Design, synthesis and antitubercular evaluation of benzothiazinones containing a piperidine moiety

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ABSTRACT

We herein report the design and synthesis of benzothiazinones containing a piperidine moiety as new antitubercular agents based on the structure feature of IMB-ZR-1 discovered in our lab. Some of them were found to have good *in vitro* activity (MIC < 1 μg/mL) against drug-susceptible Mycobacterium tuberculosis H37RV strain. After two set of modifications, compound **2i** were found to display comparable *in vitro* anti-TB activity (MIC < 0.016 μg/mL) to PBTZ169 against drug-sensitive and resistant mycobacterium tuberculosis strains. Compound **2i** also showed acceptable PK profiles. Studies to determine PK profiles in lung and *in vivo* efficacy of **2i** are currently under way.

KEYWORDS: benzothiazionones, PBTZ169, antimycobacterial activity, synthesis

1. Introduction

Tuberculosis (TB) is caused mainly by mycobacterium tuberculosis (MTB), has existed for millennia and remains a major global health problem.[1] The World Health Organization (WHO) estimated that approximately 10.4 million people were infected and 1.3 million died from TB worldwide in 2016.[2] The current guidelines for treatment of drug-susceptible TB infection recommends a combination of four front-line drugs rifampin, isoniazid, pyrazinamide and ethambutol for 6-9 months, often leading to significant side effects and poor patient compliance. In addition, the spread of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has rendered these front-line drugs ineffective. [3] Therefore, there is an urgent need for discovery of new drugs with new mechanisms of action. Although Bedaquiline (inhibition of mitochondrial ATP synthase) and Delamanid (inhibition mycolic acid biosynthesis) were approved for the treatment of MDR-TB, over a huge gap of over 40 years, [4-5] both of them have pronounced issues, including hERG toxicity concerns, as well as multiple ADME issues due to their high lipophilicity. [6]

Benzothiazinones (BTZs), a novel class of TB agents were reported to have strong inhibitory potency against drug-sensitive TB, MDR-TB and XDR-TB strains targeting on decaprenyl phosphoryl-β-D-ribose 2′-epimerase (DprE1). [7-12] The most advanced compound from this series (PBTZ169, Figure 1), developed by the Swiss Federal

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Graphic abstracts:

PBTZ169 — Previous work → IMB-ZR-1 — This work → 2i

Anti-TB activity: MIC < 0.016
Anti-MDR-TB activity: MIC <0.016

New benzothiazinones bearing a piperidine moiety were designed and synthesized as antitubercular agents based on the structure feature of IMB-ZR-1 discovered in our lab. Compound **2i** were found to display potent anti-TB activity (MIC < 0.016 μg/mL) against drug-sensitive/resistant MTB strains.

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Institute of Technology and Innovative Medicines, has progressed into Phase II clinical trials in Russian Federation for the treatment of both drug-susceptible TB and MDR-TB.[2]

<INSERT FIGURE 1 HERE>

The structure-activity relationship (SAR) and mechanistic studies of BTZs suggest that the NO_2 group at position 8 and the sulfur atom at position 1 are critical for activity, that the -CF_3 at position 6 plays an important role in maintaining activity. [7, 13-20] In our previous study, we focused on the modification at position 2, some of the resulting compounds were found to have improved activity and pharmacokinetic properties. [11, 12] Among them, IMB-ZR-1 with the piperidine ring displayed potent *in vitro* anti-TB activity against the tested MDR-TB strains (MIC = 0.016 μg/mL). [12] Inspired by the above research results, we designed and synthesized two series of new BTZs **1-2** with piperidine moiety as ring A (Figure 1.). Shorten the linker between the two cycles of IMB-ZR-1 by removing the methylene group gave the series **1**; shifting the nitrogen atom in the linker to ring B provided the series **2**. In order to explore SAR of these BTZs, the R group on series **1** could be methyl or hydrogen; the W moiety of series **2** could be piperidine, pyrrolidine, azepane, 4-methylpiperazine, diethylamino, or substituted piperidine, *et al*. The antimycobacterial activity of all the target compounds were evaluated against drug-susceptible TB strain. The potent compound **2i** was further evaluated for antimycobacterial activity against MDR-TB strains, and *in vivo* PK properties, aiming to identify alternative group at position 2 of BTZs and find more effective DprE1 inhibitors as anti-TB candidates.

<INSERT SCHEME 1 HERE>

2. Results and Discussion

2.1 Chemistry

Detailed synthetic pathways to target compounds **1a-b** and **2a-k** are outlined in scheme 1. Reductive amination of ketone **3** and *N*-methylcyclohexylamine or cyclohexylamine by $NaCNBH_3$ in MeOH afforded compounds **4a-b**. Boc deprotection by TFA in DCM gave the intermediates **5a-b**. Coupling of **5a-b** and **6** which was prepared according literature procedure [8] furnished the BTZs **1a-b**. Condensation of the acid **7** with amines in the presence of EDC and HOBt yielded compounds **8a-k**. Removal of the Boc group followed by amide reduction with $LiAlH_4$ formed intermediates **9a-k**. Installation of **9a-k** to **6** in the same manner as the preparation of **1a-b** gave targets **2a-k**.

