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Original article

Synthesis and antibacterial evaluation of rhodanine-based 5-aryloxy pyrazoles against selected methicillin resistant and quinolone-resistant *Staphylococcus aureus* (MRSA and QRSA)

Ming-Xia Song [a,b], Chang-Ji Zheng [a], Xian-Qing Deng [a], Liang-Peng Sun [a], Yan Wu [a], Lan Hong [c], Ying-Jing Li [a], Yi Liu [a], Zhi-Yu Wei [a], Ming-Jun Jin [a], Hu-Ri Piao [a,*]

[a] *Key Laboratory of Natural Resources and Functional Molecules of the Changbai Mountain, Affiliated Ministry of Education, Yanbian University College of Pharmacy, 977 Gongyuan Road, Jilin, Yanji 133002, PR China*
[b] *Jinggangshan University College of Medicine, Ji'an 343100, PR China*
[c] *Yanbian University College of Medicine, Yanji 133002, PR China*

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ABSTRACT

With an intention to synergize the anti-bacterial activity of 5-aryloxy pyrazole and rhodanine derivatives, eight series of hybrid compounds have been synthesized and evaluated for their antibacterial activity. The majority of the synthesized compounds showed good inhibitory activity against selected methicillin resistant and quinolone-resistant *Staphylococcus aureus* (MRSA, QRSA) with minimum inhibitory concentration (MIC) values in the range of 1–32 μg/mL. The cytotoxicity test suggests that these compounds exhibited in vitro antibacterial activity at non-cytotoxic concentrations. These studies therefore suggest that rhodanine-based 5-aryloxy pyrazoles are interesting scaffolds for the development of novel Gram-positive antibacterial agents.

1. Introduction

An alarming increment in pathogenic resistance to existing antimicrobial agents is a serious problem and necessitates continuing research into new classes of antimicrobials [1]. This crops up an enormous interest in antibacterial research and we strongly believe that there is an urgent call for the development of new antibacterial drugs with novel structure and a mechanism of action possibly different from that of current first-line antimicrobial drugs [2].

5-Aryloxy pyrazoles are chemically useful synthons bearing diverse biological activities, including antimicrobial [2], analgesic and anti-inflammatory activity [3]. Rhodanine and its derivatives have also been reported to exhibit a broad spectrum of biological activities, behaving as antibacterial [4–6], antifungal [7], antiparasitic [8], and anthelmintic agents [9,10].

Previously, we reported the identification of a 1,3-diarylpyrazole derivative (compound A, MIC = 4 μg/mL), which was strongly active against several strains of Gram-positive bacteria (including multidrug-resistant clinical isolates) [11]. The study of these types of compounds and their associated antimicrobial properties has been central to our ongoing research efforts. Herein, we describe further modifications that we have made to compound A, using the molecule hybrid approach. These modifications were focused on preserving the rhodanine and 1-phenylpyrazole moieties and substituting the acetic acid moiety at the N3-position of the rhodanine with several different fatty acids. Meanwhile, we introduced an aryloxy group at the 5-position of the pyrazole ring while simultaneously investigating variations to the aryloxy ring substituents. For the convenience of synthesis, we used ethyl acetoacetate as a starting material, which was eventually resulted in a methyl group at the 3-position of the pyrazole ring (Fig. 1). Thus, eight novel series of rhodanine-based 5-aryloxy pyrazoles (**5**–**12**) were synthesized, characterized and screened for their antibacterial activities. Moreover, in order to check out whether the dichlorophenyl ring on 3-position of the pyrazole ring is essential for activity or not, a series of (*Z*)-2-(5-((5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acids (**13**) were also designed.

* Corresponding author. Tel.: +86 433 2435003; fax: +86 433 2435004.
E-mail address: piaohuri@yahoo.com.cn (H.-R. Piao).

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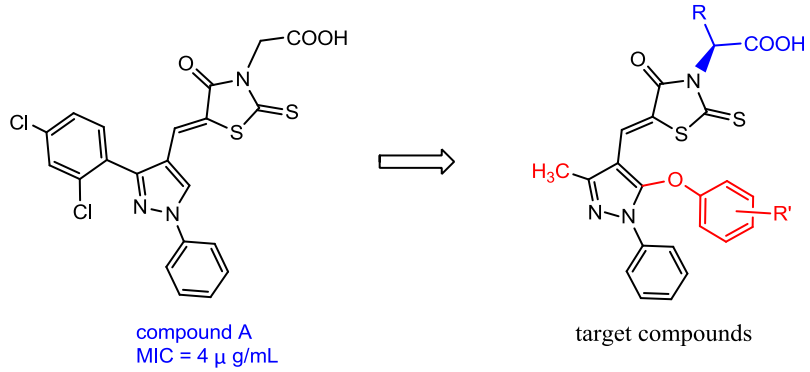


Fig. 1. Lead compound and structure-based design of the target compounds.

2. Chemistry

The target compounds were synthesized according to the route depicted in Scheme 1. Briefly, ethyl acetoacetate was reacted with phenylhydrazine to afford 3-methyl-1-phenyl-1*H*-pyrazol-5-ol (**2**). Compound **2** was treated with phosphorus oxychloride to give 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**3**), which was subsequently reacted with different substituted phenols to provide the intermediates (**4**). Compounds **3** or **4** were then subjected to a Knoevenagel condensation reaction with the appropriate rhodanine-3-fatty acids, which had themselves been synthesized using the reported procedure [11], to provide nine new series of target compounds (**5**–**13**). The structures of the desired compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral.

3. Results and discussion

3.1. Anti-bacterial activity

The antimicrobial assay was carried out using the following bacterial strains methicillin-resistant *Staphylococcus aureus CCARM* 3167 and 3506, and quinolone-resistant *S. aureus CCARM* 3505 and 3519. 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).

