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Synthesis of new chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties as potential anti-bacterial agents

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ABSTRACT

A series of chalcone derivatives bearing the 2,4-thiazolidinedione and benzoic acid moieties (**8a**–**s**) were synthesized, characterized, and evaluated for their anti-bacterial activity. Among the tested compounds, the most effective were **8a**, **8h**, **8k**, **8n** and **8q** with MIC value in the range of 0.5–4 μg/mL against six Gram-positive bacteria (including multidrug-resistant clinical isolates). None of the compounds exhibited any activity against the Gram-negative bacteria *Escherichia coli* 1356 and *E. coli* 1682 at 64 μg/mL.

1. Introduction

Bacterial infections can cause some of the most deadly diseases and widespread epidemics in the world. With the increase in resistance of bacteria to antibiotic treatment, it is significant to develop novel approaches and new anti-bacterial agents as alternatives to existing antimicrobial therapies.

Chalcones and their derivatives have been reported to have therapeutic potential including anti-inflammatory [1] and anti-bacterial activities [2]. Thiazolidine and thiazolidinone are well known as constitutional units in several agents possessing anti-microbial [3], antituberculosis [4], and anti-HIV activities [5]. In addition, benzoic acid derivatives, such as parabens, have been used as antiseptics because of their powerful anti-bacterial effects.

Previously, we reported the synthesis and antimicrobial evaluation of (E)-4-(3-phenyl-3-oxoprop-1-enyl)benzaldehyde derivatives [6] that showed a strong activity against Gram-positive strains (including multidrug-resistant clinical isolates). Herein, as part of our ongoing research, we designed and synthesized a new series of chalcone derivatives in which the chalcone moiety was reserved, 4-thioxothiazolidine-2-one was replaced with 2,4-thiazolidinedione, and the acetic acid moiety was changed to methylbenzoic acid. A total of 19 chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties (**8a**–**s**) were synthesized, characterized, and evaluated for their anti-bacterial activity.

2. Chemistry

The synthesis of chalcone derivatives is presented in Scheme 1. Thiazolidine-2,4-dione (**2**) was prepared by a previously described method [7]. (*Z*)-4-((2,4-Dioxothiazolidin-5-ylidene)methyl)benzaldehyde (**3**) was prepared *via* Knoevenagel condensation between **2** and terephthalic aldehyde (**1**). Claisen–Schmidt condensation between **3** and various substituted acetophenones (**4a**–**p**) afforded the corresponding (*Z*)-5-(4-((*E*)-3-oxo-3-phenylprop-1-en-1-yl) benzylidene)thiazolidine-2,4-dione derivatives (**5a**–**p**) [8]. Methyl 4–(((*Z*)-2,4-dioxo-5-(4-((*E*)-3-oxo-3- phenylprop-1-en-1-yl)benzylidene)thiazolidin-3-yl)methyl)benzoate derivatives (**7a**–**p**) were synthesized by reacting **5a**–**p** with methyl 4-(bromomethyl) benzoate (**6**) using potassium carbonate as a base in refluxing toluene. Hydroxy substituted derivatives (**7q**–**s**) were prepared by demethylation of the methoxy substituted derivatives (**7n**–**p**) using BBr_3 in dichloromethane. Hydrolysis of **7a**–**s** was carried out in the presence of acetic acid and hydrochloric acid to provide corresponding carboxylic acids **8a**–**s** in good yields. The structures of the desired compounds were determined by IR, ^1H NMR, ^{13}C NMR, mass spectral and elemental analyses.

