European Journal of Medicinal Chemistry 74 (2014) 405–410

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and evaluation of the antimicrobial activities of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one derivatives

Jia-Chun Liu [1], Chang-Ji Zheng [1], Meng-Xiao Wang, Ya-Ru Li, Long-xu Ma, Shao-Pu Hou, Hu-Ri Piao*

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

ARTICLE INFO

Article history:
Received 3 August 2013
Received in revised form
15 November 2013
Accepted 24 December 2013
Available online 8 January 2014

Keywords:
2-Thioxothiazolidin-4-one
1,3,4-Oxadiazole
Antibacterial activity

ABSTRACT

Two novel series of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one derivatives were designed and synthesized, and their anti-bacterial activities evaluated. These compounds showed broad-spectrum inhibitory activities against both Gram-positive and Gram-negative bacteria with minimum inhibitory concentration (MIC) values in the range of 1–64 μg/mL. The activity of compound **6c** was the more potent with MIC values of 1 μg/mL against the *MRSA* (3167 and 3506) strains than those of gatifloxacin, oxacillin, and norfloxacin. Compared to the previously reported rhodanine derivatives, 2-thioxothiazolidin-4-one derivatives exhibited an inhibition against Gram-negative strains due to the introduction of a 1,3,4-oxadiazole moiety, among which compounds **3** showed moderate activities against the Gram-negative bacteria (*Escherichiacoli* 1924) with MIC values of 16 μg/mL.

© 2014 Elsevier Masson SAS. All rights reserved.

1. Introduction

There has been a significant increase in the development of multidrug resistance (MDR) in bacteria in recent years, which has been linked to the overuse of antibiotics and antibacterial chemicals. According to worldwide studies, MDR is becoming increasingly prevalent, especially in Gram-positive pathogens such as *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* and *Enterococcus* [1]. For instance, the emergence of vancomycin resistance in the last decade, first in *Enterococcus* [2] and more recently in *S. aureus* [3], has caused considerable concern, because vancomycin is currently the drug of last-resort for the treatment of methicillin-resistant *S. aureus* (*MRSA*) infections. Furthermore, Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter* spp. and the β-lactamase-producing Enterobacteriaceae can be more difficult to treat with standard therapies. Taken together, these developments effectively highlight the fact that antibiotic resistance is becoming increasingly serious as the spectrum of bacteria that are sensitive to drugs becomes ever narrower, and the effect of combined medications becomes worse and worse. Bacterial infections are becoming increasingly difficult to treat as a result of the emergence of multidrug resistant pathogenic bacteria, with the number of infections progressing into deadly diseases and previously incogitable losses growing in number [4–8]. There is therefore an urgent need for the development of novel antimicrobial agents.

2-Thioxothiazolidin-4-one heterocyclic ring derivatives have a broad spectrum of biological activities, including their application as JSP-1 inhibitors [9], antidiabetic drugs [10], and antitubercular agents [11]. 1,3,4-Oxadiazole represents a key structural motif in heterocyclic chemistry and occupies a prominent position in antimicrobial agents, with some of its derivatives showing good activity against Gram-negative bacteria (*Escherichia coli*, MIC = 3 μg/mL) [12,13]. In our previous work [14–19], we reported the identification of a series of 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives, with all of the compounds belonging to this series showing outstanding bacteriostatic activity against Gram-positive bacteria, such as compound **A** (MIC = 2 μg/mL). Unfortunately, however, compounds belonging to this series did not show any bacteriostatic activity against Gram-negative bacteria. In contrast, compound **B** exhibited strong anti-bacterial activities against *S. aureus*, *Bacillus subtilis* and *E. coli* (MIC = 4 μg/mL) [20]. With these results in mind, we designed and synthesized twenty-one novel compounds using a structure-based design strategy with compounds **A** and **B** being used as the lead compounds. These compounds were subsequently evaluated for their antibacterial activities with the aim of finding a broader spectrum antimicrobial agent. Detailed designs are shown in Fig. 1.

* Corresponding author. Tel.: +86 433 2435003; fax: +86 433 2435004.
E-mail address: piaohuri@yahoo.com.cn (H.-R. Piao).
[1] These authors contributed equally to this work.

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.12.054

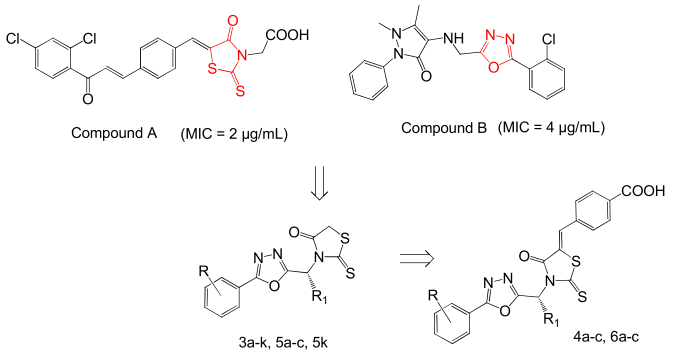


Fig. 1. Structure of the leading compounds and design of the target compounds.

