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RESEARCH ARTICLE

# Novel arylhydrazone derivatives bearing a rhodanine moiety: synthesis and evaluation of their antibacterial activities

**Wei Li · Chang-Ji Zheng · Liang-Peng Sun · Ming-Xia Song · Yan Wu · Yin-Jing Li · Yi Liu · Hu-Ri Piao**

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**Abstract** A series of arylhydrazone derivatives bearing a rhodanine moiety have been synthesized, characterized, and evaluated as antibacterial agents. Some of these compounds showed potent antibacterial activities against several different strains of Gram-positive bacteria, including multidrug-resistant clinical isolates. Of the compounds tested, **IIk** and **IIIk** were identified as the most effective, with minimum inhibitory concentration values of 2–4 μg/mL against multidrug-resistant Gram-positive organisms, including methicillin-resistant and quinolone-resistant *Staphylococcus aureus*. None of the compounds exhibited any activity against the Gram-negative bacteria *Escherichia coli* 1356 at 64 μg/mL.

**Keywords** Arylhydrazone · Rhodanine · Antibacterial activity · Methicillin-resistant *Staphylococcus aureus* · Quinolone-resistant *Staphylococcus aureus*

## Introduction

Successive annual increases in the number of infections caused by bacteria that are resistant to one or more of the available classes of antibiotics poses a significant threat to human health (Beekmann et al. 2005; Fajdetic et al. 2011) because these resistant strains could lead to treatment failure as well as other complications. A large number of different antibiotics have been developed during the course of the last 70 years and shown to be effective against a variety of infectious diseases. Unfortunately, however, Gram-positive pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Streptococcus pneumoniae* are becoming increasingly resistant to most of the existing antibiotics (Khalaj et al. 2011), and Methicillin-resistant *Staphylococcus aureus* (*MRSA*) and Vancomycin-resistant enterococci (*VRE*) in particular have had a major impact on infections in hospitals and communities throughout the world (Appelbaum 2006; Zetola et al. 2005). Although antibiotic-resistant organisms continue to become increasingly commonplace, the rate of discovery of new antibacterial agents has decreased (Walsh 2000). For this reason, there is an urgent need for new antibacterial agents that are capable of treating resistant bacterial strains.

A large number of arylhydrazone derivatives have been reported in the literature with a diverse range of pharmacological properties, including antinociceptive (Cunha et al. 2002; Silva et al. 2004), antiviral (Jin et al. 2010), anti-inflammatory (Silva et al. 2010), antiplatelet (Lima et al. 2008), and antibacterial (Wang et al. 2012) activities. Although the hydrazone and Schiff base moieties of arylhydrazones can be unstable, arylhydrazones themselves have long been regarded as privileged structures, and have been employed on several occasions to improve chemical stability during the design of new antibacterial agents. Arylhydrazones have attracted considerable levels of attention as a result of the physical, chemical, and biological activities they derive from their unique structures, and they are also known to exhibit a broad range of biological properties, including antimicrobial activities against a variety of different bacteria (Rasras et al. 2010; Metwally et al. 2006).

W. Li · C.-J. Zheng · L.-P. Sun · M.-X. Song · Y. Wu · Y.-J. Li · Y. Liu · H.-R. Piao (✉)
Key Laboratory of Natural Resources and Functional Molecules of the Changbai Mountain, Affiliated Ministry of Education, Yanbian University College of Pharmacy, Yanji 133002, Jilin, People's Republic of China
e-mail: piaohuri@yahoo.com.cn; piaohuri@aliyun.com

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In recent years, rhodanine and its derivatives have appeared in a large number of research reports focused on medicinal chemistry and drug discovery because they possess a wide variety of biological properties, including anticonvulsant (Chauhan et al. 2013), antibacterial (Zheng et al. 2012), antimalarial (Takasu et al. 2002), antiviral, and anti-diabetic (Ohishi et al. 1990) activities. The antimicrobial activities of rhodanines have been known for over 50 years, and several attempts have been made to design and synthesize anti-bacterial agents based on this unique heterocycle (Habib et al. 1997). In our previous work (Chen et al. 2010; Jin et al. 2012; Song et al. 2012), we found that several rhodanine-3-fatty acid derivatives showed strong activities against Gram-positive bacterial strains (Fig. 1). Thus, as part of our ongoing studies towards the development of novel antibacterial agents, herein we report the design, synthesis, and antimicrobial evaluation of a series of arylhydrazone derivatives bearing a rhodanine moiety as efficient antimicrobial agents.

## Chemistry

The reaction sequences employed for the synthesis of the target compounds are shown in Scheme 1. The synthesis of the aromatic hydrazines (**3a**–**p**) proceeded via the formation of the aromatic esters (**2a**–**p**), which were constructed from the corresponding acids by acid-catalyzed esterification. The aromatic esters (**2a**–**p**) were then converted to the aromatic hydrazines by their reaction with hydrazine hydrate in ethanol at reflux. The intermediates **5** were obtained in good yields either via the Knoevenagel condensation of terephthalaldehyde with rhodanine-3-fatty acid **4** or from different amino acids according to a method previously described in the literature (Chen et al. 2010; Jin et al. 2012; Song et al. 2012). The reaction of compounds **3a**–**p** with different aldehydes **5** in alcohol afforded the corresponding Schiff bases **I**–**V**. The structures of the synthesized compounds were confirmed by Fourier transform infrared, $^{1}$H NMR, $^{13}$C NMR, and mass spectroscopic analyses.

## Experimental section

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 and 75 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed in $\delta$, ppm. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). The major chemicals were purchased from Sigma-Aldrich and Fluka.

### General procedure for the preparation of rhodanine-3-fatty acid

In a round-bottomed flask equipped with a magnetic stirrer, amino acid 4 (0.03 mol) was dissolved with sodium hydroxide (0.03 mol) in water (25 mL). Then, carbon disulfide (0.03 mol) was added to the reaction mixture, which was stirred vigorously overnight. An aqueous solution of sodium chloroacetate (0.03 mol) 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, 40:1) (Hardej et al. 2010; Bursavich et al. 2007).

### General synthetic procedure for the key intermediates **5**

A mixture of terephthalaldehyde (2.68 g 0.02 mol) and *rhodanine-3-fatty acid* (0.01 mmol) reacted in ethanol under reflux for 6–7 h. The reaction was catalyzed by drops of acetic acid and piperidine. After cooling, the solvent was removed under reduced pressure, dried, and purified by silica gel column chromatography (dichloromethane/methanol, 100:1) (Tihomir et al. 2010; Wang et al. 2008).

