Bioorganic & Medicinal Chemistry Letters 20 (2010) 2461–2464

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Design, synthesis, and antibiofilm activity of 2-arylimino-3-aryl-thiazolidine-4-ones

Bin Pan [a], Ren-Zheng Huang [b], Shi-Qing Han [a,*], Di Qu [b], Ming-Li Zhu [a], Ping Wei [a], Han-Jie Ying [a]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, China
[b] Key Laboratory of Medical Molecular Virology of Ministry of Education and Ministry of Pulic Health, Institute of Medical Microbiology and Institutes of Biomedical Sciences, Shanghai Medical School of Fudan University Box 228, Shanghai 200032, China

ARTICLE INFO

*Article history:*
Received 19 November 2009
Revised 25 February 2010
Accepted 4 March 2010
Available online 7 March 2010

*Keywords:*
Thiazolidine
Antibiofilm activity

ABSTRACT

A series of novel 2-arylimino-3-aryl-thiazolidine-4-ones was designed, synthesized and tested for in vitro antibiofilm activity against *Staphylococcus epidermidis*. Among them tested, some compounds with carboxylic acid groups showed good antibiofilm activity. The antibiofilm concentration of **1x** was 6.25 μM. The structure–activity relationships revealed that incorporation of 2-phenylfuran moiety could greatly enhance antibiofilm activity of thiazolidine-4-one.

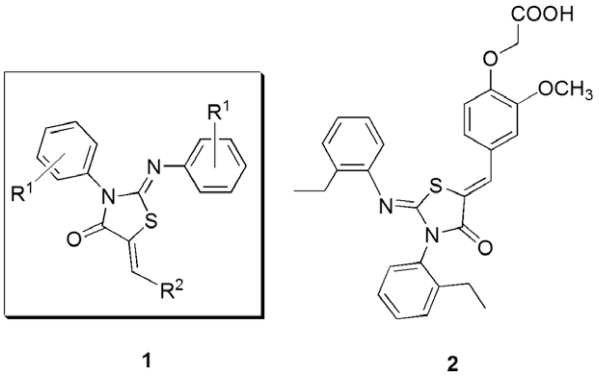


Bacterial infections are now known to predominately exist in a biofilm growth state that confers both enhanced virulence and defensive properties to the bacterium.[1] Bacteria that reside within the biofilm state also are inherently more resistant to many antibiotics and biocides that would often lead to their eradication.[2,3] Given the breadth of biofilm mediated infections, there is a significant need for new and potent antibiofilm modulators.

Recently, several compounds had been documented which had moderate biofilm inhibitory activities against RP62A,[4] a strain of the *Staphylococcus epidermidis*. It was also shown that these compounds elicited their effects through inhibition of the YycG histidine kinase, which was an important two-component system (TCS) control protein. All these inhibitors from a variety of structural classes included benzamides, furans, pyrimidinones, and thiazolidiones. Among these inhibitors, a typical compound **2**, which had a thiazolidione core substituted by aromatic ring, showed potent antibiofim activity, displayed low cytotoxicity on mammalian cells and was not hemolytic. The biofilm inhibitory activity of **2** was even better than that of vancomycin, one of the antibiotics usually used in multidrug-resistant staphylococci associated infections. In this Letter, we presented our further research results on this related compound class, thiazolidione, exemplified by the general structure **1**. (Fig. 1).

According to the interaction model which had been studied by Qin et al.[4] a four-point pharmacophore could be figured out for describing the binding of the inhibitors: each inhibitor had two hydrogen bond acceptors, hydrogen bonding to Asn503 and Lys542; the hydrophobic moieties of the inhibitors fit into the two hydrophobic cavities. In our research, a series of 2-arylimino-3-aryl-thiazolidine-4-ones (Table 1) with hydroxyl groups, ester functional and carboxylic acid groups were designed and synthesized to expect their good hydrogen bonding capacity. Moreover, from the literature survey it was revealed that some derivatives of thiazolo[2,3-*b*]dihydropyrimidinone possessing 2-phenylfuran moiety were reported to be potent antibacterial and antifungal activities.[5] Based on these observations, 2-phenylfuran moiety was introduced into thiazolidione core structure (**1l–o** and **1u–x**).