2.2 Anti-TB activity

The two series **1a-b** and **2a-e** bearing different kinds of amines to ensure the structure diversity were first synthesized (Table 1). They were preliminarily screened for in vitro activity against MTB H37Rv ATCC27395 strain, using the Microplate Alamar Blue Assay (MABA). [21] 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 MIC values of the compounds along with PBTZ169, isoniazid (INH), and rifampicin (RFP) for comparison were obtained from three independent experiments and presented in μg/mL in Table 1.

<INSERT TABLE 1 HERE>

The data revealed that these two BTZ derivatives **1a-b** and **2a-e** shown considerable anti-TB activity against this strain (MIC < 10 μg/mL), although they were less active than PBTZ169, INH and RFP. Among them, compounds **1a-b** and **2a** displayed better activity (MIC < 1 μg/mL) than other BTZs **2b-e**. Removal of the methyl group on **1a** didn't influence the potency (**1a** *vs* **1b**). Decrease or increase the ring size of the piperidine group on **2a** resulted in a loss of anti-TB activity (**2a** *vs* **2b** *vs* **2c**). Replacement of the piperidine group with *N*-methyl piperazine or diethyl amine also leaded to a decreased potency (**2a** *vs* **2d** *vs* **2e**). Specifically, compound **2d** shown more than 16-fold higher MIC value

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than that of **2a**. Consequently, this set of modification suggested that the cyclohexylamino (**1a-b**) and piperidin-1-ylmethyl (**2a**) moiety were more suitable for the Q fragment (Table 1) than other moieties. Considering compound **2a** displayed best activity (MIC = 0.481 μg/mL) among these two series, we intend to make further optimization based on **2a**.

<INSERT TABLE 2 HERE>

Next, an additional set of substituted piperidine analogues as W moiety listed in Table 2 were designed and synthesized. Our results indicated that some of them (**2g-i**) exhibited good activity (MIC < 1 μg/mL). Among them, compound **2i** (MIC < 0.016 μg/mL) is more active than INH (MIC = 0.037 μg/mL) and RFP (MIC = 0.084 μg/mL), and comparable to PBTZ169 (MIC < 0.016 μg/mL). Exploration of SAR was first conducted by introduction of methyl group to ortho-, meta-, and para-positions of piperazine ring. Introduction of methyl group to ortho-position provided decreased potency (**2a** *vs* **2f**), whereas the presence of meta- or para-methyl group afforded a slightly increased or maintained anti-TB activity (**2a** *vs* **2g** and **2h**). The installation of hydroxy or ketal groups at the para-position was detrimental to the potency (**2a** *vs* **2j** and **2k**). Surprisingly, the introduction of trifluoro group at the para position caused a dramatical improvement in potency (**2a** *vs* **2i**).

Inspired by the strong anti-TB potency against drug sensitive strain, compound **2i** was further evaluated against two clinical isolated MDR-TB strains (16995 and 16833) resistant to both INH and RFP (MIC > 40 μg/mL). As shown in table 3, compound **2i** (MIC < 0.016 μg/mL) displayed comparable anti-MDR-TB activity to PBTZ169 (MIC < 0.016 μg/mL), suggesting its promising potential for both drug-sensitive and resistant TB strains.

<INSERT TABLE 3 HERE>

2.3 Pharmacokinetics

We subsequently investigated the *in vivo* PK profiles of compound **2i** in mice after a single oral administration of 50 mg/kg. As shown in Table 4, compound **2i** showed acceptable PK properties although the AUC of **2i** (2489 h·ng/mL) is less than half of PBTZ169 (5478 h·ng/mL). The Tmax of **2i** (0.25 h) is shorter than PBTZ169 (0.83 h), indicating that **2i** was absorbed faster than PBTZ169. The Cmax is comparable to PBTZ169. We speculated that the lower AUC of **2i** probably due to extensive tissue distribution or excretion. Currently, the PK profiles of **2i** in lung is under testing, which will give the lung distribution properties of **2i**.

<INSERT TABLE 4 HERE>

3. Conclusion

In summary, two series of BTZ derivatives with piperidine ring were designed as new anti-TB agents through modifications of IMB-ZR-1. Most of them exhibited considerable in vitro inhibitory (MIC < 10 μg/mL) activity against drug sensitive strain. Compound **2i** with 4-trifuoromethyl piperidine group as the W moiety of series **2** displayed comparable *in vitro* anti-TB activity to PBTZ169 against drug-sensitive and resistant TB strains. Compound **2i** also showed acceptable PK profiles, although its AUC is less than half of PBTZ169. Studies to determine further PK profiles in lung and *in vivo* efficacy of **2i** are currently under way.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra was obtained on an MDSSCIEX Q-Tap mass spectrometer. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254).

4.2. Synthesis

4.2.1. General procedure for the preparation of compound **4a-b**

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To a stirred solution of tert-butyl 4-oxopiperidine-1-carboxylate (200 mg, 0.67 mmol) in anhydrous MeOH (5 mL) was added *N*-methylcyclohexanamine/cyclohexanamine, $NaCNBH_3$ (166 mg, 2.68 mmol) at room temperature. The mixture was adjusted to pH 6 by acetic acid, and stirred for 3 hours. The mixture quenched by H_2O (20 mL), and extracted by DCM. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by chromatography on a silica gel column to yield compound **4a-b**.

