European Journal of Medicinal Chemistry 56 (2012) 203–209

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antimicrobial evaluation of L-phenylalanine-derived C5-substituted rhodanine and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone

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ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form
17 August 2012
Accepted 20 August 2012
Available online 27 August 2012

Keywords:
Phenylpropionic acid
Rhodanine
Chalcone
Thiobarbituric acid
Antibacterial activity

ABSTRACT

Four novel series of compounds, including the L-phenylalanine-derived C5-substituted rhodanine (**6a**–**q**, **7a**–**j**) and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**) have been designed, synthesized, characterized, and evaluated for their antibacterial activity. Some of these compounds showed significant antibacterial activity against Gram-positive bacterias, especially against the strains of multidrug-resistant clinical isolates, among which compounds **6c**–**e**, **6g**, **6i**, **6j** and **6q** exhibiting high levels of antimicrobial activity against *Staphylococcus aureus* RN4220 with minimum inhibitory concentration (MIC) values of 2 μg/mL. Compound **6q** showed the most potent activity of all of the compounds against all of the test multidrug-resistant clinical isolates tested. Unfortunately, however, none of the compounds were active against Gram-negative bacteria at 64 μg/mL.

1. Introduction

Infectious diseases are responsible for a significant proportion of deaths worldwide and according to the World Health Organization, antimicrobial agents are considered to be “miracle drugs” that are the leading weapons in the treatment of infectious diseases. Unfortunately, however, a number of the current clinically efficacious antimicrobial agents are becoming less effective because of the development of antimicrobial resistance and there is evidence for the rapid global spread of resistant clinical isolates and the appearance of drug-resistant strains among community acquired infections. With all of this in mind, there is an urgent need for the discovery or optimization of novel antimicrobial agents that are active against these resistant strains [1].

In recent years, rhodanine-based molecules have been reported as small molecule inhibitors of numerous biological targets, including hepatitis C virus NS5B polymerase [2], and cathepsin D [3]. Furthermore, they have been reported as anticancer [4], antimicrobial [5], and antidiabetic [6] agents. Rhodanine and its derivatives have also been synthesized and extensively studied as antibacterial drugs. In addition, chalcones and benzyloxybenzaldehydes have been reported to exhibit a broad spectrum of biological activities, with many compounds in these structural classes showing appreciable antibacterial [7], antifungal [8], antitumor [9], anti-inflammatory [10], analgesic [11] and antiviral [12] activities. Based on our previous work, we were aware that non-functionalized chalcones exhibited low antibacterial activity, whereas chalcones functionalized with rhodanine-*N*-acetic acid or the analogous 2,4-thiazolidinedione-*N*-benzoic acid showed higher levels of antibacterial activity (Fig. 1) [13,14]. These investigations suggested that a free carboxyl group was important to the observed levels of activity. With this in mind and as part of our ongoing studies toward the development of new antimicrobial agents, structural modifications were carried out involving the substitution of the carboxyl moiety at the N-3 position of the rhodanine with a phenylpropionic acid to synthesize the L-phenylalanine-derived C5-substituted rhodanines (**6a**–**q**, **7a**–**j**).

Although the structure–activity relationships of the rhodanine-*N*-acetic acid and analogous 2,4-thiazolidinedione-*N*-benzoic acid derivatives have been studied extensively, very little is

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http://dx.doi.org/10.1016/j.ejmech.2012.08.026

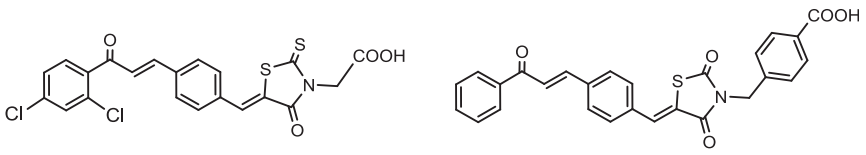
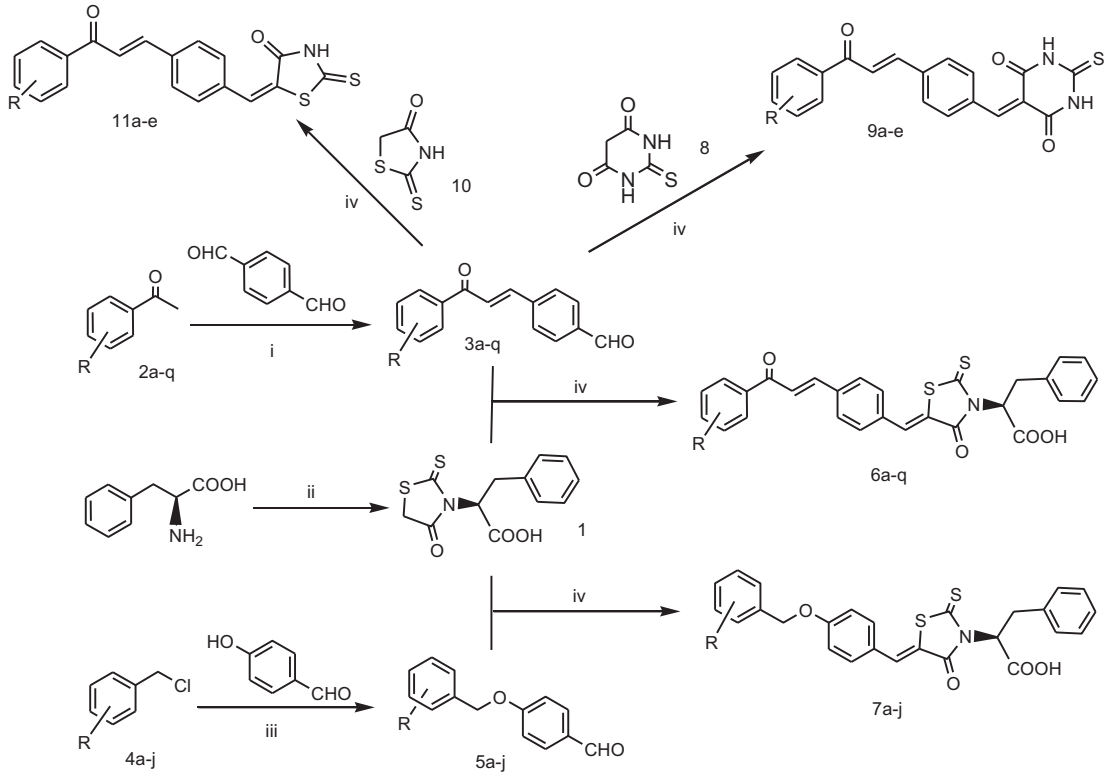


