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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 4-thiazolidione derivatives as antibacterial agents against drug-resistant *Staphylococcus epidermidis*

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Abstract A series of novel substituted 4-thiazolidione derivatives were designed, synthesized, and evaluated in vitro for their antibacterial activities, in comparison with methicillin and ampicillin. Compounds (**7d, 7h–k**) exhibit good potency in inhibiting the growth of *Staphylococcus epidermidis* (MIC: 1.57–3.13 μM). Further antibacterial effects of compounds (**7d, 7h–k**) were investigated using clinical isolates (methicillin-resistant *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus*), in comparison with methicillin and levofloxacin. Compound **7k** showed the most potent antibacterial activities among the synthesized compounds.

Keywords 4-Thiazolidione · Methicillin-resistant *Staphylococcus epidermidis* · Methicillin-resistant *Staphylococcus aureus* · Antibacterial activity

Introduction

Staphylococcus epidermidis is the most frequently isolated member of the group of coagulase-negative staphylococci (Huebner and Goldmann, 1999). In recent years, *S. epidermidis* has gained substantial interest because it has become an important cause of nosocomial infections related to indwelling medical devices such as vascular catheters, prosthetic joints, and artificial heart valves (Götz, 2002; O'Gara and Humphreys, 2001). A characteristic of many strains of this microbe is the production of a capsule or slime resulting in the formation of biofilms. In biofilms, *S. epidermidis* is protected against attacks from the immune system and against antibiotic treatment, making *S. epidermidis* infections difficult to cure (Hoffman *et al*., 2005; Davies *et al*., 1998; Vuong *et al*., 2004). The widespread use of methicillin and other semisynthetic antibiotics led to the emergence of drug-resistant *S. epidermidis* (Sieradzki *et al*., 1999; Garrett *et al*., 1999; Raad *et al*., 1998), which continues to persist in both the health care and community environments. With the appearance of multi-resistant strains increasing quickly, the need to discover novel antibiotics is urgent.

In prokaryotes, the two-component signaling systems (TCS), each pair of which are typically composed of histidine kinase (HK) and response regulator (RR), play important roles in drug-resistance, pathogenesis, and bacterial growth (West and Stock, 2001; Stephenson and Hoch, 2002). In *S. epidermidis*, at least 11 TCSs were identified; among them, YycG/YycF is essential for bacterial viability that could be a potential target for the discovery or synthesis of novel classes of antibacterial agents (Fabret and Hoch, 1998).

Not until recently, by means of a structure-based virtual screening (SBVS) method, 7 compounds were identified as potent inhibitors of histidine kinase YycG protein from the compound library of SPECS (Qin *et al*., 2006; Schreiber *et al*., 2009). A typical **1** (Fig. 1), which had a 4-thiazolidinone core substituted by aromatic rings, was validated in vitro to be active in inhibiting the growth of *S. epidermidis* without obivious cytoxicity to Vero cells or hemolysis.

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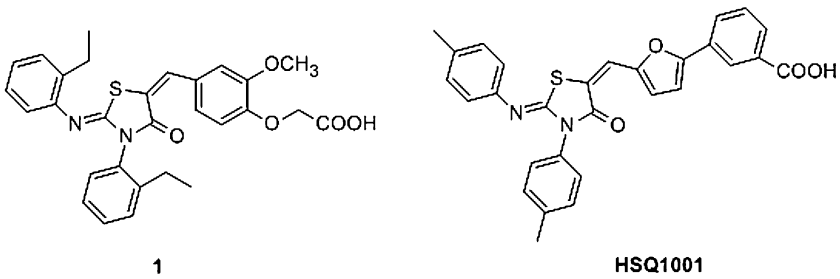


Fig. 1 The structures of compounds **1** and **HSQ1001**

In our previous work, choosing **1** as the leading compound, a series of novel 4-thiazolidinone were designed and found to have antibacterial activities, among which 3-[5-(4-Oxo-3-*p*-tolyl-2-*p*-tolylimino-thiazolidin-5-ylidenemethyl)-furan-2-yl]-benzoic acid (**HSQ1001**) showed the strongest activity with MIC values of 6.25 μM. It described the synthesis of 4-thiazolidinone derivatives with the carboxylic acid group was indispensable and the 3-(5-formylfuran-2-yl) benzoic acid fragment resulted in an increased antibiofilm and antibacterial activity (Pan *et al*., 2011; Pan *et al*., 2010).