4.2.1.1. tert-butyl 4-(cyclohexyl(methyl)amino)piperidine-1-carboxylate **4a**

Following the general procedure, employing *N*-methylcyclohexanamine yielded compound **4a** as a colorless oil in 40% yield, ^{1}HNMR (500 MHz, $CDCl_3$) δ 4.30 (2H, brs), 3.34 (1H, brs), 3.17 (1H, brs), 2.73 (2H, brs), 2.65 (3H, s), 2.20 (2H, m), 1.96 (2H, m), 1.73 (2H, m), 1.58 (2H, brs), 1.42 (9H, s), 1.25 (7H, brs); MS-ESI (m/z): 319 $(M + Na)^+$.

4.2.1.2. tert-butyl 4-(cyclohexylamino)piperidine-1-carboxylate **4b**

Following the general procedure, employing cyclohexanamine yielded compound **4b** as a colorless oil in 50% yield, MS-ESI (m/z): 283 $(M + H)^+$.

4.2.2. General procedure for the procedure of compound **5a-b**

To a stirred solution of **4a-b** (0.25 mmol) in DCM (5 mL) was added TFA, stirred for 1 hour, and concentrated. The residue was diluted by DCM, concentrated again for three times to gave the crude **5a-b** which was used directly for the next step.

4.2.3 General procedure for the preparation of compound **8a-k**

To a stirred solution of compound **7** (200 mg, 0.87 mmol) in DCM (10 mL) was added amines (1.0 mmol), EDC (186 mg, 1.2 mmol), and HOBt (153 mg, 1.2 mmol) at room temperature. The mixture was stirred for 3 hrs, quenched by H_2O (15 mL), and extracted by DCM (15 mL × 3). The organic layer was dried over anhydrous MgSO4, filtered, and concentrated. The residue was purified by chromatography on a silica gel column to afford compound 8a-l as a colorless oil.

4.2.3.1. *tert*-butyl 4-(piperidine-1-carbonyl)piperidine-1-carboxylate **8a**

Following the general procedure, employing piperidine as the amine yielded compound **8a** as a colorless oil in 85% yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.14 (brs, 2H), 3.55 (brs,2H), 3.43 (brs, 2H), 2.76 (brs, 2H), 2.62 (m, 1H), 1.66 (m, 6H), 1.55 (brs, 4H), 1.45 (s, 9H); MS-ESI (m/z): 297 $(M + H)^+$.

4.2.3.2. *tert*-butyl 4-(pyrrolidine-1-carbonyl)piperidine-1-carboxylate **8b**

Following the general procedure, employing piperidine as the amine yielded compound **8b** as a colorless oil in 90% yield, which was used directly for the next step.

4.2.3.3. tert-butyl 4-(azepane-1-carbonyl)piperidine-1-carboxylate **8c**

Following the general procedure, employing azepane as the amine yielded compound **8c** as a colorless oil in 94% yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.15(brs, 2H), 3.51(brs,2H), 3.47(t, J = 5.9 Hz, 2H), 2.75(brs, 2H), 2.60(m, 1H), 2.01(m, 2H), 1.73(m, 6H), 1.57(brs, 4H), 1.46(s, 9H); MS-ESI (m/z): 333 $(M + Na)^+$.

4.2.3.4. tert-butyl 4-(4-methylpiperazine-1-carbonyl)piperidine-1-carboxylate **8d**

Following the general procedure, employing azepane as the amine yielded compound **8d** as a colorless oil in 75% yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.14 (brs, 2H), 3.65 (brs, 2H), 3.53 (brs, 2H), 2.75 (brs, 2H), 2.6 1(m, 1H), 2.41 (d, J = 13.4 Hz, 4H), 2.32 (s, 3H), 1.67 (m, 4H), 1.45 (s, 9H); MS-ESI (m/z): 312 $(M + H)^+$, 334 $(M + Na)^+$.

4.2.3.5. tert-butyl 4-(diethylcarbamoyl)piperidine-1-carboxylate **8e**

Following the general procedure, employing diethylamine as the amine yielded compound **8e** as a colorless oil in 80% yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.15 (brs, 2H), 3.34 (q, J = 9.3 Hz, 4H), 2.74 (brs, 2H), 2.54 (t, J = 11.0 HZ, 1H), 1.75 (q, J = 10.1 Hz, 2H), 1.6 3(m, 2H), 1.45 (s, 9H), 1.20 (t, J = 6.9 HZ, 3H), 1.09 (t, J = 6.9 HZ, 3H); MS-ESI (m/z): $(M + H)^+$; MS-ESI (m/z): 285 $(M + H)^+$.

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4.2.3.6. tert-butyl 4-(2-methylpiperidine-1-carbonyl)piperidine-1-carboxylate **8f**

Following the general procedure, employing 2-methylpiperidine as the amine yielded compound **8f** as a colorless oil in 87% yield, ^1H NMR (500 MHz, $CDCl_3$) δ 4.92 (brs, 1H), 4.49 (m, 1H), 4.15 (brs, 3H), 3.66 (m, 1H), 3.13 (m, 1H), 2.76 (brs, 2H), 2.59 (brs, 1H), 1.63 (m, 8H), 1.45 (s, 9H), 1.27 (brs, 3H); MS-ESI (m/z): 333 $(M + Na)^+$.