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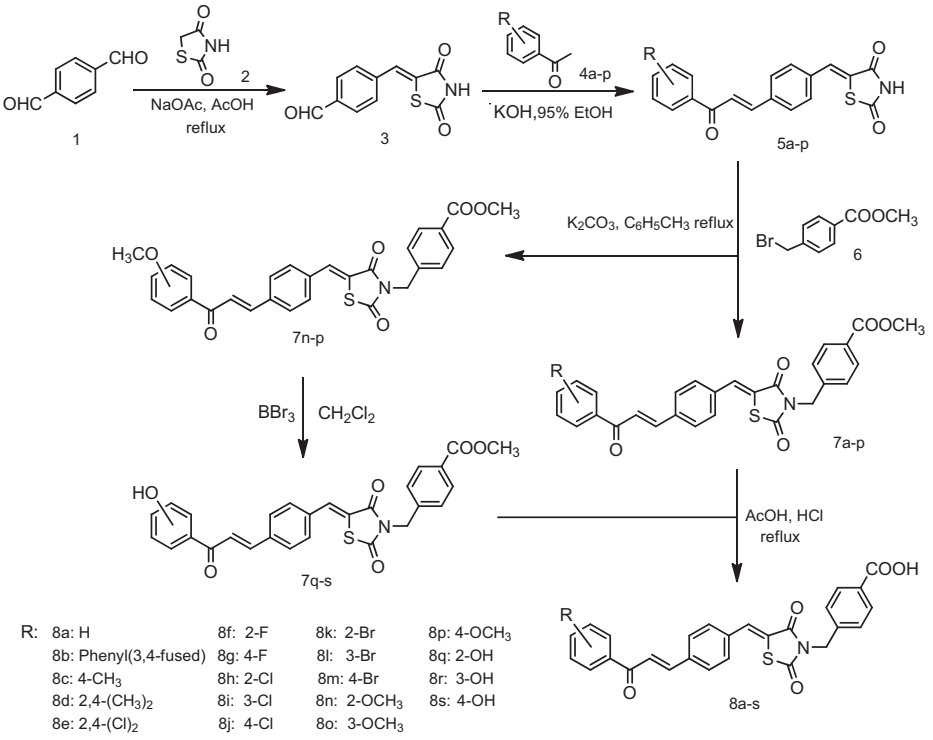

Scheme 1. Synthetic scheme for the synthesis of compounds **8a**–**s**.

3. Results and discussion

3.1. Anti-bacterial activity

The *in vitro* anti-bacterial activity was evaluated using a 96-well microtiter plate and a serial dilution method to obtain the Minimum Inhibitory Concentration (MIC) with different strains including multidrug-resistant clinical isolates. Oxacillin and norfloxacin were used as positive controls.

A preliminary *in vitro* assay showed that the intermediate esters **7a**–**s** did not exhibited any anti-bacterial activities at 64 μg/mL. Among the desired compounds, **8a**, **8h**, **8k**, **8n** and **8r** showed potent anti-bacterial activity against Gram-positive strains (*Staphylococcus aureus RN* 4220 and *S. aureus KCTC* 503) and their MIC values were in the range of 1–4 μg/mL, which was comparable to the control drugs. The test compounds did not exhibit any activity against Gram-negative strains (*Escherichia coli* 1356 and *E. coli* 1682) at 64 μg/mL (Table 1).

The MIC values against the clinical isolates of multidrug-resistant Gram-positive bacterial strains are reported in Table 2. Compounds **8a**, **8h**, **8k**, **8n** and **8r** exhibited more potent activity than the standard drugs (both oxacillin and norfloxacin) with MIC values in the range of 0.5–2 μg/mL against *MRSA CCARM* 3167 and 3506. Compound **8a**, with a MIC of 1 μg/mL and 0.5 μg/mL against *MRSA CCARM* 3167 and 3506 respectively, showed eight-fold more potency than norfloxacin (MIC = 8 μg/mL and 4 μg/mL) and 64-fold more activity than oxacillin (MIC > 64 μg/mL). For the *QRSA CCARM* 3505 and 3519 strains, these compounds also presented higher activity with MIC values of 2–4 μg/mL, slightly less active than oxacillin (MIC = 1 μg/mL) but much more potent than norfloxacin (MIC > 64 μg/mL).

Several structure-activity relationships (SAR) were recognized from the results. Firstly, it was clear that a free carboxyl group was necessary for the anti-bacterial activity against Gram-positive strains as all the esters (**7a**–**s**) were inactive but some of their corresponding carboxylic acids like **8a**, **8h**, **8k**, **8n** and **8r** exhibited significant activities. Secondly, the position of the substituent on the phenyl ring significantly influenced the anti-bacterial activity of the compounds. Among the chloro-, bromo- and methoxy substituted compounds, the *ortho*-substituted derivatives showed better anti-bacterial activity than the others. For the hydroxy substituted derivatives, the *ortho*-substituted **8q** was less active than the *meta*-substituted **8r**, which could be explained by the

Table 1
Inhibitory activity (MIC, μg/mL) of compounds **7a**–**s** and **8a**–**s** against bacteria.

