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Design, synthesis and antitubercular evaluation of benzothiazinones containing an oximido or amino nitrogen heterocycle moiety†

Rui Zhang,‡[a] Kai Lv,‡[a] Bin Wang,[b] Linhu Li,[a] Bo Wang,[a] Mingliang Liu,*[a] Huiyuan Guo,[a] Apeng Wang[a] and Yu Lu*[b]

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A series of 8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-ones (BTZs) bearing an oximido or amino nitrogen heterocycle moiety through modifications at the C-2 position of **BTZ043** and **BPTZ169** were designed and synthesized as new antitubercular agents. Many of the target compounds demonstrate excellent *in vitro* activity (MIC: <0.016–0.088 μg mL^{-1}) against the drug susceptive H37Rv strain and two clinically isolated multidrug-resistant *Mycobacterium tuberculosis* (MTB) strains. Compound **10a** displays acceptable safety, aqueous solubility and pharmacokinetic properties, opening up a new possibility for further development.

Tuberculosis (TB), one of the world's major causes of illness and death, is caused mainly by *Mycobacterium tuberculosis* (MTB).[1] The World Health Organization (WHO) estimated that approximately one third of the world population is infected with MTB, and 9.6 million people were infected and 1.5 million died from TB worldwide in 2014.[2] In particular, the spread of multidrug-resistant (MDR) TB and the emergence of extensively drug-resistant (XDR) TB have revitalized drug discovery efforts in search of novel drugs recently.[3–5] It is encouraging that bedaquiline and delamanid have been approved for the treatment of MDR-TB, after a huge gap of over 40 years.[6,7] However, there are only two new chemical entities, Q203 and TBA-354, in phase 1 clinical trials at present. Therefore, it is urgent to develop new anti-TB drugs.[8,9]

8-Nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-ones (BTZs), a novel class of TB agents targeting DprE1,[10–12] were reported to have potent activity in multiple models.[13] **BTZ043** (Fig. 1) and **PBTZ169** (Fig. 1) are being studied preclinically. Compared with **BTZ043**, **PBTZ169** is slightly more potent and not being stereoselective which makes it easier and cheaper to synthesize.[14] The structure–activity relationship (SAR) studies of BTZs show that –CF_3 and –NO_2 are the optimal groups at the C-6 and C-8 positions, respectively, so there is only one possible structural modifications at the C-2 position.

On the other hand, some of fluoroquinolones (FQs), a class of important second-line anti-TB drugs, such as ciprofloxacin, ofloxacin and levofloxacin, are frequently used for management of TB including MDR-TB.[15] Pyrrolidinyl, piperazinyl and piperidinyl are the most common groups at the C-7 position of FQs. It is interesting that recently the discovery of gemifloxacin and IMB-070593 highlights the importance of oxime-functionalized nitrogen heterocycles with respect to antibacterial activity.[16–18]

Inspired by the above research results, we intended to replace the spiroketal moiety of **BTZ043** with nitrogen cycloketone oximes and shift the terminal nitrogen on the piperazine ring of **BPTZ169** outside the ring (Fig. 1), which were expected to explore SAR of BTZs though variations on the sizes of the heterocycle and the alkyl group of the oxime. Thus, a series of novel BTZ derivatives containing an oximido or amino nitrogen heterocycle moiety were designed and synthesized in this study. Our primary objective was to identify alternative groups at the

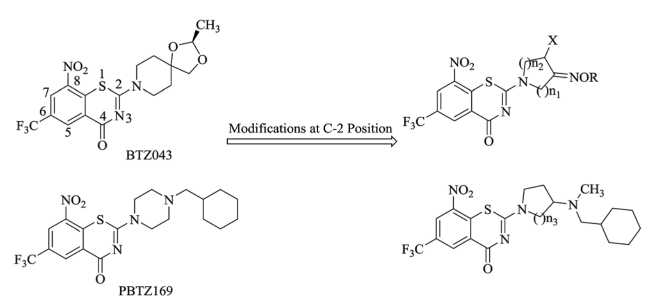

Fig. 1 Design of the new molecules.