Fig. 1. Previously reported antibacterial agents.

known about the antibacterial activity of the chalcone moiety attached to thiobarbituric acid and nonsubstituted rhodanine rings. With this in mind, we designed and synthesized two series of chalcone derivatives containing thiobarbituric acid and 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**) and evaluated their antimicrobial activities against multidrug resistant clinical isolates.

2. Chemistry

The L-phenylalanine-derived C5-substituted rhodanines (**6a**–**q**, **7a**–**j**) and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**) were synthesized as described in Scheme 1. The key intermediate (*S*)-2-(4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**1**) intermediate was prepared according to the methods reported in the literature [15–17]. The desired (*E*)-4-(3-oxo-3-phenylprop-1-en-1-yl)benzaldehydes (**3a**–**q**) were also obtained according to a previously reported literature method [13]. Briefly, the Claisen–Schmidt condensation reactions of phthalaldehyde with the substituted acetophenones (**2a**–**q**) provided the desired benzaldehydes (**3a**–**q**) under the appropriate conditions. A further series of benzyloxybenzaldehyde derivatives (**5a**–**j**) were obtained by reacting 4-hydroxybenzaldehyde with the appropriate substituted benzyl chlorides (**4a**–**j**) [9]. Compounds **6a**–**q** and **7a**–**j** were prepared via the Knoevenagel condensation reactions of the appropriate intermediates **3a**–**q** or **5a**–**j** and with compound **1** [5]. The syntheses of **9a**–**e** and **11a**–**e** were achieved by the reactions of the different chalcone intermediates **3a**–**q** with thiobarbituric acid (**8**) or 2-thioxo-4-thiazolidinone (**10**). The newly synthesized compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR, mass spectral and elemental analyses.



6a: 2-F	6g: 2-Br	6m: 3-OCH_3	7a: 3-F	7f: 2,4-$(Cl)_2$	9a: 2-F	11a: 2-F
6b: 4-F	6h: 3-Br	6n: 4-OCH_3	7b: 4-F	7g: 3-Br	9b: 4-F	11b: 4-F
6c: 2-Cl	6i: 4-Br	6o: 4-$NHCOCH_3$	7c: 2-Cl	7h: 4-Br	9c: 2-Cl	11c: 2-Cl
6d: 3-Cl	6j: 4-CH_3	6p: H	7d: 3-Cl	7i: 4-CH_3	9d: 3-Cl	11d: 3-Cl
6e: 4-Cl	6k: 2,4-$(CH_3)_2$	6q: C_6H_6(3,4-fused)	7e: 4-Cl	7j: H	9e: 4-Cl	11e: 4-Cl
6f: 2,4-$(Cl)_2$	6l: 2-OCH_3					

Scheme 1. Synthetic scheme for the synthesis of compounds **6a**–**q**, **7a**–**j**, **9a**–**e** and **11a**–**e**. Reagents and conditions: (i) NaOH, EtOH, 23 °C, 3–4 h; (ii) NaOH, $ClCH_2COONa$, HCl, reflux, 24 h; (iii) K_2CO_3, acetone, reflux, 12 h; (iv) AcOH, piperidine, EtOH, reflux, 12 h.

3. Pharmacology

3.1. Anti-bacterial activity studies

The *in vitro* antimicrobial activity was performed using the broth microdilution method and the Minimum Inhibitory Concentration (MIC) with different strains, including multidrug-resistant clinical isolates.

The newly prepared compounds were screened for their antibacterial activity against Gram-positive bacterias (*Staphylococcus aureus* RN4220, *S. aureus* KCTC 503 and *S. aureus* KCTC 209) and a Gram-negative bacteria (*Escherichia coli* 1356). 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 susceptible microbacterias were grown to mid-log phase in Mueller-Hinton broth (MHB) and diluted 1000-fold in the same medium. The stock solutions of the synthesized compounds in DMSO were poured into 96-well plates and used to obtain final concentrations of 64–0.5 μg/mL by the twofold serial dilution technique [18]. Oxacillin and norfloxacin were used as positive controls for bacteria. Suspension of microbacteria was prepared to contain approximate 10^5 CFU/mL and applied to 96-well plates with serially diluted compounds to be tested and incubated at 37 °C for 24 h. The MIC (expressed in μg/mL) was the lowest concentration of the test substance that completely inhibited growth of the microbacteria. The microbacteria growth was measured by the absorption at 630 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out three times.

4. Results and discussion

The antimicrobial activities of the synthesized compounds against selected Gram-positive and Gram-negative bacteria and multidrug-resistant bacteria are illustrated in Tables 1 and 2, respectively.