Compound	*S. aureus*		*E. coli*	
	4220	503	1356	1682
7a–**s**	>64	>64	>64	>64
8a	1	2	>64	>64
8b	>64	>64	>64	>64
8c	>64	>64	>64	>64
8d	>64	>64	>64	>64
8e	>64	>64	>64	>64
8f	>64	>64	>64	>64
8g	>64	>64	>64	>64
8h	1	2	>64	>64
8i	>64	>64	>64	>64
8j	>64	>64	>64	>64
8k	2	4	>64	>64
8l	>64	>64	>64	>64
8m	>64	>64	>64	>64
8n	2	4	>64	>64
8o	>64	>64	>64	>64
8p	>64	>64	>64	>64
8q	>64	>64	>64	>64
8r	2	4	>64	>64
8s	>64	>64	>64	>64
Norfloxacin	2	2	16	16
Oxacillin	1	1	>64	>64

S. aureus RN4220, *Staphylococcus aureus* RN4220; *S. aureus* 503, *Staphylococcus aureus* 503; *E. coli* 1356, *Escherichia coli* CCARM 1356; *E. coli* 1682, *Escherichia coli* 1682.

Table 2
Inhibitory activity (MIC, μg/mL) of compounds **8a**, **8h**, **8k**, **8n**, and **8r** against clinical isolates of multidrug-resistant Gram-positive strains.

Compound	MRSA		QRSA	
	3167	3506	3505	3519
8a	1	0.5	2	2
8h	1	1	2	2
8k	1	1	2	2
8n	2	2	4	2
8r	1	1	2	2
Norfloxacin	8	4	>64	>64
Oxacillin	>64	>64	1	1

MRSA 3167, methicillin-resistant *S. aureus CCARM* 3167; *MRSA* 3506, methicillin-resistant *S. aureus CCARM* 3506; *QRSA* 3505, quinolone-resistant *S. aureus CCARM* 3505; *QRSA* 3519, quinolone-resistant *S. aureus CCARM* 3519.

formation of an intramolecular hydrogen bond between the carboxyl group and *ortho*-hydroxy substituent.

4. Conclusion

Based on our previous work, we synthesized a new series of chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties (**8a**–**s**) and evaluated for their anti-bacterial activities against Gram-positive and Gram-negative bacteria. Some of the compounds showed anti-bacterial activities against Gram-positive bacteria, particularly against multidrug-resistant strains of clinical isolates. Compound **8a** was found to have the most potent inhibitory capacity. Furthermore, the results suggested that the carboxyl group seemed to be necessary for the activity and further development of such compounds may be of interest.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel-precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded (in KBr) on a FTIR1730. ^{1}H NMR spectra were measured on a Bruker AV-300 spectrometer using TMS as the internal standard. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses for C, H, N and S were within ±0.4% of the theoretical values and were carried out on a 204Q CHN Rapid Analyzer (Perkin–Elmer, USA). The major chemicals were purchased from Sigma–Aldrich and Fluka.

5.2. General procedure for the preparation of compounds ***7a–s***

A mixture of **5a**–**p** (3 mmol), methyl 4-(bromomethyl)benzoate (6 mmol) and potassium carbonate (9 mmol) in toluene (50 mL) was refluxed for 48 h. After cooling, the precipitate was filtered off, and the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 40:1). The hydroxy substituted derivatives (**7q**–**s**) were prepared by the hydrolysis of the corresponding methoxy substituted derivatives (**7n**–**p**). Thus, to a solution of **7n**–**p** (1 mmol) in 10 mL dichloromethane, was added dropwise boron tribromide (3–5 mmol) in 10 mL dichloromethane in an ice-bath. After stirring for 2 h at room temperature, the reaction mixture was poured into ice-water and extracted with dichloromethane. The combined solvent was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The resulting crude solid was directly used in the next step without purification.