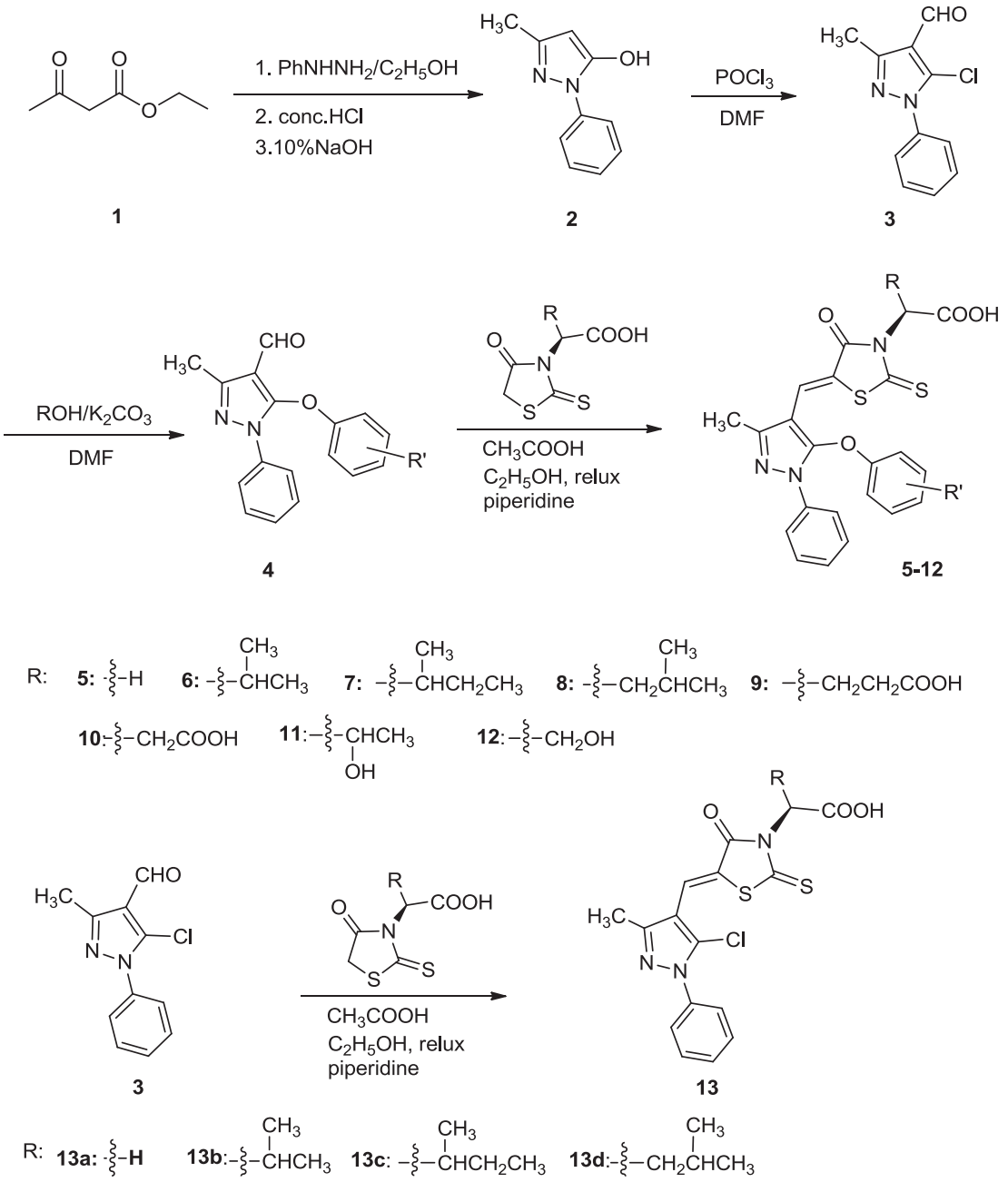
The title compounds along with the standard drugs (oxacillin and norfloxacin) for comparison were evaluated for their inhibitory activity against selected methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA). The biological results revealed that no compound in series **9**–**12** showed any inhibitory activity at 64 μg/mL against the selected drug-resistant organisms. For this reason, Tables 1 and 2 only presented the results of compounds of the series **5**–**8**. As shown in Tables 1 and 2, compounds in series **5** exhibited moderate activity, most of which with MICs of 8, 16 or 32 μg/mL. Only compound **5b** displayed much better activity against MRSA *CCARM* 3167 with a MIC value of 4 μg/mL. Compounds in series **6** and **8** presented the high activity with MIC values of 2–8 μg/mL, making them slightly less active than compounds in series **7** (MIC = 1–4 μg/mL) generally. Most compounds in series **6**, **7** and **8** presented comparable or much more potent activities than norfloxacin. Compound **7b**, in particular, showed MICs of 1 μg/mL against MRSA, representing an 8-fold increase in potency relative to norfloxacin (MIC = 8 μg/mL and 4 μg/mL) and a 64-fold increase relative to oxacillin (MIC > 64 μg/mL). For the QRSA strains, compound **7b** also presented high levels of with MIC values slightly less active than oxacillin (MIC = 1 μg/mL) but much greater than norfloxacin (MIC > 64 μg/mL).

In the present studies, three series of compounds (**5**, **6** and **7**) were firstly synthesized and evaluated for their antibacterial activity, among which compounds **5b**, **6b** and **7b** with 2,4-$(Cl)_2$ substituents, compounds **5g**, **6g** and **7g** with 4-Br substituent, and compounds **5m**, **6m** and **7m** with 2,4-$(CH_3)_2$ substituents presented much more potent activity in their respective series. Based on this fact, we inferred that the presence of 2,4-$(Cl)_2$, 2,4-$(CH_3)_2$, or 4-Br moieties may have more effects than the other substituents on the anti-microbial activity of these compounds. Therefore, in the next five series (**8**–**12**), only several compounds with 2,4-$(Cl)_2$, and/or 4-Br, and/or 2,4-$(CH_3)_2$ group were prepared to avoid a repetition.

Based on the analysis of the activities of the synthesized compounds, the following structure–activity relationships (SARs) were obtained. Introduction of some halogen groups on the phenyl ring generally increased the antimicrobial activity, relative to the non-substituted example. In the chlorine-substituted compounds, 2,4-$(Cl)_2$ groups had a good contribution to the activity (like compound **7b** with 2,4-$(Cl)_2$ groups, MIC = 2 μg/mL). A comparison of the halogen derivatives at the 4-position of the phenyl ring indicated that the different halogen atoms contributed to the antimicrobial activity generally in an order of Br > Cl > F. In the series **5**, for example, the MIC values of compound **5g** (4-Br), compound **5e** (4-Cl), and compound **5f** (4-F) were 8, 16, and >64 μg/mL, respectively. The similar trend was found in the series **6** and **7**. For the derivatives bearing electron-donating substituents, it seems that the methyl group displays much more impact on the antibacterial activity than the methoxy group with an 1–4 fold increase in potency. In the series **6**, for another example, the MIC values of the compound **6l** with 4-CH_3, compound **6o** with 4-OCH_3 were 4 and 8 μg/mL, respectively. A comparison of the activities across the eight different series of compounds, a general inhibitory activity order of series **7** > series **6**, **8** > series **5** > series **9**, **10**, **11**, **12** was obtained, which indicated that the increase of polar groups at the N3-position of the rhodanine ring did not exhibit a positive impact on the activity of the compounds. Relative to the previously reported compound bearing a rhodanine-3-acetic acid moiety (compound A), the current rhodanine-based 5-aryloxy pyrazoles generally exhibited higher levels of inhibitory activity [12]. Compared the activity of **13a**–**d** with that of the compound A and compounds **5**–**8**, it can be found that a substituted phenyl ring on pyrazole ring is essential for activity, but its position is not fixed. The substituted phenyl rings on both side of the pyrazole ring (3 or 5 position) afforded a good contribution to the activity.

3.2. Cytotoxicity

To see whether the antibacterial activity of compounds **6b**, **7b** and **8b** is selectively toxic to bacteria, their cytotoxicity was



Scheme 1. Synthetic scheme for the synthesis of the target compounds **5**–**13**.

evaluated (Table 3). Compounds **6b**, **7b** and **8b** did not affect cell viability on the Human cervical (HeLa) cells at their MICs (4, 1 or 2 μg/mL, respectively) but showed cytotoxicity at much higher concentrations. The inconsistency of compounds **6b**, **7b** and **8b** between their antibacterial activity and cytotoxicity suggests that compounds **6b**, **7b** and **8b** exhibited in vitro antibacterial activities at non-cytotoxic concentrations.

4. Conclusion

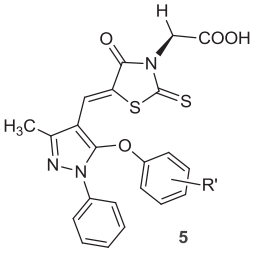
Based on our previous work, we have synthesized eight new series of rhodanine derivatives (**5**–**12**) and evaluated them for antibacterial and cytotoxic activities. Most of the compounds showed good antibacterial activities against multidrug-resistant strains of clinical isolates. Compound **7b** showed the most potent levels of activity (MIC = 1 or 2 μg/mL) against selected MRSA and QRSA strains. While the mechanism of action of this compound remains unknown, efforts to establish the cause of their antibacterial activity are ongoing and will be reported in due course.

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

Table 1
Inhibitory activity (MIC, μg/mL) of compounds **5** and **6** against clinical isolates of multidrug-resistant Gram-positive strains.



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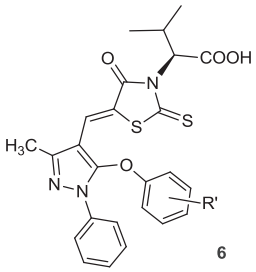

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Compound	R′	Multidrug-resistant Gram-positive strains			
		MRSA		*QRSA*	
		3167	3506	3505	3519
5a	2,6-$(Cl)_2$	32	32	32	32
5b	2,4-$(Cl)_2$	4	8	8	8
5c	2-Cl	16	16	32	16
5d	3-Cl	16	16	8	16
5e	4-Cl	16	16	16	16
5f	4-F	>64	>64	>64	>64
5g	4-Br	8	8	16	8
5h	Phenyl (3,4-fused)	8	8	8	8
5i	H	>64	>64	>64	>64
5j	2-CH_3	16	32	32	32
5k	3-CH_3	16	16	32	16
5l	4-CH_3	32	32	32	32
5m	2,4-$(CH_3)_2$	8	8	16	16
5n	3-CF_3	16	32	32	32
5o	4-OCH_3	>64	>64	>64	>64
5p	2-OCH_3	>64	>64	>64	>64
6a	2,6-$(Cl)_2$	8	16	8	16
6b	2,4-$(Cl)_2$	4	4	4	4
6c	2-Cl	4	4	8	4
6d	3-Cl	4	4	8	4
6e	4-Cl	4	4	4	4
6f	4-F	8	8	8	8
6g	4-Br	2	4	4	4
6h	Phenyl (3,4-fused)	4	8	8	8
6i	H	16	16	16	16
6j	2-CH_3	4	8	8	8
6k	3-CH_3	4	8	4	4
6l	4-CH_3	4	4	4	4
6m	2,4-$(CH_3)_2$	2	4	4	**2**
6n	3-CF_3	4	8	8	8
6o	4-OCH_3	8	8	8	8
6p	2-OCH_3	16	16	16	16
Norfloxacin	–	8	4	>64	>64
Oxacillin	–	>64	>64	1	1

an HP1100LC (Agilent Technologies, Santa Clara, CA, USA). The major chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Fluka Companies (Milwaukee, MI, USA).

