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PII: S0960-894X(16)30321-3
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.03.089
Reference: BMCL 23732

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 7 January 2016
Revised Date: 6 March 2016
Accepted Date: 25 March 2016

Please cite this article as: Cai, M-G., Wu, Y., Chang, J., Synthesis and biological evaluation of 2-arylimino-3-pyridin-thiazolineone derivatives as antibacterial agents, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.03.089

Synthesis and biological evaluation of 2-arylimino-3-pyridin-thiazolineone derivatives as antibacterial agents

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Abstract

With an intention to find more potent antibacterial agents, four halogen disubstituted thiazolineone derivatives (**2a**–**d**), five halogen monosubstituted thiazolineone derivatives (**2e**–**i**), and eleven 2-arylimino-3-pyridin-thiazolineone derivatives (**2j**–**t**) were synthesized and screened for their antibacterial activity, bactericidal activity, cytotoxicity, and erythrocyte hemolysis. Most of the synthesized derivatives showed antibacterial activity in inhibiting the growth of *S. epidermidis* and MRSA, and exhibited safety in the cytotoxicity study on the Vero cells and hemolytic activities test on healthy human erythrocytes. 2-arylimino-3-pyridin-thiazolineone derivatives not only improved the *c*log *P*, but also showed potent antibacterial activity in inhibiting the growth of *S. epidermidis* and MRSA. In particularly, several compounds (**2f**, **2i**, **2r** and **2t**) showed bactericidal activity, in which compound **2r** displayed the best inhibitory capacity among the synthesized compounds, and further druggability research is on going.

Keywords

Synthesis; biological evaluation; 2-arylimino-3-pyridin-thiazolineone derivatives; antibacterial agents

Infections caused by drug-resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus faecalis* (VRE) are still the most serious threat to global health.[1–2] NIH findings indicated that more than 80% of bacterial infections were related with biofilm.[3] Bacterial biofilm can resist not only the host immune destruction, but also most of the antibiotics, moreover, the resistance was up to a thousand times more than planktonic bacteria.[4] Most of the existing antibiotics fail to adequately penetrate the biofilm or have limited activity against surface-attached cells and cells with low metabolic activity.[5] Daptomycin and linezolid are now available for biofilm-associated infections caused by *staphylococci*, but neither was found to be bactericidal against biofilm-embedded bacteria.[6] Vancomycin is regarded as an antibiotic of last resort against MRSA, and other multiple antibiotic-resistant infections caused by Gram-positive bacteria, but it has no significant effect on the bacteria in the biofilm.[7]

Bacterial two-component systems (TCSs) were proposed as attractive targets more than 20 years ago because they are absent in mammals and essential or conditionally essential for viability in several important bacterial pathogens. WalKR system (a.k.a., YycGF, VicKR, MicAB) is an obligate essential regulatory system in Firmicutes including MRSA, VRE, and some other notorious pathogens.[8-9] It plays important roles in the growth, cell wall metabolism, and biofilm formation of pathogenic staphylococcal species.[10] We have previously described two series of YycG inhibitors that target the HK domain of *S. epidermidis* YycG and show bactericidal and antibiofilm activities against *S. epidermidis* and *S. aureus*.[7, 11-12] One of the two lead compounds is {4-[3-(2-ethylphenyl)-2-(2-ethylphenylimino)-4-oxothiazolidin-5-ylidenemethyl]-2-methoxyphenoxy}-acetic acid (**1**). In this study, we reported our further research results on the structure-activity relationships (SAR) of compound **1**.

According to the previous SAR, it was found that the introduction of halogen substituent on phenyl rings displayed more potent antibacterial activity. Considering a significant number of antibacterial agents in clinical development are chlorinated structures,[13] and the introduction of the strongly electron-withdrawing fluorine group into drugs or drug candidates can substantially enhanced binding interactions, metabolic stability, changes in physical properties, and selective reactivities,[14] we paid more attention to chlorine or fluorine substitution. Therefore, halogen disubstituted thiazolineone derivatives (**2a**–**d**) and halogen monosubstituted thiazolineone derivatives (**2e**–**i**) were synthesized. Log *P* is an important parameter in the druggability research, and *c*log *P* of the good lead compound should be between 0 and 3.0. However, *c*log *P* of most of the derivatives (**2a**–**i**) was so high that heterocyclic rings derivatives (**2j**–**t**) were designed and synthesized to increase the hydrophility by applying the principle of bioisosterism. (Figure **1**)