5.3. General procedure for the preparation of compounds ***8a–s***

A mixture of **7a**–**s** (1 mmol), glacial acetic acid (8 mL), and HCl 12 N (2 mL) was refluxed for 2 h. After evaporation *in vacuo*, the residue was refluxed again with glacial acetic acid (8 mL) and HCl 12 N (2 mL) for 2 h. The mixture was evaporated under reduced pressure, and the resulting crude solid was washed with water, recrystallized from DMSO–ethanol to provide **8a**–**s**. The yield, melting point and spectral data of each compound are given below.

5.3.1. 4-(((Z)-2,4-dioxo-5-(4-((E)-3-oxo-3-phenylprop-1-en-1-yl) benzylidene)thiazolidine-3-yl)methyl)benzoic acid (***8a***)

Yield 87%; m.p. 274–275 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.72 (d, J = 14.1 Hz, 1H, H-α), 8.03 (s, 1H, CH=C), 8.07 (d, J = 14.1 Hz, 1H, H-β), 7.43–8.20 (m, 13H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 189.55, 167.63, 167.43, 165.90, 143.00, 140.64, 137.84, 137.14, 135.12, 133.79, 133.08, 131.87, 131.07, 130.70, 130.14, 129.31, 129.06, 128.08, 124.27, 122.57, 44.92. MS *m/z* 470 (M + 1). Anal. Calcd. for $C_{27}H_{19}NO_5S$: C, 69.13; H, 4.26; N, 2.71; S, 6.79. Found: C, 69.07; H, 4.08; N, 2.98; S, 6.83.

5.3.2. 4-(((Z)-5-((4-((E)-3-(naphthalen-2-yl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (***8b***)

Yield 85%; m.p. 280–281 °C. IR (KBr) cm^{-1}: 3443 (OH), 1686 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.94 (s, 2H, CH_2), 7.85 (d, J = 15.6 Hz, 1H, H-α), 8.04 (s, 1H, CH=C), 8.26 (d, J = 15.6 Hz, 1H, H-β), 7.44–8.99 (m, 15H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 189.20, 167.62, 167.44, 165.89, 146.52, 142.85, 140.63, 137.25, 135.58, 135.16, 133.09, 132.77, 131.09, 130.73, 130.15, 129.27, 128.99, 128.19, 128.08, 127.49, 124.53, 124.29, 122.55, 113.54, 44.93. MS *m/z* 520 (M+1). Anal. Calcd. for $C_{31}H_{21}NO_5S$: C, 71.57; H, 4.13; N, 2.78; S, 6.05. Found: C, 71.66; H, 4.07; N, 2.70; S, 6.17.

5.3.3. 4-(((Z)-5-(4-((E)-3-oxo-3-p-tolylprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin -3-yl)methyl)benzoic acid (***8c***)

Yield 85%; m.p. 234–235 °C. IR (KBr) cm^{-1}: 3420 (OH), 1682 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 2.42 (s, 3H, CH_3), 4.93 (s, 2H, CH_2), 7.76 (d, J = 14.1 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.05 (d, J = 14.1 Hz, 1H, H-β), 7.40–8.11 (m, 12H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.91, 167.61, 167.40, 165.89, 144.27, 142.60, 140.64, 137.24, 135.37, 135.03, 133.09, 131.05, 130.70, 130.13, 129.85, 129.78, 129.56, 129.12, 128.08, 124.32, 122.50, 44.93, 21.66. MS *m/z* 484 (M + 1). Anal. Calcd. for $C_{28}H_{21}NO_5S$: C, 69.76; H, 4.44; N, 2.63; S, 6.68. Found: C, 69.55; H, 4.38; N, 2.90; S, 6.63.

5.3.4. 4-(((Z)-5-((4-((E)-3-(2,4-dimethylphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (***8d***)

Yield 82%; m.p. 218–219 °C. IR (KBr) cm^{-1}: 3420 (OH), 1680 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 2.35 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 7.63 (d, J = 14.1 Hz, 1H, H-α), 8.01 (s, 1H, CH=C), 7.96 (d, J = 14.1 Hz, 1H, H-β), 7.16–7.98 (m, 11H, Ar–H), 12.96 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 194.14, 167.58, 167.40, 165.87, 142.94, 141.60, 140.62, 137.73, 137.03, 135.93, 135.03, 133.05, 132.60, 131.06, 130.70, 130.13, 129.91, 129.63, 128.16, 128.08, 126.77, 122.52, 44.92, 21.39, 20.81. MS *m/z* 498 (M + 1). Anal. Calcd. for $C_{29}H_{23}NO_5S$: C, 70.18; H, 4.43; N, 2.71; S, 6.57. Found: C, 70.00; H, 4.66; N, 2.82; S, 6.44.