5.2. General procedure for the preparation of compound **2**

To a stirred solution of acetylacetic ether (0.1 mol) and 70% ethanol (5.0 mL), the solution of phenylhydrazine (0.1 mol) with absolute alcohol (3.0 mL) was added dropwise at 45 °C, and the mixture was stirred for 20 min at this temperature. Cooled to 20 °C, added concentrated hydrochloric acid (1.0 mL) dropwise and reacted for 2 h at 45 °C. Then adjusted the PH value of mixture to 7 using 10% NaOH solution, added 20 mL water and stirred for 1 h at the room temperature. The precipitated solid was filtered, washed with cold absolute alcohol to get a white solid 3-methyl-1-phenyl-1*H*-pyrazol-5-ol (**2**).

5.3. General procedure for the preparation of compound **3**

To a cold, stirred solution of dimethylformamide (1 mmol) and phosphorous oxychloride (6 mmol) was added compound **2** (1 mmol). The reaction mixture was stirred at 80 °C for 3 h, cooled to room temperature, poured into ice cold water whereupon a solid separated out that was filtered, washed with excess of cold water, dried to afford a yellow solid 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**3**).

5.4. General procedure for the preparation of compounds **4**

To a solution of compound **3** (10 mmol) and K_2CO_3 (15 mmol) in dry dimethylformamide (10 mL), corresponding substituted phenols (11 mmol) was added and mixture was stirred overnight at 80 °C. After the completion of reaction, poured the mixture into 100 mL ice-water and filtered to obtain a white solid. The resulting crude solid was directly used in the next step without purification.

5.5. General procedure for the preparation of compounds **5**

A mixture of ***4*** (3 mmol), 2-(4-oxo-2-thioxothiazolidin-3-yl) acetic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 100:1) to get a yellow solid.

5.5.1. (Z)-2-(5-((5-(2,6-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5a***)

Yield 59%; m.p. 214–216 °C. IR (KBr) cm^{-1}: 3417 (OH), 1705 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.35 (s, 3H, C$\underline{H}_3$), 4.68 (s, 2H, NC$\underline{H}_2$), 7.28–7.49 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.41 (s, 1H, COO$\underline{H}$). MS *m*/*z* 520 (M + 1).

5.5.2. (Z)-2-(5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5b***)

Yield 53%; m.p. 196–198 °C. IR (KBr) cm^{-1}: 3426 (OH), 1717 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.46 (s, 3H, C$\underline{H}_3$), 4.67 (s, 2H, NC$\underline{H}_2$), 6.70–7.73 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.40 (s, 1H, COO$\underline{H}$). MS *m*/*z* 520 (M + 1).

5.5.3. (Z)-2-(5-((5-(2-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5c***)

Yield 44%; m.p. 226–228 °C. IR (KBr) cm^{-1}: 3421 (OH), 1718 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.47 (s, 3H, C$\underline{H}_3$), 4.66 (s, 2H, NC$\underline{H}_2$), 6.65–7.58 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.40 (s, 1H, COO$\underline{H}$). MS *m*/*z* 486 (M + 1).

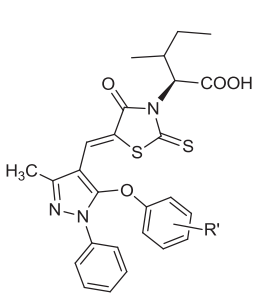
5.5.4. (Z)-2-(5-((5-(3-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5d***)

Yield 44%; m.p. 207–209 °C. IR (KBr) cm^{-1}: 3417 (OH), 1712 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.46 (s, 3H, C$\underline{H}_3$), 4.67 (s, 2H, NC$\underline{H}_2$), 6.84–7.62 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.42 (s, 1H, COO$\underline{H}$). MS *m*/*z* 486 (M + 1).

5.5.5. (Z)-2-(5-((5-(4-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (***5e***)

Yield 44%; m.p. 230–232 °C. IR (KBr) cm^{-1}: 3420 (OH), 1709 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.46 (s, 3H, C$\underline{H}_3$), 4.67 (s,

Table 2
Inhibitory activity (MIC, μg/mL) of compounds **7**, **8** and **13** against clinical isolates of multidrug-resistant Gram-positive strains.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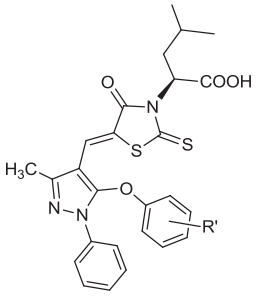
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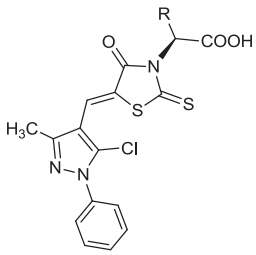
13

Compound	R′	Multidrug-resistant Gram-positive strains			
		MRSA		*QRSA*	
		3167	3506	3505	3519
7a	2,6-$(Cl)_2$	8	16	16	16
7b	2,4-$(Cl)_2$	1	1	2	2
7c	2-Cl	2	4	4	4
7d	3-Cl	4	8	8	8
7e	4-Cl	2	4	4	4
7f	4-F	2	4	8	8
7g	4-Br	2	2	2	2
7h	Phenyl(3,4-fused)	2	2	2	2
7i	H	4	4	8	4
7j	2-CH_3	2	4	4	4
7k	3-CH_3	2	4	4	2
7l	4-CH_3	2	4	4	4
7m	2,4-$(CH_3)_2$	2	2	2	2
7n	3-CF_3	4	8	8	8
7o	4-OCH_3	4	4	4	4
7p	2-OCH_3	16	16	16	16
8a	2,4-$(Cl)_2$	4	4	4	4
8b	4-Br	4	4	4	4
8c	2,4-$(CH_3)_2$	4	4	4	4
13a	–	>64	>64	>64	>64
13b	–	16	32	32	32
13c	–	16	32	16	32
13d	–	16	32	32	32
Norfloxacin	–	8	4	>64	>64
Oxacillin	–	>64	>64	1	1

2H, NC$\underline{H}_2$), 6.92–7.62 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.40 (s, 1H, COO$\underline{H}$). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.59, 167.32, 166.18, 154.53, 150.61, 144.94, 136.30, 130.14, 129.40, 128.10, 123.34, 122.48, 119.15, 117.07, 110.38, 104.80, 45.06, 12.91. MS *m*/*z* 486 (M + 1).

*5.5.6. (Z)-2-(5-((5-(4-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5f**)*

Yield 45%; m.p. 208–210 °C. IR (KBr) cm^{-1}: 3427 (OH), 1713 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.45 (s, 3H, C$\underline{H}_3$), 4.67 (s, 2H, NC$\underline{H}_2$), 6.91–7.61 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.41 (s, 1H, COO$\underline{H}$). MS *m*/*z* 470 (M + 1).

*5.5.7. (Z)-2-(5-((5-(4-Bromophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5g**)*

Yield 38%; m.p. 172–174 °C. IR (KBr) cm^{-1}: 3414 (OH), 1712 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.44 (s, 3H, C$\underline{H}_3$), 4.65 (s, 2H, NC$\underline{H}_2$), 6.85–7.60 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.42 (s, 1H, COO$\underline{H}$). MS *m*/*z* 531 (M + 1).