4.2.3.7. tert-butyl 4-(3-methylpiperidine-1-carbonyl)piperidine-1-carboxylate **8g**

Following the general procedure, employing 3-methylpiperidine as the amine yielded compound **8g** as a colorless oil in 87% yield, ^1H NMR (500 MHz, $CDCl_3$)δ4.43 (m, 1H), 4.14 (brs, 2H), 3.80 (d, J = 11.8 Hz, 1H), 3.71 (d, J = 13.4 Hz, 1H), 2.98 (m, 1H), 2.76 (brs, 2H), 2.63 (m, 1H), 2.24 (t, J = 11.2 Hz, 1H), 1.83 (d, J=12.5 Hz, 1H), 1.68 (m, 5H), 1.45 (brs, 9H), 1.25 (m, 1H), 1.15 (m, 1H), 0.92 (d, J = 10.7 Hz, 3H); MS-ESI (m/z): 333 $(M + Na)^+$.

4.2.3.8. tert-butyl 4-(4-methylpiperidine-1-carbonyl)piperidine-1-carboxylate **8h**

Following the general procedure, employing 4-methylpiperidine as the amine yielded compound **8h** as a colorless oil in 87% yield, ^1H NMR (500 MHz, $CDCl_3$) δ 4.57 (brs, 1H), 4.14 (brs, 2H), 3.85 (brs, 1H), 3.01 (brs, 1H), 2.76 (m, 2H), 2.62 (m, 1H), 2.54 (m, 1H), 1.66 (m, 7H), 1.45 (s, 9H), 1.08 (m, 2H), 0.95 (d, J = 6.5 Hz, 2H).

4.2.3.9. tert-butyl 4-(4-(trifluoromethyl)piperidine-1-carbonyl)piperidine-1-carboxylate **8i**

Following the general procedure, employing 4-trifluoromethylpiperidine as the amine yielded compound **8i** as a colorless oil in 68% yield, ^1H NMR (500 MHz, $CDCl_3$) δ 4.75 (d, J = 11.2 Hz, 1H), 4.14 (brs, 2H), 4.01 (d, J = 11.4 Hz, 1H), 3.05 (m, 1H), 2.77 (brs, 2H), 2.61 (m, 1H), 2.53 (m, 1H), 2.28 (m, 1H), 1.94 (m, 2H), 1.72 (m, 2H), 1.66 (brs, 2H), 1.45 (brs, 11H); MS-ESI (m/z): 365 $(M + H)^+$.

4.2.3.10. tert-butyl 4-(4-hydroxypiperidine-1-carbonyl)piperidine-1-carboxylate **8j**

Following the general procedure, employing 4- hydroxypiperidine as the amine yielded compound **8j** as a colorless oil in 68% yield, ^1H NMR (500 MHz, $CDCl_3$) δ 4.08 (m, 2H), 3.95 (brs,2H), 3.76 (brs, 1H), 3.23 (m, 2H), 2.76 (brs, 2H), 2.63 (brs, 1H), 2.07 (m, 2H), 1.89 (brs, 2H), 1.69 (brs, 2H), 1.50 (m, 2H), 1.45 (brs, 9H); MS-ESI (m/z): 335 $(M + Na)^+$.

4.2.3.11. tert-butyl 4-(1,4-dioxa-8-azaspiro[4.5]decane-8-carbonyl)piperidine-1-carboxylate **8k**

Following the general procedure, employing 4- hydroxypiperidine as the amine yielded compound **8k** as a colorless oil in 75% yield, ^1H NMR (500 MHz, $CDCl_3$) δ 4.14 (d, J = 11.6 Hz, 2H), 3.98 (s, 4H), 3.68 (brs, 2H), 3.56 (brs, 2H), 2.76 (t, J = 12.2 Hz, 2H), 2.64 (m, 1H), 1.69 (m, 8H), 1.45 (s, 9H); MS-ESI (m/z): 355 $(M + H)^+$.

4.2.4. General procedure for the preparation of **9a-k**

To a stirred solution of **8a-k** (0.25 mmol) in DCM (5 mL) was added TFA, and stirred for 1 hour. The mixture was concentrated, and diluted by DCM, concentrated again for three times to fully remove the TFA. The residue was dissolved in THF. To the mixture was added $LiAlH_4$ (0.5 mL,1 M solution in THF) at 0 ^oC, and stirred for 30 mins. The mixture was slowly quenched by MeOH (0.5 mL), and NaOH solution (0.2 mL, 1 M). The mixture was filtered through celite, washed by MeOH, and concentrated. The residue was concentrated and purified by chromatography on a silica gel column (DCM : MeOH : $NH_3.H_2O$ = 5 : 3 : 1) to afford crude **9a-k** as brown oils， which were used directly for the next step.

4.2.5. General procedure for the preparation of **1a-b** and **2a-k**

To a stirred solution of **5a-b** or **9a-k** (0.1 mmol) in anhydrous MeOH (5 mL) was added compound **6** (32 mg, 0.1 mmol) and Et_3N (28 µL, 0.2 mmol) at room temperature under argon. The mixture was heated to 40 ^oC, stirred for 3 hours, and concentrated. The mixture was diluted by DCM (15 mL) and washed by H_2O. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography on a silica gel column

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(DCM : MeOH : $NH_3{\cdot}H_2O$ = 10 : 1 : 0.01) or prepare TLC (DCM : MeOH : $NH_3{\cdot}H_2O$ = 10 : 1 : 0.01) to give BTZs **1a-b** or **2a-k**.