[a]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100050, China. E-mail: lmllyx@126.com; Tel: +86-10-63030965

[b]Beijing Key Laboratory of Drug Resistance Tuberculosis Research, Department of Pharmacology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China. E-mail: luyu4876@hotmail.com; Tel: +86-10-89509357

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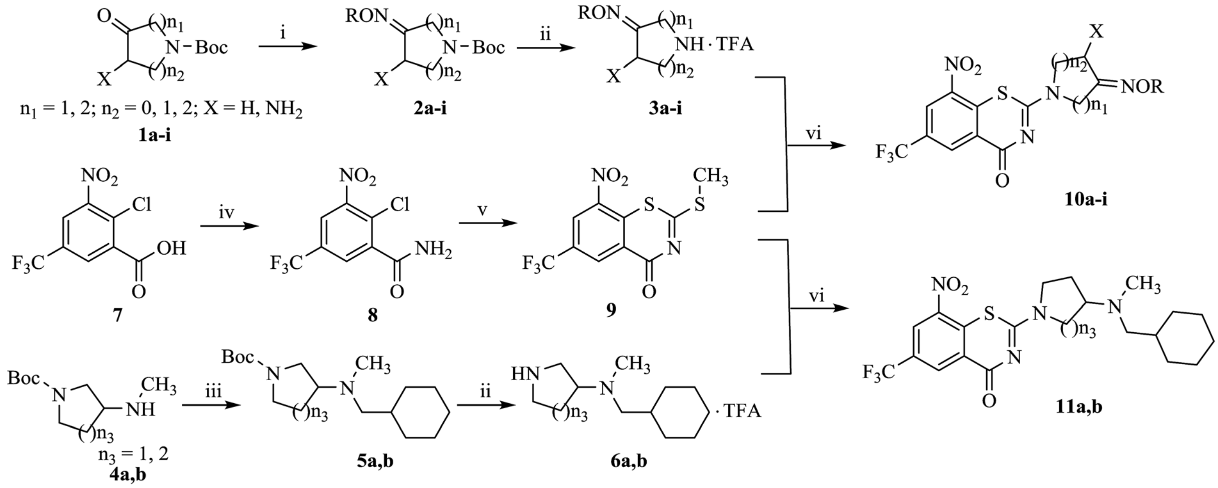
‡ These authors contributed equally to this work.



Scheme 1 Synthesis of target compounds **10a–i** and **11a**, **11b**. Reagents and conditions: (i) $RONH_2 \cdot HCl$, K_2CO_3, C_2H_5OH, rt, 40–65%; (ii) TFA, CH_2Cl_2, rt, 50–52%; (iii) c-$C_6H_{11}CH_2Br$, K_2CO_3, DMF, 80 °C, 50–52%; (iv) $SOCl_2$, $NH_3 \cdot H_2O$, reflux, 76%; (v) CS_2, CH_3I, DMSO, rt, 41%; (vi) $(C_2H_5)_3N$, C_2H_5OH, 60 °C, 45–59%.

C-2 position of BTZs with potent antimycobacterial activity and facilitate the further development of BTZs.

Detailed synthetic pathways to novel BTZ derivatives **10a–i** and **11a, 11b** are outlined in Scheme 1. 4-7-Membered nitrogen cycloketones **1a–i** were smoothly converted to the oximes **2a** by reaction with *O*-alkylhydroxyamines in ethanol at room temperature. The *N*-Boc protecting group on **2a–i** was removed with trifluoroacetic acid (TFA) in dichloromethane to afford the nitrogen cycloketone oximes **3a–i** in 40–65% yield.

Compounds **6a, 6b** were easily prepared from the corresponding *tert*-butyl 3-(methylamino) pyrrolidine/piperidine-1-carboxylate **4a, 4b** *via* nucleophilic substitution reaction with (bromomethyl)cyclohexane in the presence of K_2CO_3 in dimethyl formamide (DMF) at 80 °C, and then the resulting cyclohexylmethylates **2a, 2b** were treated with TFA.

Amidation of 2-chloro-3-nitro-5-(trifluoromethyl) benzoic acid **7** gave amide **8**, which was condensated with carbon disulfide and methyl iodide yielded BTZ core compound **9**.[10] Derivatives **10a–i** and **11a, 11b** were conveniently obtained from **9** by nucleophilic substitution with side chain compounds **3a–i** and **6a, 6b**, respectively.

The synthesized compounds **10a–i** and **11a, 11b** were preliminarily screened for *in vitro* activity against MTB H37Rv ATCC 27294 strain, using the Microplate Alamar Blue Assay (MABA).[19] The minimum inhibitory concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of >90% relative to the mean of replicate bacterium-only controls. The minimum inhibitory concentration (MIC) values of the compounds along with **PBTZ169**, isoniazid (**INH**) and rifampicin (**RFP**) for comparison are presented in Table 1.