As shown in Table 1, the *in vitro* antibacterial activities of all the compounds (**6a**–**q**, **7a**–**j**, **9a**–**e** and **11a**–**e**) were evaluated against *S. aureus* RN4220, *S. aureus* KCTC 503 and *S. aureus* KCTC 209. Their antibacterial activities were compared against the known antibacterial agents, oxacillin and norfloxacin, which were used as references. Although the rhodanine derivatives (**6a**–**q**, **7a**–**j**) exhibited significant levels of antibacterial activity with MICs of 2–16 μg/mL, they were less active than the reference drug oxacillin. In contrast, several derivatives (**6c**–**e**, **6g**, **6i**, **6j**, **6q**, **7d**–**j**) showed antibacterial activities that were very similar to that of the reference drug norfloxacin. The chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**) were the least active of the compounds tested with MIC values of 64 μg/mL. None of the compounds inhibited the growth of Gram-negative *E. coli* (MIC > 64 μg/mL).

The data in Table 2 revealed that compounds **6a**–**q** and **7a**–**j** exhibited significant activity against multidrug-resistant *S. aureus* (MRSA CCARM 3167 and MRSA CCARM 3506) and quinolone-*resistant S. aureus* (QRSA CCARM 3505 and QRSA CCARM 3519) with MIC values of 2–8 μg/mL. These values represented a 2–4-fold increase in potency relative to the standard drug norfloxacin, with compound **6q** showing the most potent levels of activity against all of the multidrug-resistant clinical isolates tested. Moreover, compounds **9a**–**e** and **11a**–**e** did not exhibit activity against the multidrug-resistant bacteria (MIC > 64 μg/mL).

Table 1
Inhibitory activity of compounds **6a**–**q**, **7a**–**j**, **9a**–**e** and **11a**–**e** expressed as MIC (μg/mL).

Compound	*S. aureus*			*E. coli*
	4220[a]	503[b]	209[c]	1356[d]
3a–**q**	>64	>64	>64	>64
6a	4	16	16	>64
6b	4	16	16	>64
6c	2	16	16	>64
6d	2	16	16	>64
6e	2	8	8	>64
6f	4	16	16	>64
6g	2	16	16	>64
6h	8	16	16	>64
6i	2	4	4	>64
6j	2	8	8	>64
6k	4	16	16	>64
6l	8	16	16	>64
6m	4	16	16	>64
6n	4	16	16	>64
6o	4	16	16	>64
6p	4	8	16	>64
6q	2	8	8	>64
5a–**j**	>64	>64	>64	>64
7a	4	16	16	>64
7b	4	8	8	>64
7c	4	8	8	>64
7d	2	8	8	>64
7e	2	8	8	>64
7f	2	8	8	>64
7g	2	4	4	>64
7h	2	8	8	>64
7i	4	16	8	>64
7j	2	8	8	>64
9a	>64	>64	>64	>64
9b	>64	>64	>64	>64
9c	>64	>64	>64	>64
9d	>64	>64	>64	>64
9e	>64	>64	>64	>64
11a	>64	>64	>64	>64
11b	>64	>64	>64	>64
11c	>64	>64	>64	>64
11d	>64	>64	>64	>64
11e	>64	>64	>64	>64
Oxacillin	1	1	1	>64
Norfloxacin	2	2	4	16

[a] *Staphylococcus aureus* RN4220.
[b] *Staphylococcus aureus* 503.
[c] *Staphylococcus aureus* 209.
[d] *Escherichia coli* CCARM 1356.

No clear structure–activity relationships were observed, indicating that the antibacterial activity was not significantly affected by the position or physicochemical properties of the different substituents on the phenyl ring. Based on all of these antibacterial activity data, the L-phenylalanine-derived C5-substituted rhodanines (**6a**–**q**, **7a**–**j**) showed good activity against the Gram-positive bacterias and multidrug-resistant bacteria, whereas the chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**) did not exhibit any antibacterial activity against these bacteria at 64 μg/mL. These data support the importance of the presence of a free carboxyl group to potent antibacterial activity. Compared to benzyloxybenzaldehyde derivatives **7a**–**j**, although compounds **6a**–**q** generally appeared to be more potent against Gram-positive bacteria, they exhibited similar levels of potency against the multidrug-resistant bacteria. Furthermore, the results demonstrated that the introduction of the phenylpropionic acid moiety at the N-3 position of the heterocycle can significantly increase the potency compared to that of the previously reported compounds, especially against the multidrug-resistant clinical isolates (Fig. 1). Further investigations are

Table 2
Inhibitory activity (MIC, μg/mL) of compounds **6a**–**q**, **7a**–**j**, **9a**–**e** and **11a**–**e** against clinical isolates of multidrug-resistant Gram-positive strains.

Compound	MRSA		QRSA	
	3167[a]	3506[b]	3505[c]	3519[d]
3a–q	>64	>64	>64	>64
6a	4	4	8	8
6b	4	4	8	8
6c	2	4	4	4
6d	2	4	8	8
6e	2	4	4	4
6f	4	4	4	16
6g	2	4	4	4
6h	8	8	8	16
6i	2	2	8	2
6j	2	2	8	2
6k	4	4	8	8
6l	8	8	4	16
6m	8	8	8	8
6n	4	4	4	4
6o	4	2	4	4
6p	4	4	8	4
6q	2	4	2	2
5a–j	>64	>64	>64	>64
7a	8	8	8	8
7b	8	8	8	8
7c	4	4	4	4
7d	4	4	4	4
7e	4	4	4	4
7f	8	16	8	8
7g	4	4	4	8
7h	4	8	8	8
7i	4	8	8	8
7j	4	8	4	8
9a	>64	>64	>64	>64
9b	>64	>64	>64	>64
9c	>64	>64	>64	>64
9d	>64	>64	>64	>64
9e	>64	>64	>64	>64
11a	>64	>64	>64	>64
11b	>64	>64	>64	>64
11c	>64	>64	>64	>64
11d	>64	>64	>64	>64
11e	>64	>64	>64	>64
Oxacillin	>64	>64	1	1
Norfloxacin	8	4	>64	>64

[a] Methicillin-resistant *S. aureus* CCARM 3167.
[b] Methicillin-resistant *S. aureus* CCARM 3506.
[c] Quinolone-resistant *S. aureus* CCARM 3505.
[d] Quinolone-resistant *S. aureus* CCARM 3519.

currently underway in our laboratory to further modify and evaluate these types of structures.

