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Original article

Synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety with potential anti-bacterial activity

Zhen-Hua Chen, Chang-Ji Zheng, Liang-Peng Sun, Hu-Ri Piao*

Key Laboratory of Natural Resources and Functional Molecules of the Changbai Mountain, Affiliated Ministry of Education, Yanbian University College of Pharmacy, Yanji 133002, PR China

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ABSTRACT

With an intention to synergize the anti-bacterial activity of chalcones and rhodanine-3-acetic acid, several hybrid compounds possessing chalcone and rhodanine-3-acetic acid moieties were synthesized and tested for their anti-bacterial activity. Some compounds presented great anti-microbial activities against Gram-positive bacteria (including the multidrug-resistant clinical isolates). This class of compounds presented high potency against *Staphylococcus aureus*, among which the derivatives **5k** with a MIC of 2 μg/mL was as active as the standard drug (norfloxacin) and less active than oxacillin. Compounds **5a**–**s** did not inhibit the growth of Gram-negative bacteria *Escherichia coli* CCARM 1924 or *E. coli* CCARM 1356 at 64 μg/mL.

1. Introduction

The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades constitutes a substantial need for new classes of anti-bacterial agents [1].

Chalcones, considered to be the precursor of flavonoids and isoflavonoids, are abundant in edible plants. They consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system. Studies revealed that compounds with a chalcone-based structure have anti-oncogenic [2], anti-inflammatory [3], anti-ulcerative [4], analgesic [5], anti-viral [6], anti-fungal [7], anti-malarial [8] and anti-bacterial activities [9]. Rhodanine-3-acetic acid and its analogs are known to possess anti-convulsant [10], anti-bacterial [11], anti-viral [12] and anti-diabetic activities [13]. The anti-microbial activity of rhodanines has been known for over 50 years, so there have been several attempts to design anti-bacterial agents based on this heterocycle [14].

Chalcone and rhodanine compounds are known to be effective anti-bacterial compounds. We initiated a program to synergize the anti-bacterial activity of chalcones and rhodanines by preparing hybrid molecules having the features of both moieties to discover potent anti-bacterial agents. In view of the facts mentioned above, 19 new chalcone derivatives containing a rhodanine-3-acetic acid moiety (**5a**–**s**) were synthesized and evaluated as anti-microbial agents.

2. Chemistry

The synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety is summarized in Scheme 1. (E)-4-(3-Substitutedphenyl-3-oxoprop-1-enyl)benzaldehydes (**3a**–**s**) were prepared by the Claisen–Schmidt condensation of 1,4-phthalaldehyde (**1**) and substituted acetophenone (**2a**–**s**) by a previously described method [15]. Compounds **5a**–**s** were prepared via a Knoevenagel condensation [16] between a range of (E)-4-(3-substitutedphenyl-3-oxoprop-1-enyl)benzaldehydes (**3a**–**s**) and rhodanine-3-acetic acid (**4**) in good yields. The structures of the desired compounds were determined by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analyses.

3. Results and discussion

3.1. Anti-microbial activity

In-vitro anti-microbial activity was evaluated using the minimum inhibitory concentration (MIC) with different strains (including multidrug-resistant clinical isolates). Oxacillin and norfloxacin were used as positive controls for bacteria.

* Corresponding author. Yanbian University College of Pharmacy, Yanji City, Jilin Province 133000, PR China. Tel.: +86 433 2660503; fax: +86 433 2659795.
E-mail address: piaohuri@yahoo.com.cn (H.-R. Piao).