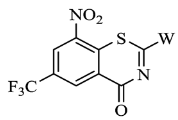
The data reveal that with a few exceptions (**10d**, **10f**, **10i**), all of the BTZ derivatives have potent *in vitro* activity against this strain (MIC: <0.1 μg mL^{-1}), which is better than **BPTZ169** (MIC: 0.116 μg mL^{-1}). In particular, the most active compounds **10b**, **10c** and **10g** (MIC: <0.016 μg mL^{-1}) were found to be >3–>7 fold more potent than **BPTZ169**, **INH** and **RFP** (MIC: 0.049–0.116 μg mL^{-1}). The potency of the oxime BTZ derivatives (**10a–h**) in this study is related to both of the sizes of the heterocycle and alkyl group (R). Generally, 4- and 6-membered heterocycles show the best activity, followed by 7- and 5-membered ones with the same R group (CH_3) successively (**10a** *vs.* **10d** *vs.* **10e** *vs.* **10h**). Moreover, the contribution of the alkyl groups of the oxime moiety to the activity is relevant to the heterocycles. The activity of the R groups is as follows: benzyl = ethyl > methyl for azetidyl-based BTZs (**10a** *vs.* **10b** *vs.* **10c**), and but benzyl > methyl ≫ ethyl for piperidinyl-based ones (**10e** *vs.* **10f** *vs.* **10g**). Further investigation also suggests that introduction of an amino group on the heterocycles is detrimental. For instance, 3-amino-4-(methoxyimino)piperidin-1-yl derivative (**10i**) displays MIC value of 0.436 μg mL^{-1} which is 15-fold less potent than **10e** (MIC: 0.029 μg mL^{-1}).

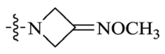
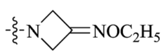
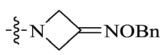
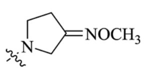
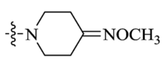
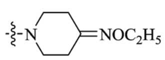
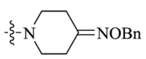
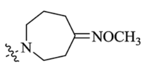
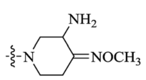
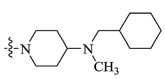
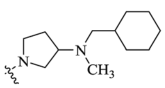
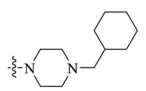
On the other hand, when the nitrogen atom of the piperazine ring of **PBTZ169** was converted to an exocyclic tertiary amino group, the resulting compound **11a** demonstrates increased activity (MIC: 0.051 μg mL^{-1}). It is clear that the piperidin-1-yl group (**11a**) could be displaced by a pyrrolidin-1-yl moiety (**11b**) without obviously affecting the potency. These results indicate that 4-aminopiperidine and 3-aminopyrrolidine rings are preferred over piperazine.

The compounds **10a–i** and **11a**, **11b** were examined for toxicity (CC_{50}) in human lung adenoma A549 cell lines by MTT assay and the results are reported in Table 1. All of them (CC_{50}: 57.66–707.33 μg mL^{-1}) are more cytotoxic than **PBTZ169** (CC_{50}: 784.20 μg mL^{-1}), but the selectivity index (SI: 7667–>44 208) of compounds **10b**, **10c**, **10g**, **11a** and **11b** are bigger than **PBTZ169** (SI: 6760) for MTB H37Rv ATCC 27294.

Encouraged by their strong potency against the drug-sensitive MTB H37Rv strain, these BTZ derivatives except compounds **10d**, **10f** and **10i** were further evaluated against two clinical isolated MDR-MTB strains 16892 (resistant to both of **INH** and **RFP**) and 16802 (resistant to **INH**, **RFP**,

Table 1 Structures and *in vitro* activity of **10a–i** and **11a**, **11b** against MTB H37Rv ATCC 2729[a]



Compd	W	MIC (μg mL^{-1})	CC_{50}[b] (μg mL^{-1})	SI[c]
10a		0.030	91.37 ± 35.18	3046
10b		<0.016	707.33 ± 133.77	>44 208
10c		<0.016	157.74 ± 66.57	>9858
10d		0.116	57.66 ± 11.34	497
10e		0.029	102.07 ± 7.13	3520
10f		0.232	191.29 ± 33.94	825
10g		<0.016	440.69 ± 102.73	>27 543
10h		0.060	58.30 ± 3.78	972
10i		0.436	241.49 ± 47.5	554
11a		0.051	530.97 ± 180.70	10 411
11b		0.088	67.46 ± 19.96	7667
PBTZ169		0.116	784.20 ± 185.8	6760
INH		0.050		
RFP		0.049		

[a] MTB H37Rv ATCC 2729 was acquired from ATCC. [b] CC_{50}: 50% cytotoxic concentration. [c] SI: selectivity index for MTB H37Rv ATCC 27294, CC_{50}/MIC; **INH**: isoniazid: **RFP**: rifampicin.

Table 2 *In vitro* activity against MDR-MTB strains, solubility and metabolic stability of selected compounds[a]

	MIC (μg mL^{-1})			
Compd	MDR-MTB 16892[b]	MDR-MTB 16802[b]	Water Solubility (mg mL^{-1})	Metabolic stability $t_{1/2}$ (min)
10a	0.016	0.031	0.24	>120
10b	0.016	0.016	0.03	NT
10c	0.016	0.016	0.05	NT
10e	0.016	0.016	0.02	NT
10g	0.016	0.016	0.05	NT
10h	0.031	0.016	0.03	NT
11a	0.016	0.016	4.05	49.1
11b	0.016	0.016	3.11	24.3
PBTZ169	0.016	0.016	0.30	19.5
INH	>40	2.5	NT	NT
RFP	>40	20	NT	NT

[a] NT: not tested. [b] MDR-MTB 16892 and MDR-MTB 16802 were isolated from patients in Beijing Chest Hospital.