5. Conclusion

In this study, we have designed and synthesized four novel series of compounds, including the L-phenylalanine-derived C5-substituted rhodanines (**6a**–**q**, **7a**–**j**) and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone (**9a**–**e**, **11a**–**e**). The results showed that some of the compounds significantly inhibited the growth of a wide spectrum of Gram positive bacteria and multidrug-resistant strains of clinical isolates. In particular, compounds **6c**–**e**, **6g**, **6i**, **6j** and **6q** exhibited high levels of antimicrobial activity against *S. aureus* RN4220 with MICs of 2 μg/mL and **6q** showed the most potent levels of activity of all of the compounds tested against the multidrug-resistant clinical isolates. The results suggest that the hybrid compounds bearing a phenylpropionic acid moiety (which plays an important role in increasing the antimicrobial properties) at the N-3 position of the heterocycle represent promising leads for the development of novel antibacterial agents. Further investigation of these compounds including the evaluation of the activities of their enantiomers is currently underway in our laboratories.

6. Experimental protocols

6.1. 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 and N 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.

6.2. General procedure for the preparation of 2-(4-oxo-2-thioxothiazolidin-3-yl)-3-L-phenylpropanoic acid (*1*)

In a round-bottomed flask equipped with a magnetic stirrer, L-phenylalanine (30.3 mmol) was dissolved with sodium hydroxide (30.3 mmol) in water (25 mL). Then, carbon disulfide (30.3 mmol) was added to the reaction mixture, which was stirred vigorously overnight. An aqueous solution of sodium chloroacetate (30.3 mmol) was added and stirring was continued at 23 °C for 3 h. Then the reaction mixture was acidified with dilute HCl until pH 1.0 and refluxed overnight. The reaction mixture was neutralized with saturated $NaHCO_3$ solution. The resultant solution was acidified again with dilute HCl. The cyclized product was extracted in ethyl acetate, dried over anhydrous sodium sulfate and evaporated under vacuum and the residue was purified by column chromatography (dichloromethane/methanol, 95:05). A brown liquid. Yield 2.40 g, (29%) [15–17].

6.2.1. (S)-2-((Z)-5-(4-((E)-3-(2-Fluorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (*6a*)

Yield 23%; m.p. 120–121 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.52 (s, 2H, CH_2), 5.90 (s, 1H, CH), 7.65 (d, J = 15.0 Hz, 1H, CH=CH), 7.84 (s, 1H, CH=), 7.95 (d, J = 15.6 Hz, 1H, CH=CH), 7.17–8.01 (m, 13H, Ar–H), 13.57 (s, 1H, COOH). MS m/z 540 (M + Na$^+$). Anal. Calcd. for $C_{28}H_{20}FNO_4S_2$: C, 64.97; H, 3.89; N, 2.71. Found: C, 65.01; H, 3.92; N, 2.66.

6.2.2. (S)-2-((Z)-5-(4-((E)-3-(4-Fluorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (*6b*)

Yield 21%; m.p. 126–127 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.52 (s, 2H, CH_2), 5.89 (s, 1H, CH), 7.73 (d, J = 15.6 Hz, 1H, CH=CH), 7.83 (s, 1H, CH=), 8.03 (d, J = 15.9 Hz, 1H, CH=CH), 7.15–8.29 (m, 13H, Ar–H), 13.49 (s, 1H, COOH). MS m/z 540 (M + Na$^+$). Anal. Calcd. for C28H20FNO4S2: C, 64.97; H, 3.89; N, 2.71. Found: C, 64.92; H, 3.92; N, 2.69.

6.2.3. (S)-2-((Z)-5-(4-((E)-3-(2-Chlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (*6c*)

Yield 56%; m.p. 102–103 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.51 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.51 (d, J = 15.3 Hz, 1H, CH=CH), 7.81 (s, 1H, CH=), 7.91 (d, J = 15.6 Hz, 1H, CH=CH), 7.13–7.96 (m, 13H, Ar–H), 13.49 (s, 1H, COOH). MS m/z 534 (M + 1). Anal. Calcd. for $C_{28}H_{20}ClNO_4S_2$: C, 62.97; H, 3.77; N, 2.62. Found: C, 62.89; H, 3.82; N, 2.65.

*6.2.4. (S)-2-((Z)-5-(4-((E)-3-(3-Chlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6d**)*

Yield 83%; m.p. 124–125 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.53 (d, J = 6.0 Hz, 2H, CH_2), 5.81 (s, 1H, CH), 7.76 (d, J = 15.6 Hz, 1H, CH=CH), 7.81 (s, 1H, CH=), 8.04 (d, J = 15.6 Hz, 1H, CH=CH), 7.14–8.21 (m, 13H, Ar–H). MS m/z 556 (M + Na^+). Anal. Calcd. for $C_{28}H_{20}ClNO_4S_2$: C, 62.97; H, 3.77; N, 2.62. Found: C, 69.99; H, 3.75; N, 2.66.

*6.2.5. (S)-2-((Z)-5-(4-((E)-3-(4-Chlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6e**)*

Yield 24%; m.p. 210–211 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.52 (d, J = 5.4 Hz, 2H, CH_2), 5.91 (s, 1H, CH), 7.75 (d, J = 15.6 Hz, 1H, CH=CH), 7.84 (s, 1H, CH=), 8.04 (d, J = 15.6 Hz, 1H, CH=CH), 7.16–8.22 (m, 13H, Ar–H), 13.55 (s, 1H, COOH). MS m/z 578 (M + Na^+). Anal. Calcd. for $C_{28}H_{20}ClNO_4S_2$: C, 62.97; H, 3.77; N, 2.62. Found: C, 62.93; H, 3.79; N, 2.70.