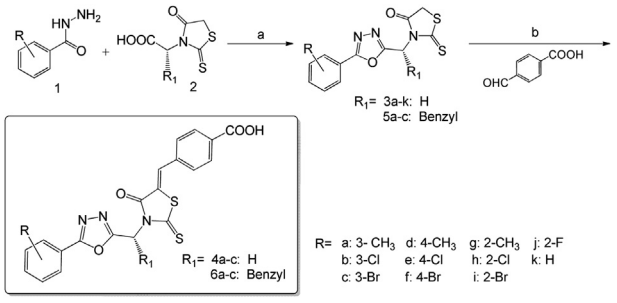
2. Chemistry

The synthetic route to the amino acid-derived 2-thioxothiazolidin-4-one derivatives **3a**–**k**, **4a**–**c**, **5a**–**c**, **5k**, **6a**–**c** is depicted in Scheme 1. Compound **1** had already been reported in a previous paper [21]. Compound **2** was synthesized through an esterification reaction followed by a hydrazinolysis reaction. Compounds **3a**–**k** and **5a**–**c** were synthesized by refluxing an equimolar mixture of **2** with **1** in phosphorous oxychloride (105–110 °C) for 6 h. Compounds **3a**–**k** and **5a**–**c** were subjected to a Knoevenagel condensation reaction with 4-formylbenzoic acid to give the target compounds **4a**–**c** and **6a**–**c**, respectively. The structures of the desired compounds were confirmed by FTIR, ^{1}H and ^{13}C NMR, and mass spectral analysis.

3. Results and discussion

All of the synthesized compounds were evaluated *in vitro* using a 96-well microtiter plate and a serial dilution method to obtain their minimum inhibitory concentration (MIC) values against a variety of different strains, including multidrug-resistant clinical isolates. The MIC is defined as the lowest concentration of antibacterial agent required to visibly inhibit the growth of the bacteria. Gatifloxacin, moxifloxacin, norfloxacin and oxacillin were used as positive controls.

The MIC values of the synthesized compounds against *S. aureus* and *Streptococcus mutans* are shown in Table 1. The majority of the compounds exhibited good MIC values in the range of 2–64 μg/mL against *S. aureus* RN 4220. By comparison, the MIC values of compounds **5a**–**c** were 2–8-fold more potent than those of **3a**–**c**, and the antibacterial activities of compounds **6a**–**c** were 4–8-fold more potent than those of **4a**–**c** against *S. aureus* RN 4220. Among these compounds, compounds **6a**–**c** showed the strongest activity with MIC values of 2 μg/mL, although the activities of these compounds



Scheme 1. Synthetic scheme for the synthesis of compounds **3**–**6**. Reagents and conditions: (a) $POCl_3$, 105–110° C reflux, 5–7 h (b) Piperidine, AcOH, EtOH, 50-60 °C, 4–6 h.

were weaker than gatifloxacin (MIC = 0.25 μg/mL) and moxifloxacin (MIC = 0.25 μg/mL) against *S. aureus* RN 4220. Compounds **3a**–**k** and **4a**–**c** exhibited weak anti-bacterial activities with MIC values in the range of 16–64 μg/mL, whereas compounds **5a**–**c**, **5k** and **6a**–**c** did not exhibit any activity (MIC > 64 μg/mL) at all against *S. aureus* KCTC 209 or *S. aureus* KCTC 503. Some compounds were sensitive towards *S. mutans* 3065 with MIC values in the range of 1–8 μg/mL. The inhibitory effects of compounds **5a**–**c** were 2–8-fold greater than those of **3a**–**c** and that the antibacterial effects of compounds **6a**–**c** were 4–16-fold greater than those of **4a**–**c** against *S. mutans* 3065. Compound **6c** showed the strongest activity of all of the compounds tested with an MIC value of 1 μg/mL, although the activity of this compounds was slightly weaker than gatifloxacin (MIC = 0.5 μg/mL) and moxifloxacin (MIC = 0.25 μg/mL) against the *S. mutans* 3065 strains.

As shown in Table 1, the antibacterial activities of all the compounds were evaluated against several different strains of Gram-negative bacteria (*E. coli*, *Salmonella typhimurium* and *P. aeruginosa*). Compounds **3a**–**k** exhibited good inhibitory activities against *E. coli* 1924 with MIC values in the range of 16–64 μg/mL, as well as weak levels of activity against *S. typhimurium* (1926 and 2421) with MIC values in the range of 32–128 μg/mL. Compounds **3a**–**c**, **3e**, **3g**–**i** showed good levels of activity against *E. coli* 1924 strains with MIC values of 16 μg/mL, although these compounds were weaker inhibitors than the standard drugs gatifloxacin (MIC = 2 μg/mL) and moxifloxacin (MIC = 2 μg/mL). Disappointingly, compounds **4a**–**c**, **5a**–**c**, **5k** and **6a**–**c** did not show any activity (MIC > 128 μg/mL) against *E. coli* 1924 or *S. typhimurium* (1926 and 2421). Furthermore, none of the compounds synthesized in the current study exhibited activity (MIC > 128 μg/mL) against *E. coli* 1356 or the *P. aeruginosa* (2742 and 2004) strains.

The inhibitory activities of the title compounds against *Candida albicans* 7535 are shown in Table 1. Compounds **3a**–**k** were only weakly active against *C. albicans* 7535 with MIC values in the range of 32–64 μg/mL. Compounds **3b**, **3c** and **3e** were the most potent of these inhibitors with MIC values of 32 μg/mL, although they were not as potent as the standard drugs gatifloxacin (MIC = 0.5 μg/mL) and moxifloxacin (MIC = 0.5 μg/mL). Compounds **5a**–**c**, **5k**, and **6a**–**c** did not exhibit any activity against the *C. albicans* 7535 strain.