In this paper, in our attempt to continue to discover new, more-effective, and less-toxic agents for the treatment of *S. epidermidis* infections, 4-thiazolidinone compounds containing carboxylic acid moieties of benzoic acid, phenoxy-acetic acid, or 4-phenoxymethyl-benzoic acid, which are similar to 3-(5-formylfuran-2-yl) benzoic acid fragment, have been synthesized and tested in vitro for their antibacterial activities against *S. epidermidis, E. coli, and Samonella* which have no homologous genes of YycG/YycF were selected as control bacteria. As halogenated 4-thiazolidinone derivatives might be expected to be more lipophilic and thus to have better absorption and distribution properties, as well as being more stable metabolically, the influence of the halogen substituent of the phenyl rings on biologic activity was studied.

Results and discussion

Chemistry

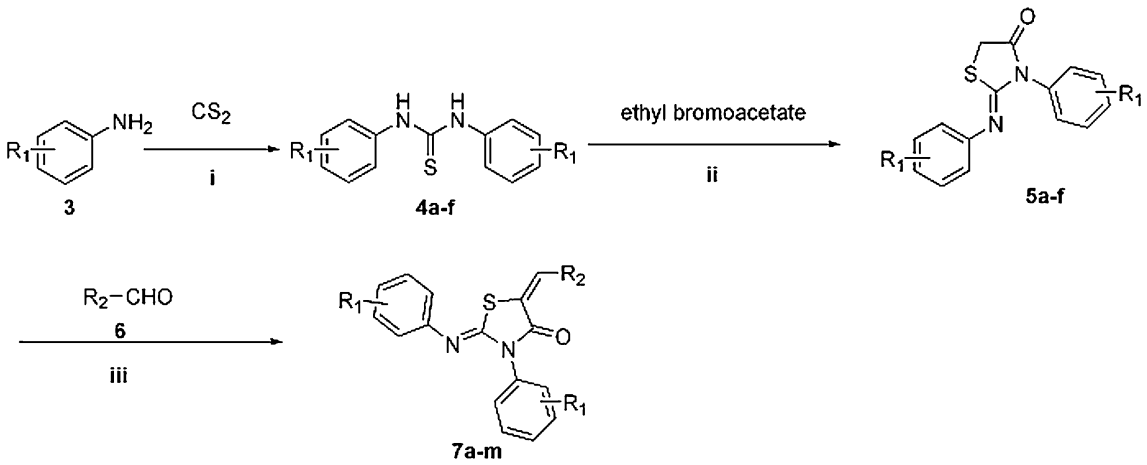
The synthetic route to the 4-thiazolidione **7a–m** is shown in Scheme 1. Treatment of amines **3** and carbon disulfide in 10 % NaOH solution at 60 °C for 4–8 h afforded *N*-substituted thioureas **4** with good yields. The construction of thiazolidione **5** was achieved by the cyclization reaction of *N*-substituted thioureas **4** with ethyl bromoacetate in EtOH, in the presence of sodium acetate (Ramla, 2007). The final step of the Knoevenagel condensation of substituted aromatic aldehydes **6** with equivalent amount of **5** was catalyzed by *β*-alanine to give the target compounds **7a–m** in good yields (Luo *et al*., 2009). A total of 13 designed compounds were successfully prepared and characterized by 1H-NMR, FTIR, and Mass spectral studies.

Antibacterial activity

All newly synthesized compounds (**7a–m**) in Table 1 were tested for their ability to inhibit bacterial growth of Gram-positive (*S. epidermidis*) and Gram-negative (*Escherichia coli and Salmonella*) species. The antibacterial activities of these compounds were also compared with two well-known antibiotics namely methicillin and ampicillin. Most of them exhibit good potency in inhibiting the growth of *S. epidermidis* which belong to low G + C Gram-positive bacterial with highly conserved YycG/YycF TCS. However, none of the molecules was able to inhibit the growth of *E. coli* and *Salmonella* (MIC > 200 μM) which have no homologous genes of YycG/YycF.