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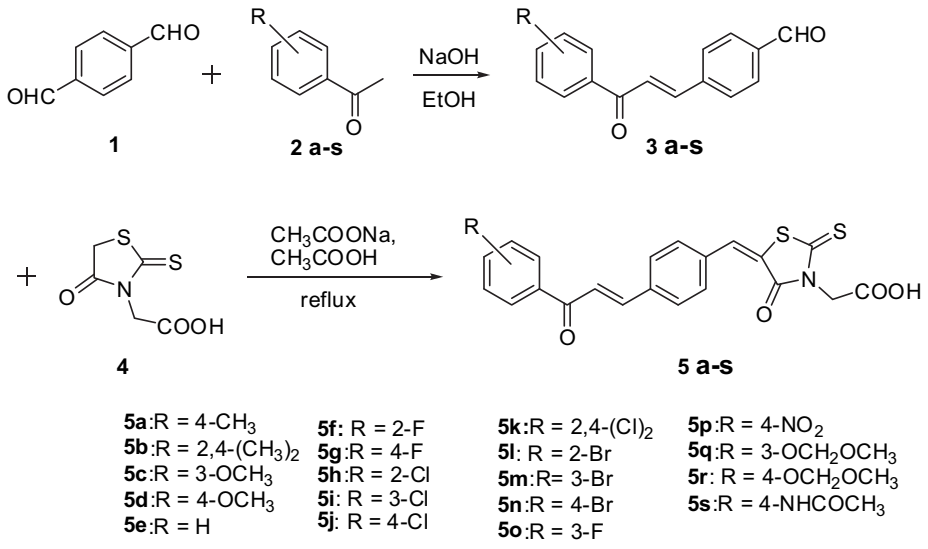

Scheme 1. Synthetic scheme for the synthesis of compounds **5a**–**s.**

Synthesized compounds **5a**–**s** and the intermediate **3k** did not show anti-bacterial activities *in vitro* against Gram-negative strains at 64 μg/mL, but some derivatives exhibited potent anti-bacterial activities against Gram-positive strains. Rhodanine-3-acetic acid (**4**) was inactive for all micro-organisms tested at 64 μg/mL (Table 1).

Most compounds exhibited MIC values in the 2–8 μg/mL range. Compounds **5b**, **5c**, **5g**, **5j**, **5m**, **5n** and **5p** were highly active against *Staphylococcus aureus* (*S. aureus* RN4220, *S. aureus* KCTC 503 and *S. aureus* KCTC 209) and *Streptococcus mutans* KCTC (*Streptococcus mutans* KCTC 3065 and *S. mutans* KCTC 3289) with a MIC of 4–8 μg/mL, but were less active than the standard drug (norfloxacin). This class of compounds presented high activity against *S. aureus*; the 2,4-Cl_2 substituted derivative **5k** with a MIC of 2 μg/mL was as active as the standard drug (norfloxacin) but less active than oxacillin.

Analyzing the activities of synthesized compounds **5a**–**s**, the following structure–activity relationships (SAR) were obtained. Seven electron-donor derivatives were designed and prepared, containing *p*-CH_3, 2,4-$(CH_3)_2$, *m*-OCH_3, *p*-OCH_3, *m*-OCH_2OCH_3, *p*-OCH_2OCH_3, *p*-$NHCOCH_3$. The pharmacology test revealed that their activities were lower than halogen-substituted derivatives, and the activity order was 2,4-$(CH_3)_2$ > *m*-OCH_3 > *p*-CH_3, *p*-OCH_3 > *m*-OCH_2OCH_3, *p*-OCH_2OCH_3, *p*-$NHCOCH_3$. Furthermore, the position of the substituent on the phenyl ring significantly influenced anti-bacterial activity, with an activity order of *p*-F > *m*-F > *o*-F for fluoro-substituted compounds, and *p*-Br > *m*-Br > *o*-Br for bromo-substituted compounds. Comparing the derivatives with different chloro-substitution positions on the phenyl ring, their activity order was 2,4-Cl_2 > *p*-Cl > *m*-Cl > *o*-Cl. Thus, *p*-F, *p*-Br and *p*-Cl compounds (**5g**, **5j**, **5n**) displayed better activity, whereas *o*-F, *o*-Br and *o*-Cl compounds (**5f**, **5h**, **5l**) were inactive for all micro-organisms tested at 64 μg/mL. Derivative **5k** (MIC = 2 μg/mL) was 32-fold more potent than the intermediate **3k** (MIC = 64 μg/mL). This suggests that hybrid compounds possessing chalcone and rhodanine-3-acetic acid moieties may have presented greater anti-bacterial properties.