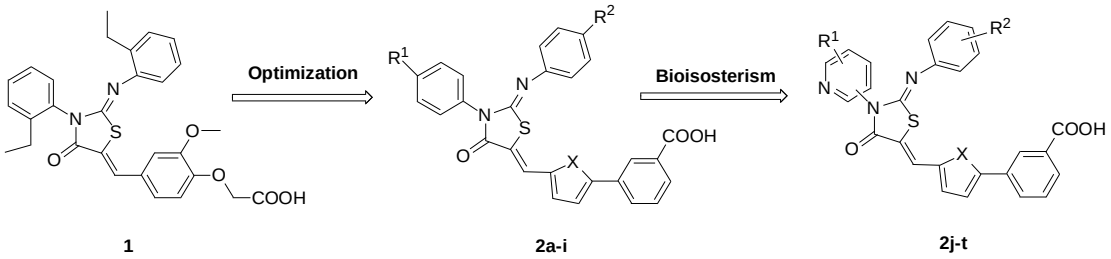
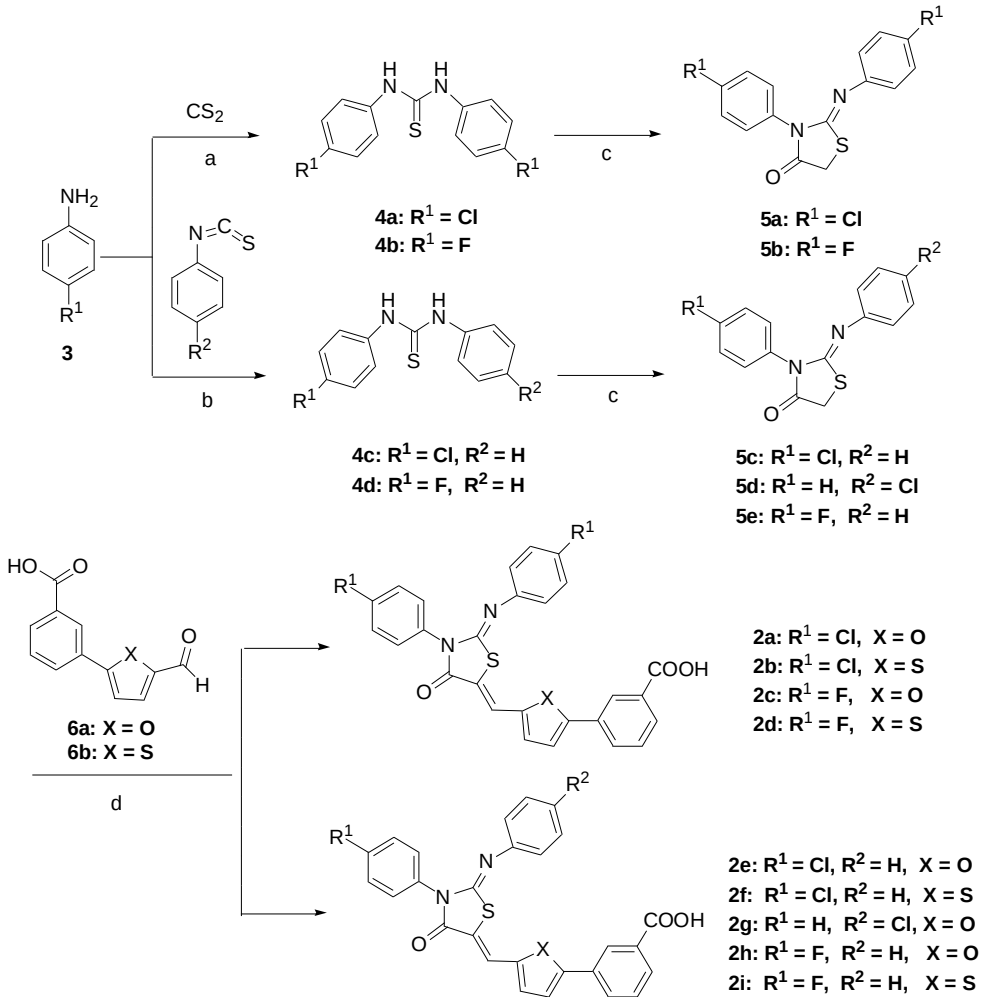


