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PII: S0960-894X(16)30598-4
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.05.088
Reference: BMCL 23947

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 7 January 2016
Revised Date: 17 May 2016
Accepted Date: 30 May 2016

Please cite this article as: Ran, K., Gao, C., Deng, H., Lei, Q., You, X., Wang, N., Shi, Y., Liu, Z., Wei, W., Peng, C., Xiong, L., Xiao, K., Yu, L., Identification of novel 2-aminothiazole conjugated nitrofuran as antitubercular and antibacterial agents, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.05.088

Identification of novel 2-aminothiazole conjugated nitrofuran as antitubercular and antibacterial agents

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Abstract: The emergence of antibiotic resistant pathogens is an ongoing main problem in the therapy of bacterial infections. In order to develop promising antitubercular and antibacterial lead compounds, we designed and synthesized a new series of derivatives of 2-aminothiazole conjugated nitrofuran with activities against both *Mycobacterium tuberculosis* and *Staphylococcus aureus*. Eight compounds **12e**, **12k**, **12l**, **12m**, **18a**, **18d**, **18e**, and **18j** emerged as promising antitubercular agents. Structure-activity relationships (SARs) were discussed and showed that the derivatives substituted at the position-3 of benzene of 5-nitro-*N*-(4-phenylthiazol-2-yl)

furan-2-carboxamide exhibited superior potency. The most potent compound **18e**, substituted with benzamide at this position, displayed minimum inhibitory concentrations (MICs) of 0.27 μg/mL against *Mtb* H37Ra and 1.36 μg/mL against *S. aureus*. Furthermore, compound **18e** had no obvious cytotoxicity to normal Vero cells (IC_{50} = 50.2 μM). The results suggest that the novel scaffolds of aminothiazole conjugated nitrofuran would be a promising class of potent antitubercular and antimicrobial agents.

Key words: *Staphylococcus aureus, Mycobacterium tuberculosis*, aminothiazole, nitrofuran, structure-activity relationships (SARs).

Due to the widespread bacterial infection and the emergence of antibiotic resistant pathogens, the infectious diseases with high rate morbidity and mortality threaten public health seriously.[1,2] The major challenges of antibacterial drug discovery in the therapy are the phenomena of drug resistance, and the critical discussions about drug resistance are *Mycobacterium tuberculosis* (*Mtb*) and *Staphylococcus aureus* (*S. aureus*) infections.[3,4] In particular, tuberculosis (TB) that infecting approximately a third of world population has become the most dangerous disease among various deadly infections.[5] However, the standard clinical antitubercular drugs, like isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and pyrazinamide (PZA), have been used for decades. The consequent emergencies of multidrug-resistant and extensively drug resistant tuberculosis (MDR and XDR-TB)

rendered the scanty drug supply to be tighter.[6] It was also clear from the WHO data that TB conjugating with AIDS lowered immunity and caused higher mortality rate, with 360000 deaths amongst HIV-sufferers in 2013.[5] Unfortunately, there was a drop in the number of authorization of new antimicrobial agents by the regulatory agencies and there was low probability to discover a novel lead compound in the pre-clinical study.[7] Hence, there is a great need for increased drug discovery efforts in this important area.

Our endeavors to develop a novel series of antibacterial agents led to the study of privileged structure-based libraries. 2-amino-1, 3-thiazole derivatives were known to exhibit a broad range of biological activities, some thiazole-2-amine agents had been used in clinical antibacterial applications successfully (e.g., Sulfatizole, Cetraxone, Aztreonam, Riluzole). [8] Of interest were compounds possessing the 4-phenylthiazol-2-amine scaffold with a benzoyl substituent on the amino group that had the inhibitory activity against *S. aureus* and *Escherichia coli* by targeting *β*-Ketoacyl-acyl carrier protein (ACP) synthase III (FabH). One of the compounds, 3-bromo-*N*-(4-(4-bromophenyl) thiazol-2-yl) benzamide (**1**) (**Figure 1**), exhibited a MIC of 6.25 μg/mL against *S. aureus* and FabH inhibitory activity IC_{50} of 5.8 μM.[9]