A simple one-pot reaction consisting of aniline or substituted aniline, carbon disulfide, water, and triethanolamine catalysed by Lac Sulfur,[6] gave 1,3-diarylthiourea (**3–7**) in good yield. Formation



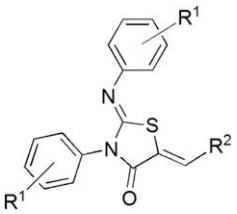

1 2

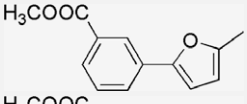
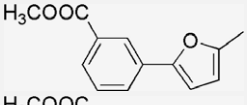
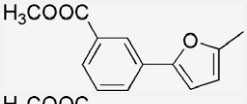
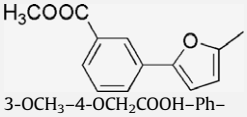
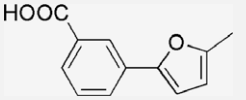
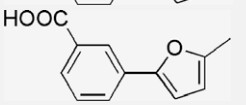
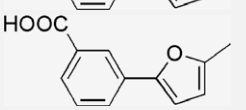
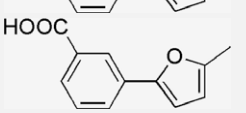
**Figure 1.** Thiazolidione core structure.

* Corresponding author. Tel.: +86 25 83587334; fax: +86 25 83587326.
*E-mail address:* hanshiqing@njut.edu.cn (S.-Q. Han).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.013

**Table 1**
Anti-bacteria and antibiofilm activities of 2-arylimino-3-aryl-thiazolidine-4-ones




| Compound | $R^1$ | $R^2$ | *S. epidermidis* RP62A MIC[a] (μM) | *S. epidermidis* RP62A MBC[b] (μM) | Antibiofilm concentration[c] (μM) |
|---|---|---|---|---|---|
| **1a** | H | 4-$N(CH_3)_2$–Ph– | >200[d] | >200 | >200 |
| **1b** | H | 3-$OCH_3$–4-OH–Ph– | >200 | >200 | >200 |
| **1c** | 2-$CH_3$ | 4-OH–Ph- | >200 | >200 | >200 |
| **1d** | 2-$CH_3$ | 3-$OCH_3$–4-OH–Ph– | >200 | >200 | >200 |
| **1e** | 2-$CH_2CH_3$ | 4-OH–Ph– | >200 | >200 | >200 |
| **1f** | 2-$CH_2CH_3$ | 3-OH–Ph– | >200 | >200 | >200 |
| **1g** | H | 3-$OCH_3$–4-$OCH_2COOCH_3$–Ph– | >200 | >200 | >200 |
| **1h** | 2-$CH_3$ | 4-$OCH_2COOCH_3$–Ph– | >200 | >200 | >200 |
| **1i** | 2-$CH_3$ | 3-$OCH_3$–4-$OCH_2COOCH_3$–Ph– | >200 | >200 | >200 |
| **1j** | 2-$CH_2CH_3$ | 4-$OCH_2COOCH_3$–Ph– | >200 | >200 | >200 |
| **1k** | 2-$CH_2CH_3$ | 3-$OCH_2COOCH_3$–Ph– | >200 | >200 | >200 |
| **1l** | 2-$CH_3$ |  | >200 | >200 | >200 |
| **1m** | 2-$CH_2CH_3$ |  | >200 | >200 | >200 |
| **1n** | 4-$CH_3$ |  | >200 | >200 | >200 |
| **1o** | 4-$OCH_3$ |  | >200 | >200 | >200 |
| **1p** | H | 3-$OCH_3$–4-$OCH_2COOH$–Ph– | >200 | >200 | >200 |
| **1q** | 2-$CH_3$ | 4-$OCH_2COOH$–Ph– | >200 | >200 | >200 |
| **1r** | 2-$CH_3$ | 3-$OCH_3$–4-$OCH_2COOH$–Ph– | >200 | >200 | >200 |
| **1s** | 2-$CH_2CH_3$ | 4-$OCH_2COOH$–Ph– | 50 | 100 | 100 |
| **1t** | 2-$CH_2CH_3$ | 3-$OCH_2COOH$–Ph– | 50 | 100 | 100 |
| **1u** | 2-$CH_3$ |  | 12.5 | 25 | 25 |
| **1v** | 2-$CH_2CH_3$ |  | 12.5 | 25 | 25 |
| **1w** | 4-$CH_3$ |  | 6.25 | 25 | 12.5 |
| **1x** | 4-$OCH_3$ |  | 6.25 | 25 | 6.25 |
| **2** | 2-$CH_2CH_3$ | 3-$OCH_3$–4-$OCH_2COOH$–Ph– | 50 | 100 | 100 |