5.3.5. 4-(((Z)-5-((4-((E)-3-(2,4-dichlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (***8e***)

Yield 83%; m.p. 256–257 °C. IR (KBr) cm^{-1}: 3420 (OH), 1692 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.90 (s, 2H, CH_2), 7.66 (d,

J = 15.6 Hz, 1H, H-α), 7.98 (s, 1H, CH=C), 7.92 (d, J = 15.6 Hz, 1H, H-β), 7.34–7.95 (m, 12H, Ar–H), 12.96 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 192.58, 167.54, 167.40, 165.86, 145.50, 141.26, 140.60, 137.60, 136.46, 136.30, 135.57, 133.40, 132.90, 131.80, 131.33, 131.07, 130.69, 130.14, 128.87, 128.09, 127.97, 122.91, 44.93. MS *m/z* 538 (M+1). Anal. Calcd. for $C_{27}H_{17}Cl_2NO_5S$: C, 60.00; H, 3.26; N, 2.50; S, 6.13. Found: C, 60.23; H, 3.18; N, 2.60; S, 5.96.

*5.3.6. 4-(((Z)-5-((4-((E)-3-(2-fluorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8f**)*

Yield 84%; m.p. 286–287 °C. IR (KBr) cm^{-1}: 3422 (OH), 1686 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.64 (d, J = 15.6 Hz, 1H, H-α), 8.01 (s, 1H, CH=C), 7.97 (d, J = 15.6 Hz, 1H, H-β), 7.37–7.99 (m, 11H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 189.12, 167.57, 167.41, 165.87, 143.47, 140.61, 136.68, 135.34, 135.00, 134.89, 132.96, 131.12, 130.71, 130.14, 130.03, 128.08, 127.53, 127.05, 125.34, 122.74, 117.29, 117.00, 44.91. MS *m/z* 488 (M + 1). Anal. Calcd. for $C_{27}H_{18}FNO_5S$: C, 66.38; H, 3.81; N, 2.99; S, 6.29. Found: C, 66.52; H, 3.72; N, 2.87; S, 6.58.

*5.3.7. 4-(((Z)-5-((4-((E)-3-(4-fluorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8g**)*

Yield 81%; m.p. 233–234 °C. IR (KBr) cm^{-1}: 3420 (OH), 1690 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.78 (d, J = 15.9 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.07 (d, J = 15.9 Hz, 1H, H-β), 7.39–8.31 (m, 13H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 187.99, 167.59, 167.40, 165.89, 143.12, 140.63, 137.10, 135.14, 134.52, 133.05, 132.14, 132.01, 131.04, 130.70, 130.14, 128.08, 124.02, 122.57, 116.45, 116.16, 44.91. MS *m/z* 488 (M + 1). Anal. Calcd. for $C_{27}H_{18}FNO_5S$: C, 66.17; H, 3.83; N, 2.95; S, 6.77. Found: C, 66.52; H, 3.72; N, 2.87; S, 6.58.

*5.3.8. 4-(((Z)-5-((4-((E)-3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8h**)*

Yield 87%; m.p. 252–253 °C. IR (KBr) cm^{-1}: 3422 (OH), 1690 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.92 (s, 2H, CH_2), 7.58 (d, J = 15.6 Hz, 1H, H-α), 8.01 (s, 1H, CH=C), 7.94 (d, J = 15.6 Hz, 1H, H-β), 7.42–7.97 (m, 13H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 192.91, 167.08, 166.93, 165.40, 144.43, 140.14, 138.41, 138.00, 136.03, 135.02, 132.04, 131.41, 130.62, 130.16, 130.03, 129.66, 129.43, 127.72, 127.61, 127.42, 122.39, 44.93. MS *m/z* 504 (M + 1). Anal. Calcd. for $C_{27}H_{18}ClNO_5S$: C, 64.54; H, 3.47; N, 2.92; S, 6.18. Found: C, 64.35; H, 3.60; N, 2.78; S, 6.36.