Among the 13 newly synthesized compounds, ten compounds (**7b**, **7c**, **7d**, **7g**, **7h**, **7i**, **7j**, **7k**, **7l**, and **7m**) were found to be much better active compounds to the leading compound **1** with MICs <50 μM. Five compounds (**7d, 7h–k**) showed much better activity than methicillin and ampicillin. It is worth mentioning that the compound {4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7k**) was found to be the most active agent against *S. epidermidis* ATCC 35984 with MIC value of 1.57 μM.

In a first effort to optimize this series, we evaluated group R_2 utilizing benzoic acid, 4-phenoxymethyl-benzoic acid, and phenoxy-acetic acid substituents. By introducing the benzoic acid moiety, compounds **7a–d** showed good activities with the MIC value of 3.13–50 μM, especially compound **7d** (MIC = 3.13 μM). However, **7e** showed no activity (MIC > 200 μM) when the big rigid fragment 4-phenoxymethyl-benzoic acid was incorporated into the 4-thiazolidione scaffold. To our delight, the activities of compounds **7f–m**, with the phenoxy-acetic acid group, were even better. The results suggested that the distance between carboxylic acid and the 4-thiazolidione ring appeared to have a direct impact on the antibacterial activity and the moieties benzoic acid or phenoxy-acetic acid had the suitable distance. As a matter of fact, the *p*-phenoxy-acetic acid derivative **7k** (MIC = 1.57 μM) was the most potent agent in this



Scheme 1 Reagents and conditions: *i* 10 % NaOH solution, 60 °C, *ii* NaOAc, absolute alcohol, reflux, *iii* β-alanine, acetic acid, reflux

Table 1 In vitro antibacterial activity screening of novel 4-thiazolidione derivatives against *S. epidermidis*, *E. coli* and *Salmonella*

Compounds	R_1	R_2	MIC (μM)[a]		
			S.epidermidis ATCC 35984	*E. coli* ATCC 25922	*Salmonella*
7a	H	4-COOH-Ph	50	>200	>200
7b	4-F	4-COOH-Ph	25	>200	>200
7c	4-Br	4-COOH-Ph	12.5	>200	>200
7d	4-Cl	4-COOH-Ph	3.13	>200	>200
7e	4-Cl	4-(4′-COOH-$PhCH_2O$)-Ph	>200	>200	>200
7f	H	4-OCH_2COOH-Ph	100	>200	>200
7g	4-F	4-OCH_2COOH-Ph	25	>200	>200
7h	4-Br	4-OCH_2COOH-Ph	3.13	>200	>200
7i	2-Cl	4-OCH_2COOH-Ph	3.13	>200	>200
7j	3-Cl	4-OCH_2COOH-Ph	3.13	>200	>200
7k	4-Cl	4-OCH_2COOH-Ph	1.57	>200	>200
7l	4-Cl	3-OCH_3-4-OCH_2COOH-Ph	6.25	>200	>200
7m	4-Cl	3-OCH_2COOH-Ph	25	>200	>200
1	2-CH_2CH_3	3-OCH_3-4-OCH_2COOH-Ph	50	>200	>200
Methicillin	–	–	5.26	>200	>200
Ampicillin	–	–	11.5	>200	>200

[a] MIC assay was performed following the broth micro-dilution method (in tubes) of the CLSI of America. In this assay, the highest concentration of each compound was 200 μM

series with a fourfold increase in potency when compared to **7l** (MIC = 6.25 μM), and was also 16 times more active than the *m*-phenoxy-acetic acid analog **7m** (MIC = 25.0 μM). The introduction of an electron-donating group 3-methoxy on the phenyl ring of phenoxy-acetic acid moiety did not improve the antibacterial activity and *m*-phenoxy-acetic acid presented a negative effect as it strongly reduced the activity.

In drug design, halogen atoms are used to improve penetration through lipid membranes and tissues. They may also present a significant reactivity depending on the structure of the molecule. In this work, the introduction of halogen substituents (F, Cl, or Br) on phenyl rings of **7b–d** and **7g–m** showed a positive effect as they enhanced the antibacterial activity (Table 1). The activities of compounds **7f**, **7g**, **7h,** and **7k** with the same phenoxy-acetic acid moiety at R_2, but different para-halogen substituents (R_1) on phenyl rings increased in the following order: H(**7f**) < F(**7g**) < Br(**7h**) < Cl(**7k**). It is noteworthy that the position of chloro group on phenyl rings also influenced the activities of the 4-thiazolidinone. Compound **7k** with para-chloro group on phenyl rings was shown to be more active than compounds **7i** and **7j** with ortho- and meta-chloro on phenyl ring, respectively. This result indicated that the para halogen substituents on