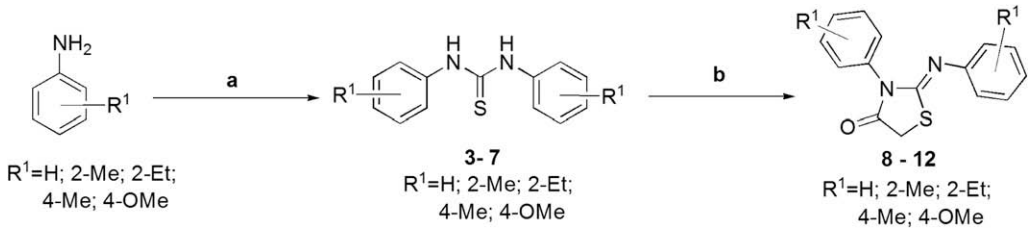
[a] MIC assays for the antibacterial activities of the compounds were performed according to the broth microdilution (in tubes) method of the Clinical and Laboratory Standards Institute (CLSI) of America.[14]

[b] MBC of the compounds was obtained by subculturing 100 μl from each negative (no visible bacterial growth) tube from the MIC assay, onto substance-free Mueller–Hinton agar plates. The plates were incubated at 37 °C for 24 h, and the MBC was defined as the lowest concentration of compounds which produced subcultures growing no more than five colonies on each plate.

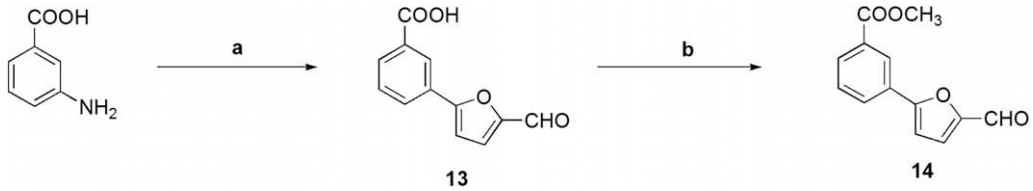
[c] In the Antibiofilm concentration experiment, overnight culture of *S. epidermidis* strain RP62A was diluted 1:10 in TSB (OD600 = 0.6–0.8), then was diluted 1:200 in MH. The bacterial suspension was inoculated into the wells of sterile 96-well polystyrene microtiter plates (Falcon) incubated at 37 °C for 6 h. The plates with young biofilm were washed gently four times with sterile PBS before adding fresh TSB containing the various concentration compounds, and incubated at 37 °C for 16 h.

[d] The initiate dilution was 200 μM.

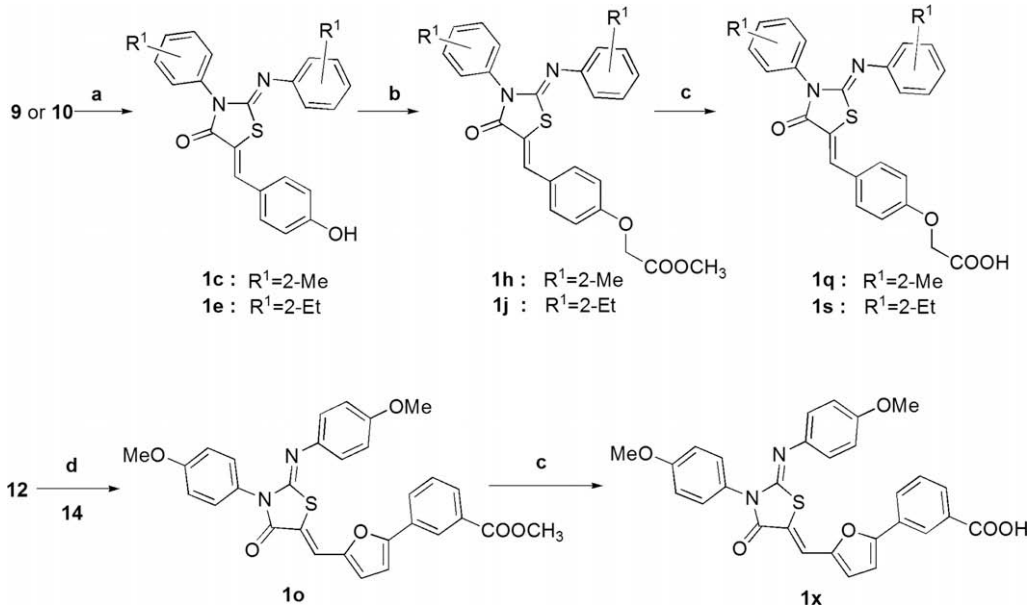
of thiazolidiones (**8–12)** from the five disubstitued symmetrical thioureas was accomplished in EtOH with chloracetic acid and NaOAc[7] (Scheme 1). The 3-(5-formylfuran-2-yl)benzoic acid **13**[8] was obtained according to the known method, then an esterification of **13** with methanol in the presence of sulfuric acid gave the **14**[9] (Scheme 2).




