Identification of benzothiazones containing a hexahydropyrrolo[3,4-c]pyrrol moiety as antitubercular agents against MDR-MTB†

Xican Ma,‡a Bing Han,‡ab Aoyu Wang,c Lu Yang,b Menghao Huang,d Kushan Chowdhury,‡d Jian Gu,*a Kai Zhang,*c and Kai Lyb

IMB1603, a spiro-benzothiazine compound discovered by our lab, displayed potent anti-MTB activity in vitro and in vivo. In this study, we reported a series of new BTZs containing the hexahydropyrrolo[3,4-c]pyrrol moiety based on the structure of IMB1603. Among them, BTZs 11 and 24 displayed potent anti-MTB (MIC < 0.035 μM) and MDR-MTB (MIC, 0.053–0.102 μM) activity, good solubility (1.82–1.85 mg mL⁻¹), and low cytotoxicity (CC₅₀ > 200 μM), suggesting BTZs 11 and 24 may serve as promising candidates for further study. The molecular docking study of 11 toward DprE was also investigated, and revealed that 11 mimicked the binding pattern of PBTZ169 in the active site of DprE1.

Tuberculosis (TB) is a chronic infectious disease caused mainly by Mycobacterium tuberculosis (MTB). The World Health Organization (WHO) estimated that approximately 10 million people were infected and 1.5 million died from TB worldwide in 2018. The current therapy for TB infected patients requires a combination of four front-line drugs for 6–9 months and does not favor patient compliance. Even worse, the prevalence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has exacerbated the situation. Although new drugs with novel mechanisms such as bedaquiline, delamanid and pretomanid have been approved in recent years for the treatment of MDR-TB, some adverse events or warnings have been noted and limited their use in the clinic. Therefore, there is still an urgent unmet medical need for safer and more effective agents for the treatment of TB.

Benzothiazinones (BTZs), a novel class of TB agents targeting decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE), exhibited exceptional inhibitory activity against MTB and MDR-MTB strains. PBTZ169 (macozinone) and BTZ043, the most advanced BTZ candidates, is currently in phase II clinical trial for the treatment of both drug-susceptible TB and MDR-TB. BTZ scaffold has become a research hot spot throughout the world for the scientists and researchers working in the field of anti-TB.

According to the binding pharmacophore revealed by the crystal structures of BTZ043/PBTZ169 complexed with DprE, the BTZ core interacted with the active site cavity, whereas the cyclohexane or spirocyclic moiety was located at the protein surface (Fig. 1). More precisely, the CF₃ group was well placed in a small pocket and interacted with Asn392; the nitro group of PBTZ169 or BTZ043 was converted to nitroso which covalently binds to Cys387 or Cys394 residue of DprE enzyme, leading to irreversible inactivation of the enzyme. It appeared that the BTZ core was crucial for the activity, but the spirocyclic or cyclohexane at the C-2 position might be open for structure modification.
Based on the binding characteristics and reported structure–activity relationship (SAR), our group focused on the discovery of alternative moieties at the C-2 position of BTZ core.\(^{22-25}\) Recently, we identified IMB1603 with a spiro-heterocyclic segment as a potent anti-TB lead by combining the structure feature of PBTZ169 and BTZ043.\(^{23}\) In this study, replacement of the spiro-heterocyclic group of IMB1603 with hexahydropyrrolo[3,4-c]pyrrole gave a new series of BTZs (Fig. 1). The anti-TB activity, solubility, and toxicity of these new BTZs were evaluated, aiming to identify alternative groups at position 2 of BTZs and find optimized potent anti-TB drug candidates with improved drug-like properties through SAR study.

The synthesis of target compounds 1–43 was shown in Scheme 1. Reductive amination of hexahydropyrrolo[3,4-c]pyrrole A in the presence of aryl aldehydes or acetophenones gave

### Table 1 The structure and anti-MTB activity of new BTZs series 1

| Compd | R     | MIC (µM) | Compd | R     | MIC (µM) |
|-------|-------|----------|-------|-------|----------|
| 1     | p-F   | 0.462    | 17    | m-NO₂ | 0.414    |
| 2     | p-Br  | 0.244    | 18    | m-CF₃ | 0.101    |
| 3     | p-CN  | 1.654    | 19    | m-MeO | 3.671    |
| 4     | p-Br  | 0.953    | 20    | o-F   | 3.595    |
| 5     | p-CN  | 0.616    | 21    | p-F, m-F | 0.111 |
| 6     | p-NO₂ | 0.451    | 22    | p-Cl, m-Cl | 0.077 |
| 7     | p-CF₃ | 0.112    | 23    | p-F, m-Cl | 0.104 |
| 8     | p-MeO | 1.832    | 24    | p-Cl, m-F | <0.035 |
| 9     | p-Bu  | 0.218    | 25    | p-F, o-Cl | 0.888 |
| 10    | p-Me  | 1.926    | 26    | p-F, o-F | 0.232    |
| 11    | p-CF₂O| <0.035   | 27    | p-Cl, o-Cl | 0.566 |
| 12    | H     | 0.796    | 28    | p-F, o-Br | 0.783    |
| 13    | m-F   | 0.222    | 29    | PBTZ169 | <0.035   |
| 14    | m-Cl  | 0.217    | 30    | INH    | 0.262    |
| 15    | m-Br  | 0.203    | 31    | RFP    | 0.166    |
| 16    | m-CN  | 0.223    | 32    |        |          |