The most active compounds, **5b**, **5c**, **5g**, **5j**, **5k**, **5m**, **5n** and **5p**, were also evaluated for anti-bacterial activity against many clinical isolates of multidrug-resistant Gram-positive bacteria (Table 2). The compounds **5b**, **5c**, **5g**, **5j**, **5m**, **5n** and **5p** were highly active against clinical isolates of multidrug-resistant Gram-positive bacteria with a MIC of 4–8 μg/mL. The compound **5k** was more potent than norfloxacin against most of the micro-organisms tested with an MIC of 2 μg/mL. Derivative **5k** showed a greater inhibitory capacity. This suggests that the introduction of two atoms of the halogens to the hybrid compound may have played an important part in increasing anti-bacterial properties, for which more compounds using **5k** as the lead compound need to be designed and synthesized for further investigation.

4. Conclusion

For the first time, we synthesized a series of novel chalcone derivatives containing a rhodanine-3-acetic acid moiety and determined their anti-bacterial activities against Gram-positive

Table 1
Inhibitory activity of compounds **3k**, **4** and **5a**–**s** expressed as MIC (μg/mL).

Compound	*S. aureus*			*S. mutans*		*E. coli*	
	4220	503	209	3065	3289	1924	1356
3k	64	64	64	>64	>64	>64	>64
4	>64	>64	>64	>64	>64	>64	>64
5a	64	64	64	64	64	>64	>64
5b	4	4	4	4	8	>64	>64
5c	8	8	8	8	8	>64	>64
5d	64	64	64	64	64	>64	>64
5e	32	32	32	64	64	>64	>64
5f	>64	>64	>64	>64	>64	>64	>64
5g	4	4	4	4	4	>64	>64
5h	>64	>64	>64	>64	>64	>64	>64
5i	64	64	64	64	64	>64	>64
5j	4	4	4	4	4	>64	>64
5k	2	2	2	2	2	>64	>64
5l	>64	>64	>64	>64	>64	>64	>64
5m	8	8	8	8	8	>64	>64
5n	4	4	4	4	4	>64	>64
5o	64	64	64	64	64	>64	>64
5p	4	4	4	8	8	>64	>64
5q	>64	>64	>64	>64	>64	>64	>64
5r	>64	>64	>64	>64	>64	>64	>64
5s	>64	>64	>64	>64	>64	>64	>64
Oxacillin	1	1	1	1	1	>64	>64
Norfloxacin	2	2	4	1	1	16	16

S. aureus RN4220, *Staphylococcus aureus* RN4220; *S. aureus* 503, *Staphylococcus aureus* 503; *S. aureus* 209, *Staphylococcus aureus* 209; *S. mutans* 3065, *Streptococcus mutans* 3065; *S. mutans* 3289, *Streptococcus mutans* 3289; *E. coli* 1924, *Escherichia coli* CCARM 1924; *E. coli* 1356, *Escherichia coli* CCARM 1356.

Table 2
MIC values (in μg/mL) against clinical isolates of multidrug-resistant Gram-positive bacterial strains.

Compound	MRSA		QRSA	
	3167	3506	3505	3519
5b	4	4	4	4
5c	8	8	8	8
5g	4	4	4	4
5j	4	4	4	4
5k	2	2	2	2
5m	8	8	8	8
5n	4	4	4	4
5p	4	4	4	4
Oxacillin	>64	>64	1	1
Norfloxacin	8	4	>64	>64

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.

and Gram-negative bacteria. Most of the compounds had potential anti-bacterial activities against Gram-positive bacteria (particularly against multidrug-resistant strains of clinical isolates). In particular, compound **5k** was found to have the most potent inhibitory capacity. This suggests that hybrid compounds possessing chalcone and rhodanine-3-acetic acid moieties may have presented greater anti-bacterial properties. These results suggested that 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 ***5a–s***

To a solution of compound **4** (0.01 mol) and anhydrous sodium acetate (0.01 mol) in glacial acetic acid were added the respective (E)-4-(3-substitutedphenyl-3-oxoprop-1-enyl)benzaldehydes (**3a–s**). The mixture was stirred under reflux for 4–6 h and then poured into ice-cold water. The precipitate was filtered, washed with water and n-hexane, dried, and purified by silica gel column chromatography (dichloromethane/methanol, 40:1). The yield, melting point and spectral data of each compound are given below.