Figure 1. Design strategy

The synthesis of halogen disubstituted thiazolineone derivatives (**2a**–**d**) commenced from corresponding halogen substituted aniline according to the previous report (Scheme **1**),[15] while halogen monosubstituted thiazolineone derivatives (**2e-i**) were synthesized from asymmetric thioureas (**4c-d**). The substituted anilines were converted to asymmetric thioureas (**4c-d**) by using phenyl isothiocyanate in CH_2Cl_2 under reflux for 2 h in 70% yields. **4c** was transformed into iminothiazolidinones **5c** and **5d** (1:1) via the reaction of ethyl bromoacetate in absolute EtOH and NaOAc,

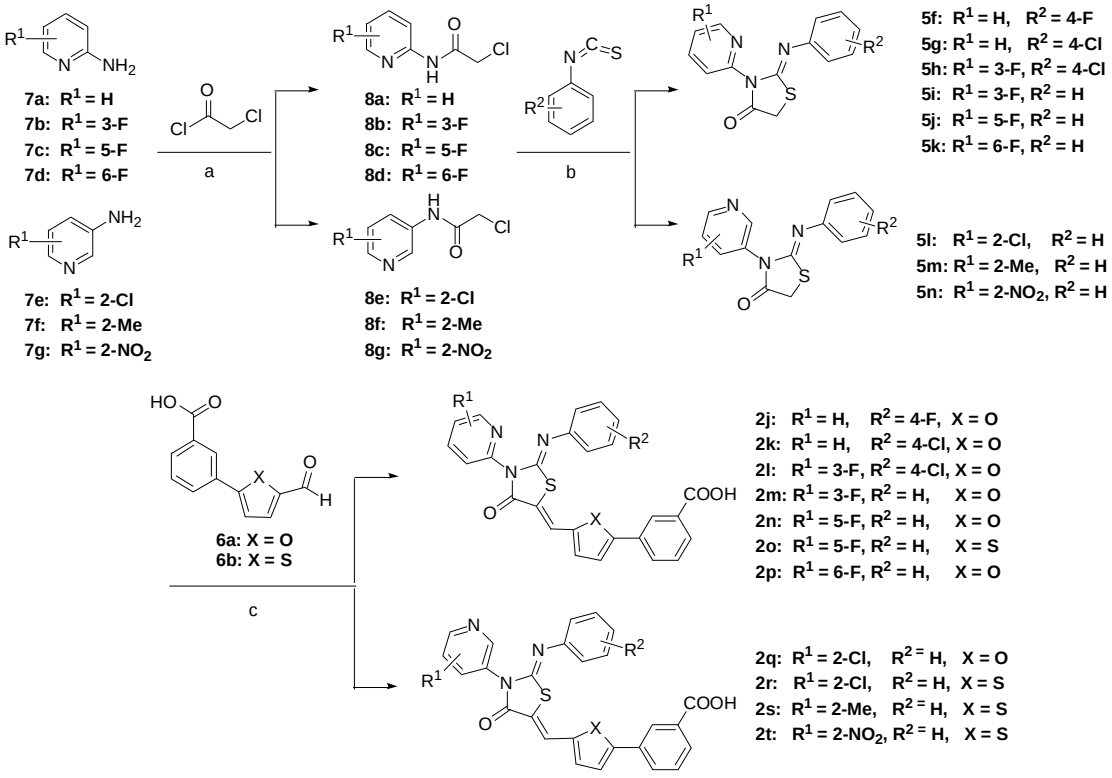
while **4d** was selectively transformed into iminothiazolidinones **5e** in 88% yield. **2e-i** were achieved by the Knoevenagel condensation of substituted iminothiazolidinones (**5c-e**) with equivalent amount of aromatic aldehydes (**6a**/**6b**) catalyzed by *β*-alanine in acetic acid under reflux for 2 h in good yields.[16]



Scheme 1. Reagents and conditions: a) 10% NaOH solution, 60 ^{o}C; b) CH_2Cl_2, reflux; c) ethyl bromoacetate, NaOAc, absolute alcohol, reflux; d) *β*-alanine, acetic acid, reflux.

To avoid of the two possible regioisomeric iminothiazolidinone products from the asymmetric thioureas, many reagents were tried, including ethyl bromoacetate, ethyl chloroacetate, chloroacetic acid, bromoacetic acid. However, unfortunately they all didn't work. At last, we found a different route to selectively synthesize **5f-n**, as illustrated in Scheme **2**.[17] The reaction of aminopyridine or substituted aminopyridine

(**7a-g**) with choloro acetylcholoride afforded the corresponding amides **8a-g**. Then the compounds **8a-g** reacted smoothly with isothiocyanates in the presence of a weak base such as K_2CO_3 in CH_3CN to produce iminothiazolidinones (**5f-n**). The final step of the Knoevenagel condensation of iminothiazolidinones (**5f-n**) with equivalent amount of substituted aromatic aldehydes (**6a**/**6b**) was catalyzed by *β*-alanine to give the target compounds **2j-t** in good yields.[18] The structures of a total of twenty synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectra.