The MIC values against the clinical isolates of multidrug-resistant Gram-positive bacterial strains are reported in Table 2. All of the compounds synthesized in the current study exhibited inhibitory activities towards the multidrug-resistant Gram-positive bacterial strains (*MRSA* CCARM 3167 and 3506, *QRSA* CCARM 3505 and 3519) with MIC values in the range of 1–64 μg/mL. The inhibitory activities of compounds **5a**–**c** were 4–8-fold greater than those of **3a**–**c**, whereas the inhibitory activities of compounds **6a**–**c** were 4–16-fold greater than that of **4a**–**c** against the different *MRSA* (CCARM 3167 and 3506) and *QRSA* (CCARM 3505 and 3519) strains. Compound **6c**, in particular, showed the greatest inhibitory effect with MIC values of 1 μg/mL against two of these strains. Furthermore, the activities of compound **6c** were found to be equivalent to those of the standard drug moxifloxacin (MIC = 1 μg/mL), and greater than those of gatifloxacin (MIC = 2 μg/mL), oxacillin (MIC > 64 μg/mL) and norfloxacin (MIC = 8 μg/mL, 4 μg/mL) against the *MRSA* (*3167* and *3506*) strains. The activities of compound **6c** were slightly weaker than those of the standard drug oxacillin (MIC = 1 μg/mL) with MIC values of 2 μg/mL, although they were stronger than those of moxifloxacin (MIC = 4 μg/mL), norfloxacin (MIC > 64 μg/mL) and gatifloxacin (MIC = 8 μg/mL, 4 μg/mL) against the *QRSA* (*3505* and *3519*) strains, respectively.

Consideration of the results revealed several structure–activity relationships. First, compounds **3a**–**k** showed that the antibacterial activities were not significantly influenced by the substituent group or the position of the substituent on the phenyl ring.

Table 1
Inhibitory activity of compounds **3**–**6** expressed as MIC (μg/mL) again strains of Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) bacteria and Gram-negative (*Escherichia coli*, *Salmonela typhinurium* and *Pseudomonas aeruginosa*) bacteria and *Candida albicans* 7535.

Compound	Substituent		Gram-positive strains				Gram-negative strains						Fungus
			S. aureus			*Streptococcus mutans*	*Escherichia coli*		*Pseudomonas aeruginosa*		*Salmonela typhinurium*		*Candida albicans*
	R	R_1	4220[a]	209[b]	503[c]	3065[d]	1924[e]	1356[f]	2742[g]	2004[h]	2421[i]	1926[j]	7535
3a	3-CH_3	H	32	16	32	8	16	>128	>128	>128	128	64	64
3b	3-Cl	H	16	16	32	16	16	>128	>128	>128	64	32	32
3c	3-Br	H	32	16	32	16	16	>128	>128	>128	128	32	32
3d	4-CH_3	H	64	64	64	64	64	>128	>128	>128	128	64	64
3e	4-Cl	H	32	32	64	16	16	>128	>128	>128	128	32	32
3f	4-Br	H	64	64	64	16	64	>128	>128	>128	128	64	64
3g	2-CH_3	H	32	16	64	16	16	>128	>128	>128	128	32	64
3h	2-Cl	H	32	16	32	16	16	>128	>128	>128	64	32	64
3i	2-Br	H	32	16	64	16	16	>128	>128	>128	128	64	64
3j	2-F	H	64	16	64	32	32	>128	>128	>128	64	64	64
3k	H	H	64	32	64	64	64	>128	>128	>128	128	64	64
4a	3-CH_3	H	16	32	32	16	>128	>128	>128	>128	>128	>128	>64
4b	3-Cl	H	8	16	16	16	>128	>128	>128	>128	>128	>128	>64
4c	3-Br	H	16	16	16	16	>128	>128	>128	>128	>128	>128	>64
5a	3-CH_3	Benzyl	8	>64	>64	4	>128	>128	>128	>128	>128	>128	>64
5b	3-Cl	Benzyl	8	>64	>64	4	>128	>128	>128	>128	>128	>128	>64
5c	3-Br	Benzyl	4	>64	>64	2	>128	>128	>128	>128	>128	>128	>64
5k	H	Benzyl	16	>64	>64	8	>128	>128	>128	>128	>128	>128	>64
6a	3-CH_3	Benzyl	2	>64	>64	4	>128	>128	>128	>128	>128	>128	>64
6b	3-Cl	Benzyl	2	>64	>64	2	>128	>128	>128	>128	>128	>128	>64
6c	3-Br	Benzyl	2	>64	>64	1	>128	>128	>128	>128	>128	>128	>64
Gatifloxacin			0.25	2	4	0.5	2	16	1	1	0.5	2	0.5
Moxifloxacin			0.25	2	2	0.25	2	128	1	2	0.5	1	0.5

[a] *Staphylococcus aureus* RN 4220.
[b] *Staphylococcus aureus* 209.
[c] *Staphylococcus aureus* 503.
[d] *Streptococcus mutans* 3065.
[e] *Escherichia coli* KCTC 1924.
[f] *Escherichia coli CCARM* 1356.
[g] *Pseudomonas aeruginosa* 2742.
[h] *Pseudomonas aeruginosa* 2004.
[i] *Salmonella typhimurium* 2421.
[j] *Salmonella typhimurium* 1926.

Second, compounds **3a**–**c**, **3e**, **3g**–**i** exhibited moderate levels of activity against *E. coli* 1924 with MIC values of 16 μg/mL. Compared with the previously reported rhodanine derivatives that were prepared in our laboratory [14–19], the introduction of a 1,3,4-oxadiazole moiety as well as the lack of a carboxylic acid group appeared to be critical to the inhibition of both Gram-negative and Gram-positive strains. Third, the introduction of a benzyl group to the methylene moiety in compounds **3a**–**k** resulted in a significant improvement in the activities against *S. aureus* RN 4220 and *S. mutans* 3065, as well as the *MRSA* CCARM (3167 and 3506) and *QRSA* CCARM (3505 and 3519) strains. Unfortunately, however, this change also resulted in a complete loss of activity against *S. aureus* KCTC 209, *S. aureus* KCTC 503, *E. coli* 1924, *S. typhimurium* (1926 and 2421) and *C. albicans* 7535. Of all of the compounds tested, compound **6c** showed the strongest antibacterial activity against *S. mutans* 3065 and *MRSA* (3167 and 3506) with MIC values of 1 μg/mL. This compound also showed high levels of activity against *S. aureus* RN 4220 and *QRSA* (3505 and 3519), with MIC values of 2 μg/mL.