*6.2.6. (S)-2-((Z)-5-(4-((E)-3-(2,4-Dichlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6f**)*

Yield 52%; m.p. 176–177 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.56 (s, 2H, CH_2), 5.74 (s, 1H, CH), 7.46 (d, J = 16.2 Hz, 1H, CH=CH), 7.77 (s, 1H, CH=), 7.91 (d, J = 15.6 Hz, 1H, CH=CH), 7.14–7.96 (m, 12H, Ar–H). MS m/z 568 (M + 1). Anal. Calcd. for $C_{28}H_{19}Cl_2NO_4S_2$: C, 59.16; H, 3.37; N, 2.46. Found: C, 59.22; H, 3.38; N, 2.45.

*6.2.7. (S)-2-((Z)-5-(4-((E)-3-(2-Bromophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6g**)*

Yield 68%; m.p. 112–113 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.49 (d, J = 5.1 Hz, 2H, CH_2), 5.88 (s, 1H, CH), 7.40 (d, J = 15.6 Hz, 1H, CH=CH), 7.81 (s, 1H, CH=), 7.76 (d, J = 15.9 Hz, 1H, CH=CH), 7.13–7.93 (m, 13H, Ar–H), 13.49 (s, 1H, COOH). MS m/z 580 (M + Na^+). Anal. Calcd. for $C_{28}H_{20}BrNO_4S_2$: C, 58.13; H, 3.48; N, 2.42. Found: C, 58.09; H, 3.51; N, 2.39.

*6.2.8. (S)-2-((Z)-5-(4-((E)-3-(3-Bromophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6h**)*

Yield 31%; m.p. 198–199 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.51 (d, J = 5.7 Hz, 2H, CH_2), 5.88 (s, 1H, CH), 7.74 (d, J = 15.6 Hz, 1H, CH=CH), 7.82 (s, 1H, CH=), 8.02 (d, J = 15.9 Hz, 1H, CH=CH), 7.15–8.32 (m, 13H, Ar–H), 13.45 (s, 1H, COOH). MS m/z 580 (M + Na^+). Anal. Calcd. for $C_{28}H_{20}BrNO_4S_2$: C, 58.13; H, 3.48; N, 2.42. Found: C, 58.20; H, 3.52; N, 2.33.

*6.2.9. (S)-2-((Z)-5-(4-((E)-3-(4-Bromophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6i**)*

Yield 41%; m.p. 211–212 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.51 (d, J = 5.4 Hz, 2H, CH_2), 5.90 (s, 1H, CH), 7.75 (d, J = 15.9 Hz, 1H, CH=CH), 7.85 (s, 1H, CH=), 8.05 (d, J = 16.0 Hz, 1H, CH=CH), 7.15–8.13 (m, 13H, Ar–H), 13.65 (s, 1H, COOH). MS m/z 602 (M + Na^+). Anal. Calcd. for $C_{28}H_{20}BrNO_4S_2$: C, 58.13; H, 3.48; N, 2.42. Found: C, 58.22; H, 3.45; N, 2.42.

*6.2.10. (S)-2-((Z)-4-Oxo-5-(4-((E)-3-oxo-3-(p-tolyl)prop-1-en-1-yl) benzylidene)-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6j**)*

Yield 54%; m.p. 229–230 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.41 (s, 3H, CH3), 3.51 (d, J = 5.7 Hz, 2H, CH_2), 5.89 (s, 1H, CH), 7.71 (d, J = 15.6 Hz, 1H, CH=CH), 7.84 (s, 1H, CH=), 8.04 (d, J = 15.6 Hz, 1H, CH=CH), 7.15–8.10 (m, 13H, Ar–H). MS m/z 514 (M + 1). Anal. Calcd. for $C_{29}H_{23}NO_4S_2$: C, 67.81; H, 4.51; N, 2.73. Found: C, 67.75; H, 4.55; N, 2.69.

*6.2.11. (S)-2-((Z)-5-(4-((E)-3-(2,4-Dimethylphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6k**)*

Yield 86%; m.p. 192–193 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.38 (s, 3H, CH3), 2.49 (s, 3H, CH3), 3.51 (s, 2H, CH_2), 5.89 (s, 1H, CH), 7.54 (d, J = 15.9 Hz, 1H, CH=CH), 7.81 (s, 1H, CH=), 7.92 (d, J = 15.6 Hz, 1H, CH=CH), 7.14–7.97 (m, 12H, Ar–H), 13.50 (s, 1H, COOH). MS m/z 550 (M + Na^+). Anal. Calcd. for $C_{30}H_{25}NO_4S_2$: C, 68.29; H, 4.78; N, 2.65. Found: C, 68.33; H, 4.75; N, 2.70.

*6.2.12. (S)-2-((Z)-5-(4-((E)-3-(2-Methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6l**)*

Yield 51%; m.p. 78–79 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.51 (s, 2H, CH_2), 3.86 (s, 3H, OCH3), 5.88 (s, 1H, CH), 7.35 (d, J = 15.0 Hz, 1H, CH=CH), 7.82 (s, 1H, CH=), 7.90 (d, J = 15.6 Hz, 1H, CH=CH), 7.03–7.97 (m, 13H, Ar–H), 13.44 (s, 1H, COOH). MS m/z 530 (M + 1). Anal. Calcd. for $C_{29}H_{23}NO_5S_2$: C, 65.77; H, 4.38; N, 2.64. Found: C, 65.80; H, 4.33; N, 2.68.