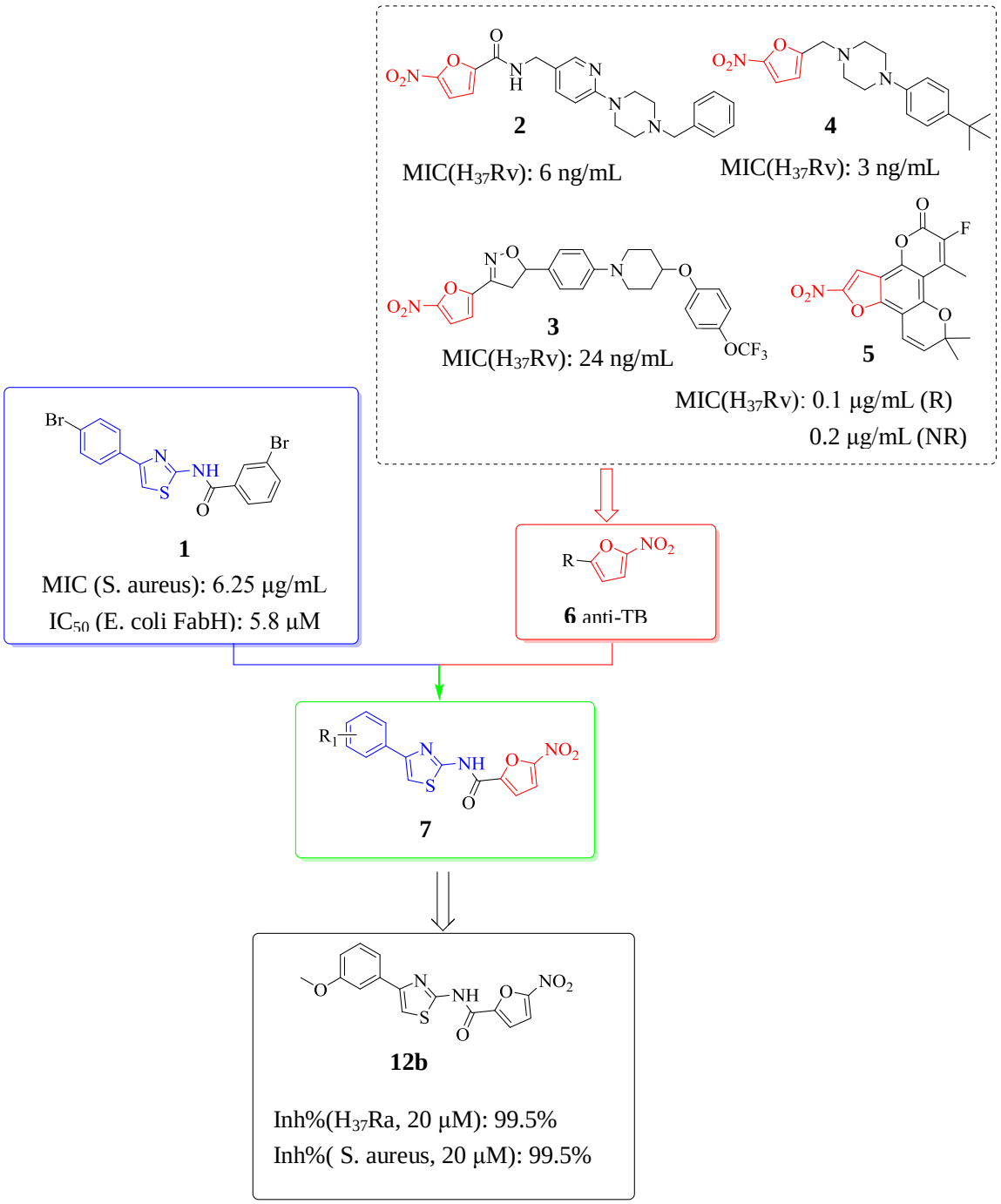


Figure 1 Aminothiazole and nitrofuranyl containing antimicrobial and antitubercular molecules.