### General synthetic procedure for the target compounds **I**–**V**

In a round-bottomed flask equipped with a magnetic stirrer, *intermediates* **5** (0.001 mol) and acylhydrazine **3a**–**p** (0.001 mol) were dissolved in ethanol (5 mL). The reaction

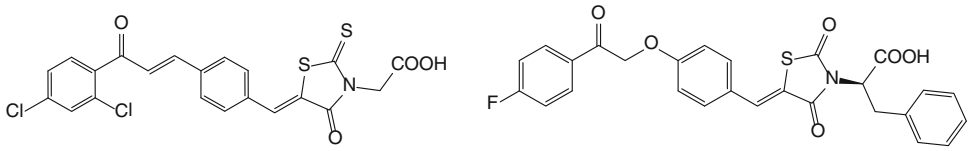
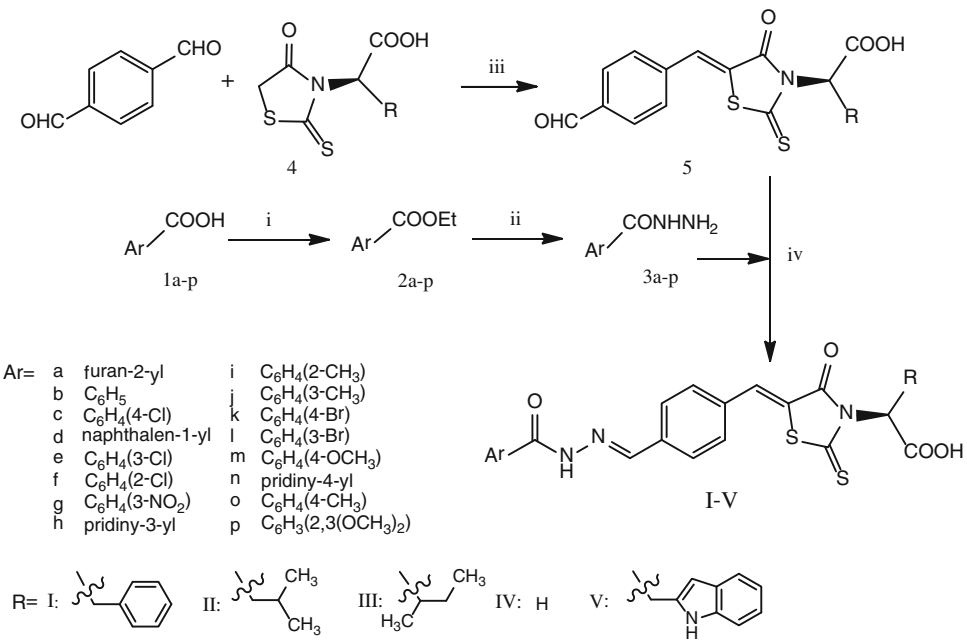



**Fig. 1** Previously reported antibacterial agents with a rhodanine moiety




**Scheme 1** Synthetic scheme for the synthesis of compounds **I–V**. Reagents and conditions: (i) $H_2SO_4$, EtOH, 110 °C, reflux, 12–24 h; (ii) hydrazine hydrate, EtOH, 100 °C, reflux, 12 h; (iii) Piperidine, AcOH, EtOH, 40 °C, 6–7 h; (iv) EtOH, 50 °C, 4–6 h

mixture was stirred at 40–50 °C, until the completion of the reaction as evidenced by TLC. After the completion of the reaction, excess solvent was removed under reduced pressure. The compound was extracted into dichloromethane, concentrated and purified by column chromatography (dichloromethane/methanol, 50:1). The yield, melting point and spectral data of each compound are given below.

*(S)-2-((Z)-5-(4-((E)-(2-(Furan-2-carbonyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ia**)*

Yield 85 %; m.p. 188–190 °C. IR (KBr) $cm^{-1}$: 3419 (OH), 1707 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.49 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.86 (s, 1H, CH), 6.70–7.95 (m, 12H, Ar–H), 7.83 (s, 1H, Ph=CH), 8.46 (s, 1H, N=CH), 12.03 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{25}H_{19}N_3O_5S_2$ ($M^+$) 505.08, found 506 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-Benzoylhydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ib**)*

Yield 86 %; m.p. 178–180 °C. IR (KBr) $cm^{-1}$: 3449 (OH), 1708 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.50 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.15–8.20 (m, 14H, Ar–H), 7.83 (s, 1H, Ph=CH), 8.36 (s, 1H, N=CH), 12.19 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{27}H_{21}N_3O_4S_2$ ($M^+$) 515.10, found 516 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ic**)*

Yield 83 %; m.p. 228–230 °C. IR (KBr) $cm^{-1}$: 3431 (OH), 1718 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.42 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.14–7.96 (m, 14H, Ar–H), 7.89 (s, 1H, Ph=CH), 8.47 (s, 1H, N=CH), 12.09 (s, 1H, NH), 13.46 (s, 1H, COOH). $^{13}C$ NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 192.61, 168.66, 166.44, 162.28, 146.73, 136.49, 133.91, 133.02, 132.24, 131.53, 131.34, 129.76, 128.97, 128.28, 127.93, 127.75, 126.73, 125.72, 121.28, 58.18, 56.02, 18.54. MS (EI) *m/z* calcd for $C_{27}H_{20}ClN_3O_4S_2$ ($M^+$) 549.06, found 550 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(1-Naphthoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Id**)*

Yield 83 %; m.p. 176–178 °C. IR (KBr) $cm^{-1}$: 3434 (OH), 1707 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.78 (s, 1H, CH), 7.14–8.77 (m, 16H, Ar–H), 7.90 (s, 1H, Ph=CH), 8.48 (s, 1H, N=CH), 12.03 (s, 1H, NH), 13.45 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{31}H_{23}N_3O_4S_2$ ($M^+$) 565.11, found 566 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(3-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ie**)*

Yield 93 %; m.p. 164–166 °C. IR (KBr) $cm^{-1}$: 3416 (OH), 1703 (C=O). $^1H$ NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 3.51

(d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.16–7.97 (m, 13H, Ar–H), 7.89 (s, 1H, Ph=CH), 8.46 (s, 1H, N=CH), 12.10 (s, 1H, NH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.06, 169.18, 166.92, 162.26, 147.41, 145.37, 136.97, 135.62, 134.43, 133.79, 133.48, 132.20, 131.81, 130.99, 129.47, 128.76, 128.44, 127.87, 127.22, 127.00, 121.76, 58.65, 33.58. MS (EI) *m/z* calcd for $C_{27}H_{20}ClN_3O_4S_2$ ($M^+$) 549.06, found 550 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(2-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**If**)*

Yield 84 %; m.p. 126–128 °C. IR (KBr) cm$^{-1}$: 3428 (OH), 1712 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.50 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.74 (s, 1H, CH), 7.14–7.88 (m, 16H, Ar–H), 8.29 (s, 1H, Ph=CH), 9.64 (s, 1H, N=CH), 12.03 (s, 1H, NH), 13.45 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{27}H_{20}ClN_3O_4S_2$ ($M^+$) 549.06, found 550 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(3-Nitrobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ig**)*

Yield 95 %; m.p. 148–150 °C. IR (KBr) cm$^{-1}$: 3416 (OH), 1704 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.89 (s, 1H, CH), 7.16–8.76 (m, 16H, Ar–H), 7.83 (s, 1H, Ph=CH), 8.46 (s, 1H, N=CH), 12.33 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{27}H_{20}N_4O_6S_2$ ($M^+$) 560.08, found 561 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-Nicotinoylhydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ih**)*