4. Conclusion

Based on our previous work, we designed and synthesized two novel series of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one derivatives (**3a**–**k**, **4a**–**c**, **5a**–**c**, **5k**, **6a**–**c**), and evaluated their antimicrobial activities against a fungus (*C. albicans*), as well as several Gram-negative and Gram-positive bacteria (including multidrug-resistant clinical isolates). In the current study, compounds **3a**–**k** showed moderate to significant levels of antibacterial activity and a broader antimicrobial spectrum than two of the leading compounds. Moreover, compound **6c** was found to be the most potent inhibitor with MIC values of 1 μg/mL against the *MRSA* (3167 and 3506) strains, which were greater than those of gatifloxacin, oxacillin, and norfloxacin. Compound **6c** was also more potent than moxifloxacin, norfloxacin, and gatifloxacin against the *QRSA* (*3505* and *3519*) strains with MIC values of 2 μg/mL. These results have demonstrated that compounds containing the 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one core structure could be used to develop new inhibitors against fungi as well as Gram-negative and Gram-positive bacteria (including multidrug-resistant clinical isolates).

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. Reaction courses were monitored by TLC on silica gel-precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded (in KBr) on a FTIR1730. ^{1}H NMR and ^{13}C NMR spectra were recorded in pure DMSO-d_6 on Bruker NMR spectrometers at 300 MHz and 75 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed in δ, ppm. High resolution mass spectroscopy were measured on a Bruker ultrafleXtreme MALDI-TOF/TOF.

Table 2
Inhibitory activity (MIC, μg/mL) of compounds **3**–**6** against clinical isolates of multidrug-resistant Gram-positive strains.

Compound	Substituent		Gram-positive strains			
			MRSA		*QRSA*	
	R	R_1	3167[a]	3506[b]	3505[c]	3519[d]
3a	3-CH_3	H	32	32	32	32
3b	3-Cl	H	32	32	32	32
3c	3-Br	H	16	32	32	32
3d	4-CH_3	H	64	64	64	64
3e	4-Cl	H	32	32	64	64
3f	4-Br	H	64	64	64	64
3g	2-CH_3	H	32	64	32	64
3h	2-Cl	H	32	32	32	32
3i	2-Br	H	16	32	32	32
3j	2-F	H	64	64	64	64
3k	H	H	64	64	64	64
4a	3-CH_3	H	32	32	32	32
4b	3-Cl	H	8	32	16	16
4c	3-Br	H	8	16	16	16
5a	3-CH_3	Benzyl	8	8	8	8
5b	3-Cl	Benzyl	4	8	8	8
5c	3-Br	Benzyl	2	4	2	4
5k	H	Benzyl	16	16	16	16
6a	3-CH_3	Benzyl	2	4	2	4
6b	3-Cl	Benzyl	2	4	2	4
6c	3-Br	Benzyl	1	1	2	2
Gatifloxacin			2	2	8	4
Moxifloxacin			1	1	4	4
Norfloxacin			8	4	>64	>64
Oxacillin			>64	>64	1	1

[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.

5.2. General synthetic procedure for the key intermediates *1* and *2*

Intermediates **1** were synthesized according to the literature [22,23]. Intermediates **2** were synthesized using the reported procedure [24].

5.3. General procedure for the target compounds *3a–k*, *5a–c* and *5k*

An equimolar mixture of *N*-carboxyl-2-(4-oxo-2-thioxothiazolidin-3-yl) **1** with the appropriate benzohydrazide **2** was refluxed with phosphorous oxychloride (105–110 °C) for 5–7 h. Reaction mixture was quenched with ice water and the solid separated was filtered off, washed with water and further purified by silica gel column chromatography (dichloromethane/petroleum ether, 80:1) to afford yellow or reddish brown compounds **3a**–**k** and **5a**–**c**. The yield, melting point and spectral data of each compound are given below.

5.3.1. 2-Thioxo-3-((5-(m-tolyl)-1,3,4-oxadiazol-2-yl)methyl) thiazolidin-4-one (*3a*)

Yield 61.7%; m.p.136–138 °C. IR (KBr) cm^{-1}: 1742 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.79–7.75 (m, 2H), 7.54–7.44 (m, 2H), 5.39 (s, 2H), 4.44 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 202.57, 173.61, 164.47, 160.71, 138.97, 132.83, 129.36, 126.80, 123.76, 122.91, 38.34, 36.21, 20.81. HRMS (MALDI) calcd for $C_{13}H_{11}N_3S_2O_2$: 306.0365, found: 306.0368 (M + 1).

5.3.2. 3-((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (*3b*)

Yield 62.4%; m.p.130–132 °C. IR (KBr) cm^{-1}: 1742 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.97–7.94 (m, 2H), 7.77–7.73 (m, 1H), 7.69–7.64 (m, 1H), 5.42 (s, 2H), 4.45 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.08, 174.08, 163.75, 161.63, 134.55, 132.51, 132.03, 126.51, 125.77, 125.37, 38.78, 36.71. HRMS (MALDI) calcd for $C_{12}H_8ClN_3S_2O_2$: 325.9819, found: 325.9829 (M + 1).