### Table 2 The structure and anti-MTB activity of new BTZs series 2

| Compd | Ar or R’ | MIC (µM) |
|-------|----------|----------|
| 12    |          | 0.796    |
| 29    |          | 1.667    |
| 30    |          | 1.849    |
| 31    |          | 1.981    |
| 32    |          | 0.463    |
| 33    |          | 0.899    |
| 34    |          | 27.350   |
| 35    | Boc      | 1.720    |
| 36    | H        | >40      |
| PBTZ169 |        | <0.035   |

### Table 3 The structure and anti-MTB activity of new BTZs series 3

| Compd | R’     | MIC (µM) |
|-------|--------|----------|
| 37    | p-CF₃  | 0.202    |
| 38    | p-OCF₃ | 0.209    |
| 39    | p-F, m-F | 0.110  |
| 40    | p-Cl, m-F | 0.219  |
| 41    | p-F, m-Cl | 0.108  |
| 42    | m-F, m-F | 0.205    |
| 43    | p-F, m-F, m-F | 0.189   |
| PBTZ169 |        | <0.035   |

This journal is © The Royal Society of Chemistry 2020

RSC Adv., 2020, 10, 14410–14414 | 14411
phenyl ring were crucial for anti-TB activity.

The target compounds 1–35 were initially screened for in vitro activity against MTB H37Rv ATCC 27294 strain using the Microplate Alamar Blue Assay (MABA).

The target compounds 1–43 were initially screened for in vitro activity against MTB H37Rv ATCC 27294 strain using the Microplate Alamar Blue Assay (MABA).

As shown in Table 1, BTZs 1 and 2 with cyclohexyl and p-fluorobenzyl group from PBTZ169 and IMB1603 were initially synthesized and evaluated. The MIC value of BTZ 2 was lower than that of 1, and comparable to that of INH. According to our previous SAR findings of spiro-BTZs, the substituents at the phenyl ring were crucial for anti-TB activity.24 In parallel with the spiro-BTZ series, we synthesized compounds 3–28 with diversity R substituents at the phenyl ring. To our delight, compounds 7, 11, 18 and 21–24 displayed increased potency (MIC < 0.15 μM). Especially, compound 11 with p-CF3O and 24 with p-Cl, m-F were found to exhibit comparable anti-TB activity to PBTZ169 (MIC < 0.035 μM). It is interesting that moving the para substituent to meta position led to an improved anti-TB activity (2–7 vs. 13–19), while transferring to the ortho position seemed to result in a decreased potency (2 vs. 20). Notably, BTZs with double substituents at the para and meta position of the benzyl moiety showed better anti-TB activity (MIC < 0.15 μM) than the corresponding mono-substituted BTZs (21–24 vs. 2–3 and 13–14), whereas introducing double substituents to the para and ortho position of benzyls were not well tolerated (25–28, MIC > 0.2 μM).

As a continuing SAR study, BTZs 29–34 with other aromatic cyclic groups as replacement of the benzyl moiety were explored. Compared to BTZ 12, the pyridyl analogues 29–31 and β-naphthalene 33 showed decreased potency, whereas the naphthalene 32 displayed a slightly increased anti-TB activity. The indole moiety was also not favored, leading to a dramatically decreased anti-TB activity (34, MIC = 27.35 μM). In addition, BTZ 35 with Boc-group was synthesized and proved to display moderate anti-TB potency (MIC < 2 μM). Removal of the R’ group, as in compound 36, led to a total loss of anti-TB potency (MIC > 40 μM), indicating the existence of N–H bond might not be tolerated at the C2-position of the BTZ core.

Finally, as shown in Table 3, BTZs with a methyl group at the linker were investigated in this set. The R’ group of 37–41 were selected from the BTZs with potent anti-TB activity in Table 1 (MIC < 0.15 μM). Compared to the corresponding BTZs in Table 1, the introduction of a methyl group resulted in a decreased anti-TB potency (37–41 vs. 7, 11, 21, 23–24). In addition, BTZs 42 with double m-F and 43 with p-F, double m-F, were designed and synthesized according above SAR findings. However, the anti-TB potency of BTZs 42–43 was also not as good as we expected, both of them displayed lower anti-TB activity than 39.