Yield 86 %; m.p. 178–180 °C. IR (KBr) cm$^{-1}$: 3429 (OH), 1713 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.43 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.14–9.07 (m, 13H, Ar–H), 7.82 (s, 1H, Ph=CH), 8.47 (s, 1H, N=CH), 12.20 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{26}H_{20}N_4O_4S_2$ ($M^+$) 516.09, found 517 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(2-Methylbenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ii**)*

Yield 91 %; m.p. 178–180 °C. IR (KBr) cm$^{-1}$: 3424 (OH), 1713 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 2.37 (s, 1H, $CH_3$), 3.51 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.14–7.86 (m, 13H, Ar–H), 7.83 (s, 1H, Ph=CH), 8.31 (s, 1H, N=CH), 11.85 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{28}H_{23}N_3O_4S_2$ ($M^+$) 529.11, found 530 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(3-Methylbenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ij**)*

Yield 86 %; m.p. 148–150 °C. IR (KBr) cm$^{-1}$: 3416 (OH), 1709 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 2.39 (s, 1H, $CH_3$), 3.51 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.82 (s, 1H, CH), 7.13–7.88 (m, 13H, Ar–H), 7.80 (s, 1H, Ph=CH), 8.48 (s, 1H, N=CH), 11.99 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{28}H_{23}N_3O_4S_2$ ($M^+$) 529.11, found 530 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(4-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ik**)*

Yield 86 %; m.p. 256–258 °C. IR (KBr) cm$^{-1}$: 3439 (OH), 1713 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.44 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.15–7.89 (m, 13H, Ar–H), 7.82 (s, 1H, Ph=CH), 8.47 (s, 1H, N=CH), 12.07 (s, 1H, NH), 13.45 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 192.62, 168.69, 167.18, 166.45, 146.15, 142.01, 136.79, 136.78, 136.50, 133.75, 133.08, 131.35, 129.00, 128.29, 127.84, 127.70, 126.75, 123.36, 121.17, 58.18, 21.04. MS (EI) *m/z* calcd for $C_{27}H_{20}BrN_3O_4S_2$ ($M^+$) 595.01, found 596 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(3-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Il**)*

Yield 82 %; m.p. 126–128 °C. IR (KBr) cm$^{-1}$: 3435 (OH), 1703 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.14–8.10 (m, 13H, Ar–H), 7.82 (s, 1H, Ph=CH), 8.47 (s, 1H, N=CH), 12.09 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{27}H_{20}BrN_3O_4S_2$ ($M^+$) 595.01, found 596 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(4-Methoxybenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Im**)*

Yield 79 %; m.p. 230–232 °C. IR (KBr) cm$^{-1}$: 3417 (OH), 1689 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.50 (d, $J = 9.0$ Hz, 2H, $CH_2$), 3.83 (s, 1H, $CH_3$), 5.88 (s, 1H, CH), 7.05–7.93 (m, 13H, Ar–H), 7.81 (s, 1H, Ph=CH), 8.47 (s, 1H, N=CH), 11.88 (s, 1H, NH), 13.44 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.08, 169.19, 166.93, 163.21, 162.59, 146.20, 137.34, 136.97, 134.15, 133.57, 131.83, 130.12, 129.47, 128.77, 128.25, 127.22, 125.67, 121.57, 114.21, 58.66, 55.90, 33.59. MS (EI) *m/z* calcd for $C_{28}H_{23}N_3O_5S_2$ ($M^+$) 545.11, found 546 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-Isonicotinoylhydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**In**)*

Yield 80 %; m.p. 256–258 °C. IR (KBr) $cm^{-1}$: 3433 (OH), 1706 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.16–8.80 (m, 13H, Ar–H), 7.83 (s, 1H, Ph=CH), 8.49 (s, 1H, N=CH), 12.22 (s, 1H, NH), 13.46 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{26}H_{20}N_4O_4S_2$ ($M^+$) 516.09, found 517 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(4-Methylbenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Io**)*

Yield 81 %; m.p. 242–244 °C. IR (KBr) $cm^{-1}$: 3426 (OH), 1702 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 2.37 (s, 3H, $CH_3$), 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 5.88 (s, 1H, CH), 7.15–7.85 (m, 13H, Ar–H), 7.82 (s, 1H, Ph=CH), 8.48 (s, 1H, N=CH), 11.93 (s, 1H, NH), 13.43 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 192.61, 168.66, 166.44, 162.28, 146.73, 136.49, 133.91, 133.02, 132.24, 131.53, 131.34, 129.76, 128.97, 128.28, 127.93, 127.75, 126.73, 125.72, 121.28, 58.18, 56.02, 18.54. MS (EI) *m/z* calcd for $C_{28}H_{23}N_3O_4S_2$ ($M^+$) 529.11, found 530 ($MH^+$).

*(S)-2-((Z)-5-(4-((E)-(2-(2,3-Dimethoxybenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**Ip**)*

Yield 82 %; m.p. 186–188 °C. IR (KBr) $cm^{-1}$: 3317 (OH), 1711 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.52 (d, $J = 9.0$ Hz, 2H, $CH_2$), 3.78 (s, 3H, $CH_3$), 3.85 (s, 3H, $CH_3$), 5.88 (s, 1H, CH), 7.07–7.86 (m, 12H, Ar–H), 7.84 (s, 1H, Ph=CH), 8.32 (s, 1H, N=CH), 11.78 (s, 1H, NH), 13.46 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 192.69, 168.68, 166.45, 162.24, 152.54, 146.22, 146.02, 136.66, 136.48, 133.84, 133.03, 131.33, 129.68, 128.98, 128.29, 127.90, 127.34, 126.75, 124.30, 120.27, 114.98, 61.19, 58.18, 56.03, 18.59. MS (EI) *m/z* calcd for $C_{29}H_{25}BrN_3O_6S_2$ ($M^+$) 575.12, found 576 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**IIc**)*

Yield 81 %; m.p. 254–256 °C. IR (KBr) $cm^{-1}$: 3294 (OH), 1724 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.92 (d, $J = 9.0$ Hz, 3H, $CH_3$), 0.97 (d, $J = 9.0$ Hz, 3H, $CH_3$), 1.54 (m, 1H, CH), 2.02–2.30 (m, 2H, $CH_2$), 5.65 (m, 1H, CH), 7.66–8.02 (m, 9H, Ar–H), 8.54 (s, 1H, N=CH), 12.14 (s, 1H, NH), 13.43 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.91, 169.83, 167.02, 162.62, 147.21, 137.24, 137.02, 134.53, 133.60, 132.33, 131.76, 130.09, 129.08, 128.43, 122.12, 56.38, 25.28, 23.34, 22.41. MS (EI) *m/z* calcd for $C_{24}H_{22}ClN_3O_4S_2$ ($M^+$) 515.07, found 516 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(1-Naphthoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**IId**)*

Yield 87 %; m.p. 268–270 °C. IR (KBr) $cm^{-1}$: 3326 (OH), 1695 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.93 (d, $J = 9.0$ Hz, 3H, $CH_3$), 0.98 (d, $J = 9.0$ Hz, 3H, $CH_3$), 1.55 (m, 1H, CH), 2.08–2.26 (m, 2H, $CH_2$), 5.66 (m, 1H, CH), 7.43–8.29 (m, 12H, Ar–H), 8.44 (s, 1H, N=CH), 12.24 (s, 1H, NH), 13.41 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{28}H_{25}N_3O_4S_2$ ($M^+$) 531.13, found 532 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(4-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-4-methylpentanoic acid (**IIk**)*

Yield 80 %; m.p. 288–290 °C. IR (KBr) $cm^{-1}$: 3318 (OH), 1689 (C=O).$^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.92 (d, $J = 9.0$ Hz, 3H, $CH_3$), 0.97 (d, $J = 9.0$ Hz, 3H, $CH_3$), 1.54 (m, 1H, CH), 2.07–2.25 (m, 2H, $CH_2$), 5.64 (m, 1H, CH), 7.80–7.95 (m, 9H, Ar–H), 8.55 (s, 1H, N=CH), 12.14 (s, 1H, NH), 13.41 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{24}H_{22}BrN_3O_4S_2$ ($M^+$) 561.02, found 562 ($MH^+$).