5.3.3. 3-((5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (*3c*)

Yield 65.2%; m.p.118–120 °C. IR (KBr) cm^{-1}: 1745 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 8.09–8.07 (t, *J* = 3.0 Hz, 1H), 7.99–7.96 (m, 1H), 7.88–7.85 (m, 1H), 7.61–7.55 (m, 1H), 5.40 (s, 2H), 4.43 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.05, 174.07, 163.63, 161.61, 135.38, 132.18, 129.31, 126.11, 125.56, 122.88, 38.78, 36.71. HRMS (MALDI) calcd for $C_{12}H_8BrN_3S_2O_2$: 369.9314, found: 369.9318 (M + 1).

5.3.4. 2-Thioxo-3-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)methyl) thiazolidin-4-one (*3d*)

Yield 63.3%; m.p.176–178 °C. IR (KBr) cm^{-1}: 1740 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.87–7.84 (d, *J* = 9.0 Hz, 2H), 7.43–7.41 (d, *J* = 6.0 Hz, 2H), 5.39 (s, 2H), 4.43 (s, 2H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO): δ 203.08, 174.09, 164.92, 160.98, 142.87, 130.49, 126.99, 120.72, 38.80, 36.63, 21.61. HRMS (MALDI) calcd for $C_{13}H_{11}N_3S_2O_2$: 306.0365, found: 306.0367 (M + 1).

5.3.5. 3-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (*3e*)

Yield 68.5%; m.p.178–180 °C. IR (KBr) cm^{-1}: 1740 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.97–7.94 (d, *J* = 9.0 Hz, 2H), 7.69–7.66 (d, *J* = 9.0 Hz, 2H), 5.39 (s, 2H), 4.41 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.07, 174.07, 164.13, 161.45, 137.40, 130.16, 128.86, 122.34, 38.77, 36.69. HRMS (MALDI) calcd for $C_{12}H_8ClN_3S_2O_2$: 325.9819, found: 325.9826 (M + 1).

5.3.6. 3-((5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (*3f*)

Yield; 70.4%; m.p.198–200 °C. IR (KBr) cm^{-1}: 1741 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.89–7.87 (d, *J* = 6.0 Hz, 2H), 7.82–7.80 (d, *J* = 6.0 Hz, 2H), 5.38 (s, 2H), 4.41 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.05, 174.07, 164.25, 161.45, 133.06, 128.95, 126.31, 122.67, 38.77, 36.69. HRMS (MALDI) calcd for $C_{12}H_8BrN_3S_2O_2$: 369.9314 found: 369.9324 (M + 1).

5.3.7. 2-Thioxo-3-((5-(o-tolyl)-1,3,4-oxadiazol-2-yl)methyl) thiazolidin-4-one (*3g*)

Yield 58.6%; m.p.140–142 °C. IR (KBr) cm^{-1}: 1741 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.86–7.84 (d, *J* = 6.0 Hz, 1H), 7.54–7.49 (m, 1H), 7.45–7.39 (m, 2H), 5.42 (s, 2H), 4.43 (s, 2H), 2.58 (s, 3H). ^{13}C NMR (75 MHz, DMSO): δ 203.09, 174.10, 165.06, 160.80, 138.10, 132.23, 132.11, 129.25, 126.97, 122.64, 38.74, 36.68, 21.77. HRMS (MALDI) calcd for $C_{13}H_{11}N_3S_2O_2$: 306.0365, found: 306.0370 (M + 1).

5.3.8. 3-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (*3h*)

Yield 59.8%; m.p.138–140 °C. IR (KBr) cm^{-1}: 1741 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.95–7.92 (d, *J* = 9.0 Hz, 1H), 7.74–7.66 (dd, *J* = 15.0, 9.0 Hz, 2H), 7.64–7.55 (m, 1H), 5.43 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 202.93, 174.05, 162.98, 161.63, 133.84, 132.33, 131.75, 131.60, 128.34, 122.66, 38.78, 36.68. HRMS (MALDI) calcd for $C_{12}H_8ClN_3S_2O_2$: 325.9819, found: 325.9814 (M + 1).

*5.3.9. 3-((5-(2-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (**3i**)*

Yield 60.5%; m.p.134–136 °C. IR (KBr) cm^{-1}: 1741 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.91–7.87 (m, 2H), 7.65–7.55 (m, 2H), 5.44 (s, 2H), 4.46 (s, 2H) ^{13}C NMR (75 MHz, DMSO): δ 202.85, 174.04, 163.64, 161.63, 134.81, 133.90, 132.18, 128.73, 124.78, 121.36, 38.80, 38.70. HRMS (MALDI) calcd for $C_{12}H_8BrN_3S_2O_2$: 369.9314, found: 369.9323 (M + 1).

*5.3.10. 3-((5-(2-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (**3j**)*

Yield 59.8%; m.p.114–116 °C. IR (KBr) cm^{-1}: 1745 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 8.01–7.95 (m, 1H), 7.75–7.68 (m, 1H), 7.55–7.42 (m, 2H), 5.43 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.02, 174.06, 161.50, 161.43, 134.97, 134.86, 130.11, 125.83, 117.75, 117.48, 38.76, 36.66. HRMS (MALDI) calcd for $C_{12}H_8FN_3S_2O_2$: 310.0115, found: 310.0112 (M + 1).

*5.3.11. 3-((5-Phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one (**3k**)*

Yield 71.2%; m.p.144–146 °C. IR (KBr) cm^{-1}: 1740 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.98–7.95 (m, 2H), 7.65–7.59 (m, 3H), 5.41 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 203.12, 174.09, 164.85, 161.27, 132.67, 129.96, 127.03, 123.45, 38.81, 36.69. HRMS (MALDI) calcd for $C_{12}H_9N_3S_2O_2$: 292.0209, found: 292.0211 (M + 1).