**Scheme 1.** Reagents and conditions: (a) $CS_2$, Lac·Sulfur, triethanolamine, water, reflux, 6 h; (b) $ClCH_2COOH$, NaOAc, absolute alcohol, reflux, 10 h.




**Scheme 2.** Reagents and conditions: (a) concentrated HCl, $H_2O$, $NaNO_2$, 0–5 °C, 30 min; 2-furanaldehyde, $CuCl_2{\cdot}2H_2O$, $H_2O$, acetone, rt, 2 d; (b) methanol, $H_2SO_4$ (catalytic), reflux, 4 h.




**Scheme 3.** Synthetic routes for representative targets. Reagents and conditions: (a) 4-hydroxybenzaldehyde, piperidine, absolute alcohol, 60 °C, 3 h; (b) $BrCH_2COOCH_3$, acetone, reflux, 4 h; (c) $K_2CO_3$, water, methanol, reflux, 10 h; (d) **14**, piperidine, absolute alcohol, 60 °C, 3 h.

Starting from intermediates **8–12**, various types of derivatives featuring different aryl fragments could be prepared. The synthesis of some representative examples was described in Scheme 3. An aldol condensation of 4-hydroxy benzaldehyde with equivalent amount of precursors **9** or **10** in EtOH was taken place in the presence of piperidine gave **1c** or **1e** without incident.[10] When equivalent amount of **1c** or **1e** and methyl bromoacetate were mixed in acetone with $K_2CO_3$ and refluxed for 4 h, compound **1h** or **1j** were formed.[11] The desired compound **1q** and **1s** were prepared by hydrolization of **1h** or **1j** in methanol and water, catalyzed by $K_2CO_3$. **1o** and **1x** were synthesized in the same manner.[12]

Subsequently, the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and antibiofilm concentration assay of compounds were determined against *S. epidermidis* using a standard tube-dilution assay (Table 1). Among tested compounds, the antibiofilm activity of **1s–x** was superior or comparable to that of **2**. Moreover, the data of **1u–x**[13] was even superior to that of vancomycin (88 μM).[4] It revealed that the 3-(5-methylfuran-2-yl) benzoic acid substitution group obviously improved the activity of 2-arylimino-3-aryl-thiazolidine-4-one. The change, that rigidity of **1u–x** is enhanced when the 3-(5-formylfuran-2-yl)benzoic acid moiety was incorporated into the scaffold, probably, led the thiazolidiones bind to the target protein more easily. The anti-bacteria and antibofilm activities may be also dependent on the substitution of the benzene ring ($R^1$). While 2-substitued inhibitors (**1q**–**t**, **1u** and **1v**) were relatively weak antibiofilm agents, their 4-substitued counterparts (**1w** and **1x**) were the most potent inhibitors among the synthesized compounds.

Unliked the good activity of **1s–x** against *S. epidermidis*, the **1j–o** showed no activity, the ester functional group of $R^2$ in these compounds might be the main reason which led the anti-bacteria and antibiofilm activities disappeared. In addition, compounds **1b–f** which contained hydroxy group of $R^2$ exhibited no activities too. The results showed that compounds contained carboxylic acid

group ($R^2$) were more likely to have anti-bacteria and antibofilm activities.