Table 2 Antibacterial activity of selected 4-thiazolidione derivatives against methicillin-resistant clinical isolates

Clinical isolates	MIC (μM)			
	MRSA8282	MRSA8166	MRSE 8311	MRSE 8354
7d	3.13	12.5	3.13	3.13
7h	3.13	6.25	3.13	3.13
7i	3.13	12.5	3.13	3.13
7j	3.13	12.5	3.13	3.13
7k	3.13	6.25	1.57	1.57
Methicillin	65.7	65.7	65.7	65.7
Levofloxacin	22.1	22.1	22.1	22.1

MRSA methicillin-resistant *Staphylococcus aureus*, *MRSE* methicillin-resistant *Staphylococcus epidermidis*

phenyl rings were more likely to inhibit the growth of *S. epidermidis*.

To examine whether the 4-thiazolidione derivatives are effective against clinical isolates, MIC values of the selected derivatives **7d, 7h–k** were determined against methicillin-resistant clinical isolates (MRSA8282, MRSA8166, MRSE 8311, and MRSE 8354) and were compared with methicillin and levofloxacin. As shown in Table 2, the antibacterial activities of **7d, 7h–k** were much superior to that of methicillin and levofloxacin. Compound **7k** demonstrated the most potent antibacterial activities among the synthesized compounds.

Conclusion

In summary, a series of 4-thaizolidinone (**7a–m**) were synthesized and evaluated in vitro for their antibacterial activities, in comparison with methicillin and ampicillin. Most of the synthesized compounds were proved to be effective agents against to *S. epidermidis*, especially **7k** showed the most active with the MIC value of 1.57 μM. The carboxylic acid moieties such as phenoxy-acetic acid fragment at R_2 afforded excellent activities. The compound bearing a chlorine atom in the para-position (R_1) of phenyl rings was favorable for achieving antibacterial activities. Further antibacterial evaluation was down on clinical isolates, a comparison of antibacterial activity of the most active compounds(**7d, 7h–k**) with that of two well-known antibiotics namely methicillin and levofloxacin indicated that compound **7k** show very good activity.

Experimental protocols

Chemistry

General remarks

All reagents and solvents were commercially available and used without further purification. Melting points were determined on an electrothermal digital apparatus model WRS-1B (ShangHai, China) without correction. Infrared spectra were recorded on an Avatar 360 ESP spectrometer (Nicolet) by potassium bromide (1 % w/w) disk scanning from 500 to 4,000 cm^{-1}. A Bruker AM-500 MHz instrument (Bruker) was used to acquire ^1H NMR spectra with TMS as internal standard. Chloroform-D and DMSO-d_6 were used as solvents. Low resolution mass spectra (LRMS) were recorded on a HP-5973 instrument (HP).

*Synthesis of 1, 3-Bis-(4-chloro-phenyl)-thiourea(**4a**). Representative procedure for N-substituted thioureas (**4a–f**)*

p-Chloroaniline (1.27 g, 0.01 mol) was dissolved in 10 % NaOH (30 mL) solution followed by addition of carbon disulfide (0.015 mol) at room temperature and the mixture was heated to 60 °C until the completion of reaction (monitored by TLC). The mixture was cooled, acidified with dilute hydrochloric acid, and extracted with CH_2Cl_2. The combined organic layer was dried over sodium sulfate and the solvent was removed completely. The residue was purified by column chromatography on silica gel to obtain 1,3-Bis-(4-chloro-phenyl)-thiourea (2.39 g, 80.7 %) as white powder.

*3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-thiazolidin-4-one(**5a**).Representative procedure for thiazolidione (**5a–f**)*

To a stirred suspension of 1,3-Bis-(4-chloro-phenyl)-thiourea (1.19 g, 4.0 mmol) and anhydrous sodium acetate (0.49 g, 6 mmol) in 20 mL of absolute ethanol was added 0.48 mL of ethyl bromoacetate (4.4 mmol). The mixture was heated at 60 °C for 3 h. After being cooled to room temperature, the reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried by anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4:1 v/v) as mobile phase to obtain pure compound as a white solid: yield, 95 %; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, $J = 8.2$ Hz, Ph–H), 7.25 (d, $J = 8.5$ Hz, Ph–H), 7.10 (d, $J = 8.1$ Hz, Ph–H), 6.80 (d, $J = 8.2$ Hz, 2H, Ph–H), 3.93 (s, 2H, CH_2).