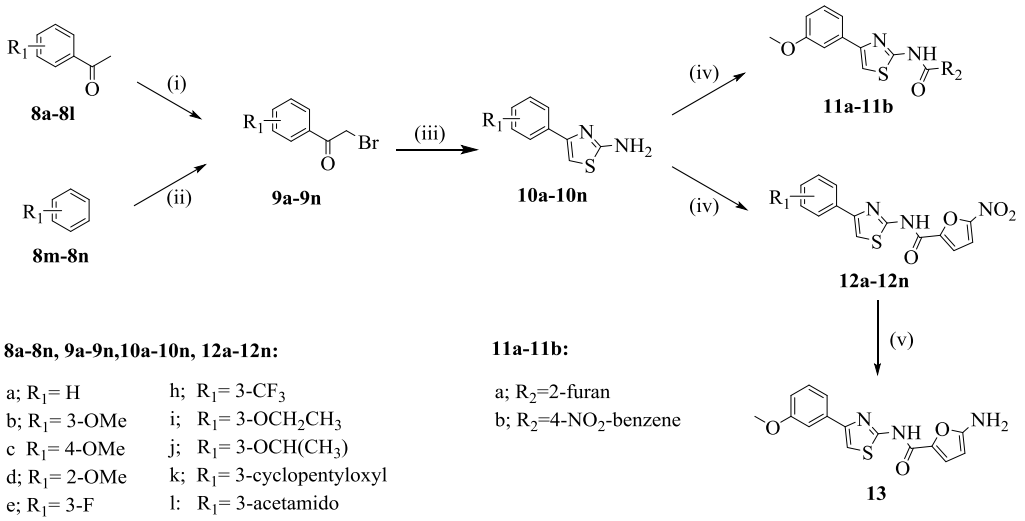
On another hand, nitrofuran derivatives were also precursors with a broad-spectrum activity against both Gram-negative and Gram-positive bacteria.[10,11] A recent literature survey revealed that nitrofuran containing compounds owed anti-TB potential.[12-16] Compound **2** (Lee-562)[13] and compound **3** (Lee-1106)[14] that

generated from the nitrofuranylamides displayed nanomolar potency against H37Rv. On the basis of Lee's study, Yempalla and co-workers had rediscovered nitrofuranyl methyl piperazie **4** with optimal pharmacokinetic property and comparatively better aqueous solubility as an effective compound against sensitive and resistant strains of MTB.[15] Likewise, the nitrofuran introduction prompted calanolides **5** to eradicate both replicating (R) and nonreplicating (NR) *Mtb*.[16]

Motivated by these findings, we adopted the strategy of combinatorial chemistry and proposed the combination of 4-phenylthiazol-2-amine and 5-nitrofuran to generate novel structure **7** (**Figure 1**), in anticipation of having the capacity of fighting both antimicrobial and anti-tubercular activity. The initial *N*-(4-(3-methoxyphenyl) thiazol-2-yl)-5-nitrofuran-2-carboxamide **12b** was found to exhibit the same inhibitory rate of 99.5% against *Mtb* and *S. aureus* at the concentration of 20 μM, which proved the practicability of our design stategy. In order to develop more potent agents, a series of 5-nitro-*N*-(4-phenylthiazol-2-yl)furan-2-carboxamide derivatives, compounds **11a-11b**, **12a-12m**, **13**, **16**, **17** and **18a-18j**, were designed, synthesized and evaluated for their antibacterial activities.

For synthesizing these designed compounds, we initially prepared a series of 4-phenylthiazol-2-amine analogues as shown in **Scheme 1**. Brominating of the commercially available acetophenone **8a-8l** with tetrabutylammonium afforded the α-brominated ketone **9a-9l** and Friedel-Crafts acylation of **8m-8n** generated **9m-9n**. Subsequently the classical Hantzsch synthesis of **9a-9n** with thiourea provided the

desired anminothiazole **10a-10n**. Then condensation of **10a-10n** with the corresponding carboxylic acid afforded the products **11a-11b** and **12a-12n**. The product **13** was synthesized by nitro-reduction of **12b**.