*5.5.8. (Z)-2-(5-((3-Methyl-5-(naphthalen-2-yloxy)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5h**)*

Yield 35%; m.p. 135–137 °C. IR (KBr) cm^{-1}: 3429 (OH), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.47 (s, 3H, C$\underline{H}_3$), 4.60 (s, 2H, NC$\underline{H}_2$), 6.52–8.53 (m, 13H, Ar–$\underline{H}$ and C$\underline{H}$), 13.37 (s, 1H, COO$\underline{H}$). MS *m*/*z* 502 (M + 1).

*5.5.9. (Z)-2-(5-((3-Methyl-5-phenoxy-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxotetrahydrothiophen-3-yl)acetic acid (**5i**)*

Yield 53%; m.p. 200–202 °C. IR (KBr) cm^{-1}: 3402 (OH), 1709 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.45 (s, 3H, C$\underline{H}_3$), 4.65 (s, 2H, NC$\underline{H}_2$), 6.85–7.61 (m, 11H, Ar–$\underline{H}$ and C$\underline{H}$), 13.38 (s, 1H, COO$\underline{H}$). MS *m*/*z* 452 (M + 1).

*5.5.10. (Z)-2-(5-((3-Methyl-1-phenyl-5-(o-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5j**)*

Yield 41%; m.p. 191–193 °C. IR (KBr) cm^{-1}: 3407 (OH), 1703 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.41 (s, 3H, ph-C$\underline{H}_3$), 2.46 (s, 3H, C$\underline{H}_3$), 4.65 (s, 2H, NC$\underline{H}_2$), 6.37–7.55 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.41 (s, 1H, COO$\underline{H}$). MS *m*/*z* 466 (M + 1).

Table 3
Cytotoxic activity of compounds **6b**, **7b** and **8b** against HeLa cell.

Compound	IC_{50} (μg/mL)[a]
6b	10.39
7b	6.43
8b	12.33

[a] IC_{50} is the concentrations required to inhibit 50% of cell growth.

*5.5.11. (Z)-2-(5-((3-Methyl-1-phenyl-5-(m-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5k**)*

Yield 39%; m.p. 158–160 °C. IR (KBr) cm^{-1}: 3408 (OH), 1702 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.16 (s, 3H, ph-CH_3), 2.45 (s, 3H, CH_3), 4.66 (s, 2H, NCH_2), 6.75–7.62 (m, 10H, Ar–H and CH), 13.41 (s, 1H, COOH). MS *m/z* 466 (M + 1).

*5.5.12. (Z)-2-(5-((3-Methyl-1-phenyl-5-(p-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5l**)*

Yield 49%; m.p. 207–209 °C. IR (KBr) cm^{-1}: 3426 (OH), 1717 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.16 (s, 3H, ph-CH_3), 2.45 (s, 3H, CH_3), 4.66 (s, 2H, NCH_2), 6.24–7.96 (m, 10H, Ar–H and CH), 13.40 (s, 1H, COOH). MS *m/z* 466 (M + 1).

*5.5.13. (Z)-2-(5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5m**)*

Yield 43%; m.p. 230–232 °C. IR (KBr) cm^{-1}: 3421 (OH), 1712 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.12 (s, 3H, ph-CH_3), 2.37 (s, 3H, ph-CH_3), 2.45 (s, 3H, CH_3), 4.65 (s, 2H, NCH_2), 5.76–7.95 (m, 9H, Ar–H and CH), 13.39 (s, 1H, COOH). MS *m/z* 480 (M + 1).

*5.5.14. (Z)-2-(5-((3-Methyl-1-phenyl-5-(3-(trifluoromethyl) phenoxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5n**)*

Yield 40%; m.p. 182–184 °C. IR (KBr) cm^{-1}: 3417 (OH), 1723 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.47 (s, 3H, CH_3), 4.66 (s, 2H, NCH_2), 7.18–7.61 (m, 10H, Ar–H and CH), 13.41 (s, 1H, COOH). MS *m/z* 520 (M + 1).

*5.5.15. (Z)-2-(5-((5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5o**)*

Yield 38%; m.p. 188–190 °C. IR (KBr) cm^{-1}: 3413 (OH), 1716 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.45 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 4.67 (s, 2H, NCH_2), 6.76–7.95 (m, 10H, Ar–H and CH), 13.42 (s, 1H, COOH). MS *m/z* 482 (M + 1).

*5.5.16. (Z)-2-(5-((5-(2-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**5p**)*

Yield 38%; m.p. 178–180 °C. IR (KBr) cm^{-1}: 3425 (OH), 1719 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.44 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.66 (s, 2H, NCH_2), 6.58–7.58 (m, 10H, Ar–H and CH), 13.42 (s, 1H, COOH). MS *m/z* 482 (M + 1).

*5.6. General procedure for the preparation of compound **6***

A mixture of ***4*** (3 mmol), (*S*)-3-methyl-2-(4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 100:1) to get a yellow solid.

*5.6.1. (S,Z)-2-(5-((5-(2,6-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6a**)*

Yield 53%; m.p. 118–120 °C. IR (KBr) cm^{-1}: 3431 (OH), 1713 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (d, 3H, *J* = 6.7 Hz, CHCH_3), 1.22 (d, 3H, *J* = 6.9 Hz, CHCH_3), 2.51 (s, 3H, CH_3), 2.61–2.78 (m, 1H, CH(CH_3)$_2$), 4.79 (s, 1H, NCH), 7.01–7.53 (m, 9H, Ar–H, and CH), 10.73 (s, 1H, COOH). MS *m/z* 563 (M + 1).

*5.6.2. (S,Z)-2-(5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6b**)*

Yield 43%; m.p. 122–124 °C. IR (KBr) cm^{-1}: 3437 (OH), 1709 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.61 (d, 3H, *J* = 6.8 Hz, CHCH_3), 1.21 (d, 3H, *J* = 6.6 Hz, CHCH_3), 2.44 (s, 3H, CH_3), 2.61–2.72 (m, 1H, CH(CH_3)$_2$), 4.80 (s, 1H, NCH), 7.19–7.72 (m, 9H, Ar–H, and CH), 11.03 (s, 1H, COOH). MS *m/z* 563 (M + 1).

*5.6.3. (S,Z)-2-(5-((5-(2-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6c**)*

Yield 54%; m.p. 91–93 °C. IR (KBr) cm^{-1}: 3427 (OH), 1706 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.64 (d, 3H, *J* = 6.8 Hz, CHCH_3), 1.21 (d, 3H, *J* = 6.4 Hz, CHCH_3), 2.46 (s, 3H, CH_3), 2.60–2.74 (m, 1H, CH(CH_3)$_2$), 5.09 (s, 1H, NCH), 6.66–7.57 (m, 10H, Ar–H, and CH), 12.66 (s, 1H, COOH). MS *m/z* 529 (M + 1).

*5.6.4. (S,Z)-2-(5-((5-(3-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6d**)*

Yield 49%; m.p. 94–96 °C. IR (KBr) cm^{-1}: 3443 (OH), 1712 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.64 (d, 3H, *J* = 6.7 Hz, CHCH_3), 1.16 (d, 3H, *J* = 6.3 Hz, CHCH_3), 2.44 (s, 3H, CH_3), 2.61–2.74 (m, 1H, CH(CH_3)$_2$), 5.07 (s, 1H, NCH), 6.85–7.61 (m, 10H, Ar–H, and CH), 12.63 (s, 1H, COOH). MS *m/z* 529 (M + 1).

*5.6.5. (S,Z)-2-(5-((5-(4-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6e**)*

Yield 46%; m.p. 102–104 °C. IR (KBr) cm^{-1}: 3407 (OH), 1711 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.62 (d, 3H, *J* = 6.8 Hz, CHCH_3), 1.17 (d, 3H, *J* = 6.3 Hz, CHCH_3), 2.44 (s, 3H, CH_3), 2.60–2.72 (m, 1H, CH(CH_3)$_2$), 4.90 (s, 1H, NCH), 6.91–7.60 (m, 10H, Ar–H, and CH), 12.77 (s, 1H, COOH). MS *m/z* 529 (M + 1).

*5.6.6. (S,Z)-2-(5-((5-(4-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6f**)*

Yield 42%; m.p. 89–91 °C. IR (KBr) cm^{-1}: 3417 (OH), 1712 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.62 (d, 3H, *J* = 6.7 Hz, CHCH_3), 1.18 (d, 3H, *J* = 6.4 Hz, CHCH_3), 2.43 (s, 3H, CH_3), 2.63–2.72 (m, 1H, CH(CH_3)$_2$), 4.87 (s, 1H, CH), 6.92–7.60 (m, 10H, Ar–H and CH), 12.67 (s, 1H, COOH). MS *m/z* 512 (M + 1).