5.2.1. 2-((5E)-5-(4-((E)-3-Oxo-3-p-tolylprop-1-enyl)benzylidene)-4-oxo-2-thioxothia-zolidin-3-yl)acetic acid (***5a***)

Yield 42%; m.p. 251–252 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.41 (s, 3H, CH_3), 4.72 (s, 2H, CH_2), 7.73 (d, J = 15.3 Hz, 1H, CH=CH), 7.98 (s, 1H, CH), 8.04 (d, J = 15.3 Hz, 1H, CH=CH), 7.38–8.08 (m, 8H, Ar–H), 13.58 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.38, 188.86, 167.70, 166.82, 144.28, 142.51, 137.60, 135.35, 134.86, 133.32, 131.63, 130.16, 129.86, 129.22, 124.49, 123.18, 45.73, 21.68. MS *m/z* 424 (M+1). Anal. Calcd. for $C_{22}H_{17}NO_4S_2$: C, 62.39; H, 4.05; N, 3.31; S, 15.14. Found: C, 62.05; H, 4.13; N, 3.28; S, 15.32.

5.2.2. 2-((5E)-5-(4-((E)-3-(2,4-Dimethylphenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5b***)

Yield 47%; m.p. 248–249 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.35 (s, 6H, CH_3), 4.75 (s, 2H, CH_2), 7.50 (d, J = 15.3 Hz, 1H, CH=CH), 7.93 (s, 1H, CH), 7.69 (d, J = 15.3 Hz, 1H, CH=CH), 7.17–8.00 (m, 7H, Ar–H), 13.52 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 194.11, 193.39, 167.71, 166.78, 142.86, 141.66, 137.78, 135.9, 134.86, 133.42, 131.66, 130.03, 129.66, 128.39, 126.8, 123.17, 45.51, 21.41, 20.84. MS *m/z* 438 (M+1). Anal. Calcd. for $C_{23}H_{19}NO_4S_2$: C, 63.14; H, 4.38; N, 3.20; S, 14.66. Found: C, 63.05; H, 4.75; N, 3.13; S, 14.49.

5.2.3. 2-((5E)-5-(4-((E)-3-(3-Methoxyphenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5c***)

Yield 49%; m.p. 280–281 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.86 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2), 7.49 (d, J = 15.3 Hz, 1H, CH=CH), 7.95 (s, 1H, CH), 8.04 (d, J = 15.3 Hz, 1H, CH=CH), 7.25–8.11 (m, 8H, Ar–H), 13.52 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.52, 187.62, 167.63, 166.76, 166.28, 142.14, 137.57, 134.98, 133.43, 131.28, 131.08, 130.55, 130.13, 124.67, 123.04, 114.59, 56.04, 45.38. MS *m/z* 440 (M+1). Anal. Calcd. for $C_{22}H_{17}NO_5S_2$: C, 60.12; H, 3.90; N, 3.19; S, 14.59. Found: C, 60.20; H, 3.78; N, 3.23; S, 14.47.

5.2.4. 2-((5E)-5-(4-((E)-3-(4-Methoxyphenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo- 2-thioxothiazolidin-3-yl)acetic acid (***5d***)

Yield 31%; m.p. 282–283 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.88 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2), 7.71 (d, J = 15.3 Hz, 1H, CH=CH), 7.94 (s, 1H, CH), 8.06 (d, J = 15.3 Hz, 1H, CH=CH), 7.12–8.21 (m, 8H, Ar–H), 13.51 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.42, 187.64, 167.72, 166.85, 166.80, 142.05, 137.78, 134.75, 133.50, 131.65, 131.51, 130.75, 130.13, 124.57, 123.06, 114.55, 56.07, 45.52. MS *m/z* 440 (M+1). Anal. Calcd. for $C_{22}H_{17}NO_5S_2$: C, 60.12; H, 3.90; N, 3.19; S, 14.59. Found: C, 60.02; H, 3.98; N, 3.06; S, 14.23.