*6.2.13. (S)-2-((Z)-5-(4-((E)-3-(3-Methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6m**)*

Yield 43%; m.p. 90–91 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.51 (s, 2H, CH_2), 3.85 (s, 3H, OCH3), 5.89 (s, 1H, CH), 7.57 (d, J = 15.6 Hz, 1H, CH=CH), 7.84 (s, 1H, CH=), 8.01 (d, J = 15.9 Hz, 1H, CH=CH), 7.17–8.07 (m, 13H, Ar–H), 13.46 (s, 1H, COOH). MS m/z 530 (M + 1). Anal. Calcd. for $C_{29}H_{23}NO_5S_2$: C, 65.77; H, 4.38; N, 2.64. Found: C, 65.75; H, 4.40; N, 2.59.

*6.2.14. (S)-2-((Z)-5-(4-((E)-3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6n**)*

Yield 46%; m.p. 119–120 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.53 (d, J = 6.3 Hz, 2H, CH_2), 3.88 (s, 3H, OCH3), 5.80 (s, 1H, CH), 7.70 (d, J = 15.6 Hz, 1H, CH=CH), 7.81 (s, 1H, CH=), 8.04 (d, J = 15.6 Hz, 1H, CH=CH), 7.01–8.20 (m, 13H, Ar–H). MS m/z 530 (M + 1). Anal. Calcd. for $C_{29}H_{23}NO_5S_2$: C, 65.77; H, 4.38; N, 2.64. Found: C, 65.79; H, 4.41; N, 2.63.

*6.2.15. (S)-2-((Z)-5-(4-((E)-3-(4-Acetamidophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6o**)*

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 2.09 (s, 3H, CH3), 3.50 (d, J = 5.7 Hz, 2H, CH_2), 5.85 (s, 1H, CH), 7.69 (d, J = 15.3 Hz, 1H, CH=CH), 7.82 (s, 1H, CH=), 8.02 (d, J = 15.6 Hz, 1H, CH=CH), 7.14–8.16 (m, 13H, Ar–H), 10.33 (s, 1H, NH). MS m/z 557 (M + 1). Anal. Calcd. for $C_{30}H_{24}N_2O_5S_2$: C, 64.73; H, 4.35; N, 5.03. Found: C, 64.79; H, 4.31; N, 5.06.

*6.2.16. (S)-2-((Z)-4-Oxo-5-(4-((E)-3-oxo-3-phenylprop-1-en-1-yl) benzylidene)-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6p**)*

Yield 64%; m.p. 106–107 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.53 (s, 2H, CH_2), 5.90 (s, 1H, CH), 7.75 (d, J = 15.6 Hz, 1H, CH=CH), 7.85 (s, 1H, CH=), 8.05 (d, J = 15.3 Hz, 1H, CH=CH), 7.15–8.19 (m, 14H, Ar–H). MS m/z 522 (M + Na^+). Anal. Calcd. for $C_{28}H_{21}NO_4S_2$: C, 67.31; H, 4.24; N, 2.80. Found: C, 67.28; H, 4.22; N, 2.83.

*6.2.17. (S)-2-((Z)-5-(4-((E)-3-(Naphthalen-2-yl)-3-oxoprop-1-en-1-yl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6q**)*

Yield 62%; m.p. 218–219 °C. IR (KBr) cm^{-1}: 3449 (OH), 1687 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.53 (s, 2H, CH_2), 5.91 (s, 1H, CH), 7.82 (d, J = 15.9 Hz, 1H, CH=CH), 7.87 (s, 1H, CH=), 8.13 (d, J = 15.6 Hz, 1H, CH=CH), 7.16–8.98 (m, 16H, Ar–H), 13.48 (s, 1H, COOH). MS m/z 572 ($M + Na^+$). Anal. Calcd. for $C_{32}H_{23}NO_4S_2$: C, 69.92; H, 4.22; N, 2.55. Found: C, 69.89; H, 4.25; N, 2.59. ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): δ 32.83, 38.35, 38.63, 38.91, 39.18, 39.46, 39.74, 40.02, 57.92, 121.21, 123.75, 126.48, 127.44, 128.02, 128.72, 129.34, 130.96, 131.99, 132.66, 134.00, 134.81, 136.22, 136.92, 141.97, 166.18, 168.47, 188.34, 192.24.

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

To a suspension of **1** (1.1 mmol) in dry ethanol (10 mL), the 4-[(substitutedbenzyl)oxy]benzaldehydes **5a–j** (1 mmol), a catalytic amount of piperidine (0.1 mmol) and glacial acetic acid (0.1 mmol) were added. The mixture was stirred and refluxed overnight. After cooling, the solvent was evaporated *in vacuo*, dried, and purified by silica gel column chromatography (dichloromethane/methanol, 200:1). The yield, melting point and spectral data of each compound are given below.

*6.3.1. (S,E)-2-(5-(4-(3-Fluorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7a**)*

Yield 51%; m.p. 84–85 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.48 (d, J = 9.0 Hz, 2H, CH_2), 5.22 (s, 2H, CH_2), 5.86 (s, 1H, CH), 7.15–7.60 (m, 13H, Ar–H), 7.75 (s, 1H, CH=), 13.42 (s, 1H, COOH). MS m/z 494 (M + 1). Anal. Calcd. for $C_{26}H_{20}FNO_4S_2$: C, 63.27; H, 4.08; N, 2.84; S, 12.99. Found: C, 63.23; H, 4.14; N, 2.87.

*6.3.2. (S,E)-2-(5-(4-(4-Fluorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7b**)*

Yield 78%; m.p. 76–77 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.49 (d, J = 9.0 Hz, 2H, CH_2), 5.02 (s, 2H, CH_2), 5.84 (s, 1H, CH), 6.97–7.63 (m, 13H, Ar–H), 7.99 (s, 1H, CH=). MS m/z 516 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{20}FNO_4S_2$: C, 63.27; H, 4.08; N, 2.84; S, 12.99. Found: C, 63.33; H, 4.04; N, 2.78.