8a-8n, 9a-9n,10a-10n, 12a-12n:

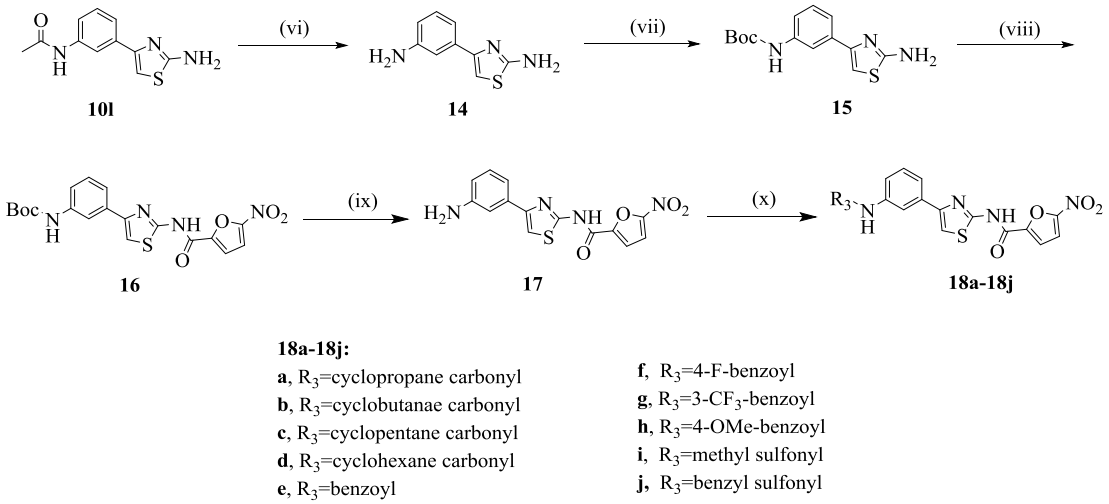
a; R_1= H
b; R_1= 3-OMe
c R_1= 4-OMe
d; R_1= 2-OMe
e; R_1= 3-F
f; R_1= 3-Cl
g; R_1= 3-Br
h; R_1= 3-CF_3
i; R_1= 3-OCH_2CH_3
j; R_1= 3-$OCH(CH_3)$
k; R_1= 3-cyclopentyloxyl
l: R_1= 3-acetamido
m; R_1= 2,4,6-trimethyl
n; R_1= 2,4,6-trifluoro

11a-11b:

a; R_2=2-furan
b; R_2=4-NO_2-benzene

Scheme 1 Reagents and conditions: (i) tetrabutylammonium tribromide, acetonitrile, 12 h; (ii) bromoacetyl bromide, $AlCl_3$, DCM, 0 ℃, 5 min; (iii) thiourea, EtOH, reflux, 3 h, 50%-96%; (iv) the corresponding carboxylic acid, EDCI, DMAP, DCM, r.t., 1 d; (vi) Fe, NH_4Cl, EtOH, H_2O, reflux, 6 h.

Another series of compounds **18a-18j** were started from the above **10l** (**Scheme 2**), which was hydrolyzed firstly via hydrochloric acid to produce the de-acetylated **14**. After the process of *Boc*-protection, **15** condensed with 5-nitro-2-furoic acid to generate **16**. The de-protection of **16** afforded compound **17**. Finally, the uncovered phenylamine **17** was reacted with various alkyl, aromatic acyl chloride or sulfuryl chloride to generate the products **18a-18j**.

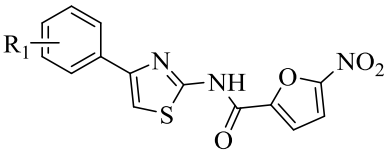


Scheme 2 Agents and conditions: (vi) HCl, H_2O, reflux, 12 h; (vii) $(Boc)_2O$, TEA, MeOH, r.t., 12 h; (viii) 5-nitro-2-furoic acid, EDCI, DMAP, DCM, r.t., 1 d; (ix) TFA, 0-5 ℃, 5 h; (x) the corresponding chloride or sulfonyl chloride, TEA, DCM, r.t., overnight.