5.2.5. 2-((5E)-5-(4-((E)-3-Oxo-3-phenylprop-1-enyl)benzylidene)-4-oxo-2-thioxothia- zolidin-3-yl)acetic acid (***5e***)

Yield 43%; m.p. 235–236 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.76 (s, 2H, CH_2), 7.57 (d, J = 15.3 Hz, 1H, CH=CH), 7.95 (s, 1H, CH), 8.01 (d, J = 15.3 Hz, 1H, CH=CH), 7.60–8.20 (m, 9H, Ar–H), 13.58 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.37, 188.76, 167.60, 166.72, 144.27, 142.57, 137.67, 135.38, 134.87, 133.32, 131.64, 130.16, 129.76, 129.22, 124.49, 123.18, 45.72. MS *m/z* 410 (M+1). Anal. Calcd. for $C_{21}H_{15}NO_4S_2$: C, 61.60; H, 3.69; N, 3.42; S, 15.66. Found: C, 61.65; H, 3.81; N, 3.40; S, 15.59.

5.2.6. 2-((5E)-5-(4-((E)-3-(2-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5f***)

Yield 36%; m.p. 262–263 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.51 (s, 2H, CH_2), 7.56 (d, J = 15.3 Hz, 1H, CH=CH), 7.97 (s, 1H, CH), 7.82 (d, J = 15.3 Hz, 1H, CH=CH), 7.36–8.00 (m, 8H, Ar–H), 13.50 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.35, 189.17, 167.44, 167.04, 143.48, 135.86, 135.38, 134.90, 132.41, 131.60, 131.06, 130.13, 127.68, 127.21, 125.37, 123.99, 117.31, 47.07. MS *m/z* 428 (M+1). Anal. Calcd. for $C_{21}H_{14}FNO_4S_2$: C, 59.00; H, 3.30; N, 3.28; S, 15.00. Found: C, 59.09; H, 3.47; N, 3.19; S, 15.01.

5.2.7. 2-((5E)-5-(4-((E)-3-(4-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5g***)

Yield 58%; m.p. 267–268 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.71 (s, 2H, CH_2), 7.76 (d, J = 15.3 Hz, 1H, CH=CH), 7.94 (s, 1H, CH), 8.08 (d, J = 15.3 Hz, 1H,

CH=CH), 7.40–8.31 (m, 8H, Ar–H), 13.57 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.46, 189.37, 167.28, 167.07, 143.48, 135.85, 135.28, 134.80, 132.31, 131.69, 131.02, 130.35, 127.25, 127.11, 125.37, 123.98, 117.21, 47.03. MS *m/z* 428 (M+1). Anal. Calcd. for $C_{21}H_{14}FNO_4S_2$: C, 59.00; H, 3.30; N, 3.28; S, 15.00. Found: C, 58.97; H, 3.46; N, 3.19; S, 15.1.

5.2.8. 2-((5E)-5-(4-((E)-3-(2-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5h)***

Yield 40%; m.p. 281–282 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.75 (s, 2H, CH_2), 7.39 (d, J = 15.3 Hz, 1H, CH=CH), 7.96 (s, 1H, CH), 7.93 (d, J = 15.3 Hz, 1H, CH=CH), 7.41–7.98 (m, 8H, Ar–H), 13.50 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.38, 188.34, 167.72, 166.87, 143.36, 138.59, 137.42, 136.25, 135.06, 133.33, 131.67, 130.58, 130.29, 129.39, 124.18, 123.16, 45.59. MS *m/z* 444 (M+1). Anal. Calcd. for $C_{21}H_{14}ClNO_4S_2$: C, 56.82; H, 3.18; N, 3.16; S, 14.45. Found: C, 56.76; H, 3.25; N, 3.10; S, 14.40.