On the bases of above studies, BTZs 7, 11, 18, 21–24, 39, and 41 with potent anti-TB activity (MIC < 0.15 μM) were evaluated against two clinical isolated MDR-MTB (16833 and 16995) strains resistant to both INH and RFP.8 As shown in Table 4, although the MIC values of these new BTZs were all lower than that of PBTZ169 (MIC < 0.035 μM), most of them displayed considerable anti-MDR-TB activity (MIC < 0.15 μM).

These compounds were then tested for mammalian cell cytotoxicity using Vero cells measured as a concentration

| Compd | MDR-MTB1 | MDR-MTB2 | CC50 | Water solubility |
|-------|----------|----------|------|------------------|
| 7     | 0.080    | 0.053    | 166.37 | 2.30 |
| 11    | 0.085    | 0.053    | >200  | 1.85 |
| 18    | 0.209    | 0.112    | 55.77 | 2.29 |
| 21    | 0.119    | 0.058    | 38.63 | 3.50 |
| 22    | 0.110    | 0.056    | 27.31 | 1.94 |
| 23    | 0.115    | 0.058    | 40.79 | 2.01 |
| 24    | 0.102    | 0.073    | >200  | 1.82 |
| 39    | 0.121    | 0.087    | 54.98 | 2.97 |
| 41    | 0.188    | 0.097    | 50.68 | 2.55 |
| PBTZ169 | <0.035  | <0.035   | >200  | 0.90 |
| INH   | >40      | >40      | ND    | NT   |
| RFP   | >40      | >40      | ND    | NT   |

a MDR-MTB1 (MDR-MTB 16833) and MDR-MTB2 (MDR-MTB 16995) were obtained from the State Laboratory of Tuberculosis Reference of China.

b The 50% cytotoxic concentration. c The water solubility was tested in 0.01 M HCl solution (approximate pH 2.0). d This data was from ref. 24.
Fig. 2 Overlay of the docking hydroxylamine intermediate of 11 (carbons are green) on the crystallized semimercaptal adduct (carbons are off white). (A) The overall view of the binding pattern; (B) close-up view of the DprE1 active site.

Conclusions

In summary, a series of new BTZs containing hexahydropyrrolo[3,4-c]pyrrol moiety were designed and synthesized based on the spiro-BTZ IMB1603 discovered in our lab. Many of them exhibited potent in vitro anti-TB activity. Especially, compounds 11 and 24 were found to display excellent anti-MTB activity against the drug-sensitive MTB strain H37Rv (MIC < 0.035 μM), and also potent anti-MDR-MTB activity against the two drug-resistant clinical isolates (MIC, 0.053–0.102 μM). In addition, BTZs 11 and 24 showed low cytotoxicity (CC50 > 200 μM), and exhibited better water solubility than PBTZ169, suggesting both of them may serve as new and promising candidates for further antitubercular drug discovery. The molecular docking results suggested that 11 mimicked the binding pattern of PBTZ169 in the active site of DprE1. Studies to determine the PK profiles and in vivo efficacy of 11 and 24 are currently under way.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work is supported by the National Mega-project for Innovative Drugs (2018ZX09721001-004-007; 2018ZX09711001-007-002), National Natural Science Foundation of China (81872753).

References

1 C. L. Daley, Thorac. Surg. Clin., 2019, 29, 19–25.
2 World Health Organization, Global Tuberculosis Report, 2019, who.int/tb/publications/global_report/en/.
3 M. AlMatar, H. AlMandeal, I. Var, B. Kayar and F. Koksal, Biomed. Pharmacother., 2017, 91, 546–558.
4 J. Herrmann, J. Rybniker and R. Muller, Curr. Opin. Biotechnol., 2017, 48, 94–101.
5 S. Tiberi, N. du Plessis, G. Walzl, M. J. Vjecha, M. Rao, F. Ntoumi, S. Mfinanga, N. Kapata, P. Mwaba, T. D. McHugh, G. Ippolito, G. B. Migliori, M. J. Mauerer and A. Zumla, Lancet Infect. Dis., 2018, 18, e183–e198.
6 D. T. Hoagland, J. Liu, R. B. Lee and R. E. Lee, Adv. Drug Delivery Rev., 2016, 102, 55–72.
7 N. J. Ryan and J. H. Lo, Drugs, 2014, 74, 1041–1045.
8 Side Effects of bedaquiline and pretomanid, https://www.drugs.com/sfx/bedaquiline-side-effects.html, https://www.drugs.com/sfx/pretomanid-side-effects.html.
9 M. Brecik, I. Centarova, R. Mukherjee, G. S. Kolly, S. Huszar, A. Bobovska, E. Kilacska, V. Mokosova, Z. Svetlikova, M. Sarkan, J. Neres, J. Kordulakova, S. T. Cole and K. Mikusova, ACS Chem. Biol., 2015, 10, 1631–1636.
10 R. V. Chikhale, M. A. Barmade, P. R. Murumkar and M. R. Yadav, J. Med. Chem., 2018, 61, 8563–8593.
11 V. Makarov, G. Manina, K. Mikusova, U. Mollmann, O. Ryabova, B. Saint-Joanis, N. Dhar, M. R. Pasca, S. Buroni, A. P. Lucarelli, A. Milano, E. De Rossi, M. Belanova, A. Bobovska, P. Dianiskova, J. Kordulakova,
12 V. Makarov, S. T. Cole and K. Johnsson, *J. Am. Chem. Soc.*, 2010, 132, 13663–13665.