In conclusion, new 2-arylimino-3-aryl-thiazolidine-4-ones were designed and synthesized. Incorporation of a 2-phenylfuran moiety had been found to greatly enhance antibiofilm activity. In addition, the chain length and the substituted position of $R^1$ also influenced antibiofilm activity and the carboxylic acid group of $R^2$ was indispensable. All these results suggested that the thiazolidione scaffold was clearly a good chemotype for exploration of antibiofilm drugs. Further studies on these compounds and optimization of their structures leading to novel analogues with superior biological properties are on going in our laboratories.

## Acknowledgment

This work was supported by National Natural Science Foundation of China (Grant No. 20942006).

## References and notes

1. Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. *Science* **1999**, *284*, 1318.
2. Davies, D. *Nat. Rev. Drug Disc.* **2003**, *2*, 114.
3. Musk, D. J.; Hergenrother, P. J. *Curr. Med. Chem.* **2006**, *13*, 2163.
4. Qin, Z. Q.; Zhang, J.; Xu, B.; Chen, L. L.; Wu, Y.; Yang, X. M.; Shen, X.; Molin, S.; Danchin, A.; Jiang, H. L.; Qu, D. *BMC Microbiology* **2006**, *6*, 96.
5. Mithun, A.; Bantwal, S. H.; Nalilu, S. K. *Eur. J. Med. Chem.* **2007**, *42*, 380.
6. Ramadas, K.; Janarthanan, N.; Velmathi, S. *Synth. Commun.* **1997**, *27*, 2255.
7. Sule, E.; Ilknur, D. *J. Org. Chem.* **2007**, *72*, 2494.
8. Livio, R.; Vesna, T. K.; David, W. B.; Grace, K. Z. *Molecules* **2003**, *8*, 342.
9. Narges, H.; Latifeh, N.; Hooman, S.; Mhsen, A.; Nasrin, S.; Mohammad, A. F.; Abbas, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6354.
10. Rosaria, O.; Rosanna, M.; Rosella, C. *Bioorg. Med. Chem.* **2007**, *15*, 7618.
11. (a) Rosanna, M.; Rosaria, O.; Rosella, C.; Maria, G. V.; Dietmar, R.; Theodora, S.; Thierry, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3886; (b) Rosanna, M.; Paolo, P.; Rosaria, O.; Michela, J.; Rosella, C.; Giampaolo, M.; Theodora, S.; Thierry, L.; Maria, G. V.; Guido, C. *Bioorg. Med. Chem.* **2007**, *15*, 5137.
12. Wang, S. F.; Ye, Y. H.; Zhang, Z.; Tan, R. X. *Ultrason. Sonochem.* **2006**, *13*, 28.
13. The selected data of representative compounds **1u**–**x** were as follows: compound **1u**: light yellow solid; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.23 (s, 1H, CH), 7.93–7.91 (d, 1H, furan-H), 7.83–7.81 (d, 1H, furan-H), 7.69–6.96 (m, 12H, Ph-H), 2.25 (s, 3H, $CH_3$), 2.05 (s, 3H, $CH_3$); HRMS $m/z$ 495.1372 [calcd for $C_{29}H_{23}N_2O_4S$ $(M+H)^+$, 495.1373]; compound **1v**: white yellow solid; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.24 (s, 1H, CH), 7.93–7.91 (d, 1H, furan-H), 7.85–7.83 (d, 1H, furan-H), 7.70–6.97 (m, 12H, Ph-H), 2.60–2.55 (q, 2H, $CH_2$), 2.42–2.37 (q, 2H, $CH_2$), 1.19–1.14 (t, 3H, $CH_3$), 1.01–0.96 (t, 3H, $CH_3$); EI-HRMS: calcd for $C_{31}H_{26}N_2O_4S$ $(M^+)$, 522.1613; found, 522.1614; compound **1w**: light yellow solid; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.27 (s, 1H, CH), 7.94–7.92 (d, 1H, furan-H), 7.87–7.85 (d, 1H, furan-H), 7.64–6.91 (m, 12H, Ph-H), 2.38 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$); compound **1x**: light yellow solid; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.30 (s, 1H, CH), 7.94–7.92 (d, 1H, furan-H), 7.89–7.87 (d, 1H, furan-H), 7.64–6.99 (m, 12H, Ph-H), 3.82 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$).
14. Clinical and Laboratory Standards Institute. (**2006**). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved standard M7–A7. Clinical and Laboratory Standards Institute, Wayne, PA.