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Original article

Synthesis and antibacterial activity of novel 1,3-diphenyl-1*H*-pyrazoles functionalized with phenylalanine-derived rhodanines

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ABSTRACT

In the present study, a series of novel 1,3-diphenyl-1*H*-pyrazoles functionalized with phenylalanine-derived rhodanine derivatives were synthesized and evaluated for their antibacterial activity. Compounds **4**, **6**, **9**, **10**, **12** and **15** exhibited stronger activity than the standard drugs, norfloxacin and oxacillin, with MIC values of 1 μg/mL against methicillin-resistant *Staphylococcus aureus* and quinolone-resistant *S. aureus*. None of the compounds showed any activity against Gram-negative bacteria.

1. Introduction

The treatment of bacterial infections remains an important and challenging therapeutic problem for many reasons, including the emergence of new infectious diseases and the increasing number of multidrug-resistant microbial pathogens [1–4]. For these reasons, the development of novel antimicrobial drugs is still necessary and very much in demand. Pyrazoles represent a key structural motif in heterocyclic chemistry and occupy a significant position in medicinal and pesticide chemistry because of their capability to exhibit a wide range of bioactivities, including their antimicrobial activity [5–10]. In our previous work, we reported the identification of a rhodanine-3-acetic acid derivatives (compound A, MIC = 2 μg/mL) bearing a 2,4-Cl_2 substituent chalcone moiety that showed high levels of inhibitory activity against Gram-positive bacterial strains (Fig. 1) [11]. Following on from this, we reported the identification of a 1,3-diarylpyrazole derivative (compound B, MIC = 2 μg/mL) bearing a rhodanine-3-pentanoic acid moiety that exhibited potent antibacterial activity against multidrug-resistant Gram-positive bacterial strains [12]. In addition, Diane Hardej et al. reported a phenylalanine-derived rhodanine derivative (compound C, MIC = 1.95 μg/mL) that showed high levels of inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [13]. Herein, we describe further modifications that we have made to compound B. These changes were focused on preserving the rhodanine and 1,3-diarylpyrazole moieties and substituting the fatty acid moiety with a phenylalanine while simultaneously investigating variations to the phenyl ring substituents. Thus, using the molecule hybrid approach, we designed and synthesized 15 novel 1,3-diphenyl-1*H*-pyrazole derivatives bearing phenylalanine-derived rhodanine moieties and evaluated their antibacterial activity.

2. Chemistry

The synthetic route to the phenylalanine-derived rhodanine derivatives **3**–**17** is depicted in Scheme 1. A series of 1,3-diaryl-4-formylpyrazoles **1** and the phenylalanine-derived rhodanine **2** had already been reported in a previous paper [13]. The 1,3-diaryl-4-formylpyrazoles **1** were subjected to a Knoevenagel condensation reaction with **2** to give the target compounds **3**–**17**. The identities of all of the synthesized compounds were confirmed by FTIR, ^{1}H and ^{13}C NMR, and mass spectral and elemental analyses.

3. Results and discussion

3.1. Evaluation of antibacterial activity

All of the synthesized compounds were evaluated *in vitro* with different antibacterial strains, including multidrug-resistant clinical isolates, using the minimum inhibitory concentration (MIC),

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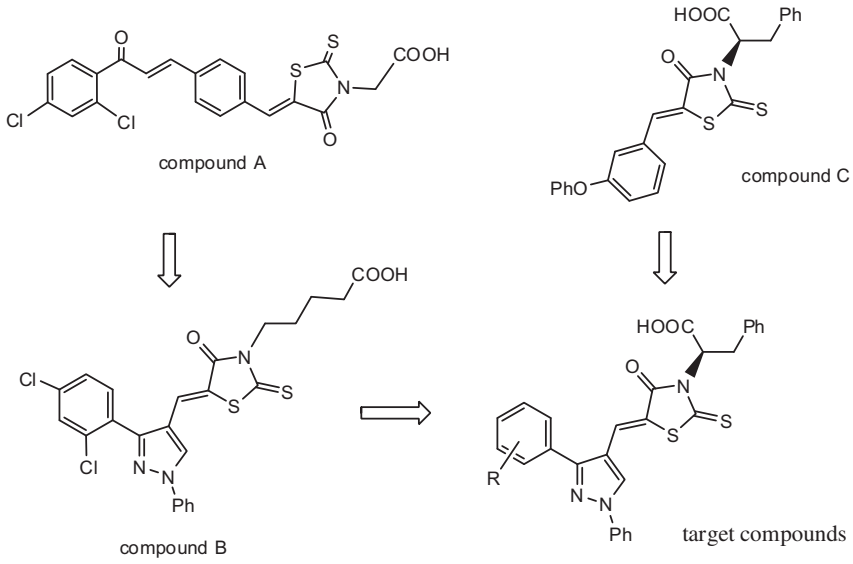