*5.3.9. 4-(((Z)-5-((4-((E)-3-(3-chlorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8i**)*

Yield 83%; m.p. 250–251 °C. IR (KBr) cm^{-1}: 3422 (OH), 1687 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.62 (d, J = 15.9 Hz, 1H, H-α), 8.03 (s, 1H, CH=C), 7.81 (d, J = 15.9 Hz, 1H, H-β), 7.41–8.24 (m, 12H, Ar–H), 13.00 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.24, 167.59, 167.40, 165.89, 143.74, 140.63, 138.82, 136.32, 135.29, 134.35, 133.03, 131.23, 131.15, 131.02, 130.65, 130.29, 130.13, 128.08, 127.00, 123.82, 122.67, 120.65, 44.93. MS *m/z* 504 (M + 1). Anal. Calcd. for $C_{27}H_{18}ClNO_5S$: C, 64.24; H, 3.47; N, 3.09; S, 6.13. Found: C, 64.35; H, 3.60; N, 2.78; S, 6.36.

*5.3.10. 4-(((Z)-5-((4-((E)-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8j**)*

Yield 84%; m.p. 258–259 °C. IR (KBr) cm^{-1}: 3422 (OH), 1690 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.95 (s, 2H, CH_2), 7.81 (d, J = 15.6 Hz, 1H, H-α), 8.04 (s, 1H, CH=C), 8.08 (d, J = 15.6 Hz, 1H, H-β), 7.45–8.24 (m, 12H, Ar–H), 13.01 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.38, 167.58, 167.40, 165.88, 143.42, 140.62, 138.76, 137.04, 136.49, 135.20, 133.04, 131.04, 130.96, 130.70, 130.14, 129.38, 128.08, 123.91, 122.61, 44.92. MS *m/z* 504 (M + 1). Anal. Calcd. for $C_{27}H_{18}ClNO_5S$: C, 64.22; H, 3.93; N, 2.75; S, 6.28. Found: C, 64.35; H, 3.60; N, 2.78; S, 6.36.

*5.3.11. 4-(((Z)-5-((4-((E)-3-(2-bromophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8k**)*

Yield 81%; m.p. 265–266 °C. IR (KBr) cm^{-1}: 3422 (OH), 1690 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.92 (s, 2H, CH_2), 7.54 (d, J = 15.0 Hz, 1H, H-α), 8.01 (s, 1H, CH=C), 7.94 (d, J = 15.0 Hz, 1H, H-β), 7.41–7.96 (m, 12H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 194.33, 167.60, 167.40, 165.89, 143.47, 140.63, 137.05, 136.82, 135.23, 133.04, 132.34, 132.14, 131.73, 131.06, 130.70, 130.57, 130.44, 130.13, 129.85, 128.09, 123.93, 122.65, 44.94. MS *m/z* 548 (M + 1). Anal. Calcd. for $C_{27}H_{18}BrNO_5S$: C, 59.30; H, 3.07; N, 2.62; S, 5.71. Found: C, 59.13; H, 3.31; N, 2.55; S, 5.85.

*5.3.12. 4-(((Z)-5-((4-((E)-3-(3-bromophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8l**)*

Yield 82%; m.p. 285–286 °C. IR (KBr) cm^{-1}: 3420 (OH), 1687 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.80 (d, J = 15.6 Hz, 1H, H-α), 8.03 (s, 1H, CH=C), 8.09 (d, J = 15.6 Hz, 1H, H-β), 7.43–8.37 (m, 12H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.16, 167.59, 167.40, 165.88, 143.74, 140.63, 139.84, 137.00, 136.37, 135.28, 133.04, 131.57, 131.48, 131.01, 130.70, 130.30, 130.14, 128.08, 123.79, 122.84, 122.65, 44.93. MS *m/z* 548 (M + 1). Anal. Calcd. for $C_{27}H_{18}BrNO_5S$: C, 59.24; H, 3.37; N, 2.29; S, 5.73. Found: C, 59.13; H, 3.31; N, 2.55; S, 5.85.

