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: benzothiazinones, 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 1.5 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
Graphic abstracts:

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.
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]

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.

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-methylecyclohexylamine 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.

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
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.

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.

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.

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-trifluoromethyl 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. 1H NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d6 or CDCl3 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
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, NaCNBH₃ (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 H₂O (20 mL), and extracted by DCM. The organic layer was dried over anhydrous MgSO₄, 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, ¹H NMR (500 MHz, CDCl₃) 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 H₂O (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, ¹H NMR (500 MHz, CDCl₃) 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, ¹H NMR (500 MHz, CDCl₃) 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, ¹H NMR (500 MHz, CDCl₃) δ 4.14 (brs, 2H), 3.65 (brs, 2H), 3.53 (brs, 2H), 2.75 (brs, 2H), 2.61 (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, ¹H NMR (500 MHz, CDCl₃) 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), 1.63 (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)⁺.
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. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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).^7

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. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.43 (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 (brs, 3H); MS-ESI (m/z): 333 (M + Na).^7

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. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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).^7

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. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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).^7

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 °C, 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\(_2\)O = 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\(_3\)N (28 µL, 0.2 mmol) at room temperature under argon. The mixture was heated to 40 °C, stirred for 3 hours, and concentrated. The mixture was diluted by DCM (15 mL) and washed by H\(_2\)O. The organic layer was dried over anhydrous MgSO\(_4\), filtered, and concentrated. The residue was purified by chromatography on a silica gel column...
Following the general procedure, employing 5a yielded compound 1a as yellow solid in 40% yield; HPLC purity, 98.1%; 1H NMR (500 MHz, CDCl₃) 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)+.

Following the general procedure, employing 5b yielded compound 1b as yellow solid in 40% yield; HPLC purity, 99.6%; 1H NMR (500 MHz, CDCl₃) δ 9.08 (1H, s), 8.73 (1H, s), 4.99 (1H, brs), 3.35 (1H, 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)+.

Following the general procedure, employing 9a yielded compound 2a as yellow solid in 40% yield; HPLC purity, 99.9%; 1H NMR (500 MHz, CDCl₃) δ (ppm) 9.10 (1H, s), 8.74 (1H, s), 5.25 (3H, brs), 4.37 (3H, brs), 3.29 (brs, 1H), 3.01 (brs, 1H), 2.35 (brs, 4H), 2.18-1.95 (m, 7H), 1.57-1.27 (m, 6H); 13C NMR (400 MHz, CDCl₃) δ (ppm) 166.64, 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): 471 (M + H)+.

Following the general procedure, employing 9b yielded compound 2b as yellow solid in 20% yield; HPLC purity, 96.8%; 1H NMR (500 MHz, CDCl₃) δ (ppm) 9.09 (1H, s), 8.74 (1H, s), 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); 13C NMR (400 MHz, CDCl₃) 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)+.

Following the general procedure, employing 9c yielded compound 2c as yellow solid in 28% yield; mp: 141-143 °C; HPLC purity, 92.8%; 1H NMR (500 MHz, CDCl₃) δ (ppm) 9.10 (1H, s), 8.74 (1H, s), 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); 13C NMR (400 MHz, CDCl₃) 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)+.

Following the general procedure, employing 9d yielded compound 2d as yellow solid in 28% yield; mp: 143-145 °C; HPLC purity, 95.1%; 1H NMR (500 MHz, CDCl₃) 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 = 6.6 Hz, 2H), 1.99-1.90 (m, 3H), 1.28-1.26 (m, 2H); 13C NMR (400 MHz, CDCl₃) 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)+.