*6.3.3. (S,E)-2-(5-(4-(2-Chlorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7c**)*

Yield 66%; m.p. 98–99 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.49 (d, J = 9.0 Hz, 2H, CH_2), 5.26 (s, 2H, CH_2), 5.88 (s, 1H, CH), 7.14–7.63 (m, 13H, Ar–H), 7.77 (s, 1H, CH=). MS m/z 531 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{20}ClNO_4S_2$: C, 61.23; H, 3.95; N, 2.75; S, 12.57. Found: C, 61.29; H, 3.90; N, 2.77.

*6.3.4. (S,E)-2-(5-(4-(3-Chlorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7d**)*

Yield 57%; m.p. 74–75 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.49 (d, J = 9.0 Hz, 2H, CH_2), 5.23 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.14–7.62 (m, 13H, Ar–H), 7.76 (s, 1H, CH=), 13.44 (s, 1H, COOH). MS m/z 531 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{20}ClNO_4S_2$: C, 61.23; H, 3.95; N, 2.75; S, 12.57. Found: C, 61.25; H, 4.01; N, 2.71.

*6.3.5. (S,E)-2-(5-(4-(4-Chlorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7e**)*

Yield 29%; m.p. 70–71 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.49 (d, J = 9.0 Hz, 2H, CH_2), 5.21 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.14–7.61 (m, 13H, Ar–H), 7.76 (s, 1H, CH=), 13.43 (s, 1H, COOH). MS m/z 531 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{20}ClNO_4S_2$: C, 61.23; H, 3.95; N, 2.75; S, 12.57. Found: C, 61.19; H, 3.92; N, 2.77.

*6.3.6. (S,E)-2-(5-(4-(2,4-Dichlorobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-p-henylpropanoic acid (**7f**)*

Yield 33%; m.p. 80–81 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.50 (d, J = 9.0 Hz, 2H, CH_2), 5.25 (s, 2H, CH_2), 5.88 (s, 1H, CH), 7.14–7.71 (m, 13H, Ar–H), 7.77 (s, 1H, CH=), 13.45 (s, 1H, COOH). MS m/z 567 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{19}Cl_2NO_4S_2$: C, 57.35; H, 3.52; N, 2.57; S, 11.78. Found: C, 57.39; H, 3.51; N, 2.55.

*6.3.7. (S,E)-2-(5-(4-(3-Bromobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7g**)*

Yield 56%; m.p. 78–79 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.48 (d, J = 9.0 Hz, 2H, CH_2), 5.21 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.13–7.70 (m, 13H, Ar–H), 7.76 (s, 1H, CH=), 13.43 (s, 1H, COOH). MS m/z 556 (M + 1). Anal. Calcd. for $C_{26}H_{20}BrNO_4S_2$: C, 56.32; H, 3.64; N, 2.53; S, 11.57. Found: C, 56.39; H, 3.62; N, 2.59.

*6.3.8. (S,E)-2-(5-(4-(4-Bromobenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phen-ylpropanoic acid (**7h**)*

Yield 53%; m.p. 82–83 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.48 (d, J = 9.0 Hz, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.86 (s, 1H, CH), 7.15–7.60 (m, 13H, Ar–H), 7.74 (s, 1H, CH=). MS m/z 556 (M + 1). Anal. Calcd. for $C_{26}H_{20}BrNO_4S_2$: C, 56.32; H, 3.64; N, 2.53; S, 11.57. Found: C, 56.29; H, 3.66; N, 2.59.

*6.3.9. (S,Z)-2-(5-(4-(p-Methylbenzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**7i**)*

Yield 76%; m.p. 94–95 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.30 (s, 3H, CH3), 3.49 (d, J = 9.0 Hz, 2H, CH_2), 5.15 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.16–7.60 (m, 13H, Ar–H), 7.75 (s, 1H, CH=). MS m/z 512 ($M + Na^+$). Anal. Calcd. for $C_{27}H_{23}NO_4S_2$: C, 66.23; H, 4.73; N, 2.86; S, 13.10. Found: C, 66.28; H, 4.69; N, 2.59.

*6.3.10. (S,E)-2-(5-(4-(Benzyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenyl-propa-noic acid (**7j**)*

Yield 55%; m.p. 158–159 °C. IR (KBr) cm^{-1}: 3420 (OH), 1686 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 3.48 (d, J = 9.0 Hz, 2H, CH_2), 5.19 (s, 2H, CH_2), 5.87 (s, 1H, CH), 7.16–7.59 (m, 14H, Ar–H), 7.75 (s, 1H, CH=), 13.45 (s, 1H, COOH). MS m/z 498 ($M + Na^+$). Anal. Calcd. for $C_{26}H_{21}NO_4S_2$: C, 65.66; H, 4.45; N, 2.95; S, 13.48. Found: C, 65.60; H, 4.59; N, 2.93.

*6.4. General procedure for the preparation of compounds **9a–e***

To a suspension of thiobarbituric acid (**8**) (1.2 mmol) in dry ethanol (10 mL), the chalcone intermediate **3a–q** (1 mmol), a catalytic amount of piperidine (0.1 mmol) and glacial acetic acid (0.1 mmol) were added. The mixture was stirred and refluxed overnight. After cooling, the solvent was evaporated *in vacuo*, dried, and purified by silica gel column chromatography (dichloromethane/methanol, 200:1). The yield, melting point and spectral data of each compound are given below.

*6.4.1. (E)-5-(4-(3-(2-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)-dione (**9a**)*

Yield 47%; m.p. 264–265 °C. IR (KBr) cm^{-1}: 1668 (C=O), 2650 (NH). 1H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.89 (d, J = 8.4 Hz, 1H, CH=CH), 8.16 (d, J = 8.4 Hz, 1H, CH=CH), 7.37–8.18 (m, 8H, Ar–H), 8.29 (s, 1H, CH=), 12.39 (s, 1H, NH), 12.50 (s, 1H, NH). MS m/z 381 (M + 1).