5.2.9. 2-((5E)-5-(4-((E)-3-(3-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5i)***

Yield 36%; m.p. 286–287 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 M Hz, ppm): δ 4.52 (s, 2H, CH_2), 7.60 (d, J = 15.3 Hz, 1H, CH=CH), 7.88 (s, 1H, CH), 7.78 (d, J = 15.3 Hz, 1H, CH=CH), 7.62–8.24 (m, 8H, Ar–H), 13.55 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 192.36, 187.38, 166.71, 165.80, 143.32, 138.75, 137.48, 136.43, 135.03, 133.31, 131.64, 130.97, 130.28, 129.36, 124.14, 123.25, 45.68. MS *m/z* 444 (M+1). Anal. Calcd. for $C_{21}H_{14}ClNO_4S_2$: C, 56.82; H, 3.18; N, 3.16; S, 14.45. Found: C, 56.80; H, 3.29; N, 3.06; S, 14.63.

5.2.10. 2-((5E)-5-(4-((E)-3-(4-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5j)***

Yield 44%; m.p. 284–285 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.76 (s, 2H, CH_2), 7.78 (d, J = 15.3 Hz, 1H, CH=CH), 7.95 (s, 1H, CH), 8.06 (d, J = 15.3 Hz, 1H, CH=CH), 7.65–8.23 (m, 8H, Ar–H), 13.45 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.36, 188.36, 167.70, 166.80, 143.33, 138.79, 137.42, 136.45, 135.03, 133.32, 131.62, 130.98, 130.29, 129.39, 124.10, 123.27, 45.64. MS *m/z* 444 (M+1). Anal. Calcd. for $C_{21}H_{14}ClNO_4S_2$: C, 56.82; H, 3.18; N, 3.16; S, 14.45. Found: C, 56.73; H, 3.10; N, 3.29; S, 14.56.

5.2.11. 2-((5E)-5-(4-((E)-3-(2,4-Dichlorophenyl)-3-oxoprop-1-enyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5k)***

Yield 53%; m.p. 235–236 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.75 (s, 2H, CH_2), 7.43 (d, J = 15.3 Hz, 1H, CH=CH), 7.96 (s, 1H, CH), 7.80 (d, J = 15.3 Hz, 1H, CH=CH), 7.38–7.99 (m, 7H, Ar–H), 13.50 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.37, 192.62, 167.71, 166.78, 145.44, 137.59, 136.88, 136.33, 135.40, 133.24, 131.82, 131.67, 130.28, 128.16, 123.57, 45.52. MS *m/z* 479 (M+1). Anal. Calcd. for $C_{21}H_{13}Cl_2NO_4S_2$: C, 52.73; H, 2.74; N, 2.93; S, 13.41. Found: C, 52.48; H, 2.92; N, 2.63; S, 13.27.

5.2.12. 2-((5E)-5-(4-((E)-3-(2-Bromophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5l)***

Yield 32%; m.p. 281–282 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.75 (s, 2H, CH_2), 7.51 (d, J = 15.3 Hz, 1H, CH=CH), 7.95 (s, 1H, CH), 7.92 (d, J = 15.3 Hz, 1H, CH=CH), 7.36–8.02 (m, 8H, Ar–H), 13.58 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.35, 188.40, 167.18, 167.06, 143.72, 139.83, 137.16, 136.39, 135.82, 132.74, 131.55, 130.40, 128.04, 123.93, 122.86, 47.19. MS *m/z* 489 (M+1). Anal. Calcd. for $C_{21}H_{14}BrNO_4S_2$: C, 51.65; H, 2.89; N, 2.87; S, 13.13. Found: C, 51.23; H, 2.92; N, 2.48; S, 13.05.