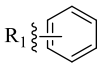
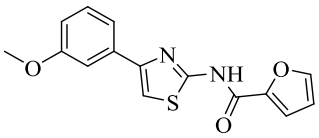
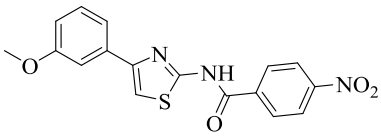
The structure-activity relationships of all compounds were studied for their inhibitory rate (%Inh) against *M. tuberculosis* H37Ra at the concentration of 10 μM and 1μM *in vitro*, while ciprofloxacin was used as positive control. The cytotoxicity was tested on Vero cells by a MTT assay (**Table 1**). The role of nitrofuran was investigated firstly by synthesizing analogues **11a-11b**, **13**, in which the nitrofuran group was replaced by furan, 4-nitrobenzene and 2-aminofuran, respectively. Although the three compounds showed less toxicity than **12b**, they lost anti-TB activity. When the methoxyl of **12b** was replaced by a hydrogen atom (**12a)**, a sharp loss of activity was observed at 1μM concentration, suggesting that the substituent on benzene ring was very important. Then moving the methoxyl from the *meta*-position to the *para*- and the *ortho*-position, the obviously declined tendency of **12c** and **12d** (%Inh = -8.3% and %Inh = -3.0% respectively at 1 μM concentration) indicated that the *meta*-position substitute was beneficial. Next, we introduced different substituents

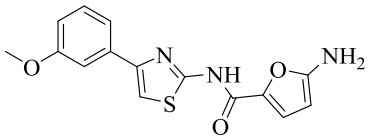
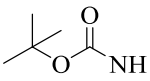
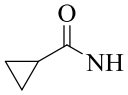
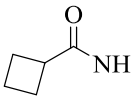
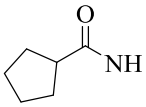
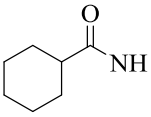
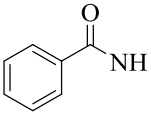
in this position to investigate the polar and electronic effects on the potency. Among the electron-withdrawing halogenic substituted **12e-12g**, trifluoromethyl substituted **12h** and acetamide **12l**, the smallest 3-fluoro substituted **12e** (%Inh = 95.7% at 1 μM concentration) had better activity than **12b** and **12h** remained the equal grade, while others were less potent in general. Among the electron-donating alkyoxy substituted analogs, ethoxyl, isopropoxyl and cyclopentyloxyl substituted **12i**, **12j**, **12k** (%Inh = 95.7%, 97.8% and 97.9% respectively at 1 μM concentration) resulted in the enormous improvement of activity and lower cytotoxicity (IC_{50} > 90 μM, = 69.5 and 81.1 μM respectively) in comparison to **12b**. Besides, the 2, 4, 6-trimethyl and trifluoro substituted **12m** and **12n** showed no activity at all at 10 μM concentration.

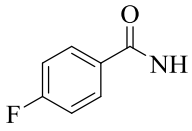
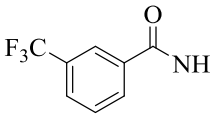
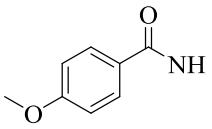
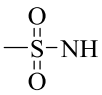
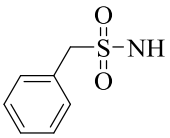
Table 1 Inhibition rate (%Inh) against TB and Vero cell toxicity of all synthesized compounds *in vitro*.