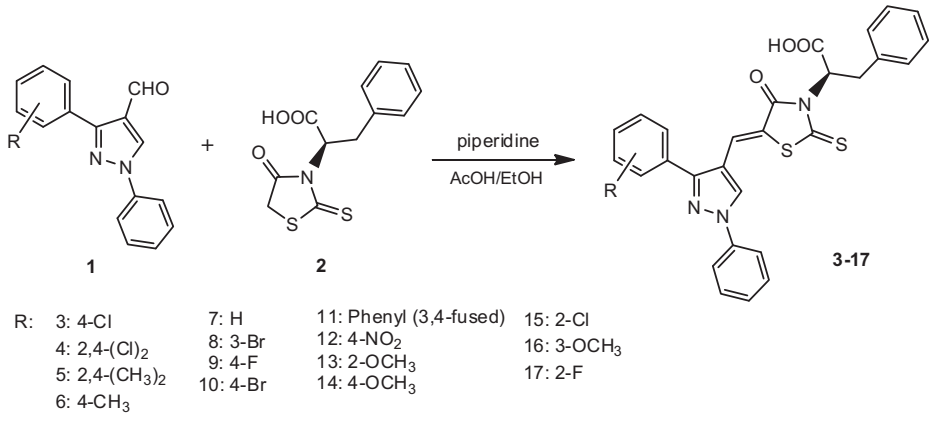
Fig. 1. Previously reported compounds and structure-based design of the target compounds.

which is defined as the lowest concentration of antibacterial agent required to visibly inhibit the growth of the bacteria. Oxacillin and norfloxacin were used as positive controls.

The MIC values of the synthesized compounds against *S. aureus* have been provided in Table 1. It is clear from the data that the majority of the compounds exhibited MIC values in the 2–8 μg/mL range. Compounds **3**, **4**, **5**, **6**, and **15** were highly active against *S. aureus* (*S. aureus* RN4220 and *S. aureus* KCTC 503) showing MIC values of 2 μg/mL. Furthermore, the activities of these compounds were comparable to norfloxacin, although they were less active than oxacillin. This class of compounds did not show any antibacterial activity *in vitro* against the Gram-negative (*Escherichia coli* 1924 and *E. coli* 1356) strains at 64 μg/mL.

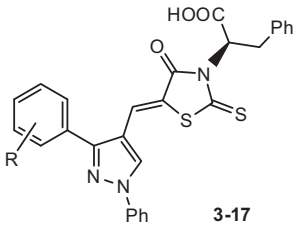
The MIC values of the synthesized compound against clinical isolates of multidrug-resistant Gram-positive bacterial strains are shown in Table 2. It is clear from the data that all of the compounds exhibited higher levels of inhibitory activity than oxacillin against MRSA 3167 (methicillin-resistant *S. aureus* CCARM 3167) and MRSA 3506 (methicillin-resistant *S. aureus* CCARM 3506). Compounds **4**, **6**, **9**, **10**, **12** and **15** were 8-fold more active against MRSA 3167 and 4-fold more active against MRSA 3506 than norfloxacin, with all of the compounds having a MIC value of 1 μg/mL against both bacterial strains. Against QRSA 3505 (quinolone-resistant *S. aureus* CCARM 3505) and QRSA 3519 (quinolone-resistant *S. aureus* CCARM 3519), all of the synthesized compounds exhibited higher levels of inhibitory activity than norfloxacin. Compounds **6** and **9** showed similar levels of activity to oxacillin against QRSA 3505 and QRSA 3519, with both compounds providing a MIC value of 1 μg/mL.

From an analysis of the activities of the synthesized compounds, some structure–activity relationships emerged. The position of the substituent on the phenyl ring did not have any impact on the antibacterial activity. Most of the derivatives bearing electron-donating substituents (except for the methyl group) showed lower levels of activity against multidrug-resistant clinical isolates than the corresponding derivatives bearing electron withdrawing groups. There was no clear correlation between the effect of different substituents and the observed activity. In comparison to the previously reported compounds bearing a rhodanine-3-fatty acid moiety [12], these 1,3-diphenyl-1*H*-pyrazole derivatives bearing a phenylalanine moiety generally exhibited higher levels of inhibitory activity. The current result therefore supports the hypothesis reported by Diane Hardej et al. that the presence of a phenylalanine moiety at the N3-position would increase the likelihood of bacterial cell wall penetration and therefore potentially improve the anti-MRSA activity of such compounds.