4.2.5.1. 2-(4-(cyclohexyl(methyl)amino)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **1a**

Following the general procedure, employing **5a** yielded compound **1a** as yellow solid in 40% yield, mp:146-148 ^oC; HPLC purity, 98.1%; ^1H NMR (500 MHz, $CDCl_3$) δ 9.09 (1H, s), 8.75 (1H, s), 5.24 (1H, brs), 4.43 (1H, brs), 3.18 (2H,brs), 3.05 (1H, brs), 2.68 (1H,brs), 2.35 (3H, s), 2.05 (2H, brs), 1.83 (4H, brs), 1.65 (2H, d, J = 11.7 Hz), 1.37 (2H, m), 1.26 (2H, m), 1.12 (2H, m); ESI-MS (m/z): 471 $(M + H)^+$.

4.2.5.2. 2-(4-(cyclohexylamino)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **1b**

Following the general procedure, employing **5b** yielded compound **1b** as yellow solid in 40% yield, mp:171-173 ^oC; HPLC purity, 96.3%; ^1H NMR (500 MHz, $CDCl_3$) δ 9.09 (1H, s), 8.75 (1H, s), 4.99 (1H, brs), 4.34 (1H, brs), 3.48 (1H, s), 3.38 (2H, brs), 3.11 (1H, brs), 2.62 (1H, brs), 2.09 (2H, brs), 1.91 (2H, d, J = 9.65 Hz), 1.76 (2H, d, J = 12.3 Hz), 1.63 (2H, d, J = 11.8 Hz), 1.53 (2H, brs), 1.26 (2H, m), 1.16 (2H, m); ESI-MS (m/z): 457 $(M + H)^+$.

4.2.5.3. 8-nitro-2-(4-(piperidin-1-ylmethyl)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **2a**

Following the general procedure, employing **9a** yielded compound **2a** as yellow solid in 40% yield, mp:165-167 ^oC; HPLC purity, 99.0%; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.10 (s, 1H), 8.74 (s, 1H), 5.25 (brs, 1H), 4.37 (brs, 1H), 3.29 (brs, 1H), 3.01 (brs, 1H), 2.35 (brs, 4H), 2.18-1.95 (m, 7H), 1.57-1.27 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.46, 161.62, 144.03, 134.28, 133.26 (q, J = 3.5 Hz), 129.61 (q, J = 35.4 Hz), 126.88, 125.85 (q, J = 3.6 Hz), 122.44 (q, J = 272.95 Hz), 64.36, 55.20, 46.94, 33.63, 30.84, 25.63, 24.16; ESI-MS (m/z): 457 $(M + H)^+$.

4.2.5.4. 8-nitro-2-(4-(pyrrolidin-1-ylmethyl)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **2b**

Following the general procedure, employing **9b** yielded compound **2b** as yellow solid in 20% yield, mp:156-158 ^oC; HPLC purity, 93.9%; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.09 (s, 1H), 8.74 (s, 1H), 5.30-5.24 (m, 1H), 4.38 (brs, 1H), 3.91-3.05 (m, 2H), 2.71 (brs, 4H), 2.52 (brs, 3H), 2.21-2.00 (m, 4H), 1.87 (brs, 2H), 1.35-1.25 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.57, 161.74, 143.94, 134.30, 133.34 (q, J = 3.4 Hz), 129.60(q, J = 35.4 Hz), 126.73, 126.00 (q, J = 3.7 Hz), 122.42(q, J = 273.5 Hz), 61.72, 54.76, 46.81, 35.08, 30.67, 23.48; ESI-MS (m/z): 443 $(M + H)^+$.

4.2.5.5. 2-(4-(azepan-1-ylmethyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **2c**

Following the general procedure, employing **9c** yielded compound **2c** as yellow solid in 28% yield, mp: 141-143 ^oC; HPLC purity, 92.8%; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.10 (s, 1H), 8.74 (s, 1H), 5.30-5.24 (m, 1H), 4.38 (brs, 1H), 3.29 (brs, 1H), 3.04 (brs, 1H), 2.67 (brs, 4H), 2.40 (brs, 2H), 2.22-1.88 (m, 5H), 1.62 (d, J = 26.0 Hz, 6H), 1.28-1.25 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.61, 161.62, 143.95, 134.39, 133.34(q, J = 35.3 Hz), 126.78, 125.97 (q, J = 3.6 Hz), 122.44 (q, J = 272.4 Hz), 63.29, 55.98, 47.16, 34.81, 30.75, 27.83, 27.17; ESI-MS (m/z): 471.6 $(M + H)^+$.

4.2.5.6. 2-(4-((4-methylpiperazin-1-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3] thiazin-4-one **2d**

Following the general procedure, employing 9d yielded compound 2d as yellow solid in 28% yield, mp: 143-145 ^oC; HPLC purity, 95.1%; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.09 (s, 1H), 8.74 (s, 1H), 5.25 (brs, 1H), 4.37 (brs, 1H), 3.28-3.20 (m, 1H), 3.11-3.00 (m, 1H), 2.53 (brs, 8H), 2.37 (s,3H), 2.24 (d, J = 6.6Hz, 2H), 1.99-1.90 (m, 3H), 1.28-1.26 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.57, 161.67, 143.95, 134.32, 133.35 (q, J = 3.5 Hz), 129.59 (q, J = 35.5 Hz), 126.76, 125.98 (q, J = 3.6 Hz), 122.42 (q, J = 271.6 Hz), 63.53, 54.90, 52.96, 46.96, 45.58, 33.60, 30.67; ESI-MS (m/z): 472.6 $(M + H)^+$.