5.2.13. 2-((5E)-5-(4-((E)-3-(3-Bromophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5m)***

Yield 41%; m.p. 289–290 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.53 (s, 2H, CH_2), 7.53 (d, J = 15.3 Hz, 1H, CH=CH), 7.87 (s, 1H, CH), 8.03 (d, J = 15.3 Hz, 1H, CH=CH), 7.55–8.36 (m, 8H, Ar–H), 13.56 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.33, 188.20, 167.19, 167.06, 143.72, 139.83, 137.16, 136.39, 135.32, 132.44, 131.50, 130.40, 128.04, 123.92, 122.83, 47.15. MS *m/z* 489 (M+1). Anal. Calcd. for $C_{21}H_{14}BrNO_4S_2$: C, 51.65; H, 2.89; N, 2.87; S, 13.13. Found: C, 51.45; H, 2.92; N, 2.63; S, 13.07.

5.2.14. 2-((5E)-5-(4-((E)-3-(4-Bromophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5n)***

Yield 36%; m.p. 286–287 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.75 (s, 2H, CH_2), 7.75 (d, J = 15.3 Hz, 1H, CH=CH), 7.94 (s, 1H, CH), 8.04 (d, J = 15.3 Hz, 1H, CH=CH), 7.78–8.14 (m, 8H, Ar–H), 13.58 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.28, 188.27, 167.16, 167.11, 143.72, 139.28, 137.08, 136.36, 135.35, 132.44, 131.52, 130.43, 128.09, 123.93, 122.85, 47.35. MS *m/z* 489 (M+1). Anal. Calcd. for $C_{21}H_{14}BrNO_4S_2$: C, 51.65; H, 2.89; N, 2.87; S, 13.13. Found: C, 51.27; H, 2.96; N, 2.53; S, 13.03.

5.2.15. 2-((5E)-5-(4-((E)-3-(3-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5o)***

Yield 36%; m.p. 262–263 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.50 (s, 2H, CH_2), 7.55 (d, J = 15.3 Hz, 1H, CH=CH), 7.97 (s, 1H, CH), 7.82 (d, J = 15.3 Hz, 1H, CH=CH), 7.36–8.03 (m, 8H, Ar–H), 13.56 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.25, 189.16, 167.45, 167.09, 143.47, 135.88, 135.34, 134.90, 132.41, 131.61, 131.05, 130.18, 127.69, 127.22, 125.38, 123.96, 117.38, 47.06. MS *m/z* 428 (M+1). Anal. Calcd. for $C_{21}H_{14}FNO_4S_2$: C, 59.00; H, 3.30; N, 3.28; S, 15.00. Found: C, 59.09; H, 3.46; N, 3.17; S, 15.11.

5.2.16. 2-((5E)-5-(4-((E)-3-(4-Nitrophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5p)***

Yield 39%; m.p. 293–294 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 4.75 (s, 2H, CH_2), 7.76 (d, J = 15.3 Hz, 1H, CH=CH), 7.94 (s, 1H, CH), 8.07 (d, J = 15.3 Hz, 1H, CH=CH), 7.79–8.39 (m, 8H, Ar–H), 13.56 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.35, 188.63, 169.67, 167.59, 166.76, 150.35, 144.31, 142.51, 137.20, 135.25, 133.31, 131.64, 130.42, 124.33, 124.11, 123.39, 45.47. MS *m/z* 455 (M+1). Anal. Calcd. for $C_{21}H_{14}N_2O_6S_2$: C, 55.50; H, 3.10; N, 6.16; S, 14.11. Found: C, 55.28; H, 3.45; N, 6.03; S, 14.07.