*3-(4-Chloro-phenyl)-2-(3-chloro-phenylimino)-thiazolidin-4-one (**5b**)*

3-(4-Chloro-phenyl)-2-(3-chloro-phenylimino)-thiazolidin-4-one was prepared from 1, 3-Bis-(3-chloro-phenyl)-thiourea and ethyl bromoacetate according to the procedure described above: yield 92 %, white powder; ^1H NMR

(300 MHz, $CDCl_3$) δ 7.93 (s, 1H, Ph–H), 7.82 (s, 1H, Ph-H), 7.56–7.43 (m, $J = 13.3$ Hz, 2H, Ph–H), 7.35 (t, $J = 7.4$ Hz, 1H, Ph–H, 7.27 (d, $J = 7.4$ Hz, 1H, Ph–H), 7.20 (d, $J = 7.3$ Hz, 1H, Ph–H), 7.13 (d, $J = 7.0$ Hz, 1H, Ph–H), 4.16 (s, 2H, CH_2).

*3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-thiazolidin-4-one (**5c**)*

3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-thiazolidin-4-one was prepared from 1,3-Bis-(4-Fluoro-phenyl)-thiourea and ethyl bromoacetate according to the procedure described above: yield 97 %, white powder; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.45 (m, 2H, Ph–H), 7.30–7.23 (m, 2H, Ph–H), 7.13 (t, $J = 7.7$ Hz, 2H, Ph–H), 7.06 (t, $J = 7.7$ Hz, 2H, Ph–H), 4.12 (s, 2H, CH_2).

*3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-thiazolidin-4-one (**5d**)*

3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-thiazolidin-4-one was prepared from 1,3-Bis-(4-Fluoro-phenyl)-thiourea and ethyl bromoacetate according to the procedure described above: yield 90 %, white powder; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 7.4$ Hz, 2H, Ph–H), 7.62 (q, $J = 7.6$ Hz, 4H, Ph–H), 7.03 (d, $J = 7.4$ Hz, 2H, Ph–H), 4.16 (s, 2H, CH_2).

*General procedure for preparation of compounds (**7a–m**)*

The corresponding aldehyde **6** (1 equiv), β-alanine (0.2 equiv), and thiazolidione **5** (1 equiv) were heated at 100 °C for 1 h in glacial acetic acid. Upon completion of the reaction, the mixture was cooled, the reaction was quenched with water, and the precipitate was filtered off. The solid products were filtered and recrystallized in methanol to obtain pure compound.

*4-(4-Oxo-3-phenyl-2-phenylimino-thiazolidin-5-ylidenemethyl)-benzoic acid (**7a**)*

4-(4-Oxo-3-phenyl-2-phenylimino-thiazolidin-5-ylidenemethyl)-benzoic acid was prepared from 3-phenyl-2-phenylimino-thiazolidin-4-one and 4-formyl-benzoic acid according to the procedure described above: White powder, yield 69 %; mp: 168–169 °C; IR(KBr): $\nu/cm^{-1} = 3410$(CO–OH), 3034(C–H), 1671(C=O), 1587 (C=C), 1322(C–S); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.11 (s, 1H, COOH), 8.03 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.85 (s, 1H, CH), 7.67 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.57 (d, $J = 4.3$ Hz, 4H, Ph–H), 7.53–7.44 (m, 1H, Ph–H), 7.39 (t, $J = 7.9$ Hz, 2H, Ph–H), 7.18 (t, $J = 7.4$ Hz, 1H, Ph–H), 6.98 (dd, $J = 8.3$, 1.0 Hz, 2H, Ph–H); ESI–MS: m/z 401.0$(M+1)^+$.