4.2.5.7. 2-(4-((diethylamino)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **2e**

Following the general procedure, employing **9e** yielded compound **2e** as yellow solid in 38% yield, mp: 117-118 ^oC; HPLC purity, 92.9%; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.07 (s, 1H), 8.72 (s, 1H), 5.28 (brs, 1H), 4.36 (brs, 1H),

3.28 (brs, 1H), 3.06-2.99 (m, 1H), 2.51 (q, J = 7.0 Hz, 4H), 2.27 (d, J = 6.5 Hz, 2H), 2.02-1.99 (m, 2H), 1.86-1.81 (m, 1H), 1.30-1.21 (m, 2H), 0.99 (t, 6H); ESI-MS (m/z): 445 $(M + H)^+$.

4.2.5.8. 2-(4-((2-methylpiperidin-1-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3] thiazin-4-one **2f**

Following the general procedure, employing **9e** yielded compound **2e** as yellow solid in 36% yield, mp: 128-130 ^{o}C; HPLC purity, 98.1%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.09 (s, 1H), 8.74 (s, 1H), 5.29-5.25 (m, 1H), 4.37 (brs, 1H), 3.29 (brs, 1H), 3.00 (brs, 1H), 2.84 (brs, 1H), 2.54 (brs, 1H), 2.62-1.89 (m, 4H), 1.63 (brs,4H), 1.28-1.25 (m, 6H), 1.02 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.58, 161.57, 143.39, 133.34(q, J = 3.5 Hz), 129.54 (q, J = 35.2 Hz), 126.79, 125.96 (q, J = 3.7 Hz), 122.44 (q, J = 272.9 Hz), 59.31, 56.88, 53.12, 47.19, 34.71, 30.90, 25.93, 23.57, 19.05, 14.15; ESI-MS (m/z): 471 $(M + H)^+$.

4.2.5.9. 2-(4-((3-methylpiperidin-1-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3] thiazin-4-one **2g**

Following the general procedure, employing **9g** yielded compound **2g** as yellow solid in 36% yield, mp: 127-128 ^{o}C; HPLC purity, 98.1%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.10 (s, 1H), 8.74 (s, 1H), 5.34-5.25 (m, 1H), 4.37 (brs, 1H), 3.30 (brs, 1H), 3.02 (brs, 1H), 2.72 (brs, 2H), 2.16 (brs, 2H), 2.04-1.85 (m, 5H), 1.70-1.55 (m, 5H), 1.26 (brs, 2H), 0.86 (d, J = 5.7 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.60, 161.62, 143.95, 134.39, 133.34(q, J = 3.4 Hz), 129.56(q, J = 35.5 Hz), 126.79, 125.96 (q, J = 3.7 Hz), 122.44 (q, J = 273.8 Hz), 64.36, 62.79, 54.78, 47.12, 33.81, 32.98, 31.09, 29.35, 25.51, 19.71; ESI-MS (m/z): 471 $(M + H)^+$.

4.2.5.10. 2-(4-((4-methylpiperidin-1-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3] thiazin-4-one **2h**

Following the general procedure, employing **9h** yielded compound **2h** as yellow solid in 36% yield, mp: 130-131 ^{o}C; HPLC purity, 97.6%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.14 (s, 1H), 8.79 (s, 1H), 5.39-5.28 (m, 1H), 4.41 (brs, 1H), 3.33 (brs, 1H), 3.06 (brs, 1H), 2.84 (brs, 1H), 2.22 (d, J = 4.0 Hz, 2H), 1.96 (brs, 5H), 1.66-1.63 (m, 3H), 1.30 (brs, 4H), 0.97 (d, J = 6.3 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.58, 161.62, 143.94, 133.33 (q, J = 3.5 Hz), 129.54 (q, J = 35.4 Hz), 126.79, 125.95(q, J = 3.6 Hz), 122.44(q, J = 273.2 Hz), 63.21, 54.65, 53.46, 47.06, 34.27, 33.84, 30.78, 21.87; ESI-MS (m/z): 471 $(M + H)^+$.

4.2.5.11. 8-nitro-6-(trifluoromethyl)-2-(4-((4-(trifluoromethyl)piperidin-1-yl)methyl)piperidin-1-yl)-4H-benzo[e][1,3] thiazin-4-one **2i**

Following the general procedure, employing **9i** yielded compound **2i** as yellow solid in 36% yield, mp: 158-159 ^{o}C; HPLC purity, 98.4%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.14 (s, 1H),8.79 (s, 1H),5.30 (brs, 1H), 4.43 (brs, 1H), 3.33 (brs, 1H), 2.98 (brs, 4H), 2.26 (brs, 2H), 2.01 (brs, 3H), 1.87 (brs, 4H), 1.66 (brs,2H), 1.32 (d, J = 7.2 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.58, 161.70, 143.95, 134.30, 133.35 (q, J = 3.5 Hz), 129.61 (q, J = 35.5 Hz), 126.75, 126.00 (q, J = 3.6 Hz), 122.42 (q, J = 272.5 Hz),63.73, 53.08, 46.92, 40.33 (q, J = 28.4 Hz), 33.83, 30.69, 24.63; ESI-MS (m/z): 525.6 $(M + H)^+$.