*(R)-4-methyl-2-((Z)-5-(4-((E)-(2-(4-Methylbenzoyl) hydrazono)methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**IIo**)*

Yield 90 %; m.p. 232–234 °C. IR (KBr) $cm^{-1}$: 3460 (OH), 1712 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.92 (d, $J = 9.0$ Hz, 3H, $CH_3$), 0.97 (d, $J = 9.0$ Hz, 3H, $CH_3$), 1.54 (m, 1H, CH), 2.07–2.26 (m, 2H, $CH_2$), 2.44 (s, 3H, $CH_3$), 5.65 (m, 1H, CH), 7.38–7.96 (m, 9H, Ar–H), 8.55 (s, 1H, N=CH), 12.00 (s, 1H, NH), 12.92 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.35, 169.31, 166.54, 163.03, 146.15, 141.97, 136.77, 133.89, 133.14, 131.26, 128.99, 127.83, 127.71, 121.52, 55.92, 24.82, 22.83, 21.93, 21.04. MS (EI) *m/z* calcd for $C_{25}H_{25}N_3O_4S_2$ ($M^+$) 515.07, found 516 ($MH^+$).

*(2R)-2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**IIIc**)*

Yield 79 %; m.p. 256–258 °C. IR (KBr) $cm^{-1}$: 3466 (OH), 1707 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.81 (t, $J = 7.5$ Hz, 3H, $CH_3$), 0.96–1.22 (m, 2H,

$CH_2$), 1.17 (d, $J = 6.0$ Hz, 2H, $CH_3$), 1.29 (m, 1H, CH), 5.24 (d, $J = 9.0$ Hz, 1H, CH), 7.66–8.02 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 12.14 (s, 1H, NH), 13.29 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.76, 169.08, 167.12, 162.61, 147.19, 137.24, 137.09, 134.48, 133.95, 132.34, 131.82, 130.09, 129.07, 128.42, 121.74, 62.06, 33.56, 25.36, 18.04, 11.38. MS (EI) *m/z* calcd for $C_{24}H_{22}ClN_3O_4S_2$ ($M^+$) 515.07, found 516 ($MH^+$).

*(2R)-2-((Z)-5-(4-((E)-(2-(1-Naphthoyl)hydrazono)methyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**IIId**)*

Yield 82 %; m.p. 278–280 °C. IR (KBr) cm$^{-1}$: 3429 (OH), 1694 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.86 (t, $J = 7.5$ Hz, 3H, $CH_3$), 0.97–1.09 (m, 2H, $CH_2$), 1.22 (d, $J = 6.0$ Hz,2H, $CH_3$), 1.30 (m, 1H, CH), 5.30 (d, $J = 9.0$ Hz, 1H, CH), 7.65–8.29 (m, 12H, Ar–H), 8.44 (s, 1H, N=CH), 12.24 (s, 1H, NH), 13.27 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{28}H_{25}N_3O_4S_2$ ($M^+$) 531.13, found 532 ($MH^+$).

*(2R)-2-((Z)-5-(4-((E)-(2-(4-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-methylpentanoic acid (**IIIk**)*

Yield 73 %; m.p. 266–267 °C. IR (KBr) cm$^{-1}$: 3421 (OH), 1702 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.81 (t, $J = 7.5$ Hz, 3H, $CH_3$), 0.96–1.08 (m, 2H, $CH_2$), 1.17 (d, $J = 6.0$ Hz, 2H, $CH_3$), 1.24 (m, 1H, CH), 5.24 (d, $J = 9.0$ Hz, 1H, CH), 7.76–7.79 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 12.09 (s, 1H, NH), 13.21 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{24}H_{22}BrN_3O_4S_2$ ($M^+$) 561.02, found 562 ($MH^+$).

*(2R)-3-Methyl-2-((Z)-5-(4-((E)-(2-(4-methylbenzoyl) hydrazono)methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (**IIIo**)*

Yield 88 %; m.p. 186–188 °C. IR (KBr) cm$^{-1}$: 3427 (OH), 1701 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 0.81 (t, $J = 7.5$ Hz, 3H, $CH_3$), 0.96–1.05 (m, 2H, $CH_2$), 1.17 (d, $J = 6.0$ Hz, 2H, $CH_3$), 1.24 (m, 1H, CH), 2.39 (s, 3H, $CH_3$), 5.24 (d, $J = 9.0$ Hz, 1H, CH), 7.33–7.89 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 11.95 (s, 1H, NH), 13.18 (s, 1H, COOH). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, ppm): $\delta$ 193.53, 168.59, 166.67, 162.97, 146.15, 142.02, 136.85, 133.86, 133.50, 131.33, 130.35, 129.01, 127.84, 127.74, 121.19, 61.61, 33.09, 24.90, 21.04, 17.55, 10.88. MS (EI) *m/z* calcd for $C_{25}H_{25}N_3O_4S_2$ ($M^+$) 515.07, found 516 ($MH^+$).

*2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**IVc**)*

Yield 90 %; m.p. 223–225 °C. IR (KBr) cm$^{-1}$: 3418 (OH), 1698 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 4.81 (s, 2H, $CH_2$), 7.68–8.01 (m, 9H, Ar–H), 8.54 (s, 1H, N=CH), 12.13 (s, 1H, NH), 13.42 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{20}H_{14}ClN_3O_4S_2$ ($M^+$) 459.01, found 460 ($MH^+$).

*2-((Z)-5-(4-((E)-(2-(1-Naphthoyl)hydrazono)methyl) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**IVd**)*

Yield 90 %; m.p. 261–264 °C. IR (KBr) cm$^{-1}$: 3418 (OH), 1697 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 4.81 (s, 2H, $CH_2$), 7.44–8.29 (m, 12H, Ar–H), 8.44 (s, 1H, N=CH), 12.23 (s, 1H, NH), 13.47 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{24}H_{17}N_3O_4S_2$ ($M^+$) 475.07, found 476 ($MH^+$).