12a-12n, 16, 17, 18a-18j

Comps		R_1	TB[a]（%Inh）		Vero IC_{50} (μM)
			10 μM	1 μM	
11a			-0.9	1.9	>90
11b			-1.5	-0.4	>90
12a		H	99.5	37.2	41.7
12b	3	CH_3O	100.2	68.8	58.7

12c	4	CH_3O	99.3	-8.3	63.0
12d	2	CH_3O	99.5	-3.0	42.0
12e	3	F	99.6	95.7	51.6
12f	3	Cl	99.2	40.5	43.7
12g	3	Br	98.9	70.4	71.0
12h	3	CF_3	98.6	67.0	23.9
12i	3	CH_3CH_2O	99.4	95.7	>90
12j	3	$(CH_3)_2CHO$	99.2	97.8	69.5
12k	3		99.3	97.9	81.1
12l	3		99.4	40.6	57.6
12m	2, 4, 6	CH_3	-4.3	0.9	30.4
12n	2, 4, 6	F	-10.1	-4.1	19.9
13			NT[b]	5.2	NT
16	3		NT	40.0	NT
17	3	NH_2	NT	-1.1	NT
18a	3		99.6	99.3	35. 7
18b	3		NT	1.9	NT
18c	3		NT	0.2	NT
18d	3		99.1	98.9	21.9
18e	3		99.5	98.9	50.2

18f	3		NT	39.8	NT
18g	3		NT	13.7	NT
18h	3		NT	-2.7	NT
18i	3		82.1	-4.1	<2.8125
18j	3		NT	93.9	59.3
Ciprofloxacin			99.7	99.6	NT

[a] Inhibitory rate (%Inh) test against *M. tuberculosis* H37Ra (ATCC25177).
[b] Not tested

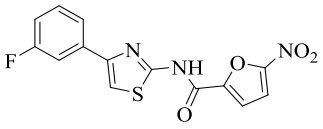
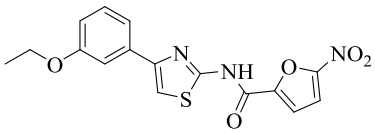
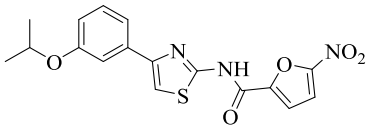
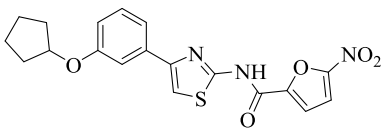
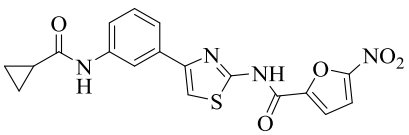
Now that the prolonged *O*-alkylation, increasing compounds' lipophilicity but leading to poor aqueous solubility, contributed to anti-TB activity, we had a try of the increasing lipophilic derivation with alkyl/aryl amide or sulfamide on the basis of compound **12l** as well. The first compound in this series we got was the *Boc*-protection compound **16** (%Inh = 40.0%), which did not have any improved activity. The barely amino-substituted compound **17** (%Inh = -1.1%) exhibited no inhibition against *Mtb*. Compounds **18a** and **18d** among the C3-C6 cycloalkyl substituted analogues emerged comparatively better inhibition of 99.3% and 98.9%, respectively. In the further aryl amide modifications, compound **18e**, with the benzamide group on the benzene ring, inhibited the 98.9% strain growth at 1 μM

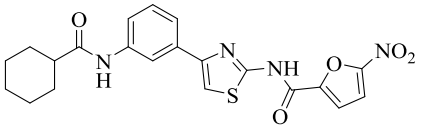
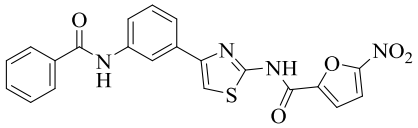
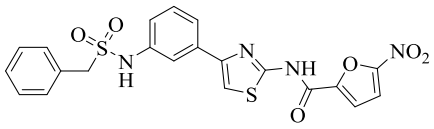
concentration. However, compounds **18f-18h**, with some usually substituted groups on benzamide, showed decreased activity in different degree, in which **18f** and **18g** indicated lower inhibition (%Inh = 39.8% and 13.7% respectively) and **18h** had no activity. Therefore, we did not continue to explore the effect of other substituted benzamide. In addition, two sulfamide compounds **18i** and **18j**, with methane sulfamide and benzenemethane sulfamide respectively, were -4.1% and 93.9% inhibition rate against H37Ra strain, which also clearly verified that the hydrophobic substituent at the *meta*-position of benzene of structure **7** was beneficial for the activity improvement.