*5.3.12. (S,Z)-3-(2-Phenyl-1-(5-(m-tolyl)-1,3,4-oxadiazol-2-yl) ethyl)-2-thioxothiazolidin-4-one (**5a**)*

Yield 74.4%; m.p.72–74 °C. IR (KBr) cm^{-1}: 1738 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 7.80–7.77 (d, J = 9.0 Hz, 2H), 7.52–7.45 (q, 2H), 7.31–7.25 (m, 5H), 6.61–6.55 (t, J = 9.0, 1H), 4.39–4.20 (dd, J = 36.0 Hz, J = 18.0 Hz, 2H), 3.85–3.68 (m, 2H), 2.41 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 202.86, 173.96, 164.26, 162.97, 139.02, 135.65, 135.49, 132.87, 129.38, 128.44, 127.13, 126.83, 123.84, 122.94, 52.12, 34.98, 33.21, 20.81. HRMS (MALDI) calcd for $C_{20}H_{17}N_3S_2O_2$: 396.0835, found: 396.0836 (M + 1).

*5.3.13. (S,Z)-3-(1-(5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-phenylethyl)-2-thioxothiazolidin-4-one (**5b**)*

Yield 73.6%; m.p.108–110 °C. IR (KBr) cm^{-1}: 1737 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 8.00–7.95 (m, 2H), 7.75–7.72 (m, 1H), 7.67–7.62 (m, 1H), 7.31–7.25 (m, 5H), 6.60–6.55 (t, J = 7.5 Hz, 1H), 4.38–4.19 (dd, J = 39.0, 18.0 Hz, 2H), 3.85–3.67 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 203.17, 174.39, 163.90, 163.56, 135.92, 134.59, 132.51, 132.00, 129.80, 128.91, 127.61, 126.54, 125.83, 125.38, 52.57, 35.51, 33.73. HRMS (MALDI) calcd for $C_{19}H_{14}ClN_3S_2O_2$: 416.0289, found: 416.0296 (M + 1).

*5.3.14. (S,Z)-3-(1-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-2-phenylethyl)-2-thioxothiazolidin-4-one (**5c**)*

Yield 74.8%; m.p.82–84 °C. IR (KBr) cm^{-1}: 1740 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 8.12 (s, 1H), 8.03–7.99 (m, 1H), 7.87–7.83 (m, 1H), 7.60–7.54 (t, J = 9.0 Hz, 1H), 7.32–7.25 (m, 5H), 6.61–6.56 (t, J = 6.0 Hz, 1H), 4.39–4.20 (dd, J = 39.0, 18.0 Hz, 2H), 3.89–3.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 202.77, 173.93, 163.43, 162.96, 135.44, 134.96, 131.73, 129.33, 128.84, 128.44, 127.13, 125.72, 125.09, 122.46, 52.08, 35.04, 33.21. HRMS (MALDI) calcd for $C_{19}H_{14}BrN_3S_2O_2$: 459.9784, found: 459.9777 (M + 1).

*5.3.15. (S,Z)-3-(2-Phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl)-2-thioxothiazolidin-4-one (**5k**)*

Yield 77.5%; m.p.90–92 °C. IR (KBr) cm^{-1}: 1742 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 8.00–7.98 (d, J = 6.0 Hz, 2H), 7.65–7.61 (m, 3H), 7.31–7.26 (m, 5H), 6.61–6.56 (t, J = 7.5 Hz, 1H), 4.38–4.20 (dd, J = 18.0, 9.0 Hz, 2H), 3.85–3.68 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 203.17, 174.41, 164.40, 163.50, 135.96, 132.64, 129.92, 129.80, 128.92, 127.60, 127.09, 123.49, 52.62, 35.46, 33.73. HRMS (MALDI) calcd for $C_{19}H_{15}N_3S_2O_2$: 382.0678, found: 382.0684 (M + 1).

*5.4. General procedure for the target compounds **4a–c** and **6a–c***

To a solution of compounds **3a–c** or **5a–c** (1.0 mmoL) and 4-formylbenzoic acid (1.5 mmol) in absolute ethanol (10.0 mL) were added 5 drops glacial acetic acid and 5 drops piperidine. The reaction mixture was stirred at 50–60 °C for 4–6 h, until the completion of the reaction as evidenced by TLC. The resulting reaction mixture was concentrated to dryness, recrystallized with water to give yellow solid **4a–c** and purified by silica gel column chromatography (dichloromethane/methanol, 150:1) to afford pure yellow products **6a–c**. The yield, melting point and spectral data of each compound are given below.

*5.4.1. (Z)-4-((4-Oxo-2-thioxo-3-((5-(m-tolyl)-1,3,4-oxadiazol-2-yl) methyl)thiazolidin-5-ylidene)methyl)benzoic acid (**4a**)*

Yield 88.7%; m.p.246–248 °C. IR (KBr) cm^{-1}: 3500 (OH), 1724 (C=O), 1608 (C=O). ^1H NMR: ^1H NMR (300 MHz, DMSO-d_6): δ 13.31 (s, 1H), 8.10–8.08 (d, J = 6.0 Hz, 2H), 7.98 (s, 1H), 7.82–7.76 (m, 4H), 7.52–7.44 (m, 2H), 5.59 (s, 2H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ192.81, 166.53, 166.15, 164.61, 160.58, 138.97, 136.62, 132.86, 132.50, 132.32, 130.74, 130.17, 129.37, 126.83, 124.18, 123.80, 122.91, 38.69, 20.80. HRMS (MALDI) calcd for $C_{21}H_{15}N_3O_4S_2$: 438.0577, found: 438.0570 (M + 1).