*5.3.13. 4-(((Z)-5-((4-((E)-3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8m**)*

Yield 82%; m.p. 206–207 °C. IR (KBr) cm^{-1}: 3422 (OH), 1690 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.79 (d, J = 14.1 Hz, 1H, H-α), 8.03 (s, 1H, CH=C), 8.09 (d, J = 14.1 Hz, 1H, H-β), 7.43–8.14 (m, 12H, Ar–H), 13.01 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.63, 167.56, 167.41, 165.87, 145.10, 140.94, 140.60, 136.50, 135.51, 133.70, 132.92, 132.48, 131.09, 130.72, 130.13, 129.76, 128.34, 128.08, 122.88, 119.12, 44.94. MS *m/z* 548 (M + 1). Anal. Calcd. for $C_{27}H_{18}BrNO_5S$: C, 59.35; H, 3.26; N, 2.63; S, 5.69. Found: C, 59.13; H, 3.31; N, 2.55; S, 5.85.

*5.3.14. 4-(((Z)-5-((4-((E)-3-(2-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8n**)*

Yield 85%; m.p. 219–220 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 3.89 (s, 3H, OCH_3), 4.93 (s, 2H, CH_2), 7.55 (d, J = 15.6 Hz, 1H, H-α), 8.01 (s, 1H, CH=C), 7.93 (d, J = 15.6 Hz, 1H, H-β), 7.08–7.95 (m, 12H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 192.24, 167.59, 167.40, 165.89, 158.39, 141.27, 140.62, 137.14, 134.94, 133.81, 133.07, 131.16, 131.07, 130.70, 130.14, 129.74, 129.04, 128.62, 128.09, 122.46, 121.05, 112.85, 56.33, 44.92. MS *m/z* 500 (M + 1). Anal. Calcd. for $C_{28}H_{21}NO_6S$: C, 67.00; H, 4.32; N, 2.15; S, 6.49. Found: C, 67.32; H, 4.24; N, 2.08; S, 6.42.

*5.3.15. 4-(((Z)-5-((4-((E)-3-(3-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8o**)*

Yield 88%; m.p. 250–251 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^{1}H NMR (DMSO–d_6, 300 MHz, ppm): δ 3.87 (s, 3H, OCH_3), 4.93 (s, 2H, CH_2), 7.76 (d, J = 14.4 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.05 (d, J = 14.4 Hz, 1H, H-β), 7.25–8.10 (m, 12H, Ar–H), 13.04 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 189.21, 167.59, 167.44, 165.89, 160.04, 143.04, 140.60, 139.29, 137.14, 136.33, 135.11, 133.07, 131.04, 130.78, 130.42, 130.14, 128.07, 124.28, 122.54, 121.59, 119.83, 113.52, 55.85, 44.93. MS *m/z* 500 (M + 1). Anal. Calcd. for $C_{28}H_{21}NO_6S$: C, 67.19; H, 4.05; N, 2.31; S, 6.36. Found: C, 67.32; H, 4.24; N, 2.08; S, 6.42.

*5.3.16. 4-(((Z)-5-((4-((E)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8p**)*

Yield 87%; m.p. 273–274 °C. IR (KBr) cm^{-1}: 3420 (OH), 1682 (C=O). ^1H NMR (DMSO–d_6, 300 MHz, ppm): δ 3.88 (s, 3H, OCH_3), 4.93 (s, 2H, CH_2), 7.74 (d, J = 15.6 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.07 (d, J = 15.6 Hz, 1H, H-β), 7.09–8.21 (m, 12H, Ar–H), 13.02 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 187.20, 167.63, 167.42, 165.90, 163.83, 142.30, 142.13, 140.64, 137.34, 134.90, 133.13, 131.49, 131.06, 130.74, 130.15, 130.00, 128.07, 124.29, 122.41, 114.53, 56.05, 44.92. MS *m/z* 500 (M+1). Anal. Calcd. for $C_{28}H_{21}NO_6S$: C, 67.10; H, 4.17; N, 2.23; S, 6.58. Found: C, 67.32; H, 4.24; N, 2.08; S, 6.42.