4.2.5.12. 2-(4-((4-hydroxypiperidin-1-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3] thiazin-4-one **2j**

Following the general procedure, employing **9j** yielded compound **2j** as yellow solid in 36% yield, mp: 78-80 ^{o}C; HPLC purity, 93.4%; ^{1}H NMR (500 MHz, $CDCl_3$)δ(ppm) 9.09(s, 1H),8.7 5(s, 1H), 5.26 (brs, 1H), 4.39 (brs, 1H), 3.71-3.52 (m, 1H), 3.29-3.04 (m, 5H), 2.73 (brs, 2H), 2.33-2.20 (m, 4H), 2.00-1.90 (m, 3H), 1.56 (brs, 2H), 1.26 (brs, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.60, 161.71, 143.95, 134.32, 133.36 (q, J = 3.3 Hz), 129.60 (q, J = 34.9 Hz), 126.75, 126.00 (q, J = 3.3 Hz), 122.42 (q, J = 273.1 Hz), 63.51, 51.60, 46.98, 34.47, 33.91, 30.79, 29.72, 29.35, 27.24; ESI-MS (m/z): 473.6 $(M + H)^+$.

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4.2.5.13. 2-(4-((1,4-dioxa-8-azaspiro[4.5]decan-8-yl)methyl)piperidin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one **2k**

Following the general procedure, employing **9k** yielded compound **2k** as yellow solid in 36% yield, mp: 78-80 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.75 (s, 1H), 5.34-5.25 (m, 1H), 4.41 (brs, 1H), 3.96 (brs, 4H), 3.28 (brs, 1H), 3.05 (brs, 1H), 2.59 (brs, 4H), 2.40-2.33 (m, 2H), 2.23-2.20 (m, 1H), 1.79-1.77 (brs, 4H), 1.63-1.58 (m, 2H), 1.27 (brs, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.58, 161.72, 143.95, 134.30, 133.36 (q, J = 3.6 Hz), 129.60 (q, J =35.9 Hz), 126.75, 126.00(q, J = 3.6 Hz), , 122.42 (q, J = 272.9 Hz), 64.37, 63.13, 51.99, 46.78,34.48, 33.80, 30.84, 27.24; ESI-MS (m/z): 515.6 $(M + H)^+$.

4.3. MIC determination

MICs against replicating M. tuberculosis were determined by the microplate Alamar blue assay (MABA). RIF and INH were included as positive controls. M. tuberculosis H37Rv and clinical isolate strains was grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 Broth (catalog no. 271310) supplemented with 0.2% (vol/vol) glycerol, 0.05% Tween 80, and 10% (vol/vol) albumin-dextrosecatalase (BBL Middlebrook ADC Enrichment, catalog no. 212352) (7H9-ADCTG). Cultures were centrifuged, washed twice, and then suspended in phosphate phosphate-buffered saline. Suspensions were then passed through an 8 µm-pore-size filter to remove clumps, and aliquots were frozen at -80 °C. Two fold dilutions of target compounds were prepared in 7H9-ADC-TG in a volume of 100 µl in 96-well, black, clear-bottom microplates (BD Biosciences, Franklin Lakes, NJ). *M. tuberculosis* (100 µl containing 2×10^5 CFU) was added, yielding a final testing volume of 200 µl. The plates were incubated at 37°C; on day 7 of incubation, 12.5 µl of 20% Tween 80 and 20 µl of Alamar blue were added to all wells. After incubation at 37 °C for 16 to 24 h, the fluorescence was read at an excitation of 530 nm and an emission of 590 nm. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacterium-only controls.

4.4. Pharmacokinetic Profiles determination

SPF female ICR mice weighing 20-25 g were used in the pharmacokinetic study. The rats were fasted overnight before dosing. Every treatment group contained 3 mice. Mice were dosed with the tested compounds suspension at 50 mg/kg (p.o.). Compounds were suspended in 0.5% CMC for oral administration. Blood was collected from the jugular vein of each animal at the following times after administration of drugs: 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after a single oral dosing. All blood samples were centrifuged at 3000 r/min for 10 min to obtain serum which was then stored at −20 °C. 150 µL of the serum was added to 500 µL of acetonitrile and the mixture was centrifuged at 13000 r/min for 10 min to remove protein. The supernatant was dried and dissolved in 100 µL of acetonitrile, the solution was centrifuged at 13000 r/min for 10 min. The supernatant was moved to a sample bottle for HPLC analysis. Total area under the concentration time curve (AUC), the elimination half-time ($t_{1/2}$), the peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of samples were determined directly from the experimental data using WinNonlin V6.2.1.

4.5 HPLC purity determination.

All samples were performed on an Agilent 1260 HPLC-UV system. Conditions (solvent A = methanol, solvent B = 0.1% TFA + H_2O): Zorbax SB-C18 column (250 mm × 4.6 mm, 5 µm, PN: 883975-902). Injection volumn: 10 µL. Flow: 1.3 mL/min. Gradient elution: 0.00 min, 10% A; 3 min, 50% A; 15 min, 100% A; 16 min, 10% A; 18 min 10% A. UV at 254 nm.