*5.4.2. (Z)-4-((3-((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzoic acid (**4b**)*

Yield 85.9%; m.p.254–256 °C. IR (KBr) cm^{-1}: 3500 (OH), 1725 (C=O), 1608 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 13.32 (s, 1H), 8.10–8.08 (d, J = 6.0 Hz, 2H), 7.98–7.93 (m, 3H), 7.82–7.79 (d, J = 9.0 Hz, 2H), 7.74–7.72 (d, J = 6.0 Hz, 1H), 7.67–7.62 (m, 1H), 5.59 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 193.33, 167.02, 166.63, 163.91, 161.49, 137.10, 134.53, 132.93, 132.84, 132.53, 132.04, 131.21, 130.66, 126.55, 125.83, 125.38, 124.72, 39.02. HRMS (MALDI) calcd for $C_{20}H_{12}ClN_3O_4S_2$: 458.0031, found: 458.0032 (M + 1).

*5.4.3. (Z)-4-((3-((5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl) methyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzoic acid (**4c**)*

Yield 87.3%; m.p.262–264 °C. IR (KBr) cm^{-1}: 3500 (OH), 1724 (C=O), 1605 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 13.32 (s, 1H), 8.10–8.07 (m, 3H), 7.99–7.97 (m, 2H), 7.87–7.79 (dd, J = 15.0, 6.0 Hz, 3H), 7.60–7.55 (m, 1H), 5.58 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 193.32, 167.02, 166.63, 163.76, 161.48, 137.10, 135.40, 132.92, 132.80, 132.21, 131.21, 130.66, 129.34, 126.16, 125.59, 124.71, 122.86, 39.00. HRMS (MALDI) calcd for $C_{20}H_{12}BrN_3O_4S_2$: 501.9525, found: 501.9526 (M + 1).

*5.4.4. (S,Z)-4-((4-Oxo-3-(2-phenyl-1-(5-(m-tolyl)-1,3,4-oxadiazol-2-yl)ethyl)-2-thioxothiazolidin-5-ylidene)methyl)benzoic acid (**6a**)*

Yield 88.3%; m.p.110–112 °C. IR (KBr) cm^{-1}: 3500 (OH), 1718 (C=O), 1604 (C=O). ^1H NMR (300 MHz, DMSO-d_6): δ 13.27 (s, 1H), 8.07 (s, 1H), 8.04 (s, 1H), 7.84–7.79 (m, 3H), 7.74 (s, 1H), 7.72 (s, 1H), 7.51–7.46 (m, 2H), 7.30–7.21 (m, 5H), 6.81–6.76 (t, J = 7.5 Hz, 1H), 3.90–3.86 (m, 2H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 193.08, 166.97, 166.71, 164.88, 163.25, 139.46, 136.88, 135.77, 133.34, 133.21, 132.84, 131.30, 130.61, 129.83, 129.74, 128.95, 127.70, 127.32, 124.34, 123.38, 123.27, 52.78, 33.84, 21.27. HRMS (MALDI) calcd for $C_{28}H_{21}N_3O_4S_2$: 528.1046, found: 528.1047 (M + 1).

*5.4.5. (S,Z)-4-((3-(1-(5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-phenylethyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzoic acid (**6b**)*

Yield 89.2%; m.p.120–122 °C. IR (KBr) cm^{-1}: 3500 (OH), 1725 (C═O), 1605 (C═O). ^1H NMR (300 MHz, DMSO-d_6): δ 13.33 (s, 1H), 8.08–8.05 (d, J = 9.0 Hz, 3H), 8.02–8.00 (d, J = 6.0 Hz, 1H), 7.85 (s, 1H), 7.76–7.73 (d, J = 9.0 Hz, 3H), 7.67–7.62 (m, 1H), 7.31–7.25 (m, 5H), 6.81–6.76 (t, J = 7.5 Hz, 1H), 3.93–3.82 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 192.67, 168.24, 166.52, 166.24, 163.26, 136.41, 135.27, 134.12, 132.67, 132.09, 131.56, 130.83, 130.15, 129.27, 128.50, 127.25, 126.12, 126.05, 125.43, 124.85, 122.88, 52.24, 33.32. HRMS (MALDI) calcd for $C_{27}H_{18}ClN_3O_4S_2$: 548.0500, found: 548.0513 (M + 1).

*5.4.6. (S,Z)-4-((3-(1-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-2-phenylethyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzoic acid (**6c**)*

Yield 84.6%; m.p.128–130 °C. IR (KBr) cm^{-1}: 3500 (OH), 1718 (C═O), 1605 (C═O). ^1H NMR (300 MHz, DMSO-d_6): δ 13.24 (s, 1H), 8.07–7.99 (m, 4H), 7.84 (s, 1H), 7.75–7.72 (d, J = 9.0 Hz, 3H), 7.66–7.61 (m, 1H), 7.30–7.26 (d, J = 13.3 Hz, 5H), 6.81–6.76 (t, J = 7.5 Hz, 1H), 3.90–3.87 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 192.64, 166.50, 166.23, 163.22, 163.10, 136.40, 135.24, 134.95, 132.63, 132.33, 131.66, 130.81, 130.14, 129.26, 128.87, 128.48, 127.24, 125.73, 125.03, 122.87, 122.46, 52.23, 33.31. HRMS (MALDI) calcd for $C_{27}H_{18}BrN_3O_4S_2$: 591.9995, found: 591.9985 (M + 1).