*5.3.17. 4-(((Z)-5-((4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8q**)*

Yield 73%; m.p. 254–256 °C. IR (KBr) cm^{-1}: 3069 (OH), 1686 (C=O). ^1H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.86 (d, J = 15.6 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.13 (d, J = 15.6 Hz, 1H, H-β), 7.00–8.27 (m, 12H, Ar–H), 12.42 (s, 1H, OH), 12.98 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 192.05, 167.62, 167.43, 165.90, 157.41, 140.61, 139.68, 137.23, 134.86, 133.83, 131.43, 131.07, 130.70, 130.14, 129.70, 129.66, 128.98, 128.73, 128.08, 122.60, 121.03, 114.25, 44.91. MS *m/z* 486 (M + 1). Anal. Calcd. for $C_{27}H_{19}NO_6S$: C, 66.66; H, 3.83; N, 2.75; S, 6.87. Found: C, 66.79; H, 3.94; N, 2.88; S, 6.60.

*5.3.18. 4-(((Z)-5-((4-((E)-3-(3-hydroxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8r**)*

Yield 77%; m.p. 296–297 °C. IR (KBr) cm^{-1}: 3341 (OH), 1682 (C=O). ^1H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.45 (d, J = 15.1 Hz, 1H, H-αβ), 8.02 (s, 1H, CH=C), 8.05 (d, J = 15.1 Hz, 1H, H-β), 7.07–8.07 (m, 12H, Ar–H), 9.84 (s, 1H, OH), 12.98 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 188.04, 167.62, 167.46, 165.90, 159.45, 142.33, 139.13, 137.23, 136.62, 135.55, 133.05, 131.05, 130.77, 130.20, 130.14, 129.52, 128.06, 124.42, 122.51, 121.57, 119.56, 114.90, 44.92. MS *m/z* 486 (M+1). Anal. Calcd. for $C_{27}H_{19}NO_6S$: C, 66.89; H, 3.17; N, 2.62; S, 6.54. Found: C, 66.79; H, 3.94; N, 2.88; S, 6.60.

*5.3.19. 4-(((Z)-5-((4-((E)-3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid (**8s**)*

Yield 72%; m.p. 296–297 °C. IR (KBr) cm^{-1}: 3341 (OH), 1682 (C=O). ^1H NMR (DMSO–d_6, 300 MHz, ppm): δ 4.93 (s, 2H, CH_2), 7.71 (d, J = 14.7 Hz, 1H, H-α), 8.02 (s, 1H, CH=C), 8.09 (d, J = 14.7 Hz, 1H, H-β), 6.90–8.12 (m, 12H, Ar–H), 10.50 (s, 1H, OH), 13.01 (s, 1H, COOH). ^{13}C NMR (DMSO–d_6, 75 MHz, ppm): δ 187.45, 167.65, 167.45, 165.91, 162.82, 141.73, 140.63, 137.43, 134.82, 133.15, 131.76, 131.07, 130.73, 130.15, 129.92, 129.44, 128.06, 124.43, 122.38, 115.91, 44.91. MS *m/z* 486 (M + 1). Anal. Calcd. for $C_{27}H_{19}NO_6S$: C, 66.55; H, 3.73; N, 2.96; S, 6.92. Found: C, 66.79; H, 3.94; N, 2.88; S, 6.60.

5.4. Evaluation of anti-bacterial activity in vitro

The micro-organisms used in the present study were *S. aureus* (*S. aureus RN* 4220, *S. aureus KCTC* 503), and *E. coli* (*E. coli* 1356 and *E. coli* 1682). The strains of multidrug-resistant clinical isolates were multidrug-resistant *S. aureus* (*MRSA CCARM* 3167 and *MRSA CCARM* 3506) and quinolone-resistant *S. aureus* (*QRSA CCARM* 3505 and *QRSA CCARM* 3519). Clinical isolates were collected from various patients hospitalized in several clinics.

Test bacteria were grown to mid-log phase in Mueller-Hinton broth (MHB) and diluted 1000-fold in the same medium. The bacteria of 10^5 CFU/mL were inoculated into MHB and dispensed at 0.2 mL/well in a 96-well microtiter plate. As positive controls, oxacillin and norfloxacin were used. Test compounds were prepared in DMSO, the final concentration of which did not exceed 0.05%. A twofold serial dilution technique [9] was used to obtain final concentrations of 64–0.25 μg/mL. The MIC was defined as the concentration of a test compound that completely inhibited bacteria growth during 24 h incubation at 37 °C. Bacteria growth was determined by measuring the absorption at 650 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out three times.

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