13 J. Neres, F. Pojer, E. Molteni, L. R. Chiarelli, N. Dhar, S. Boy-Rottger, S. Buroni, E. Fullam, G. Degiacomi, A. P. Lucarelli, R. J. Read, G. Zanoni, D. E. Edmondson, E. De Rossi, M. R. Pasca, J. D. McKinney, P. J. Dyson, G. Riccardi, A. Mattevi, S. T. Cole and C. Binda, *Sci. Transl. Med.*, 2012, 4, 150ra121.

14 Phase 2a study of **PBTZ169**, https://clinicaltrials.gov/ct2/show/NCT03334734.

15 Phase 1 study of **BTZ043**, https://clinicaltrials.gov/ct2/show/NCT04044001.

16 V. Makarov, B. Lechartier, M. Zhang, J. Neres, A. M. van der Sar, S. A. Raadtsen, R. C. Hartkoorn, O. B. Ryabova, A. Vocat, L. A. Decosterd, N. Widmer, T. Buclin, W. Bitter, K. Andries, F. Pojer, P. J. Dyson and S. T. Cole, *EMBO Mol. Med.*, 2014, 6, 372–383.

17 R. Tiwari, P. A. Miller, L. R. Chiarelli, G. Mori, M. Sarkan, I. Centarova, S. H. Cho, K. Mikusova, S. G. Franzblau, A. G. Oliver and M. J. Miller, *ACS Med. Chem. Lett.*, 2016, 7, 266–270.

18 L. Xiong, C. Gao, Y. J. Shi, X. Tao, J. Rong, K. L. Liu, C. T. Peng, N. Y. Wang, Q. Lei, Y. W. Zhang, L. T. Yu and Y. Q. Wei, *RSC Adv.*, 2018, 8, 11163–11176.

19 P. Li, B. Wang, X. Zhang, S. M. Batt, G. S. Besra, T. Zhang, C. Ma, D. Zhang, Z. Lin, G. Li, H. Huang and Y. Lu, *Eur. J. Med. Chem.*, 2018, 160, 157–170.

20 R. Tiwari, P. A. Miller, S. Cho, S. G. Franzblau and M. J. Miller, *ACS Med. Chem. Lett.*, 2015, 6, 128–133.

21 T. Karoli, B. Becker, J. Zuegg, U. Mollmann, S. Ramu, J. X. Huang and M. A. Cooper, *J. Med. Chem.*, 2012, 55, 7940–7944.

22 R. Zhang, K. Lv, B. Wang, L. Li, B. Wang, M. Liu, H. Guo, A. Wang and Y. Lu, *RSC Adv.*, 2017, 7, 1480–1483.

23 K. Lv, X. You, B. Wang, Z. Wei, Y. Chai, B. Wang, A. Wang, G. Huang, M. Liu and Y. Lu, *ACS Med. Chem. Lett.*, 2017, 8, 636–641.

24 K. Lv, Z. Tao, Q. Liu, L. Yang, B. Wang, S. Wu, A. Wang, M. Huang, M. Liu and Y. Lu, *Eur. J. Med. Chem.*, 2018, 151, 1–8.

25 A. Wang, K. Lv, Z. Tao, J. Gu, L. Fu, M. Liu, B. Wan, S. G. Franzblau, C. Ma, X. Ma, B. Han, A. Wang, S. Xu and Y. Lu, *Eur. J. Med. Chem.*, 2019, 181, 111595.

26 C. Trefzer, M. Rengifo-Gonzalez, M. J. Hinner, P. Schneider, V. Y. Lu, M. Zheng, B. Wang, L. Fu, W. Zhao, P. Li, J. Xu, H. Zhu, H. Jin, D. Yin, H. Huang, A. M. Upton and Z. Ma, *Antimicrob. Agents Chemother.*, 2011, 55, 5185–5193.