Notes

The authors declare no competing financial interest.

Abbreviations

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TB, tuberculosis; MTB, Mycobacterium tuberculosis; MDR, multidrug-resistant; XDR, extensively drug-resistant; WHO, World Health Organization; ATP, adenosine triphosphate; hERG, human ether-a-go-go related gene; ADME, absorption, distribution, metabolism, excretion, toxicity; BTZs, nitrobenzothiazinones; DprE1, Decaprenyl phosphoryl-β-D-ribose 2/-epimerase; SAR, structure-activity relationship; PK, pharmacokinetic; MeOH, methanol; NaCNBH3, sodium cyanoborohydride; TFA, trifluoroacetic acid; DCM, methylene chloride; MIC, minimum inhibitory concentration; HOBt, hydroxybenzotriazole; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; LiAlH4, lithium aluminum hydride; MABA, microplate alamar blue assay; INH, isoniazid; RFP, rifampicin; T1/2, half-life; Cmax, the maximum serum concentration; Tmax, the time at which the Cmax is observed; AUC, area under the curve; MRT, mean residence time.

Ethical statement

All animal experiments were carried out in accordance with the guidelines of the Chinese Association for Laboratory Animal Sciences, and approved by the institutional ethical committee (IEC) of Peking Union Medical College.

Acknowledgment

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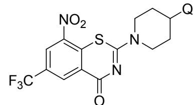
Table 1. Structures and activities of **1a-b** and **2a-e**



Compd.	Q	MIC
1a		0.973
1b		0.959
2a		0.481
2b		3.578
2c		1.320
2d		7.844
2e		1.825
PBTZ169		<0.016
INH		0.037
RFP		0.084

INH: isoniazid; RFP: rifampicin

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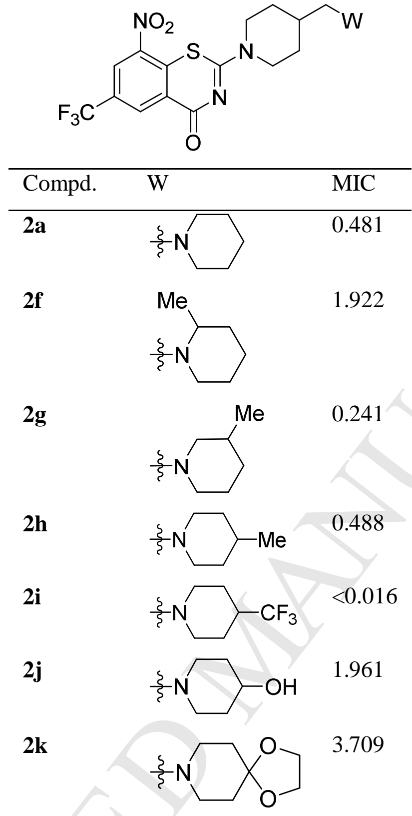
Table 2. Structures and activities of **1a-b** and **2a-e**



Compd.	W	MIC
2a		0.481
2f		1.922
2g		0.241
2h		0.488
2i		<0.016
2j		1.961
2k		3.709
PBTZ169		<0.016

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Table 3. Anti-MDR TB activity of compound **2i**

Compd.	MIC (μg/mL)	
	MDR-TB 16995[a]	MDR-TB 16883[a]
2i	<0.016	<0.016
PBTZ169	<0.016	<0.016
INH	>40	>40
RFP	>40	>40

INH: isoniazid; RFP: rifampicin; [a] MDR-TB 16995 and MDR-TB 16883 were isolated from patients in Beijing Chest Hospital.

Table 4. PK profiles of compound **2i** dosed orally in mice at 50 mg/kg (n = 3)

PK parameters	**2i**	PBTZ169
$T_{1/2}$ (h)	3.3 ± 3.01	2.87 ± 1.03
Tmax (h)	0.25 ± 0	0.83 ± 0.29
Cmax (ng/mL)	1165 ± 223	1300 ± 422
$AUC_{0\text{-}inf}$ (h·ng/mL)	2489 ± 1273	5478 ± 1730
MRT (h)	4.26 ± 3.74	3.73 ± 0.94

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Figure 1. Design of new BTZs

a) $NaCNBH_3$, MeOH, cyclohexylamine or *N*-methylcyclohexylamine, AcOH, rt, 2 hrs; b) TFA, DCM, 0 ^{o}C, 0.5 h; c) Et_3N, MeOH, rt, 3 hrs; d) EDC, HOBt, Et_3N, DCM, rt, 5 hrs; e) i: TFA, DCM, 0 ^{o}C, 0.5 h, ii: 1.0 M $LiAlH_4$ in THF, THF,0 ^{o}C, 1 h.

Scheme 1. Synthesis of BTZs **1a-b** and **2a-k**

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Highlights:

1. Two series of BTZs **1-2** with piperidine moiety were designed and synthesized.

2. Some targets showed considerable *in vitro* activity (MIC<1 μg/mL) against MTB strain.

3. Compound **2i** displayed potent *in vitro* anti-MDR-TB activity (MIC < 0.016 μg/mL).

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