Following the general procedure, employing 9e yielded compound 2e as yellow solid in 38% yield; mp: 117-118 °C; HPLC purity, 92.9%; 1H NMR (500 MHz, CDCl₃) δ (ppm) 9.07 (s, 1H), 8.72 (s, 1H), 5.28 (brs, 1H), 4.36 (brs, 1H), 3.44-3.35 (m, 1H), 2.98-2.77 (m, 12H), 2.04-1.77 (m, 12H), 1.74-1.20 (m, 30H); ESI-MS (m/z): 818 (M + H)+.
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 °C; HPLC purity, 98.1%; 1H NMR (500 MHz, CDCl3) 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), 2.54 (brs, 1H), 2.62-1.89 (m, 4H), 1.63 (brs, 4H), 1.28-1.25 (m, 6H), 1.02 (s, 3H); 13C NMR (400 MHz, CDCl3) 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 = 72.9 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 °C; HPLC purity, 98.1%; 1H NMR (500 MHz, CDCl3) 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); 13C NMR (400 MHz, CDCl3) 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 = 72.9 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 °C; HPLC purity, 97.6%; 1H NMR (500 MHz, CDCl3) 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); 13C NMR (400 MHz, CDCl3) δ (ppm) 166.58, 161.62, 143.95, 134.39, 133.34(q, J = 3.4 Hz), 129.54 (q, J = 35.4 Hz), 126.79, 125.95 (q, J = 3.6 Hz), 122.44 (q, J = 72.9 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 °C; HPLC purity, 98.4%; 1H NMR (500 MHz, CDCl3) δ (ppm) 9.14 (s, 1H), 8.79 (s, 1H), 5.39-5.28 (m, 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); 13C NMR (400 MHz, CDCl3) δ (ppm) 166.68, 161.70, 143.95, 134.30, 133.35 (q, J = 3.5 Hz), 129.61 (q, J = 35.6 Hz), 127.65, 125.95 (q, J = 3.6 Hz), 122.44 (q, J = 72.9 Hz), 63.21, 54.65, 53.46, 47.06, 34.27, 33.84, 30.78, 21.87; 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 °C; HPLC purity, 93.4%; 1H NMR (500 MHz, CDCl3) δ (ppm) 9.09 (s, 1H), 8.75 (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); 13C NMR (400 MHz, CDCl3) δ (ppm) 166.60, 161.71, 143.95, 134.32, 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 = 72.5 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).
4.2.5.13. 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 °C; 1H NMR (500 MHz, CDCl₃) 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); 13C NMR (400 MHz, CDCl₃) δ (ppm) 166.58, 161.72, 143.95, 134.30, 133.36 (q, J = 3.6 Hz), 129.60 (q, J = 3.6 Hz), 126.75, 126.00(q, J = 3.5 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-dextrose-catalase (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 × 10⁵ 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 (t₁/₂), the peak concentration (Cmax) and the time to reach peak concentration (Tmax) 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 + H₂O; Zorbax SB-C18 column (250 mm × 4.6 mm, 5 µm, PN: 883975-902). Injection volume: 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%. UV at 254 nm.

Notes
The authors declare no competing financial interest.

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

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<th>MIC</th>
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<td>1a</td>
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<tr>
<td>1b</td>
<td>H</td>
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</tr>
<tr>
<td>2a</td>
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</tr>
<tr>
<td>2b</td>
<td>N</td>
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<tr>
<td>2c</td>
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<td>2d</td>
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<tr>
<td>2e</td>
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<tr>
<td>RFP</td>
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INH: isoniazid, RFP: rifampicin
Table 2. Structures and activities of 1a-b and 2a-e

![Structure](image)

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<th>Compd.</th>
<th>W</th>
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Table 3. Anti-MDR TB activity of compound 2i

<table>
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<tr>
<th>Compd.</th>
<th>MIC (µg/mL)</th>
<th>MDR-TB 16995&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MDR-TB 16883&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>&gt;40</td>
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<tr>
<td>RFP</td>
<td>&gt;40</td>
<td>&gt;40</td>
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</table>

INH: isoniazid; RFP: rifampicin;<sup>a</sup> 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)

<table>
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<th>PK parameters</th>
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<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>3.3 ± 3.01</td>
<td>2.87 ± 1.03</td>
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<tr>
<td>Tmax (h)</td>
<td>0.25 ± 0</td>
<td>0.83 ± 0.29</td>
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<tr>
<td>Cmax (ng/mL)</td>
<td>1165 ± 223</td>
<td>1300 ± 422</td>
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<tr>
<td>AUC&lt;sub&gt;0-inf&lt;/sub&gt; (h·ng/mL)</td>
<td>2489 ± 1273</td>
<td>5478 ± 1730</td>
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<tr>
<td>MRT (h)</td>
<td>4.26 ± 3.74</td>
<td>3.73 ± 0.94</td>
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Figure 1. Design of new BTZs

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

1a R = Me
1b R = H
2a R = Me
2b R = H
2c R = Ph
2d R = 4-ClC6H4
2e R = 4-NO2C6H4
2f R = 4-NC6H4
2g R = 4-BrC6H4
2h R = 3-ClC6H4
2i R = 2-ClC6H4
2j R = 2-BrC6H4
2k R = 4-BrC6H4

Detailed structures of 2a-k seen in the tables.

a) NaCNBH3, MeOH, cyclohexylamine or 1-methylcyclohexylamine, AcOH, rt, 2 hrs;
b) TFA, DCM, 0°C, 0.5 hr; c) EJN, MeOH, rt, 3 hrs; d) EDC, HOBr, EJN, DCM, rt, 5 hrs;
e) TFA, DCM, 0°C, 0.5 hr; f) 1.0 M LAH, in THF, THF, 0°C, 1 hr.
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).