*5.6.7. (S,Z)-2-(5-((5-(4-Bromophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6g**)*

Yield 46%; m.p. 120–122 °C. IR (KBr) cm^{-1}: 3414 (OH), 1717 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (d, 3H, *J* = 6.6 Hz, CHCH_3), 1.16 (d, 3H, *J* = 6.3 Hz, CHCH_3), 2.44 (s, 3H, CH_3), 2.60–2.70 (m, 1H, CH(CH_3)$_2$), 5.01 (s, 1H, CH), 6.86–7.60 (m, 10H, Ar–H and CH), 12.70 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 193.98, 172.07, 168.83, 166.48, 154.95, 150.58, 144.93, 136.33, 132.97, 129.38, 128.10, 123.17, 122.47, 117.58, 116.05, 104.70, 62.95, 21.97, 21.11, 19.08, 12.90. MS *m/z* 573 (M + 1).

*5.6.8. (S,Z)-3-Methyl-2-(5-((3-methyl-5-(naphthalen-1-yloxy)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (**6h**)*

Yield 36%; m.p. 91–93 °C. IR (KBr) cm^{-1}: 3397 (OH), 1708 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.55 (d, 3H, *J* = 6.8 Hz, CHCH_3), 1.16 (d, 3H, *J* = 6.4 Hz, CHCH_3), 2.48 (s, 3H, CH_3), 2.54–2.65 (m, 1H, CH(CH_3)$_2$), 4.74 (s, 1H, CH), 6.51–8.51 (m, 13H, Ar–H and CH), 12.35 (s, 1H, COOH). MS *m/z* 544 (M + 1).

*5.6.9. (S,Z)-3-Methyl-2-(5-((3-methyl-5-phenoxy-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (**6i**)*

Yield 50%; m.p. 133–135 °C. IR (KBr) cm^{-1}: 3421 (OH), 1702 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.60 (d, 3H, J = 6.6 Hz, CHC$\underline{H}_3$), 1.20 (d, 3H, J = 6.2 Hz, CHC$\underline{H}_3$), 2.43 (s, 3H, C$\underline{H}_3$), 2.60–2.71 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 4.80 (s, 1H, C$\underline{H}$), 6.85–8.15 (m, 11H, Ar–$\underline{H}$ and C$\underline{H}$), 11.41 (s, 1H, COO$\underline{H}$). MS m/z 494 (M + 1).

*5.6.10. (S,Z)-3-Methyl-2-(5-((3-methyl-1-phenyl-5-(o-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) butanoic acid (**6j**)*

Yield 56%; m.p. 96–98 °C. IR (KBr) cm^{-1}: 3399 (OH), 1715 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.62 (d, 3H, J = 6.7 Hz, CHC$\underline{H}_3$), 1.16 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.40 (s, 3H, ph-C$\underline{H}_3$), 2.45 (s, 3H, C$\underline{H}_3$), 2.58–2.71 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 4.99 (s, 1H, NC$\underline{H}$), 6.37–7.54 (m, 10H, Ar–$\underline{H}$, and C$\underline{H}$), 12.27 (s, 1H, COO$\underline{H}$). MS m/z 508 (M + 1).

*5.6.11. (S,Z)-3-Methyl-2-(5-((3-methyl-1-phenyl-5-(m-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) butanoic acid (**6k**)*

Yield 53%; m.p. 132–134 °C. IR (KBr) cm^{-1}: 3407 (OH), 1719 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (d, 3H, J = 6.7 Hz, CHC$\underline{H}_3$), 1.16 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.15 (s, 3H, ph-C$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 2.59–2.71 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 5.02 (s, 1H, NC$\underline{H}$), 6.27–7.61 (m, 10H, Ar–$\underline{H}$, and C$\underline{H}$), 12.80 (s, 1H, COO$\underline{H}$). MS m/z 508 (M + 1).

*5.6.12. (S,Z)-3-Methyl-2-(5-((3-methyl-1-phenyl-5-(p-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) butanoic acid (**6l**)*

Yield 48%; m.p. 140–142 °C. IR (KBr) cm^{-1}: 3405 (OH), 1709 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.64 (d, 3H, J = 6.8 Hz, CHC$\underline{H}_3$), 1.14 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.14 (s, 3H, ph-C$\underline{H}_3$), 2.43 (s, 3H, C$\underline{H}_3$), 2.62–2.70 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 5.08 (s, 1H, C$\underline{H}$), 6.74–7.60 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 12.93 (s, 1H, COO$\underline{H}$). MS m/z 508 (M + 1).

*5.6.13. (S,Z)-2-(5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6m**)*

Yield 44%; m.p. 124–126 °C. IR (KBr) cm^{-1}: 3395 (OH), 1701 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (d, 3H, J = 6.5 Hz, CHC$\underline{H}_3$), 1.15 (d, 3H, J = 6.2 Hz, CHC$\underline{H}_3$), 2.10 (s, 3H, ph-C$\underline{H}_3$), 2.35 (s, 3H, ph-C$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 2.63–2.73 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 5.03 (s, 1H, C$\underline{H}$), 6.74–7.60 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.27 (s, 1H, COO$\underline{H}$). MS m/z 522 (M + 1). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 194.87, 167.51, 152.14, 150.57, 145.72, 136.50, 132.81, 132.27, 129.23, 128.08, 127.66, 125.14, 123.59, 122.54, 112.48, 109.51, 104.92, 62.87, 44.28, 27.79, 21.97, 19.95, 19.06, 15.79, 12.88.

*5.6.14. (S,Z)-3-Methyl-2-(5-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (**6n**)*

Yield 41%; m.p. 128–130 °C. IR (KBr) cm^{-1}: 3410 (OH), 1706 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.61 (d, 3H, J = 6.7 Hz, CHC$\underline{H}_3$), 1.15 (d, 3H, J = 6.3 Hz, CHC$\underline{H}_3$), 2.45 (s, 3H, C$\underline{H}_3$), 2.62–2.70 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 5.03 (s, 1H, C$\underline{H}$), 7.18–7.60 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.45 (s, 1H, COO$\underline{H}$). MS m/z 562 (M + 1).

*5.6.15. (S,Z)-2-(5-((5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6o**)*

Yield 41%; m.p. 129–121 °C. IR (KBr) cm^{-1}: 3394 (OH), 1703 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (d, 3H, J = 6.6 Hz, CHC$\underline{H}_3$), 1.16 (d, 3H, J = 6.3 Hz, CHC$\underline{H}_3$), 2.43 (s, 3H, C$\underline{H}_3$), 2.61–2.71 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.62 (s, 3H, OC$\underline{H}_3$), 5.00 (s, 1H, C$\underline{H}$), 6.80–7.61 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.67 (s, 1H, COO$\underline{H}$). MS m/z 524 (M + 1).

*5.6.16. (S,Z)-2-(5-((5-(2-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**6p**)*

Yield 41%; m.p. 122–124 °C. IR (KBr) cm^{-1}: 3411 (OH), 1716 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.65 (d, 3H, J = 6.5 Hz, CHC$\underline{H}_3$), 1.16 (d, 3H, J = 6.3 Hz, CHC$\underline{H}_3$), 2.42 (s, 3H, C$\underline{H}_3$), 2.63–2.74 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.83 (s, 3H, OC$\underline{H}_3$), 5.05 (s, 1H, C$\underline{H}$), 6.60–7.56 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.27 (s, 1H, COO$\underline{H}$). MS m/z 524 (M + 1).

*5.7. General procedure for the preparation of compounds **7***

A mixture of ***4*** (3 mmol), (2*S*)-3-methyl-2-(4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 80:1) to get a yellow solid.

*5.7.1. (2S)-2-((Z)-5-((5-(2,6-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7a**)*

Yield 53%; m.p. 106–108 °C. IR (KBr) cm^{-1}: 3417 (OH), 1743 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.63 (t, 3H, J = 7.4 Hz, CH$_2$C$\underline{H}_3$), 0.80–0.89 (m, 2H, CH$_3$C$\underline{H}_2$), 1.22 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.15–2.24 (m, 1H, CH$_3$C$\underline{H}$), 2.29 (s, 1H, C$\underline{H}_3$), 4.73 (br.s, 1H, NC$\underline{H}$), 7.00–7.53 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 12.72 (s, 1H, COO$\underline{H}$). MS m/z 577 (M + 1).