Based on the initially measured inhibitory levels against *Mtb*, the eight most active compounds, **12e**, **12k**, **12l**, **12m**, **12a**, **18d**, **18e**, and **18j**, whose inhibitory rate higher than 90% against H37Ra, were further tested for MIC against *Mtb* and *S. aureus* and aqueous solubility was also studied (**Table 2**). All the tested compounds showed the MICs of 0.27-1.00 μg/mL against *Mtb* and 1.36-4.99 μg/mL against *S. aureus*. Among them, compound **18e** exhibited the lowest anti-TB MIC of 0.27 μg/mL and the lowest anti-*S. aureus* MIC of 1.36 μg/mL. Compound **12e** had the highest aqueous solubility (14.83 μg/mL). The 3-alkoxyl substituted compounds **12k, 12l, 12m** and sulfamide compound **18j** remained the weaker inhibition, and the poor aqueous solubility (<1 μg/mL) confirmed our previous prediction. Compound **18d** almost showed the equivalent activity against *Mtb* and 2-fold lower against *S. aureus* than **18e**. By the way, **18e** was safer by comparing the cytotoxity of compound **18d** and **18e** (IC_{50} = 21.9 and 50.2 μM respectively). These results declared that the

nitrofuranyl introduction of structure **7** satisfied our original design of inhibiting both *Mtb* and *S. aureus*. Moreover, the *meta*-amide substitutions on the benzene ring of the key structure **7** exhibited good activity. The most potent compound **18e** exhibited about 5-fold improvement than lead **1** against *S. aureus*, as well as possessed the submicromolar anti-tubercular activity. Overall, these scaffolds could be potential single antitubercular agents or broadly antibacterial agents.

Table 2 The solubility, MIC value against *Mtb* and *S. aureus* of compounds **12e**, **12i**, **12j**, **12k**, **12a**, **18d**, **18e** and **18j**.

Comps	Structure	Solubility (μg/mL)[a]	MIC(μg/mL) *Mtb*[b]	MIC(μg/mL) *S. aureus*[c]
12e		14.83	0.83	4.17
12i		0.34	0.90	4.49
12j		0.27	0.47	4.67
12k		0.027	1.00	4.99
18a		0.47	0.50	2.49

18d		3.46	0.28	2.75
18e		1.27	0.27	1.36
18j		0.48	0.61	1.52
Ciprofloxacin			1.04	0.26

[a] Aqueous solubility at room temperature.
[b] MIC test against *M. tuberculosis* H37Ra (ATCC 25177).
[c] Inhibitory rate (%Inh) against *S. aureus* (ATCC 6538).

In conclusion, we had designed and synthesized novel scaffolds of 2-aminothiazole conjugating nitrofuran to effectively inhibit both the strains of *Mtb* and *S. aureus*. The SARs indicated that substitutions at the *meta*-position of benzene ring of 5-nitro-N-(4-phenylthiazol-2-yl) furan-2-carboxamide exhibited superior activity. Eight compounds had the inhibitory rate > 90% against *Mtb* at 1 μM concentration, and compound **18e** showed the lowest antitubercular MIC of 0.27 μg/mL against H37Ra and the lowest antibacterial MIC of 1.36 μg/mL against *S. aureus*. According to the above data, the novel scaffolds conjugating aminothiazole with nitrofuran could be potential antitubercular and antibacterial agents and the more potent molecules were currently underway.

Acknowledgments

This work was supported by The National Science and Technology Major Project of China (2012ZX09103101-021). We thank Shanghai Chempartner for in *vitro* antitubercular and antimicrobial screening. We also thank Lihua Zhou of State Key Laboratory of Biotherapy (Sichuan University) for NMR measurements.

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