*2-((Z)-5-(4-((E)-(2-(4-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**IVk**)*

Yield 85 %; m.p. 257–259 °C. IR (KBr) cm$^{-1}$: 3418 (OH), 1698 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 4.80 (s, 2H, $CH_2$), 7.80–7.97 (m, 9H, Ar–H), 8.54 (s, 1H, N=CH), 12.13 (s, 1H, NH), 13.52 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{20}H_{14}BrN_3O_4S_2$ ($M^+$) 504.96, found 506 ($MH^+$).

*2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**IVo**)*

Yield 92 %; m.p. 199–201 °C. IR (KBr) cm$^{-1}$: 3421 (OH), 1696 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 4.79 (s, 2H, $CH_2$), 7.37–7.95 (m, 9H, Ar–H), 8.53 (s, 1H, N=CH), 12.01 (s, 1H, NH). MS (EI) *m/z* calcd for $C_{21}H_{17}N_3O_4S_2$ ($M^+$) 439.07, found 440 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(4-Chlorobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-(1H-indol-2-yl)propanoic acid (**Vc**)*

Yield 89 %; m.p. 278–280 °C. IR (KBr) cm$^{-1}$: 3428 (OH), 1705 (C=O). $^1$H NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.64–3.76 (m, 2H, $CH_2$), 5.93 (m, 1H, CH), 6.93–8.02 (m, 14H, Ar–H), 8.53 (s, 1H, N=CH), 10.85 (s, 1H, NH), 12.12 (s, 1H, NNH), 13.45 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{29}H_{21}ClN_4O_4S_2$ ($M^+$) 588.07, found 589 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(1-Naphthoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-(1H-indol-2-yl)propanoic acid (**Vd**)*

Yield 90 %; m.p. 281–283 °C. IR (KBr) $cm^{-1}$: 3432 (OH), 1706 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.66–3.77 (m, 2H, $CH_2$), 5.89 (m, 1H, CH), 6.96–8.26 (m, 17H, Ar–H), 8.43 (s, 1H, N=CH), 10.84 (s, 1H, NH), 12.23 (s, 1H, NNH). MS (EI) *m/z* calcd for $C_{33}H_{24}N_4O_4S_2$ ($M^+$) 604.12, found 605 ($MH^+$).

*(R)-2-((Z)-5-(4-((E)-(2-(4-Bromobenzoyl)hydrazono) methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-(1H-indol-2-yl)propanoic acid (**Vk**)*

Yield 81 %; m.p. 283–285 °C. IR (KBr) $cm^{-1}$: 3432 (OH), 1706 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 3.36–3.75 (m, 2H, $CH_2$), 5.93 (m, 1H, CH), 6.93–7.94 (m, 14H, Ar–H), 8.55 (s, 1H, N=CH), 10.84 (s, 1H, NH), 12.14 (s, 1H, NNH), 13.41 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{29}H_{21}BrN_4O_4S_2$ ($M^+$) 632.02, found 633 ($MH^+$).

*(R)-3-(1H-Indol-2-yl)-2-((Z)-5-(4-((E)-(2-(4-Methylbenzoyl)hydrazono)methyl)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (**Vo**)*

Yield 83 %; m.p. 241–243 °C. IR (KBr) $cm^{-1}$: 3427 (OH), 1701 (C=O). $^1H$ NMR (DMSO-$d_6$, 300 MHz, ppm): $\delta$ 2.43 (s, 3H, $CH_3$), 3.38–3.81 (m, 2H, $CH_2$), 5.94 (m, 1H, CH), 6.93–7.90 (m, 14H, Ar–H), 8.53 (s, 1H, N=CH), 10.84 (s, 1H, NH), 11.99 (s, 1H, NNH), 13.45 (s, 1H, COOH). MS (EI) *m/z* calcd for $C_{30}H_{24}N_4O_4S_2$ ($M^+$) 568.12, found 569 ($MH^+$).

### In vitro evaluation of the antibacterial activity of the compounds

The anti-bacterial activities of the compounds were evaluated in vitro in 96-well microtiter plates. A serial dilution method was used to obtain the minimum inhibitory concentration (MIC) values of the synthesized compounds against several different bacterial strains, including multidrug-resistant clinical isolates. Oxacillin and norfloxacin were used as positive controls. The test bacteria were grown to the mid-log phase in Mueller–Hinton broth (MHB) and subsequently diluted 1,000-fold in the same medium. The bacteria of 105 CFU/mL were inoculated into MHB and dispensed at 0.2 mL/well in a 96-well microtiter plate. Oxacillin and norfloxacin were used as positive controls. The test compounds were prepared in dimethyl sulfoxide (DMSO), with the final concentrations of the compounds not exceeding 0.05 %. A 2-fold serial dilution technique was used to obtain final concentrations of 64–1 μg/mL. The MIC value was defined as the concentration of test compound required to completely inhibit the growth of the bacteria during a 24 h incubation period at 37 °C. The growth of the bacteria was determined by measuring the absorption at 650 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All of the experiments were conducted in triplicate.

### Evaluation of cytotoxicity

Human cervical (HeLa) cell monolayers were used as an in vitro model of the cervico-vaginal epithelium for testing the cytotoxicities of the new compounds. HeLa cells were grown in Dulbecco modified Eagle medium (DMEM) 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 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 $\sim 1 \times 10^4$ cells were seeded into each well of a 96-well plate and incubated overnight to allow the cells to become attached to the substrate. After 24 h, the medium was replaced with DMEM supplemented with 10 % FBS containing a variety of different concentrations of the test compounds and incubated for 48 h. A 10 μL portion of an MTT solution (5 mg/mL in PBS) was then added to each well. Following a 4 h period of incubation, the medium was removed and the resulting formazan crystals were dissolved with in DMSO (100 μL). Following a period of shaking for 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 the cell growth by 50 %.

## Result and discussion

### Anti-microbial activity

Compounds **I–V** were evaluated for their antibacterial activities against three strains of Gram-positive bacteria (*S. aureus RN*4220, *S. aureus KCTC* 503 and *S. aureus KCTC* 209), four strains of multidrug-resistant Gram-positive bacteria (Methicillin-resistant *S. aureus* (*MRSA CCARM* 3167 and *MRSA CCARM* 3506), Quinolone-resistant *S. aureus* (*QRSA CCARM* 3505 and *QRSA CCARM* 3519)) and one strain of Gram-negative bacteria (*Escherichia coli CCARM* 1356) using a conventional agar-dilution method. The MIC values of the compounds were subsequently determined and compared with those of oxacillin and norfloxacin, which were used as reference inhibitors.

For series **I**, derivatives that contained a heterocyclic aromatic ring (Ar groups) did not exhibit any activity against the bacterial strains tested, indicating that the phenyl ring was critical for the activity. Based on these results, the corresponding derivatives were not considered for synthesis in any of the other series.

A preliminary in vitro assay revealed that most of the derivatives did not show any anti-bacterial activity against the Gram-negative strain (*E. coli CCARM* 1356) at 64 μg/mL, whereas most of the derivatives exhibited potent antibacterial activities against the Gram-positive strains. Of the compounds tested, compounds from series **II** and **III** showed excellent levels of inhibition against the three Gram-positive strains (*S. aureus RN* 4220, *S. aureus KCTC* 209 and *S. aureus KCTC* 503) with MIC values in the range of 2–8 μg/mL. Compounds **IId**, **IIk**, **IIId** and **IIIk** (MIC = 2 μg/mL) were all 2-fold more potent than the positive control norfloxacin (MIC = 4 μg/mL) against the *S. aureus KCTC* 209, but were also 2- to 4-fold less potent against *S. aureus RN* 4220 and *S. aureus KCTC* 503, and showed lower levels of potency than the positive control oxacillin (MIC = 1 μg/mL) against the three Gram-positive strains (Table 1).