Scheme 2. Reagents and conditions: a) Et_3N, CH_2Cl_2, rt; b) K_2CO_3, CH_3CN, rt; c) *β*-alanine, acetic acid, reflux.

The *in vitro* antibacterial activity (MIC) was evaluated with Gram-positive bacterial strains (*S. epidermidis* ATCC35984 and clinical bacterial strain MRSA 8282) and Gram-negative bacterial strain (*E. Coli* ATCC25922) according to the broth micro-dilution (in tubes) methods of the clinical and Laboratory Standards Institute (CLSI) of America. Most of the synthesized compounds showed good potency in

inhibiting the growth of *S. epidermidis* ATCC35984 and MRSA 8282, while none of them was able to inhibit the growth of *E. Coli*.

Table 1. Biological activity of the compounds **2a-t**

Compounds	*S. epidermidis* ATCC 35984 MIC (μM) [a]	MRSA 8282 MIC (μM) [a]	*S. epidermidis* ATCC 35984 MBC (μM) [b]	*E. Coli* ATCC 25922 MIC (μM) [a]	CC_{50} (μM) [c]	Hemolysis [d]
1	25	25	100	>200	50	—
2a	25	25	>200	>200	>200	—
2b	12.5	25	>200	>200	>200	—
2c	100	100	>200	>200	>200	—
2d	6.25	6.25	100	>200	>200	—
2e	6.25	6.25	50	>200	>200	—
2f	1.56	3.13	25	>200	>200	—
2g	6.25	6.25	100	>200	>200	—
2h	25	25	50	>200	>200	—
2i	6.25	12.5	25	>200	>200	—
2j	12.5	12.5	>200	>200	>200	—
2k	6.25	6.25	50	>200	>200	—
2l	12.5	12.5	>200	>200	>200	—
2m	12.5	12.5	>200	>200	>200	—
2n	12.5	12.5	>200	>200	>200	—
2o	3.13	3.13	50	>200	>200	—
2p	6.25	6.25	100	>200	>200	—
2q	3.13	6.25	50	>200	>200	—
2r	1.56	3.13	6.3	>200	>200	—
2s	12.5	12.5	100	>200	>200	—
2t	1.56	3.13	12.6	>200	>200	—
methicillin	3.13	100	>200	>200	>200	—

[a] MIC represented the minimal inhibitory concentration of the compounds against *S. epidermidi* ATCC 35984, MRSA 8282, and *E. Coli* ATCC 25922. [b] MBC represented minimal bactericidal concentration of the compounds against *S. epidermidi* ATCC 35984. [c] CC_{50} represented the concentration of the compounds which produces 50% cytotoxicity effect on Vero cells. [d] Hemolytic activities of the compounds on healthy human erythrocytes were tested at 200 μM, "—": no hemolytic activity at 200 μM.

The results showed that halogen monosubstituted series (**2e-i**) exhibited more potent antibacterial activity than the corresponding disubstituted series (**2a-d**) (Table **1**). The replacement of furan moiety with thiophene moiety based on the bioisosterism improved the inhibitory activity compared with the corresponding furan derivatives. **2e** showed same inhibitory activity as **2g**, which suggested that the site of halogen monosubstituted on the phenyl ring connected with amide or imino didn't influence the inhibitory activity. Heterocyclic rings derivatives (**2j**–**t**) still maintain the antibacterial activity. Similar as the above SAR, compound **2j** with the halogen substituted on the phenyl ring exhibited comparable antibacterial activity to compounds **2m** and **2n**, with the halogen substituted on the pyridine ring. It was noteworthy that although the positions of fluorine and nitrogen on the pyridine didn't influence the activity significantly, chlorine substitution derivatives showed more potent antibacterial activity than fluorine substitution derivatives. Compound **2s** with the methyl substituted on the pyridine ring significantly reduced the antibacterial activity, compared with compound **2t** with the nitro group substituted. Moreover, compounds **2a-2t** exhibited similar antibacterial activity against clinical bacterial strain MRSA 8282. It was worth mentioning that compounds **2f**, **2i**, **2r** and **2t** also showed bactericidal activity. In the cytotoxicity study on the Vero cells, the CC_{50} values of all the derivatives were greater than 200 μM. At the MIC concentrations, all the derivatives lysed healthy human erythrocytes by less than 1%, and showed no obvious hemolysis even at the highest concentration 200 μM.