*4-[3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid (**7b**)*

4-[3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid was prepared from 3-(4-fluoro-phenyl)-2-(4-fluoro-phenylimino)-thiazolidin-4-one and 4-formyl-benzoic acid according to the procedure described above: White powder, yield 87 %; mp: 224–225 °C; IR(KBr): $\nu/cm^{-1} = 3412$(CO–OH), 3032(C–H), 1667(C=O), 1573(C=C), 1321(C–S); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.10(s, 1H, COOH), 8.04 (d, $J = 8.3$ Hz, 2H, Ph–H), 7.89 (s, 1H, CH), 7.68 (d, $J = 8.3$ Hz, 2H, Ph–H), 7.63 (s, 4H, Ph–H), 7.41 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.01 (d, $J = 8.4$ Hz, 2H, Ph–H); ESI–MS: m/z 436.9$(M+1)^+$.

*4-[3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid (**7c**)*

4-[3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid was prepared from 3-(4-bromo-phenyl)-2-(4-bromo-phenylimino)-thiazolidin-4-one and 4-formyl-benzoic acid according to the procedure described above: White powder, yield 82 %; mp: 188–190 °C; IR(KBr): $\nu/cm^{-1} = 3389$(CO–OH), 3022(C–H), 1687(C=O), 1556(C=C), 1330(C–S); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.22 (s, 1H, COOH), 8.04 (d, $J = 8.3$ Hz, 2H, Ph–H), 7.89 (s, 1H, CH), 7.78 (d, $J = 8.6$ Hz, 2H, Ph–H), 7.70 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.57 (t, $J = 8.5$ Hz, 4H, Ph–H), 6.96 (d, $J = 8.6$ Hz, 2H, Ph–H); ESI–MS: m/z 556.9$(M+1)^+$.

*4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid (**7d**)*

4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-benzoic acid was prepared from 3-(4-chloro-phenyl)-2-(4-chloro-phenylimino)-thiazolidin-4-one and 4-formyl-benzoic acid according to the procedure described above: White powder, yield 67 %; mp: 214–215 °C; IR(KBr): $\nu/cm^{-1} = 3403$(CO–OH), 2984(C–H), 1707(C=O), 1571(C=C), 1236(C–S); ^{1}H NMR (300 MHz, DMSO-d_6) : δ(ppm) = 13.15 (s, 1H, COOH), 8.03 (d, $J = 8.3$ Hz, 2H, Ph–H), 7.88 (s, 1H, CH), 7.68 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.63 (s, 4H, Ph–H), 7.44 (d, $J = 8.6$ Hz, 2H, Ph-H), 7.01 (d, $J = 8.6$ Hz, 2H, Ph–H); ESI–MS: m/z 469.0 $(M+1)^+$.

*4-{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxymethyl}-benzoic acid (**7e**)*

4-{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxymethyl}-benzoic acid was prepared from 3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino) -thiazolidin-4-one and 4-(4-Formyl-phenoxymethyl)-benzoic acid according to the procedure described above: White powder, yield 48 %; mp: 256–257 °C; IR(KBr): ν/cm^{-1} = 3387 (CO–OH), 3010 (C–H), 1698 (C=O), 1549 (C=C), 1329 (C–S), 1182 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 12.89 (s, 1H, COOH), 7.96 (d, J = 8.2 Hz, 2H, Ph–H), 7.79 (s, 1H, CH), 7.64–7.52 (m, 9H, Ph–H), 7.44 (d, J = 8.5 Hz, 2H, Ph–H), 7.16 (d, J = 8.8 Hz, 2H, Ph–H), 7.01 (d, J = 8.6 Hz, 2H, Ph–H), 6.91 (d, J = 8.6 Hz, 1H, Ph–H), 5.25 (s, 2H, CH_2); ESI–MS: *m/z* 575.0$(M+1)^+$.

*[4-(4-Oxo-3-phenyl-2-phenylimino-thiazolidin-5-ylidenemethyl)-phenoxy]-acetic acid (**7f**)*