All of the newly synthesized compounds were also tested for their inhibitory activities against the clinical isolates of several different multidrug-resistant Gram-positive bacterial strains, including Methicillin-resistant *S. aureus* (*MRSA CCARM* 3167 and *MRSA CCARM* 3506) and Quinolone-resistant *S. aureus* (*QRSA CCARM* 3505 and *QRSA CCARM* 3519), as shown in Table 2. The results revealed that the newly synthesized compounds provided similar levels of inhibitory activity in both the conventional and multidrug-resistant bacterial strains. Compounds from series **II** and **III** exhibited higher levels of inhibitory activity against the different multidrug-resistant Gram-positive bacterial strains, with compounds **IIk** and **IIIk** in particular showing excellent levels of inhibitory activity against *MRSA CCARM* 3167 and 3506 with an MIC value of 4 μg/mL. This value represented a 2-fold increase in potency relative to the standard drug norfloxacin and a 16-fold increase relative to oxacillin (MIC > 64 μg/mL). For the *QRSA CCARM* 3505 and 3519 strains, although these compounds exhibited lower levels of inhibitory activity with an MIC value of 4 μg/mL (oxacillin, MIC = 1 μg/mL), they showed much higher levels of activity than norfloxacin (MIC > 64 μg/mL).

Among the tested compounds in the five series, the compounds in series **II** and **III** showed excellent inhibition against the seven Gram-positive Strains (including drug-resistance bacteria), with MIC values in the range of 2–16 μg/mL. Compounds in series **I** displayed moderate to good inhibition against the seven Gram-positive Strains. However, the activity of compounds in series **IV** and **V** was low. The compounds from series **V** were less active than those from the other four series of compounds.The mechanism of action of the compounds tested in this study will be done in the next study.

No clear correlations were found for any of these compounds in terms of their anti-bacterial activity and the

**Table 1** Inhibitory activity of compounds **I–V** expressed as MIC (μg/mL) (μM)

| Compound | Ar | *S.aureus* | | | *E.coli* |
|---|---|---|---|---|---|
| | | 4220[a] | 503[b] | 209[c] | 1356[d] |
| **Ia** | Furan-2-yl | >64 | >64 | >64 | >64 |
| **Ib** | $C_6H_5$ | 32 (62) | >64 | 64 (124) | >64 |
| **Ic** | $C_6H_4$(4-Cl) | 4 (7) | 8 (14) | 16 (29) | >64 |
| **Id** | Naphthalen-1-yl | 8 (14) | 16 (29) | 8 (14) | >64 |
| **Ie** | $C_6H_4$(3-Cl) | 8 (14) | 16 (29) | 8 (14) | >64 |
| **If** | $C_6H_4$(2-Cl) | 64 (117) | 64 (117) | 64 (117) | >64 |
| **Ig** | $C_6H_4$(3-$NO_2$) | 32 (57) | 32 (57) | 32 (57) | >64 |
| **Ih** | Pyridin-3-yl | >64 | >64 | >64 | >64 |
| **Ii** | $C_6H_4$(2-$CH_3$) | 32 (60) | 32 (60) | 32 (60) | >64 |
| **Ij** | $C_6H_4$(3-$CH_3$) | 32 (60) | 16 (30) | 32 (60) | >64 |
| **Ik** | $C_6H_4$(4-Br) | 16 (27) | 8 (13) | 4 (7) | >64 |
| **Il** | $C_6H_4$(3-Br) | 16 (27) | 16 (27) | 16 (27) | >64 |
| **Im** | $C_6H_4$(4-$OCH_3$) | 16 (29) | 16 (29) | 16 (29) | >64 |
| **In** | Pyridin-4yl | >64 | 64 (124) | >64 | >64 |
| **Io** | $C_6H_4$(4-$CH_3$) | 32 (60) | 8 (15) | 8 (15) | >64 |
| **Ip** | $C_6H_3$(2,3-($OCH_3$)$_2$) | 64 (111) | 32 (56) | 64 (111) | >64 |
| **IIc** | $C_6H_4$(4-Cl) | 8 (16) | 8 (16) | 8 (16) | >64 |
| **IId** | Naphthalen-1-yl | 8 (15) | 4 (8) | 2 (4) | >64 |
| **IIk** | $C_6H_4$(4-Br) | 4 (7) | 4 (7) | 2 (4) | >64 |
| **IIo** | $C_6H_4$(4-$CH_3$) | 8 (16) | 8 (16) | 4 (8) | >64 |
| **IIIc** | $C_6H_4$(4-Cl) | 8 (16) | 8 (16) | 4 (8) | >64 |
| **IIId** | Naphthalen-1-yl | 8 (15) | 4 (8) | 2 (4) | >64 |
| **IIIk** | $C_6H_4$(4-Br) | 4 (7) | 4 (7) | 2 (4) | >64 |
| **IIIo** | $C_6H_4$(4-$CH_3$) | 8 (16) | 4 (8) | 4 (8) | >64 |
| **IVc** | $C_6H_4$(4-Cl) | >64 | >64 | >64 | >64 |
| **IVd** | Naphthalen-1-yl | >64 | >64 | >64 | >64 |
| **IVk** | $C_6H_4$(4-Br) | >64 | >64 | >64 | >64 |
| **IVo** | $C_6H_4$(4-$CH_3$) | >64 | >64 | >64 | >64 |
| **Vc** | $C_6H_4$(4-Cl) | 64 (109) | 64 (109) | 32 (54) | >64 |
| **Vd** | Naphthalen-1-yl | 64 (106) | 64 (106) | 64 (106) | >64 |
| **Vk** | $C_6H_4$(4-Br) | 64 (101) | 64 (101) | 64 (101) | >64 |
| **Vo** | $C_6H_4$(4-$CH_3$) | 64 (113) | 64 (113) | 64 (113) | >64 |
| Oxacillin | | 1 (2) | 1 (2) | 1 (2) | >64 |
| Norfloxacin | | 2 (6) | 2 (6) | 4 (13) | 16 (50) |

Numbers in parentheses represent the MIC converted to molar concentrations (μM)