Table 3 PK Profiles of **10a**, **11a** and **11b** dosed orally in mice[a] at 50 mg kg^{-1} ($n = 3$)

Compd	**10a**	**11a**	**11b**	**PBTZ169**
C_{max} (ng mL^{-1})	5767 ± 3190	173 ± 76.8	327 ± 137	1512 ± 696
T_{max} (h)	0.333 ± 0.144	1.000 ± 0.866	0.250 ± 0	0.583 ± 0.382
$AUC_{0-\infty}$ (h ng mL^{-1})	7678 ± 4395	627 ± ND	646 ± 460	5681 ± 1756
$t_{1/2}$ (h)	1.15 ± 0.540	1.76 ± ND	1.69 ± 0.483	3.31 ± 0.187
MRT (h)	1.85 ± 0.634	2.92 ± ND	2.42 ± 0.171	4.48 ± 0.484

[a] Male CD-1 mice was acquired from WuXi AppTec (Shanghai) CO., Ltd; ND: not determined (parameters not determined due to inadequately defined terminal elimination phase).

streptomycin, ethambutol and levofloxacin) (Table 2). The results indicate that all of **10a–c**, **10e**, **10g**, **10h** and **11a**, **11b** have excellent activity against both of the two strains with similar MIC values (0.016–0.031 μg mL^{-1}) to **PBTZ169** (MIC: 0.016 μg mL^{-1}), suggesting their promising potential for both drug-sensitive (MIC: <0.1 μg mL^{-1}) and resistant MTB strains (Tables 1 and 2).

The BTZ derivatives were initially evaluated for their water solubility which was determined by HPLC measurement of the concentration of a micromembrane filtered saturated solution.[18] Compared with other oxime derivatives (0.02–0.05 mg mL^{-1}, clog P: 3.28–4.98), compound **10a** (clog P: 2.83) has much greater solubility (0.24 mg mL^{-1}) which is only slightly smaller than **PBTZ169** (0.30 mg mL^{-1}). As expected, exocyclic tertiary amino derivatives **11a**, **11b** possess excellent aqueous solubility (3.11–4.05 mg mL^{-1}) which is more than ten times that of **BPTZ169** (Table 2). Moreover, all of **10a**, **11a** and **11b** show better metabolic stability ($t_{1/2}$: 24.3–>120 min) in human liver microsomes compared to **BPTZ169** ($t_{1/2}$: 19.5 min).

Based on the measured activity levels against the tested strains, solubility and metabolic stability, **10a**, **11a** and **11b** were further tested for *in vivo* pharmacokinetic (PK) profiles in mice after a single oral administration of 50 mg kg^{-1}. As shown in Table 3, compound **10a**, with a 3-(methoxyimino) azetidine moiety, displays good PK properties, with C_{max} of 5767 ng mL^{-1}, $AUC_{0-\infty}$ of 7678 h ng mL^{-1} and $t_{1/2}$ of 1.15 h. Compounds **11a** and **11b** have a relatively longer $t_{1/2}$ of 1.69–1.76 h and MRT of 2.42–2.92 h, but a poor C_{max} of 173–327 ng mL^{-1} and $AUC_{0-\infty}$ of 627–646 h ng mL^{-1}. Unexpectedly, all of the three compounds have a shorter $t_{1/2}$ of 1.15–1.76 h compared to **BPTZ169** ($t_{1/2}$: 3.31 h). The *in vitro* and *in vivo* half-times of inconsistency may be due to differences in species between human liver microsomes and mice.

Conclusions

A series of novel BTZ derivatives bearing an oximido or amino nitrogen heterocycle moiety were designed as new antitubercular agents through modifications at the C-2 position of **BTZ043** and **BPTZ169**. Many of them exhibit excellent *in vitro* inhibitory activity against both drug-sensitive MTB strain H37Rv and drug-resistant clinical isolates. Compound **10a** displays acceptable safety, aqueous solubility and PK properties, and it may serve as a new and promising lead compound for further antitubercular drug discovery. Studies to determine the *in vivo* efficacy of **10a** are currently underway.

Ethical statement

Animals were maintained in accordance with the guidelines of the Chinese Association for Laboratory Animal Sciences, Beijing, China, and approved by the Institutional Ethical Committee (IEC) of Peking Union Medical College.

Acknowledgements

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