Scheme 1. Synthesis of compounds **3**–**17**.

Table 1
Inhibitory activity of compounds **3**–**17** expressed as MIC (μg/mL).

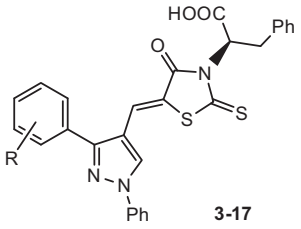


3-17

Compound	R	*S. aureus*[a]		*E. coli*[b]	
		4220	503	1924	1356
3	4-Cl	2	2	>64	>64
4	2,4-$(Cl)_2$	2	2	>64	>64
5	2,4-$(CH_3)_2$	2	2	>64	>64
6	4-CH_3	2	1	>64	>64
7	H	2	8	>64	>64
8	3-Br	2	8	>64	>64
9	4-F	4	16	>64	>64
10	4-Br	4	16	>64	>64
11	Phenyl(3,4-fused)	2	16	>64	>64
12	4-NO_2	4	16	>64	>64
13	2-OCH_3	2	16	>64	>64
14	4-OCH_3	4	16	>64	>64
15	2-Cl	2	2	>64	>64
16	3-OCH_3	4	16	>64	>64
17	2-F	4	16	>64	>64
Oxacillin		1	1	>64	>64
Norfloxacin		2	2	16	16

[a] *S. aureus* RN4220, *Staphylococcus aureus* RN4220; *S. aureus* 503, *Staphylococcus aureus* 503.
[b] *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.



3-17

compound	R	MRSA[a]		QRSA[b]	
		3167	3506	3505	3519
3	4-Cl	2	2	2	2
4	2,4-$(Cl)_2$	1	1	2	1
5	2,4-$(CH_3)_2$	2	2	2	1
6	4-CH_3	1	1	1	1
7	H	4	4	4	4
8	3-Br	4	4	4	4
9	4-F	1	1	1	1
10	4-Br	1	1	1	16
11	Phenyl (3,4-fused)	16	16	16	16
12	4-NO_2	1	1	1	1
13	2-OCH_3	16	16	16	16
14	4-OCH_3	4	4	4	8
15	2-Cl	1	1	2	1
16	3-OCH_3	16	16	16	16
17	2-F	4	4	2	4
Oxacillin		>64	>64	1	1
Norfloxacin		8	4	>64	>64

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

3.2. Evaluation of cytotoxicity

Human cervical (HeLa) cell monolayers were used as an *in vitro* model of the cervicovaginal epithelium to test the cytotoxicity of the synthesized compounds. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics (penicillin-streptomycin mixture [100 U/ml]). Cells at 80–90% confluence were split by trypsin (0.25% in phosphate buffered saline [PBS]; pH 7.4), and the medium was changed at 24 h intervals. The cells were cultured at 37 °C in a 5% CO_2 incubator. The cells were grown for three passages and approximately 1×10^4 cells were seeded into each well of a 96-well plate and allowed to incubate overnight to allow the cells to attach to the substrate. After 24 h, the medium was replaced with DMEM that was supplemented with 10% FBS and contained various concentrations of test compounds and incubated for 48 h. A 10 μl aliquot of MTT solution (5 mg/ml in PBS) was then added to each well. Following 4 h of incubation, the medium was removed and the resulting formazan crystals were dissolved in DMSO (100 μL). Following a 10-min period of shaking, the optical density of the solutions was measured at 570 nm using a microtiter enzyme-linked immunosorbent assay reader. The assay was conducted four times. The IC_{50} values were defined as the concentrations at which the cell growth was inhibited by 50%.

The cytotoxicity of compounds **6** and **15** was evaluated to determine if their observed antibacterial activity was caused by selective toxicity towards the bacterial cells (Table 3). Compounds **6** and **15** did not affect cell viability in the HeLa cells at their MIC values (1 μg/mL) but showed cytotoxicity at much higher concentrations. These results suggested that both compounds **6** and **15** could exhibit *in vitro* antibacterial activity at non-cytotoxic concentrations.