[a] *Staphylococcus aureus* RN4220

[b] *Staphylococcus aureus* 503

[c] *Staphylococcus aureus* 209

[d] *Escherichia coli* CCARM 1356

**Table 2** Inhibitory activity (MIC, μg/mL) (μm) of compounds **I–V** against clinical isolates of multidrug-resistant Gram-positive strains

| Compound | Ar | MRSA | | QRSA | |
|---|---|---|---|---|---|
| | | 3167[a] | 3506[b] | 3505[c] | 3519[d] |
| **Ia** | Furan-2-yl | >64 | >64 | >64 | >64 |
| **Ib** | $C_6H_5$ | 32 (62) | 64 (116) | 32 (62) | 32 (62) |
| **Ic** | $C_6H_4$(4-Cl) | 16 (29) | 8 (15) | 8 (15) | 8 (15) |
| **Id** | Naphthalen-1-yl | 8 (14) | 16 (28) | 8 (14) | 16 (28) |
| **Ie** | $C_6H_4$(3-Cl) | 8 (15) | 16 (29) | 16 (29) | 16 (29) |
| **If** | $C_6H_4$(2-Cl) | 16 (29) | 32 (58) | 32 (58) | 32 (58) |
| **Ig** | $C_6H_4$(3-$NO_2$) | 32 (57) | 32 (57) | 32 (57) | 32 (57) |
| **Ih** | Pyridin-3-yl | >64 | >64 | >64 | >64 |
| **Ii** | $C_6H_4$(2-$CH_3$) | 32 (60) | 32 (60) | 32 (60) | 32 (60) |
| **Ij** | $C_6H_4$(3-$CH_3$) | 8 (15) | 8 (15) | 8 (15) | 8 (15) |
| **Ik** | $C_6H_4$(4-Br) | 16 (27) | 8 (13) | 8 (13) | 4 (7) |
| **Il** | $C_6H_4$(3-Br) | 8 (13) | 16 (27) | 8 (13) | 8 (13) |
| **Im** | $C_6H_4$(4-$OCH_3$) | 16 (29) | 32 (59) | 16 (29) | 16 (29) |
| **In** | Pyridin-4yl | >64 | >64 | >64 | >64 |
| **Io** | $C_6H_4$(4-$CH_3$) | 8 (15) | 8 (15) | 8 (15) | 8 (15) |
| **Ip** | $C_6H_3$(2,3-($OCH_3$)$_2$) | 32 (56) | 32 (56) | 32 (56) | 32 (56) |
| **IIc** | $C_6H_4$(4-Cl) | 4 (8) | 8 (16) | 8 (16) | 16 (31) |
| **IId** | Naphthalen-1-yl | 2 (4) | 4 (8) | 8 (15) | 4 (8) |
| **IIk** | $C_6H_4$(4-Br) | 4 (7) | 4 (7) | 4 (7) | 4 (7) |
| **IIo** | $C_6H_4$(4-$CH_3$) | 8 (16) | 8 (16) | 8 (16) | 8 (16) |
| **IIIc** | $C_6H_4$(4-Cl) | 4 (8) | 8 (16) | 4 (8) | 8 (16) |
| **IIId** | Naphthalen-1-yl | 4 (8) | 4 (8) | 8 (16) | 8 (16) |
| **IIIk** | $C_6H_4$(4-Br) | 4 (7) | 4 (7) | 4 (7) | 4 (7) |
| **IIIo** | $C_6H_4$(4-$CH_3$) | 4 (8) | 8 (16) | 8 (16) | 4 (8) |
| **IVc** | $C_6H_4$(4-Cl) | >64 | >64 | >64 | >64 |
| **IVd** | Naphthalen-1-yl | >64 | >64 | >64 | >64 |
| **IVk** | $C_6H_4$(4-Br) | >64 | >64 | >64 | >64 |
| **IVo** | $C_6H_4$(4-$CH_3$) | >64 | >64 | >64 | >64 |
| **Vc** | $C_6H_4$(4-Cl) | 64 (102) | 32 (51) | 64 (102) | 16 (26) |
| **Vd** | Naphthalen-1-yl | 32 (53) | 64 (106) | 64 (106) | 32 (53) |
| **Vk** | $C_6H_4$(4-Br) | 32 (51) | 64 (101) | 32 (51) | 64 (101) |
| **Vo** | $C_6H_4$(4-$CH_3$) | 32 (56) | 64 (113) | 64 (113) | 32 (56) |
| Oxacillin | | >64 | >64 | 1 (2) | 1 (2) |
| Norfloxacin | | 8 (25) | 4 (13) | >64 | >64 |

Numbers in parentheses represent the MIC converted to molar concentrations (μM)

[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

position or the physicochemical properties of the different substituents on their phenyl ring.

### Cytotoxicity activity

To determine whether the antibacterial activities of compounds **IIk** and **IIIk** were selectively toxic to bacteria, their cytotoxicities were evaluated (Table 3). Compounds **IIk** and **IIIk** did not affect the cell viability of Human cervical (HeLa) cells at their MICs (4 and 2 μg/mL, respectively) but showed cytotoxicity at much higher concentrations ($IC_{50}$ = 18.62 or 9.01 μg/mL, respectively). The differences between the antibacterial and cytotoxic activities of these two compounds suggested that compounds **IIk** and **IIIk** exhibited in vitro antibacterial activities at non-cytotoxic concentrations.

**Table 3** Cytotoxic activity of compounds **IIk** and **IIIk** against HeLa cell

| Compound | MIC(μg/mL) | $IC^a_{50}$ (μg/mL) |
|---|---|---|
| **IIk** | 2 or 4 | 18.62 |
| **IIIk** | 2 or 4 | 9.01 |

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

## Conclusion

In the present study, a series of arylhydrazone derivatives bearing a rhodanine moiety were synthesized and their antimicrobial activities evaluated against a variety of Gram-positive and Gram-negative bacteria. Some of the synthesized compounds showed potent anti-bacterial activities against Gram-positive bacteria, particularly against the multidrug-resistant strains of clinical isolates. Compound **IIk** and **IIIk** were found to possess the most potent inhibitory activities of the compounds synthesized in the current study. These results suggested that the introduction of an arylhydrazone moiety to the 5-benzylidenethiazolidine-2,4-dione scaffold would not lead to a reduction in the antibacterial activity of these compounds, and the further development of such compounds could be of significant interest, especially in relation to the search for new derivatives against Methicillin-resistant *S. aureus* and Quinolone-resistant *S. aureus*.

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## References

Appelbaum, P.C. 2006. MRSA—the tip of the iceberg. *Clinical Microbiology and Infectious* 12(Suppl 2): 3–10.

Beekmann, S.E., K.P. Heilmann, S.S. Richter, J. Garcia-de-Lomas, and G.V. Doern. 2005. Antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and group A β-haemolytic streptococci in 2002–2003 Results of the multinational GRASP Surveillance Program. *International Journal of Antimicrobial Agents* 25: 148–156.

Bursavich, M.G., A.M. Gilbert, S. Lombardi, K.E. Georgiadis, E. Reifenberg, C.R. Flannery, and E.A. Morris. 2007. Synthesis and evaluation of aryl thioxothiazolidinone inhibitors of ADAMTS-5 (Aggrecanase-2). *Bioorganic & Medicinal Chemistry Letters* 17: 1185–1188.

Chauhan, K., M. Sharma, J. Saxena, S.V. Singh, P. Trivedi, K. Srivastava, S.K. Puri, J.K. Saxena, V. Chaturvedi, and P.M.S. Chauhan. 2013. Synthesis and biological evaluation of a new class of 4-aminoquinoline-rhodanine hybrid as potent anti-infective agents. *European Journal of Medicinal Chemistry* 62: 693–704.