*6.4.2. (E)-5-(4-(3-(4-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)-dione (**9b**)*

Yield 53%; m.p. 281–282 °C. IR (KBr) cm^{-1}: 1668 (C=O), 2650 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.99 (d, J = 8.4 Hz, 1H, CH=CH), 8.19 (d, J = 8.4 Hz, 1H, CH=CH), 7.76–8.30 (m, 8H, Ar–H), 8.31 (s, 1H, CH=), 12.39 (s, 1H, NH), 12.50 (s, 1H, NH). MS m/z 381 (M + 1).

*6.4.3. (E)-5-(4-(3-(2-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)-dione (**9c**)*

Yield 38%; m.p. 253–254 °C. IR (KBr) cm^{-1}: 1668 (C=O), 2650 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.82 (d, J = 8.4 Hz, 1H, CH=CH), 8.08 (d, J = 8.4 Hz, 1H, CH=CH), 7.65–8.20 (m, 8H, Ar–H), 8.22 (s, 1H, CH=), 12.39 (s, 1H, NH), 12.50 (s, 1H, NH). MS m/z 397 (M + 1).

*6.4.4. (E)-5-(4-(3-(3-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)-dione (**9d**)*

Yield 48%; m.p. 279–280 °C. IR (KBr) cm^{-1}: 1668 (C=O), 2650 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.87 (d, J = 8.4 Hz, 1H, CH=CH), 8.13 (d, J = 8.4 Hz, 1H, CH=CH), 7.47–8.16 (m, 8H, Ar–H), 8.28 (s, 1H, CH=), 12.38 (s, 1H, NH), 12.50 (s, 1H, NH). MS m/z 397 (M + 1).

*6.4.5. (E)-5-(4-(3-(4-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)-dione (**9e**)*

Yield 42%; m.p. 285–286 °C. IR (KBr) cm^{-1}: 1668 (C=O), 2650 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.02 (d, J = 8.4 Hz, 1H, CH=CH), 8.20 (d, J = 8.4 Hz, 1H, CH=CH), 7.61–8.25 (m, 8H, Ar–H), 8.31 (s, 1H, CH=), 12.39 (s, 1H, NH), 12.50 (s, 1H, NH). MS m/z 397 (M + 1).

*6.5. General procedure for the preparation of compounds **11a–e***

To a suspension of 2-thioxo-4-thiazolidinone (**10**) (1.2 mmol) in dry ethanol (10 mL), the chalcone intermediate **3a–q** (1 mmol), a catalytic amount of piperidine (0.1 mmol) and glacial acetic acid (0.1 mmol) were added. The mixture was stirred and refluxed overnight. After cooling, the solvent was evaporated *in vacuo*, dried, and purified by silica gel column chromatography (dichloromethane/methanol, 200:1). The yield, melting point and spectral data of each compound are given below.

*6.5.1. (E)-5-((E)-4-((E)-3-(2-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxothiazolidin-4-one (**11a**)*

Yield 39%; m.p. 271–272 °C. IR (KBr) cm^{-1}: 1775 (C=O), 2690 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.54 (d, J = 6.9 Hz, 1H, CH=CH), 7.75 (d, J = 6.9 Hz, 1H, CH=CH), 7.40–7.92 (m, 8H, Ar–H), 7.95 (s, 1H, CH=), 13.91 (s, 1H, NH). MS m/z 370 (M + 1).

*6.5.2. (E)-5-((E)-4-((E)-3-(4-Fluorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxothiazolidin-4-one (**11b**)*

Yield 29%; m.p. 290–291 °C. IR (KBr) cm^{-1}: 1775 (C=O), 2690 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.53 (d, J = 6.9 Hz, 1H, CH=CH), 7.65 (d, J = 6.9 Hz, 1H, CH=CH), 7.46–7.94 (m, 8H, Ar–H), 7.97 (s, 1H, CH=), 13.91 (s, 1H, NH). MS m/z 370 (M + 1).

*6.5.3. (E)-5-((E)-4-((E)-3-(2-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxothiazolidin-4-one (**11c**)*

Yield 36%; m.p. 263–264 °C. IR (KBr) cm^{-1}: 1775 (C=O), 2690 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.42 (d, J = 6.9 Hz, 1H, CH=CH), 7.58 (d, J = 6.9 Hz, 1H, CH=CH), 7.37–7.92 (m, 8H, Ar–H), 7.95 (s, 1H, CH=), 13.91 (s, 1H, NH). MS m/z 386 (M + 1).

*6.5.4. (E)-5-((E)-4-((E)-3-(3-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxothiazolidin-4-one (**11d**)*

Yield 31%; m.p. 288–289 °C. IR (KBr) cm^{-1}: 1775 (C=O), 2690 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.42 (d, J = 6.9 Hz, 1H, CH=CH), 8.20 (d, J = 6.9 Hz, 1H, CH=CH), 7.56–8.13 (m, 8H, Ar–H), 8.22 (s, 1H, CH=), 13.89 (s, 1H, NH). MS m/z 386 (M + 1).

*6.5.5. (E)-5-((E)-4-((E)-3-(4-Chlorophenyl)-3-oxoprop-1-enyl) benzylidene)-2-thioxothiazolidin-4-one (**11e**)*

Yield 35%; m.p. 294–295 °C. IR (KBr) cm^{-1}: 1775 (C=O), 2690 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.43 (d, J = 6.9 Hz, 1H, CH=CH), 7.60 (d, J = 6.9 Hz, 1H, CH=CH), 7.42–7.97 (m, 8H, Ar–H), 7.99 (s, 1H, CH=), 13.91 (s, 1H, NH). MS m/z 386 (M + 1).

Acknowledgment

This work was supported by the National Science Foundation of China (grant number 20962021).

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