for ($C_{10}H_7NO_5S$) C, 47.43; H, 2.79; N, 5.53. Found: C, 47.40; H, 2.77; N, 5.50.

*(Z)-2-(2,4-dioxo-5-(thiophen-2-ylmethylene)thizolidin-3-yl)-acetic acid (**3l**)* This compound was prepared according to general procedure and it was obtained as light orange solid, mp190–192 °C. IR (KBr, cm^{-1}) 3340 (OH of COOH), 1725 (C=O), 1695 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 8.17 (s, 1H, –CH=), 8.14 (d, 1H, thiophene), 7.80 (d, 1H, thiophene), 7.33 (dd, 1H, thiophene), 4.68 (s, 2H, CH_2) ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6) 169.43 (C=O, TZD), 167.19 (C=O, –CH_2C=O), 166.05 (C=O, TZD), 137.22, 136.23, 135.13, 129.43, 126.82, 119.11, 45.37 (CH_2). Elemental Anal. Calcd for ($C_{10}H_7NO_4S_2$) C, 44.60; H, 2.62; N, 5.20. Found: C, 44.61; H, 2.60; N, 5.19.

*(Z)-2-(5-((1-methyl-1H-pyrrol-2-yl)methylene-2,4-dioxo-thizolidin-3-yl)acetic acid (**3m**)* This compound was prepared according to general procedure and it was obtained as orange solid, mp185–186 °C. IR (KBr, cm^{-1}) 3370 (OH of COOH), 1720 (C=O), 1680 (C=O). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 7.65 (s, 1H, –CH=), 7.29 (d, 1H, pyrrol), 6.61 (d, 1H, pyrrol), 6.36 (dd, 1H, pyrrol), 4.59 (s, 2H, CH_2), 3.80 (s, 3H, CH_3); ^{13}C NMR (400 MHz, δ, ppm, DMSO-d_6): 168.63 (C=O, TZD), 167.34 (C=O, –CH_2C=O), 166.25 (C=O, TZD), 130.88, 127.86, 121.09, 116.71, 113.97, 111.36, 45.81(CH_2), 38.87 (N–CH_3). Elemental Anal. Calcd for ($C_{11}H_{10}N_2O_4S$) C, 49.62; H, 3.79; N, 10.52. Found: C, 49.60; H, 3.78; N, 10.50.

Microbiology

For the antibacterial and antifungal activity, the compounds were dissolved in dimethylsulfoxide (DMSO). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 128, 64, 32, 16, 8, 4, 2, and 1 μg/ml concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. The MICs were determined using the twofold serial dilution technique (National Committee for Clinical Laboratory Standards, 2000). A control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in the culture medium.

In vitro assay for antibacterial and antifungal 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 inoculum 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 replicated twice.

In vitro assay for cytotoxic activity

In vitro cytotoxicity was determined using a standard MTT assay (Mosmann, 1983) with protocol appropriate for the individual test system. Test compounds were prepared prior to 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.

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