4. Conclusion

In conclusion, a series of novel 1,3-diphenyl-1*H*-pyrazoles that have been functionalized with phenylalanine-derived rhodanine derivatives have been synthesized and evaluated for their antibacterial activity. All of the compounds displayed antibacterial activity. Compounds **4**, **6**, **9**, **10**, **12** and **15** (MIC = 1 μg/mL) exhibited stronger activity against MRSA and QRSA than the standard drugs, norfloxacin and oxacillin. Compounds **6** and **15** were evaluated for their cytotoxicity and exhibited no significant influence on cell viability in the HeLa cells at their MIC (1 μg/mL). None of the compounds showed any activity against Gram-negative bacteria.

Table 3
Cytotoxic activity of compounds **6** and **15** against HeLa cell.[a]

Compound	IC_{50}[b] (μg/mL)
6	20.16
15	30.24

[a] Hela cell (Human cervical) monolayers were used as an in vitro model of cervicovaginal epithelium for testing the cytotoxicity of the new compounds.
[b] IC_{50} is defined as the concentration at which 50% growth is observed.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open glass capillaries in an electrical melting point apparatus 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 and ^{13}C NMR spectra were recorded in pure DMSO-d_6 on Bruker NMR spectrometers at 300 MHz and 75 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed in δ, ppm. 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 synthetic procedure for the key intermediates

Compound **1** was synthesized according to the literature [5] and compound **2** was already reported in a previous paper [13–16].

5.3. General synthetic procedure for the target compounds **3**–**17**

To a solution of compound **1** (1.0 mmol) and compound **2** (1.0 mmol) in absolute ethanol (8.0 ml) was added drops of glacial acetic acid and piperidine. The reaction mixture was stirred at 40–50 °C for 2–4 h, until the completion of the reaction as evidenced by TLC. The resulting reaction mixture was concentrated to dryness, purified by silica gel column chromatography (dichloromethane/methanol, 40:1) to afford pure products **3**–**17**. The yield, melting point and spectral data of each compound are given below.

5.3.1. (R,Z)-2-(5-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**3**)

Yield 85%; m.p. 202–204 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.77 (d, J = 7.5 Hz, 2H), 7.65 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.56–7.48 (m, 5H), 7.41 (d, J = 7.2 Hz, 1H), 7.20 (d, J = 6.8 Hz, 4H), 6.02 (m, 1H), 3.62 (m, 2H). MS m/z 545 (M + 1). Anal. calcd. for $C_{28}H_{20}ClN_3O_3S_2$: C, 61.59; H, 3.69; N, 7.70; S, 11.74. Found: C, 61.57; H, 3.67; N, 7.68; S, 11/72.

5.3.2. (R,Z)-2-(5-((3-(2,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**4**)

Yield 88%; m.p. 212–214 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.76 (d, J = 7.7 Hz, 2H), 7.53 (m, 3H), 7.45–7.36 (m, 3H), 7.30 (s, 1H), 7.19 (t, J = 8.2 Hz, 6H), 5.98 (m, 1H), 3.59 (m, 2H). MS m/z 579 (M + 1). Calcd. for $C_{28}H_{19}Cl_2N_3O_3S_2$: C, 57.93; H, 3.30; N, 7.24; S, 11.05. Found: C, 57.91; H, 3.28; N, 7.22; S, 11.03.

5.3.3. (R,Z)-2-(5-((3-(2,4-dimethylphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**5**)

Yield 80%; m.p. 217–219 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.77 (d, J = 8.2 Hz, 2H), 7.50 (t, J = 7.8 Hz, 3H), 7.41–7.34 (m, 2H), 7.21–7.14 (m, 7H), 5.98 (m, 1H), 3.58 (m, 2H), 2.39 (s, 3H), 2.27 (s, 3H). MS m/z 539 (M + 1). Calcd. for $C_{20}H_{25}N_3O_3S_2$: C, 66.77; H, 4.67; N, 7.79; S, 11.88. Found: C, 66.76; H, 4.66; N, 7.78; S, 11.86.

5.3.4. (R,Z)-2-(4-oxo-5-((1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl) methylene)-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**6**)

Yield 86%; m.p. 218–220 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.77 (d, J = 7.8 Hz, 2H), 7.72 (s, 1H), 7.52 (m, 4H), 7.39 (d, J = 7.4 Hz, 1H), 7.32 (d, J = 7.9 Hz, 2H), 7.19 (t, J = 6.4 Hz, 5H), 6.01 (m, 1H), 3.61 (m, 2H), 2.44 (s, 3H). MS m/z 525 (M + 1). Calcd. for $C_{29}H_{23}N_3O_3S_2$: C, 66.26; H, 4.41; N, 7.99; S, S, 12.20. Found: C, 66.25; H, 4.40; N, 7.97; S, 12.19.