5.2.17. 2-((5E)-5-(4-((E)-3-(3-(Methoxymethoxy)phenyl)-3-oxoprop-1-enyl)benzylid-ene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid ***(5q)***

Yield 46%; m.p. 292–293 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.16 (s, 3H, OCH_3), 4.57 (s, 2H, CH_2), 5.31 (s, 2H, OCH_2), 7.35 (d, J = 15.3 Hz, 1H, CH=CH), 7.97 (s, 1H, CH), 7.70 (d, J = 15.3 Hz, 1H, CH=CH), 7.33–8.10 (m, 8H, Ar–H), 13.54 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.44, 187.88, 167.52, 166.89, 161.23, 142.34, 136.63, 134.37, 133.25, 131.68, 131.39, 130.17, 130.18, 124.46, 123.33, 116.33, 94.18, 56.35, 46.08. MS *m/z* 470 (M+1). Anal. Calcd. for $C_{23}H_{19}NO_6S_2$: C, 58.83; H, 4.08; N, 2.98; S, 13.66. Found: C, 58.79; H, 4.37; N, 2.72; S, 13.46.

5.2.18. 2-((5E)-5-(4-((E)-3-(4-(Methoxymethoxy)phenyl)-3-oxoprop-1-enyl)benzylid-ene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid ***(5r)***

Yield 53%; m.p. 295–296 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.41 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2), 5.33 (s, 2H, OCH_2), 7.71 (d, J = 15.3 Hz, 1H, CH=CH), 7.88 (s, 1H, CH), 8.04 (d, J = 15.3 Hz, 1H, CH=CH), 7.16–8.20 (m, 8H, Ar–H), 13.59 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.40, 187.83, 167.51, 166.89, 161.28, 142.24, 137.63, 134.87, 133.15, 131.61, 131.38, 130.13, 130.13, 124.49, 123.34, 116.34, 94.16, 56.36, 46.03. MS *m/z* 470 (M+1). Anal. Calcd. for $C_{23}H_{19}NO_6S_2$: C, 58.83; H, 4.08; N, 2.98; S, 13.66. Found: C, 58.63; H, 4.36; N, 2.69; S, 13.42.

5.2.19. 2-((E)-5-(4-((E)-3-(4-Acetamidophenyl)-3-oxoprop-1-enyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***(5s)***

Yield 58%; m.p. 281–282 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.10 (s, 3H, CH_3), 4.76 (s, 2H, CH_2), 7.72 (d, J = 15.3 Hz, 1H, CH=CH), 7.94 (s, 1H, CH), 8.01 (d, J = 15.3 Hz, 1H, CH=CH), 7.74–8.18 (m, 8H, Ar–H), 10.34 (s, 1H, NH), 13.51 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.39, 187.77, 169.40, 167.63, 166.79, 144.24, 142.18, 137.72, 134.77, 133.47, 132.39, 131.65, 130.46, 130.11, 129.75, 124.48, 123.08, 118.67, 45.49, 24.61. MS *m/z* 467 (M+1). Anal. Calcd. for $C_{23}H_{18}N_2O_5S_2$: C, 59.21; H, 3.89; N, 6.00; S, 13.75. Found: C, 59.09; H, 3.97; N, 5.83; S, 13.63.

5.3. Evaluation of anti-bacterial activity in vitro

The micro-organisms used in the present study were *S. aureus* (*S. aureus* RN4220, *S. aureus* KCTC 503 and *S. aureus* KCTC 209), *S. mutans* (*S. mutans* KCTC 3065 and *S. mutans* KCTC 3289), and *Escherichia coli* (*E. coli* 1924 and *E. coli* 1356). The strains of multi-drug-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.

A twofold serial dilution technique [17] was followed to determine the minimum inhibitory concentration (MIC) of the compounds against the susceptible micro-organisms in the preliminary test (Gram-positive bacteria and Gram-negative bacteria) and against strains of clinical isolates of multidrug-resistant Gram-positive bacteria. Test compounds dissolved in DMSO were added to culture media (Brain Heart Infusion for *S. mutans* and Müller–Hinton agar for other bacteria) to obtain final concentrations of 64–0.5 μg/mL. The final amount applied was of 10^5 CFU/mL for bacteria. MIC values were read after incubation at 37 °C for 20 h. The lowest concentration of the test substance that completely inhibited growth of the micro-organism was recorded as the MIC (expressed in μg/mL). Oxacillin and norfloxacin were used as the standard drugs. All experiments were carried out three times.

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