5.5. Evaluation of anti-bacterial activity in vitro

The micro-organisms used in the present study were *S. aureus* (*S. aureus* RN 4220, *S. aureus* KCTC 503 and *S. aureus* KCTC 209), *S. mutans* KCTC 3065, *E. coli KCTC* 1924, *E. coli* CCARM 1356, *Salmonella typhimurium* 1926 and 2421, *P. aeruginosa* 2742 and 2004, *C. albicans* 7535. The strains of multidrug-resistant clinical isolates were multidrug-resistant *S. aureus* (*MRSA* CCARM 3167 and *MRSA* CCARM 3506) and Quinolone-resistant *S. aureus* (*QRSA* CCARM 3505 and *QRSA* CCARM 3519). Clinical isolates were collected from various patients hospitalized in several clinics.

A two-fold serial dilution technique [25] was followed to determine the minimum inhibitory concentration (MIC) of the compounds against the susceptible micro-organisms in the preliminary test (Gram-positive bacteria and Gram-negative bacteria) and against strains of clinical isolates of multidrug-resistant Gram-positive bacteria. Test compounds dissolved in DMSO were added to culture media (Brain Heart Infusion for *S. mutans* and Müller-Hinton agar for other bacteria) to obtain final concentrations of 0.5–64 μg/mL. The final amount applied was of 10^5 CFU/mL for bacteria. MIC values were read after incubation at 37 °C for 20 h. The lowest concentration of the test substance that completely inhibited growth of the micro-organism was recorded as the MIC (expressed in μg/mL). Gatifloxacin, moxifloxacin, norfloxacin and oxacillin were used as the standard drugs. All experiments were carried out three times.

Acknowledgments

This work was supported by the National Science Foundation of China (81260468).

References

[1] A.W. Karchmer, Clin. Infect. Dis. 31 (2000) 139–143.
[2] Y. Gholizadeh, P. Courvalin, Int. J. Antimicrob. Agents 16 (2001) 11–17.
[3] C. Ruef, Infection 32 (2004) 315–327.
[4] R. Haque, C.D. Huston, M. Hughes, E. Houpt, W.A. Petri Jr., N. Engl. J. Med. 348 (2003) 1565–1573.
[5] N. Harnett, S. Brown, C. Krishnan, Antimicrob. Agents Chemother. 35 (1991) 1911–1913.
[6] L.J.V. Piddock, Drugs 58 (1999) 11–18.
[7] P.C. Appelbaum, P.A. Hunter, Int. J. Antimicrob. Agents 16 (2000) 5–15.
[8] A.K. Zafer, T.Z. Gulhan, O. Ahmet, R. Gilbert, Eur. J. Med. Chem. 43 (2008) 155–159.
[9] N.S. Cutshall, C. O'Day, M. Prezhdo, Bioorg. Med. Chem. Lett. 15 (2005) 3374–3379.
[10] M. Sortino, P. Delgado, S. Juarez, J. Quiroqa, R. Abonia, B. Insuasty, M. Noqueras, L. Rodero, F.M. Garibatto, R.D. Enriz, S.A. Zacchino, Bioorg. Med. Chem. 15 (2007) 484–494.
[11] R. Nurugan, S. Anbazhagan, S.S. Narayanan, Eur. J. Med. Chem. 44 (2009) 3272–3279.
[12] R.A. Rane, S.D. Gutte, N.U. Sahu, Bioorg. Med. Chem. Lett. 22 (2012) 6429–6432.
[13] B. Chandrakantha, P. Shetty, V. Nambiyar, N. Isloor, A.M. Isloor, Eur. J. Med. Chem. 45 (2010) 1206–1210.
[14] L.L. Xu, C.J. Zheng, L.P. Sun, J. Miao, H.R. Piao, Eur. J. Med. Chem. 48 (2012) 174–178.
[15] C.J. Zheng, L.L. Xu, L.P. Sun, J. Miao, H.R. Piao, Eur. J. Med. Chem. 58 (2012) 112–116.
[16] Z.H. Chen, C.J. Zheng, L.P. Sun, H.R. Piao, Eur. J. Med. Chem. 45 (2010) 5739–5743.
[17] M.X. Song, C.J. Zheng, X.Q. Deng, Q. Wang, S.P. Hou, T.T. Liu, X.L. Xing, H.R. Piao, Eur. J. Med. Chem. 54 (2012) 403–412.
[18] M.X. Song, C.J. Zheng, X.Q. Deng, L.P. Sun, Y. Wu, L. Hong, Y.J. Li, Y. Liu, Z.Y. Wei, M.J. Jin, H.R. Piao, Eur. J. Med. Chem. 60 (2013) 376–385.
[19] X. Jin, C.J. Zheng, M.X. Song, Y. Wu, L.P. Sun, Y.J. Li, L.J. Yu, H.R. Piao, Eur. J. Med. Chem. 56 (2012) 203–209.
[20] M.J. Ahsan, J.G. Samy, H. Khalilullah, M.S. Nomani, P. Saraswat, R. Gaur, A. Singh, Bioorg. Med. Chem. Lett. 21 (2011) 7246–7250.
[21] D. Hardej, C.R. Ashby Jr., N.S. Khadtare, S.S. Kulkarni, S. Singh, T.T. Talele, Eur. J. Med. Chem. 45 (2010) 5827–5832.
[22] L.Y. Wang, F.S. Kong, C.L. Kokoski, D.W. Andrewsb, C.G. Xing, Bioorg. Med. Chem. Lett. 18 (2008) 236–240.
[23] C.G. Xing, L. Wang, X.H. Tang, Y.Y. Sham, Bioorg. Med. Chem. Lett. 15 (2007) 2167–2176.
[24] D. Kumar, N.M. Kumar, S. Ghosh, K. Shah, Bioorg. Med. Chem. Lett. 22 (2012) 212–215.
[25] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard M7-A6, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2003.