[4-(4-Oxo-3-phenyl-2-phenylimino-thiazolidin-5-ylidenemethyl)-phenoxy]-acetic acid was prepared from 3-phenyl-2-phenylimino-thiazolidin-4-one and 4-formyl-phenoxy-acetic acid according to the procedure described above: White powder, yield 45 %; mp: 176–177 °C; IR(KBr): ν/cm^{-1} = 3334(CO–OH), 2984(C–H), 1715(C=O), 1548 (C=C), 1244(C–S), 1175(C–O); ^{1}H NMR (300 MHz, DMSO-d_6) : δ(ppm) = 13.00 (s, 1H, COOH), 7.78 (s, 1H, CH), 7.55 (s, 4H, Ph–H), 7.52 (d, J = 8.8 Hz, 2H, Ph–H), 7.50–7.45 (m, 1H, Ph–H), 7.39 (t, J = 7.8 Hz, 2H, Ph–H), 7.17 (t, J = 7.4 Hz, 1H, Ph–H), 7.06 (d, J = 8.8 Hz, 2H, Ph–H), 6.98 (d, J = 7.4 Hz, 2H, Ph–H), 4.74 (s, 2H, CH_2); ESI–MS: *m/z* 431.0$(M+1)^+$.

*{4-[3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7g**)*

{4-[3-(4-Fluoro-phenyl)-2-(4-fluoro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(4-fluoro-phenyl)-2-(4-fluoro-phenylimino)-thiazolidin-4-one and (4-formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 69 %; mp: 178–179 °C; IR(KBr): ν/cm^{-1} =3418(CO–OH), 2984 (C–H), 1707(C=O), 1541(C=C), 1236(C–S), 1175(C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.00 (s, 1H, COOH), 7.77 (s, 1H, CH), 7.60 (dd, J = 7.6, 5.1 Hz, 2H, Ph–H), 7.52 (d, J = 8.1 Hz, 2H, Ph–H), 7.37 (t, J = 8.4 Hz, 2H, Ph–H), 7.21 (t, J = 8.4 Hz, 2H, Ph–H), 7.05 (d, J = 8.1 Hz, 2H, Ph–H), 7.00 (dd, J = 7.5, 5.1 Hz, 2H, Ph–H), 4.73 (s, 2H, CH_2); ESI–MS: *m/z* 467.1 $(M+1)^+$.

*{4-[3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7h**)*

{4-[3-(4-Bromo-phenyl)-2-(4-bromo-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(4-bromo-phenyl)-2-(4-bromo-phenylimino)-thiazolidin-4-one and (4-formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 77 %; mp: 192–193 °C; IR(KBr): ν/cm^{-1} = 3418(CO–OH), 2904(C–H), 1700(C=O), 1548(C=C), 1235(C–S), 1160(C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 12.94 (s, 1H, COOH), 7.79 (d, J = 5.4 Hz, 2H, Ph–H), 7.75 (s, 1H, CH), 7.56 (dd, J = 10.5, 8.6 Hz, 6H, Ph–H), 7.06 (d, J = 8.9 Hz, 2H, Ph–H), 6.96 (d, J = 8.6 Hz, 2H, Ph–H), 4.74 (s, 2H, CH_2); ESI–MS: *m/z* 586.8$(M+1)^+$.

*{4-[3-(2-Chloro-phenyl)-2-(2-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7i**)*

{4-[3-(2-Chloro-phenyl)-2-(2-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(2-Chloro-phenyl)-2-(2-chloro-phenylimino)-thiazolidin-4-one and (4-Formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 64 %; mp: 186–187 °C; IR(KBr): ν/cm^{-1} = 3368 (CO–OH), 3023 (C–H), 1678 (C=O), 1521 (C=C), 1316 (C–S), 1173 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.21 (s, 1H, COOH), 7.45 (s, 1H, CH), 7.42–7.23 (m, 7H, Ph–H), 7.23–7.16 (m, 2H, Ph–H), 7.10 (td, J = 7.5, 1.5 Hz, 1H, Ph–H), 6.88 (d, J = 7.6 Hz, 2H, Ph–H), 4.82 (s, 2H, CH_2).

*{4-[3-(3-Chloro-phenyl)-2-(3-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7j**)*

{4-[3-(3-Chloro-phenyl)-2-(3-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(3-Chloro-phenyl)-2-(3-chloro-phenylimino)-thiazolidin-4-one and (4-Formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 58 %; mp: 176–177 °C; IR(KBr): ν/cm^{-1} = 3321(CO–OH), 3023(C–H), 1723(C=O), 1639, 1578 (C=C), 1236 (C–S), 1160(C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ(ppm) = 13.35 (s, 1H, COOH), 7.66 (s, 1H, CH), 7.54 (s, 1H, Ph–H), 7.39 (d, J = 7.8 Hz, 2H, Ph–H), 7.35–7.28 (m, 4H, Ph–H), 7.28–7.23 (m, 2H, Ph–H), 7.16 (d, J = 7.4 Hz, 1H, Ph–H), 6.89 (d, J = 7.5 Hz, 2H, Ph–H), 4.82 (s, 2H, CH_2).