*5.7.2. (2S)-2-((Z)-5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7b**)*

Yield 43%; m.p. 126–128 °C. IR (KBr) cm^{-1}: 3418 (OH), 1745 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.74 (t, 3H, J = 7.1 Hz, CH$_2$C$\underline{H}_3$), 0.81–0.88 (m, 2H, CH$_3$C$\underline{H}_2$), 1.11 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.37–2.44 (m, 1H, CH$_3$C$\underline{H}$), 2.45 (s, 1H, C$\underline{H}_3$), 5.16 (br.s, 1H, NC$\underline{H}$), 6.72–7.73 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.18 (s, 1H, COO$\underline{H}$). MS m/z 577 (M + 1).

*5.7.3. (2S)-2-((Z)-5-((5-(2-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7c**)*

Yield 54%; m.p. 136–138 °C. IR (KBr) cm^{-1}: 3422 (OH), 1748 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.74 (t, 3H, J = 7.1 Hz, CH$_2$C$\underline{H}_3$), 0.83–0.94 (m, 2H, CH$_3$C$\underline{H}_2$), 1.11 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$), 2.38–2.45 (m, 1H, CH$_3$C$\underline{H}$), 2.46 (s, 1H, C$\underline{H}_3$), 5.15 (d, 1H, J = 8.6 Hz, NC$\underline{H}$), 6.67–7.56 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.15 (s, 1H, COO$\underline{H}$). MS m/z 543 (M + 1).

*5.7.4. (2S)-2-((Z)-5-((5-(3-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7d**)*

Yield 49%; m.p. 127–129 °C. IR (KBr) cm^{-1}: 3425 (OH), 1749 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.74 (t, 3H, J = 7.4 Hz, CH$_2$C$\underline{H}_3$), 0.81–0.92 (m, 2H, CH$_3$C$\underline{H}_2$), 1.17 (d, 3H, J = 6.3 Hz, CHC$\underline{H}_3$), 2.34–2.42 (m, 1H, CH$_3$C$\underline{H}$), 2.43 (s, 1H, C$\underline{H}_3$), 4.84 (br.s, 1H, NC$\underline{H}$), 6.83–7.61 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 12.55 (s, 1H, COO$\underline{H}$). MS m/z 543 (M + 1).

*5.7.5. (2S)-2-((Z)-5-((5-(4-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7e**)*

Yield 46%; m.p. 186–188 °C. IR (KBr) cm^{-1}: 3420 (OH), 1741 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.72 (t, 3H, J = 7.8 Hz, CH$_2$C$\underline{H}_3$), 0.84–1.02 (m, 2H, CH$_3$C$\underline{H}_2$), 1.17 (d, 3H, J = 6.3 Hz, CHC$\underline{H}_3$),

2.36–2.42 (m, 1H, CH_3CH), 2.43 (s, 1H, CH_3), 4.81 (br.s, 1H, NCH), 6.90–7.60 (m, 10H, Ar–H and CH), 12.59 (s, 1H, COOH). MS *m/z* 543 (M + 1).

*5.7.6. (2S)-2-((Z)-5-((5-(4-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7f**)*

Yield 42%; m.p. 164–166 °C. IR (KBr) cm^{-1}: 3413 (OH), 1738 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.74 (t, 3H, *J* = 7.6 Hz, CH_2CH_3), 0.82–0.89 (m, 2H, CH_3CH_2), 1.11 (d, 3H, *J* = 6.3 Hz, $CHCH_3$), 2.38–2.43 (m, 1H, CH_3CH), 2.44 (s, 1H, CH_3), 5.16 (d, 1H, *J* = 7.8 Hz, NCH), 6.91–7.60 (m, 10H, Ar–H and CH), 13.17 (s, 1H, COOH). MS *m/z* 526 (M + 1).

*5.7.7. (2S)-2-((Z)-5-((5-(4-Bromophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7g**)*

Yield 46%; m.p. 188–190 °C. IR (KBr) cm^{-1}: 3426 (OH), 1749 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.75 (t, 3H, *J* = 7.9 Hz, CH_2CH_3), 0.86–1.02 (m, 2H, CH_3CH_2), 1.11 (d, 3H, *J* = 6.4 Hz, $CHCH_3$), 2.37–2.43 (m, 1H, CH_3CH), 2.44 (s, 1H, CH_3), 5.15 (d, 1H, *J* = 7.4 Hz, NCH), 6.86–7.60 (m, 10H, Ar–H and CH), 13.17 (s, 1H, COOH). MS *m/z* 587 (M + 1).

*5.7.8. (2S)-3-Methyl-2-((Z)-5-((3-methyl-5-(naphthalen-1-yloxy)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**7h**)*

Yield 36%; m.p. 89–91 °C. IR (KBr) cm^{-1}: 3424 (OH), 1743 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.83 (t, 3H, *J* = 7.9 Hz, CH_2CH_3), 1.41 (d, 3H, *J* = 6.4 Hz, $CHCH_3$), 1.50–1.59 (m, 2H, CH_3CH_2), 2.42–2.48 (m, 1H, CH_3CH), 2.49 (s, 1H, CH_3), 4.72 (br.s, 1H, NCH), 6.06–8.51 (m, 13H, Ar–H and CH), 13.26 (s, 1H, COOH). MS *m/z* 558 (M + 1).

*5.7.9. (2S)-2-((Z)-5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7i**)*

Yield 44%; m.p. 188–190 °C. IR (KBr) cm^{-1}: 3420 (OH), 1744 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.74 (t, 3H, *J* = 7.9 Hz, CH_2CH_3), 1.11 (d, 3H, *J* = 6.6 Hz, $CHCH_3$), 1.15–1.26 (m, 2H, CH_3CH_2), 2.11 (s, 1H, ph-CH_3), 2.36 (s, 1H, ph-CH_3), 2.38–2.43 (m, 1H, CH_3CH), 2.44 (s, 1H, CH_3), 5.15 (d, 1H, *J* = 8.1 Hz, NCH), 6.25–7.54 (m, 9H, Ar–H and CH), 13.17 (s, 1H, COOH). MS *m/z* 536 (M + 1).

*5.7.10. (2S)-3-Methyl-2-((Z)-5-((3-methyl-1-phenyl-5-(o-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**7j**)*

Yield 56%; m.p. 185–187 °C. IR (KBr) cm^{-1}: 3409 (OH), 1738 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.75 (t, 3H, *J* = 7.8 Hz, CH_2CH_3), 1.11 (d, 3H, *J* = 6.5 Hz, $CHCH_3$), 1.21–1.29 (m, 2H, CH_3CH_2), 2.40 (s, 1H, ph-CH_3), 2.41–2.44 (m, 1H, CH_3CH), 2.45 (s, 1H, CH_3), 5.14 (d, 1H, *J* = 8.9 Hz, NCH), 6.38–7.55 (m, 10H, Ar–H and CH), 13.15 (s, 1H, COOH). MS *m/z* 522 (M + 1).

*5.7.11. (2S)-3-Methyl-2-((Z)-5-((3-methyl-1-phenyl-5-(m-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**7k**)*

Yield 53%; m.p. 184–186 °C. IR (KBr) cm^{-1}: 3420 (OH), 1752 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.75 (t, 3H, *J* = 7.6 Hz, CH_2CH_3), 0.80–0.91 (m, 2H, CH_3CH_2), 1.12 (d, 3H, *J* = 6.3 Hz, $CHCH_3$), 2.16 (s, 1H, ph-CH_3), 2.40–2.44 (m, 1H, CH_3CH), 2.45 (s, 1H, CH_3), 5.16 (br.s, 1H, NCH), 6.75–7.61 (m, 10H, Ar–H and CH), 13.17 (s, 1H, COOH). MS *m/z* 522 (M + 1).