Chen, Z.H., C.J. Zheng, L.P. Sun, and H.R. Piao. 2010. Synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety with potential anti-bacterial activity. *European Journal of Medicinal Chemistry* 45: 5739–5743.

Cunha, A.C., J.L.M. Tributino, A.L.P. Miranda, C.A.M. Fraga, and E.J. Barreiro. 2002. Synthesis and pharmacological evaluation of novel antinociceptive Nsubstituted-phenylimidazolyl-4-acylhydrazone derivatives. *IL Farmaco* 57: 999–1007.

Fajdetic, A., A. Vinter, H.C. Paljetak, J. Padovan, I.P. Jakopovic, S. Kapic, S. Alihodzic, D. Filic, M. Modric, N. Kosutic-Hulita, R. Antolovic, Z.I. Schoenfel, S. Mutak, V.E. Haber, and R. Spaventi. 2011. Synthesis, activity and pharmacokinetics of novel antibacterial 15-membered ring macrolones. *European Journal of Medicinal Chemistry* 46: 3388–3397.

Habib, N.S., S.M. Rida, E.A.M. Badawey, H.T.Y. Fahmy, and H.A. Ghozlan. 1997. Synthesis and antimicrobial activity of rhodanine derivatives. *European Journal of Medicinal Chemistry* 32: 759–762.

Hardej, D., C.R. Ashby Jr, N.S. Khadtare, S.S. Kulkarni, S. Singh, and T.T. Talele. 2010. The synthesis of phenylalanine-derived C5-substituted rhodanines and their activity against selected methicillin-resistant Staphylococcus aureus (MRSA) strains. *European Journal of Medicinal Chemistry* 45: 5827–5832.

Jin, X., C.J. Zheng, M.X. Song, Y. Wu, L.P. Sun, Y.J. Li, L.J. Yu, and H.R. Piao. 2012. Synthesis and antimicrobial evaluation of L-phenylalanine-derived C5-substituted rhodanine and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone. *European Journal of Medicinal Chemistry* 56: 203–209.

Jin, Y.X., Z.W. Tan, M.Z. He, B.H. Tian, S.X. Tang, I. Hewlett, and M. Yang. 2010. SAR and molecular mechanism study of novel acylhydrazone compounds targeting HIV-1 CA. *Bioorganic & Medicinal Chemistry* 18: 2135–2140.

Khalaj, A., M. Nakhjiri, A.S. Negahbani, M. Samadizadeh, L. Firoozpour, S. Rajabalian, N. Samadi, M.A. Faramarzi, N. Adibpour, A. Shafiee, and A. Foroumadi. 2011. Discovery of a novel nitroimidazolyleoxazolidinone hybrid with potent anti Gram-positive activity: Synthesis and antibacterial evaluation. *European Journal of Medicinal Chemistry* 46: 65–70.

Lima, L.M., F.S. Frattani, J.L. Santos, H.C. Castro, C.A.M. Fraga, R.B. Zingali, and E.J. Barreiro. 2008. Synthesis and anti-platelet activity of novel arylsulfonate-acylhydrazone derivatives, designed as antithrombotic candidates. *European Journal of Medicinal Chemistry* 43: 348–356.

Metwally, K.A., L.M. Abdel-Aziz, E.M. Lashine, M.I. Husseiny, and R.H. Badawy. 2006. Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. *Bioorganic & Medicinal Chemistry* 14: 8675–8682.

Ohishi, Y., T. Mukai, M. Nagahara, M. Yajima, N. Kajikawa, K. Miyahara, and T. Takano. 1990. Preparations of 5-alkylmethylidene-3-carboxymethylrhodanine derivatives and their aldose reductase inhibitory activity. *Chemical & Pharmaceutical Bulletin* 38: 1911–1919.

Rasras, A.J.M., T.H. Al-Tel, A.F. Al-Aboudi, and R.A. Al-Qawasmeh. 2010. Synthesis and antimicrobial activity of cholic acid hydrazone analogues. *European Journal of Medicinal Chemistry* 45: 2307–2313.

Silva, G.A., L.M.M. Costa, F.C.F. Brito, A.L.P. Miranda, E.J. Barreiroa, and C.A.M. Fraga. 2004. New class of potent antinociceptive and antiplatelet 10 *H*-phenothiazine-1-acylhydrazone derivatives. *Bioorganic & Medicinal Chemistry* 12: 3149–3158.

Silva, Y.K.C., C.V. Augusto, M.L.C. Barbosa, G.M.A. Melo, A.C. Queiroz, T.L.M.F. Dias, W.B. Junior, E.J. Barreiro, L.M. Lima, and M.S. Alexandre-Moreira. 2010. Synthesis and pharmacological evaluation of pyrazine *N*-acylhydrazone derivatives designed as novel analgesic and anti-inflammatory drug candidates. *Bioorganic & Medicinal Chemistry* 18: 5007–5015.

Song, M.X., C.J. Zheng, X.Q. Deng, Q. Wang, S.P. Hou, T.T. Liu, X.L. Xing, and H.R. Piao. 2012. Synthesis and bioactivity evaluation of rhodanine derivatives as potential anti-bacterial agents. *European Journal of Medicinal Chemistry* 54: 403–412.

Takasu, K., H. Inoue, H.S. Kim, M. Suzuki, T. Shishido, Y. Wataya, and M. Ihara. 2002. Rhodacyanine dyes as antimalarials. 1. preliminary evaluation of their activity and toxicity. *Journal of Medicinal Chemistry* 45: 995–998.

Tihomir, T., Z. Nace, M.P. Manica, K. Danijel, and P.M. Lucija. 2010. Synthesis and antibacterial activity of 5-ylidenethiazolidin-4-ones and 5-benzylidene-4,6-pyrimidinediones. *European Journal of Medicinal Chemistry* 45: 1667–1672.

Walsh, C. 2000. Molecular mechanisms that confer antibacterial drug resistance. *Nature* 406: 775–781.

Wang, L.Y., F.S. Kong, C.L. Kokoski, D.W. Andrewsb, and C.G. Xing. 2008. Development of dimeric modulators for anti-apoptotic Bcl-2 proteins. *Bioorganic & Medicinal Chemistry Letters* 18: 236–240.

Wang, X.L., Y.B. Zhang, R.Q. Chen, Y.S. Yang, J.F. Tang, F. Zhang, H.B. Gong, and H.L. Zhu. 2012. Design, synthesis and antibacterial activities of vanillic acylhydrazone derivativesas potential β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitors. *European Journal of Medicinal Chemistry* 57: 373–382.

Zetola, N., J.S. Francis, E.L. Nuermberger, and W.R. Bishai. 2005. Community-acquired meticillin-resistant *Staphylococcus aureus*: An emerging threat. *Lancet Infect Dis* 5: 275–286.

Zheng, C.J., L.L. Xu, L.P. Sun, J. Miao, and H.R. Piao. 2012. Synthesis and antibacterial activity of novel 1,3-diphenyl-1*H*-pyrazoles functionalized with phenylalanine-derived rhodanines. *European Journal of Medicinal Chemistry* 58: 112–116.