5.3.5. (R,Z)-2-(5-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**7**)

Yield 79%; m.p. 224–226 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.15 (s, 1H), 7.75–7.50 (m, 8H), 7.44–7.31 (m, 3H), 7.26–6.95 (m, 5H), 6.01 (m, 1H), 3.60 (m, 2H). MS m/z 511 (M + 1). Calcd. for $C_{28}H_{21}N_3O_3S_2$: C, 65.73; H, 4.14; N, 8.21; S, S, 12.53. Found: C, 65.72; H, 4.12; N, 8.20; S, 12.52.

5.3.6. (R,Z)-2-(5-((3-(3-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**8**)

Yield 90%; m.p. 199–201 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.12 (s, 1H), 7.87 (s, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.67–7.59 (m, 2H), 7.53 (t, J = 7.9 Hz, 3H), 7.44–7.38 (m, 2H), 7.23–7.16 (m, 5H), 6.01 (m, 1H), 3.62 (m, 2H). MS m/z 591 (M + 1). Calcd. for $C_{28}H_{20}BrN_3O_3S_2$: C, 56.95; H, 3.41; N, 7.12; S, 10.86. Found: C, 56.93; H, 3.39; N, 7.10; S, 10.84.

5.3.7. (R,Z)-2-(5-((3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**9**)

Yield 87%; m.p. 210–212 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.12 (s, 1H), 7.77 (d, J = 7.8 Hz, 2H), 7.64 (m, 3H), 7.52 (t, J = 7.6 Hz, 3H), 7.41 (d, J = 7.4 Hz, 1H), 7.20 (d, J = 6.9 Hz, 6H), 6.01 (m, 1H), 3.62 (m, 2H). MS m/z 529 (M + 1). Calcd. for $C_{28}H_{20}FN_3O_3S_2$: C, 63.50; H, 3.81; N, 7.93; S, S, 12.11. Found: C, 63.51; H, 3.83; N, 7.92; S, 12.10.

5.3.8. (R,Z)-2-(5-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**10**)

Yield 84%; m.p. 207–209 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.77 (d, J = 7.7 Hz, 2H), 7.66 (d, J = 8.1 Hz, 3H), 7.54 (d, J = 8.3 Hz, 4H), 7.41 (d, J = 7.5 Hz, 1H), 7.23–7.15 (m, 5H), 6.01 (m, 1H), 3.62 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): δ 190.54, 166.03, 153.50, 138.48, 135.26, 131.73, 130.49, 129.94, 129.70, 128.74, 127.55, 126.62, 119.18, 115.94, 76.10, 29.32. MS m/z 591 (M + 1). Calcd. for $C_{28}H_{20}BrN_3O_3S_2$: C, 56.95; H, 3.41; N, 7.12; S, 10.86. Found: C, 56.96; H, 3.42; N, 7.11; S, 10.87.

5.3.9. (R,Z)-2-(5-((3-(naphthalen-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**11**)

Yield 82%; m.p. 223–224 °C. IR (KBr) cm^{-1}: 3429 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.15 (s, 1H), 8.12 (s, 1H), 8.04–7.88 (m, 4H), 7.81 (d, J = 7.3 Hz, 3H), 7.54 (m, 4H), 7.41 (d, J = 7.4 Hz, 1H), 7.20 (m, 5H), 6.01 (m, 1H), 3.61 (m, 2H). MS m/z 501 (M + 1). Calcd. for $C_{32}H_{23}N_3O_3S_2$: C, 68.43; H, 4.13; N, 7.48; S, 11.42. Found: C, 68.42; H, 4.12; N, 7.46; S, 11.43.

5.3.10. (R,Z)-2-(5-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**12**)

Yield 81%; m.p. 205–207 °C. IR (KBr) cm^{-1}: 3419 (OH), 1697 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.38 (d, J = 8.9 Hz, 2H), 8.15 (s, 1H), 7.91–7.84 (m, 2H), 7.78 (m, 2H), 7.64 (s, 1H), 7.54 (t, J = 7.7 Hz, 2H),

7.44 (d, $J = 7.3$ Hz, 1H), 7.24–7.16 (m, 5H), 6.01 (m, 1H,), 3.62 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): δ 190.30, 172.24, 165.99, 151.90, 147.64, 138.32, 137.21, 135.18, 129.38, 128.72, 128.10, 126.67, 123.73, 122.31, 116.28, 76.54, 30.07. MS m/z 556 (M + 1). Calcd. for $C_{28}H_{20}N_4O_5S_2$: C, 60.42; H, 3.62; N, 10.07; S, 11.52. Found: C, 60.41; H, 3.63; N, 10.05; S, 11.53.