In conclusion, four halogen disubstituted thiazolineone derivatives (**2a**–**d**), five halogen monosubstituted thiazolineone derivatives (**2e**–**i**), and nine 2-arylimino-3-pyridin-thiazolineone derivatives (**2j**–**t**) were synthesized and screened for their antibacterial activity, bactericidal activity, cytotoxicity, and erythrocyte hemolysis. Most of the synthesized derivatives showed antibacterial activity in inhibiting the growth of *S. epidermidis* and clinical bacterial strain MRSA 8282, and

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Acknowledgments

Financial support by the National Natural Science Foundation of China (No: 81573268), and the Shanghai Municipal Committee of Science and Technology (No: 14431900300, 15431900400) are acknowledged.

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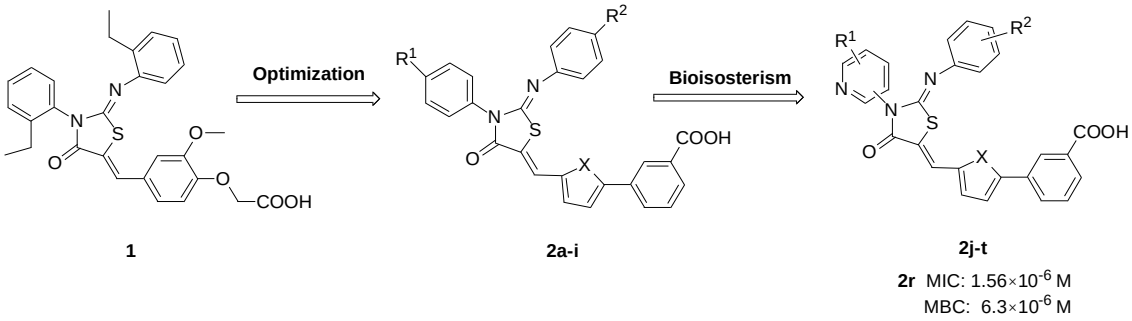
18. *Representative experiment for the preparation of* 3-{5-{[2-phenylimino-3-(2-chloropyridin-3-yl)-4-oxothiazolidin-5-ylidene]methyl}thiophene-2-yl}-benzoic acid (**2r**): choloro acetylcholoride (24 mmol) and Et_3N (24 mmol) was added to a solution of 2-chloro-3-aminopyridine **7e** (20 mmol) in CH_2Cl_2 (20 mL) at room temperature. The mixture was stirred for 5 hrs, and the solvent was evaporated under vacuum. The residue was purified by column chromatography (CH_2Cl_2:CH_3OH: 30:1) on silica gel to obtain pure compound **8e** as a white powder in 72% yield. To a solution of amide derivative **8e** (5 mmol) and potassium carbonate (7.5 mmol) in acetonitrile (20 ml) was added isothiocyanate (6 mmol) during about 5 minutes. The reaction mixture was stirred at room temperature overnight, and the solvent was evaporated under vacuum. The residue was extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The obtained residue was purified by silica gel flash chromatography column (CH_2Cl_2:CH_3OH: 30:1) to afford **5l** as a white solid in

82% yield. To a solution of **5l** (1 mmol) in glacial acetic acid (5 mL) were added aldehyde **6b** (1 mmol) and *β*-alanine (1 mmol). The resulting mixture was stirred under reflux for 2 h. Upon completion of the reaction, the mixture was cooled, the reaction was quenched with water, and the precipitate was filtered off, then the residue was purified by column chromatography (CH_2Cl_2:CH_3OH: 15:1) on silica gel to obtain pure compound **2r** as a faint yellow powder in 80% yield. Mp 230-232 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23(s, 1H), 8.59 (s, 1H), 8.17 (m, 2H), 7.97 (d, J = 7.5 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.80 (m, 1H), 7.75 (m, 1H), 7.56 (m, 4H), 7.41 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.86, 163.42, 154.79, 149.70, 148.83, 148.54, 148.07, 146.17, 140.02, 130.99, 128.95, 128.64 (3), 128.54, 128.26, 127.12, 124.33, 123.61, 123.50, 120.03 (3), 116.83, 116.73, 109.71. ESI-MS *m/z* 518.0 $[M + H]^+$; HR-ESIMS *m/z* calcd. for $C_{26}H_{17}ClN_3O_3S_2$ $[M + H]^+$: 518.0400, found 518.0402.

Graphic Abstract



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