*{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7k**)*

{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-thiazolidin-4-one and (4-Formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 56 %; mp: 187–188 °C; IR(KBr): ν/cm^{-1} = 3367 (CO–OH), 2988 (C–H), 1679 (C=O), 1565 (C=C), 1321 (C–S), 1204 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6) : δ(ppm) = 13.11 (s, 1H, COOH), 7.79 (s, 1H, CH), 7.61 (d, J = 1.2 Hz, 4H, Ph–H), 7.54 (d, J = 8.8 Hz, 2H, Ph–H), 7.44 (d, J = 8.6 Hz, 2H, Ph–H), 7.03 (dd, J = 14.0, 8.7 Hz, 4H, Ph–H), 4.74 (s, 2H, CH_2); ESI–MS: m/z 498.9 $(M+1)^+$.

*{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-2-methoxy-phenoxy}-acetic acid (**7l**)*

{4-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-2-methoxy-phenoxy}-acetic acid was prepared from 3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-thiazolidin-4-one and (4-Formyl-2-methoxy-phenoxy)-acetic acid according to the procedure described above: White powder, yield 64 %; mp: 196–197 °C; IR(KBr): ν/cm^{-1} = 3321 (CO–OH), 3056 (C–H), 1711 (C=O), 1568 (C=C), 1323 (C–S), 1195 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 13.36 (s, 1H, COOH), 7.45 (s, 1H, CH), 7.44–7.36 (m, 4H, Ph–H), 7.31 (d, J = 3.4 Hz, 4H, Ph–H), 7.02 (dd, J = 7.5, 1.3 Hz, 1H, Ph–H), 6.95 (d, J = 1.4 Hz, 1H, Ph–H), 6.84 (d, J = 7.5 Hz, 1H, Ph–H), 4.82 (s, 2H, CH_2), 3.79 (s, 3H, CH_3); ESI–MS: m/z 529.4 $(M+1)^+$.

*{3-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid (**7m**)*

{3-[3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-4-oxo-thiazolidin-5-ylidenemethyl]-phenoxy}-acetic acid was prepared from 3-(4-Chloro-phenyl)-2-(4-chloro-phenylimino)-thiazolidin-4-one and (3-Formyl-phenoxy)-acetic acid according to the procedure described above: White powder, yield 35 %; mp: 156–158 °C; IR(KBr): ν/cm^{-1} = 3403(CO–OH), 2992(C–H), 1700(C=O),1609 (C=C), 1244(C–S), 1175(C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ(ppm) =12.98 (s, 1H, COOH), 7.67(s, 1H, CH)7.48 (d, J = 7.5 Hz, 2H, Ph–H), 7.41 (d, J = 7.5 Hz, 2H, Ph–H), 7.34–7.29 (m, 2H, Ph–H), 7.29–7.20 (m, 3H, Ph–H), 7.05 (d, J =7.5 Hz, 1H, Ph–H), 6.96 (s, 1H, Ph–H), 6.83 (d, J = 7.5 Hz, 1H, Ph–H), 4.82 (s, 2H, CH_2).

Minimal inhibitory concentration (MIC)

MIC assays for the antibacterial activities of the derivatives were performed according to the broth microdilution (in tubes) method of the Clinical and Laboratory Standards Institute (CLSI) of America. In brief, a serial twofold dilution of derivatives was added to eight tubes containing 4 mL Mueller–Hinton Broth (OXOID, England), making the final concentration from 200 μM to 0.78 μM. The turbidity of 6-h strain cultures is adjusted to match that of a 0.5 McFarland standard ($\sim$108142 CFU mL^{-1}), and then 0.02 mL of the bacterial inoculum was added to each tube. An inoculated broth containing no antibiotic was included as a bacterial growth control and a tube of un-inoculated broth was used as a sterility control. These bacteria were incubated at 37 °C for 12 h, the lowest concentration which completely inhibits visible growth of the organism as detected by the unaided eye is recorded as the MIC.

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