*5.3.11. (R,Z)-2-(5-((3-(2-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**13**)*

Yield 89%; m.p. 209–211 °C. IR (KBr) cm^{-1}: 3419 (OH), 1697 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.10 (s, 1H), 7.77–7.59 (m, 5H), 7.47 (m, 3H), 7.34 (d, $J = 6.4$ Hz, 1H), 7.18 (d, $J = 6.1$ Hz, 4H), 7.08 (d, $J = 7.8$ Hz, 2H), 6.02 (m, 1H), 3.81 (s, 3H), 3.61 (m, 2H). MS m/z 541 (M + 1). Calcd. for $C_{29}H_{23}N_3O_4S_2$: C, 64.31; H, 4.28; N, 7.76; S, 11.84. Found: C, 64.29; H, 4.26; N, 7.77; S, 11.83.

*5.3.12. (R,Z)-2-(5-((3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**14**)*

Yield 80%; m.p. 226–228 °C. IR (KBr) cm^{-1}: 3419 (OH), 1697 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.10 (s, 1H), 7.77 (d, $J = 7.9$ Hz, 2H), 7.71 (s, 1H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.51 (m, 3H), 7.39 (d, $J = 6.9$ Hz, 1H), 7.20 (d, $J = 6.4$ Hz, 4H), 7.04 (d, $J = 8.3$ Hz, 2H), 6.02 (m, 1H), 3.88 (s, 3H), 3.61 (m, 2H). MS m/z 541 (M + 1). Calcd. for $C_{29}H_{23}N_3O_4S_2$: C, 64.31; H, 4.28; N, 7.76; S, 11.84. Found: C, 64.33; H, 4.29; N, 7.75; S, 11.83.

*5.3.13. (Z)-2-(5-((3-(2-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**15**)*

Yield 78%; m.p. 265–267 °C. IR (KBr) cm^{-1}: 3419 (OH), 1697 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.10 (s, 1H), 7.46–7.58 (m, 5H), 7.49–7.37 (m, 5H), 7.33 (d, $J = 6.9$ Hz, 1H), 7.16 (d, $J = 6.5$ Hz, 4H), 6.01 (m, 1H), 3.61 (m, 2H). MS m/z 545 (M + 1). Calcd. for $C_{21}H_{14}ClN_3O_3S_2$: C, 61.59; H, 3.69; N, 7.70; S, 11.74. Found: C, 61.58; H, 3.68; N, 7.71; S, 11.73.

*5.3.14. (R,Z)-2-(5-((3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**16**)*

Yield 82%; m.p. 227–229 °C. IR (KBr) cm^{-1}: 3419 (OH), 1707 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.10 (s, 1H), 7.74 (d, $J = 7.1$ Hz, 2H), 7.61 (m, 3H), 7.48 (m, 3H), 7.28 (m, 5H), 7.08 (d, $J = 7.7$ Hz, 2H), 6.02 (m, 1H), 3.86 (s, 3H), 3.61 (m, 2H). MS m/z 541 (M + 1). Calcd. for $C_{29}H_{23}N_3O_4S_2$: C, 64.31; H, 4.28; N, 7.76; S, 11.84. Found: C, 64.29; H, 4.26; N, 7.77; S, 11.83.

*5.3.15. (R,Z)-2-(5-((3-(2-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**27**)*

Yield 91%; m.p. 234–236 °C. IR (KBr) cm^{-1}: 3419 (OH), 1707 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.11 (s, 1H), 7.79 (d, $J = 7.8$ Hz, 2H), 7.61 (m, 3H), 7.48 (m, 4H), 7.17 (m, 4H), 7.08 (d, $J = 6.9$ Hz, 2H), 6.01 (m, 1H), 3.60 (m, 2H). MS m/z 529 (M + 1). Calcd. for $C_{28}H_{20}FN_3O_3S_2$: C, 63.50; H, 3.81; N, 7.93; S, 12.11. Found: C, 63.49; H, 3.80; N, 7.94; S, 12.12.

5.4. Evaluation of antibacterial activity in vitro

In vitro antibacterial activity assay method was referred in Ref. [11].

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