*5.7.12. (2S)-3-Methyl-2-((Z)-5-((3-methyl-1-phenyl-5-(p-tolyloxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**7l**)*

Yield 48%; m.p. 189–191 °C. IR (KBr) cm^{-1}: 3418 (OH), 1755 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.73 (t, 3H, *J* = 7.6 Hz, CH_2CH_3), 0.84–1.03 (m, 2H, CH_3CH_2), 1.10 (d, 3H, *J* = 6.3 Hz, $CHCH_3$), 2.14 (s, 1H, ph-CH_3), 2.38–2.42 (m, 1H, CH_3CH), 2.43 (s, 1H, CH_3), 5.14 (d, 1H, *J* = 8.7 Hz, NCH), 6.73–7.59 (m, 10H, Ar–H and CH), 13.07 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 194.04, 168.67, 166.54, 153.82, 150.65, 145.73, 136.47, 133.25, 130.55, 129.32, 127.97, 124.03, 122.34, 117.41, 114.99, 104.79, 61.54, 32.97, 24.81, 20.01, 17.58, 12.89, 10.84. MS *m/z* 522 (M + 1).

*5.7.13. (2S)-3-Methyl-2-((Z)-5-((3-methyl-5-phenoxy-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) pentanoic acid (**7m**)*

Yield 50%; m.p. 183–185 °C. IR (KBr) cm^{-1}: 3421 (OH), 1751 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.73 (t, 3H, *J* = 7.8 Hz, CH_2CH_3), 0.84–1.02 (m, 2H, CH_3CH_2), 1.11 (d, 3H, *J* = 6.3 Hz, $CHCH_3$), 2.38–2.43 (m, 1H, CH_3CH), 2.44 (s, 1H, CH_3), 5.11 (d, 1H, *J* = 7.8 Hz, NCH), 6.86–8.02 (m, 11H, Ar–H and CH), 13.20 (s, 1H, COOH). MS *m/z* 508 (M + 1).

*5.7.14. (2S)-3-Methyl-2-((Z)-5-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**7n**)*

Yield 41%; m.p. 79–81 °C. IR (KBr) cm^{-1}: 3425 (OH), 1754 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.73 (t, 3H, *J* = 7.8 Hz, CH_2CH_3), 0.89–1.07 (m, 2H, CH_3CH_2), 1.11 (d, 3H, *J* = 6.2 Hz, $CHCH_3$), 2.39–2.45 (m, 1H, CH_3CH), 2.46 (s, 1H, CH_3), 5.15 (d, 1H, *J* = 8.3 Hz, NCH), 7.19–7.60 (m, 10H, Ar–H and CH), 13.15 (s, 1H, COOH). MS *m/z* 576 (M + 1).

*5.7.15. (2S)-2-((Z)-5-((5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7o**)*

Yield 41%; m.p. 124–126 °C. IR (KBr) cm^{-1}: 3414 (OH), 1747 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.75 (t, 3H, *J* = 7.6 Hz, CH_2CH_3), 0.90–1.04 (m, 2H, CH_3CH_2), 1.11 (d, 3H, *J* = 6.4 Hz, $CHCH_3$), 2.37–2.43 (m, 1H, CH_3CH), 2.44 (s, 1H, CH_3), 3.73 (s, 1H, ph-CH_3), 5.16 (d, 1H, *J* = 7.4 Hz, NCH), 6.81–7.61 (m, 10H, Ar–H and CH), 13.16 (s, 1H, COOH). MS *m/z* 538 (M + 1).

*5.7.16. (2S)-2-((Z)-5-((5-(2-Methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**7p**)*

Yield 38%; m.p. 109–111 °C. IR (KBr) cm^{-1}: 3419 (OH), 1752 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.84 (t, 3H, *J* = 7.9 Hz, CH_2CH_3), 0.94–1.08 (m, 2H, CH_3CH_2), 1.23 (d, 3H, *J* = 6.3 Hz, $CHCH_3$), 2.33–2.40 (m, 1H, CH_3CH), 2.41 (s, 1H, CH_3), 3.73 (s, 1H, ph-CH_3), 4.82 (br.s, 1H, NCH), 6.57–7.57 (m, 10H, Ar–H and CH), 12.92 (s, 1H, COOH). MS *m/z* 538 (M + 1).

5.8. General procedure for the preparation of compound **8**

A mixture of appropriate 5-(substituted phenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3 mmol), (*S*)-4-methyl-2-(4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 80:1) to get a yellow solid.

*5.8.1. (S,Z)-2-(5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**8a**)*

Yield 35%; m.p. 120–122 °C. IR (KBr) cm^{-1}: 3425 (OH), 1718 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.80 (d, 3H, J = 6.51 Hz, CHC$\underline{H}_3$), 0.85 (d, 3H, J = 6. 45 Hz, CHC$\underline{H}_3$), 1.15 (m, 1H, CHC$\underline{H}_2$-Ha), 1.37 (m, 1H, CHC$\underline{H}_2$-Hb), 2.14 (m, 1H, C$\underline{H}$ $(CH_3)_2$), 2.44 (s, 3H, C$\underline{H}_3$), 5.51 (br.s, 1H, NC$\underline{H}$), 7.17–7.71 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.27 (s, 1H, COO$\underline{H}$). MS m/z 576 (M + 1).

*5.8.2. (S,Z)-2-(5-((5-(4-Bromophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**8b**)*

Yield 42%; m.p. 98–100 °C. IR (KBr) cm^{-1}: 3426 (OH), 1717 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.80 (d, 3H, J = 6.51 Hz, CHC$\underline{H}_3$), 0.86 (d, 3H, J = 6. 36 Hz, CHC$\underline{H}_3$), 1.36 (m, 1H, CHC$\underline{H}_2$-Ha), 1.97 (m, 1H, CHC$\underline{H}_2$-Hb), 2.12 (m, 1H, C$\underline{H}$ $(CH_3)_2$), 2.44 (s, 3H, C$\underline{H}_3$), 5.51 (br.s, 1H, NC$\underline{H}$), 7.29–7.59 (m, 10H, Ar–$\underline{H}$ and C$\underline{H}$), 13.30 (s, 1H, COO$\underline{H}$). MS m/z 587 (M + 1).

*5.8.3. (S,Z)-2-(5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**8c**)*

Yield 32%; m.p. 81–83 °C. IR (KBr) cm^{-1}: 3429 (OH), 1713 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.82 (d, 3H, J = 6.36 Hz, CHC$\underline{H}_3$), 0.87 (d, 3H, J = 6.54 Hz, CHC$\underline{H}_3$), 1.24 (m, 1H, CHC$\underline{H}_2$-Ha), 1.37 (m, 1H, CHC$\underline{H}_2$-Hb), 1.96 (m, 1H, C$\underline{H}$ $(CH_3)_2$), 2.11 (s, 3H, C$\underline{H}_3$), 2.37 (s, 3H, ph-C$\underline{H}_3$), 2.45 (s, 3H, ph-C$\underline{H}_3$), 5.51 (br.s, 1H, NC$\underline{H}$), 6.23–7.66 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 13.35 (s, 1H, COO$\underline{H}$). MS m/z 536 (M + 1).

*5.9. General procedure for the preparation of compound **9***

A mixture of 5-(2,4-dimethylphenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3 mmol), (*S*)-2-(4-oxo-2-thioxothiazolidin-3-yl)pentanedioic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol/acetic acid, 50:1:0.5) to get a yellow solid.

*5.9.1. (S,Z)-2-(5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) pentanedioic acid (**9a**)*

Yield 20%; m.p. 144–146 °C. IR (KBr) cm^{-1}: 3406 (OH), 1713 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.11 (s, 3H, ph-C$\underline{H}_3$), 2.17–3.23 (m, 2H, CHC$\underline{H}_2$), 2.36 (s, 3H, ph-C$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 3.36–3.43 (m, 2H, COC$\underline{H}_2$), 5.28 (br.s, 1H, NC$\underline{H}$), 6.21–7.55 (m, 9H, Ar–H and C$\underline{H}$), 13.18 (s, 2H, COO$\underline{H}$). MS m/z 552 (M + 1).

*5.10. The procedure for the preparation of compound **10***

A mixture of 5-(2,4-dichlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3 mmol), (*S*)-2-(4-oxo-2-thioxothiazolidin-3-yl)succinic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol/acetic acid, 50:1:0.5) to get a yellow solid.

*5.10.1. (S,Z)-2-(5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) succinic acid (**10a**)*

Yield 23%; m.p. 131–133 °C. IR (KBr) cm^{-1}: 3406 (OH), 1713 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.45 (s, 3H, C$\underline{H}_3$), 2.94–3.02 (m, 2H, CHC$\underline{H}_2$), 5.49 (br.s, 1H, CH_2C$\underline{H}$), 6.67–7.73 (m, 9H, Ar–$\underline{H}$ and C$\underline{H}$), 10.75 (s, 1H, N$\underline{H}$), 13.22 (s, 2H, COO$\underline{H}$). MS m/z 579 (M + 1).

*5.11. General procedure for the preparation of compound **11***

A mixture of appropriate 5-(substituted phenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3 mmol), (2*S*)-3-hydroxy-2-(4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol/acetic acid, 100:1:0.5) to get a yellow solid.

*5.11.1. (2S)-2-((Z)-5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-hydroxybutanoic acid (**11a**)*

Yield 23%; m.p. 107–109 °C. IR (KBr) cm^{-1}: 3414, 2924 (OH), 1674 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 1.33 (d, 3H, J = 6.0 Hz, CHC$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 4.26–4.33 (m, 1H, CH_3C$\underline{H}$), 5.02 (br.s, 1H, NC$\underline{H}$), 6.66–7.56 (m, 10H, Ar–$\underline{H}$), 7.72 (s, 1H, O$\underline{H}$), 13.14 (s, 1H, COO$\underline{H}$). MS m/z 565 (M + 1).

*5.11.2. (2S)-2-((Z)-5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-hydroxybutanoic acid (**11b**)*

Yield 19%; m.p. 110–112 °C. IR (KBr) cm^{-1}: 3412, 2921 (OH), 1676 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 1.34 (d, 3H, J = 6.1 Hz, CHC$\underline{H}_3$), 2.11 (s, 3H, ph-C$\underline{H}_3$), 2.38 (s, 3H, ph-C$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 4.32–4.41 (m, 1H, CH_3C$\underline{H}$), 5.05 (br.s, 1H, NC$\underline{H}$), 7.01 (s, 1H, O$\underline{H}$), 6.76–7.60 (m, 9H, Ar–$\underline{H}$), 13.13 (s, 1H, COO$\underline{H}$). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 186.17, 152.27, 150.36, 150.18, 145.32, 138.31, 136.57, 132.68, 132.33, 129.23, 128.99, 128.82, 127.97, 127.68, 125.95, 125.03, 122.42, 112.17, 105.15, 43.39, 21.72, 19.96, 15.83, 13.09. MS m/z 524 (M + 1).

*5.12. General procedure for the preparation of compound **12***

A mixture of appropriate 5-(substituted phenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3 mmol), (*S*)-3-hydroxy-2-(4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol/acetic acid, 100:1:0.5) to get a yellow solid.

*5.12.1. (S,Z)-2-(5-((5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-hydroxypropanoic acid (**12a**)*

Yield 21%; m.p. 191–193 °C. IR (KBr) cm^{-1}: 3448, 2904 (OH), 1703 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.45 (s, 3H, C$\underline{H}_3$), 4.01–4.11 (m, 2H, CHC$\underline{H}_2$), 5.46 (s, 1H, CH_2C$\underline{H}$), 5.76 (s, 1H, CH_2O$\underline{H}$), 6.67–7.72 (m, 9H, Ar–H), 13.24 (s, 1H, COO$\underline{H}$). MS m/z 551 (M + 1).

*5.12.2. (S,Z)-2-(5-((5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-hydroxypropanoic acid (**12b**)*

Yield 19%; m.p. 199–201 °C. IR (KBr) cm^{-1}: 3453, 2901 (OH), 1709 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 1.99 (s, 3H, ph-C$\underline{H}_3$), 2.38 (s, 3H, ph-C$\underline{H}_3$), 2.45 (s, 3H, C$\underline{H}_3$), 4.01–4.10 (m, 2H, CHC$\underline{H}_2$), 5.50 (s, 1H, CH_2C$\underline{H}$), 5.76 (s, 1H, CH_2O$\underline{H}$), 6.21–7.56 (m, 9H, Ar–$\underline{H}$), 13.21 (s, 1H, COO$\underline{H}$). ^{13}C NMR (DMSO-d_6, 300 MHz, ppm):

δ 186.17, 152.27, 150.36, 150.18, 145.32, 138.31, 136.57, 132.68, 132.33, 129.23, 128.99, 128.82, 127.97, 127.68, 125.95, 125.03, 122.42, 112.17, 105.15, 43.39, 21.72, 19.96, 15.83, 13.09. MS *m*/*z* 510 (M + 1).

5.13. General procedure for the preparation of compound **13**

A mixture of compound **3** (3 mmol), appropriate rhodanines (3 mmol), 10 drops glacial acetic acid and 10 drops piperidine in ethanol (20 mL) was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo*, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol, 100:1) to get a yellow solid.

5.13.1. (Z)-2-(5-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**13a**)

Yield 76%; m.p. 146–148 °C. IR (KBr) cm^{-1}: 3427 (OH), 1709 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 2.46 (s, 3H, CH_3), 4.54 (s, 2H, NCH_2), 7.45–7.73 (m, 6H, Ar–H and CH), 13.40 (s, 1H, COOH). MS *m*/*z* 395 (M + 1).

5.13.2. (S,Z)-2-(5-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylbutanoic acid (**13b**)

Yield 43%; m.p. 112–114 °C. IR (KBr) cm^{-1}: 3421 (OH), 1704 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.78 (d, 3H, *J* = 6.72 Hz, $CHCH_3$), 0.93 (m, 1H, CH_3CH), 1.26 (d, 3H, *J* = 6.36 Hz, $CHCH_3$), 2.44 (s, 3H, CH_3), 5.13 (br.s, 1H, CH_2CH), 7.44–8.02 (m, 6H, Ar–H and CH), 13.71 (s, 1H, COOH). MS *m*/*z* 436 (M + 1).

5.13.3. (2S)-2-((Z)-5-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**13c**)

Yield 50%; m.p. 106–108 °C. IR (KBr) cm^{-1}: 3418 (OH), 1702 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 0.85 (t, 3H, *J* = 7.23 Hz, $CHCH_3$), 0.99 (m, 2H, CH_3CH_2), 1.20 (d, 3H, *J* = 6.48 Hz, $CHCH_3$), 2.45 (s, 3H, CH_3), 5.27 (br.s, 1H, CH_2CH), 7.56–7.99 (m, 6H, Ar–H and CH), 13.30 (s, 1H, COOH). MS *m*/*z* 450 (M + 1).

5.13.4. (S,Z)-2-(5-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**13d**)

Yield 38%; m.p. 109–111 °C. IR (KBr) cm^{-1}: 3423 (OH), 1715 (C=O). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 0.92 (t, 3H, *J* = 6.57 Hz, $CHCH_3$), 0.97 (t, 3H, *J* = 6.48 Hz, $CHCH_3$), 5.63 (br.s, 1H, CH_2CH), 7.56–7.67 (m, 6H, Ar–H and CH), 13.44 (s, 1H, COOH). MS *m*/*z* 436 (M + 1).

5.14. Evaluation of anti-bacterial activity in vitro

The micro-organisms used in the present study were methicillin-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 two-fold serial dilution technique [13] was used to obtain final concentrations of 64–0.5 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.

5.15. Evaluation of cytotoxicity in vitro

Human cervical (HeLa) cell monolayers were used as an in vitro model of cervicovaginal epithelium for testing the cytotoxicity of the new compounds. HeLa cells were grown in Dulbecco modified Eagle medium supplemented with fetal bovine serum (10%), and antibiotics (penicillin–streptomycin mixture [100 U/mL]). Cells at 80–90% confluence were split by trypsin (0.25% in 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 to 3 passages and approximately 1×10^4 cells were seeded into each well of a 96-well plate and allowed to incubate overnight to allow cells to attach to the substrate. After 24 h, the medium was replaced with DMEM supplemented with 10% FBS containing various concentrations of test compounds and incubated for 48 h. Then 10 μl of MTT solution (5 mg/mL in PBS) was added to each well. After incubation for 4 h, the medium was removed and the resulting formazan crystals were dissolved with 100 μl DMSO. After shaking 10 min, the optical density was measured at 570 nm using a microtiter ELISA reader. The assay was conducted four times. The IC_{50} values were defined as the concentrations inhibiting 50% of cell growth.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.007.

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