Communication

# Design, synthesis and antimycobacterial activity of novel nitrobenzamide derivatives

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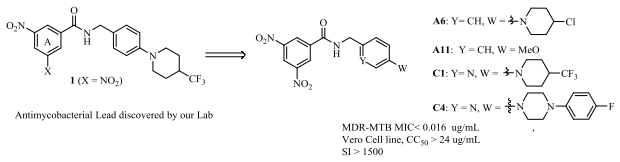
*Graphical abstract*




We report herein the design and synthesis of a series of novel nitrobenzamide derivatives. Results reveal that **A6**, **A11**, **C1** and **C4** have not only the same excellent MIC values of <0.016 μg/mL against drug-resistant clinical isolates as lead **1**, but also acceptable safety indices (SI>1500), opening a new direction for further development.

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ABSTRACT

We report herein the design and synthesis of a series of novel nitrobenzamide derivatives. Results reveal that many of them display considerable *in vitro* antitubercular activity. Four *N*-benzyl or *N*-(pyridine-2-yl)methyl 3,5-dinitrobenzamides **A6**, **A11**, **C1** and **C4** have not only the same excellent MIC values of <0.016 μg/mL against both drug-sensitive MTB strain H37Rv and two drug-resistant clinical isolates as PBTZ169 and the lead **1**, but also acceptable safety indices (SI>1500), opening a new direction for further development.

Tuberculosis (TB) has existed for millennia and remains a major global health problem [1]. It is a widespread infectious disease predominantly caused by *Mycobacterium tuberculosis* (MTB), which can be transmitted through the air as droplets and affects the lungs [2]. The World Health Organization (WHO) estimated that approximately 10.4 million people were infected and 1.3 million died from TB worldwide in 2016 [1]. The spread of multidrug-resistant TB (MDR-TB) and the emergence of extensively drug-resistant TB (XDR-TB) have reinvigorated drug discovery efforts in search of novel agents [3-6]. Despite the introduction of Bedaquiline [7] and Delamanid [8] to the repertoire of anti-TB therapies for MDR-TB, some adverse events have been noted [9]. Therefore, it is urgently needed to develop antimycobacterial molecules with new mechanisms of action and that are active against MDR- and XDR-TB [10].

Decaprenyl phosphoryl-*β*-D-ribose 2′-epimerase (DprE1) was identified as a potential target for developing potent and safer anti-TB agents [11-13]. Some new chemical entities (NCEs) were found to have potent activity against MDR/XDR-MTB as covalent or noncovalent inhibitors of the DprE1 enzyme [14-22], such as nitroaromatic compounds DNB1, MTX and PBTZ 169 (Fig. S1 in Supporting information). As the most advanced scaffold among

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these NCEs, nitrobenzothiazinones (BTZs) have garnered great interest recently, and many series of BTZ derivatives were reported [23-26]. Above all, candidate PBTZ169 entered in Phase II clinical trials in 2017 [1].

In our previous studies, many BTZs containing various cyclic ketoximes, spiro-heterocycles and piperidines moieties were found to have considerable antitubercular activity [27-29]. Recently, *N*-(4-(4-trifluoromethyl)piperidin-1-yl)benzyl nitrobenzamides **1** and **2** (Fig. 1) were identified as new anti-TB agents by the thiazinone ring opening of PBTZ169 in our lab [30]. Both of them with simpler structures than PBTZ169, show potent activity against MTB H37Rv strain (MIC ≤ 0.016 μg/mL). Moreover, compound **1** also displays acceptable safety and better PK properties than PBTZ169.

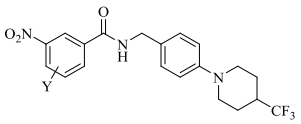
Inspired by the above research results, compounds **1** and **2** were employed as lead compounds, and the three moieties (A, B and C ring) were all explored in this study. We started with the modification of A ring and B ring. Replacement of X group on ring A with various substituents (Y) leaded to 3-nitrobenzamides bearing *N*-benzyl (**A1-4**); introduction of pyridine as A ring while reserving the nitro group gave 5-nitronicotinamides **A5**. Subsequently, the B ring was changed to pyrin-3-yl or pyrin-2-yl leading to compounds **B1-5** or **C1-3** (Fig. 1). After identifying the optimal A and B rings, C ring was then further investigated. Our primary objective was to find optimized benzamides with potent antimycobacterial activity. A preliminary structure-activity relationship (SAR) study was also explored to facilitate the further development of these compounds.

Detailed synthetic pathways to side chains **6–8**, leads **1**, **2** and targets **A–C** are shown in Schemes S1 and S2 (Supporting information), respectively. Commercially unavailable benzylamines and pyridinylmethylamines **6–8** were first prepared according to Scheme S1. 4-Fluorobenzonitrile **3**, 6-fluoronicotinonitrile **4** and 5-fluoropicolinonitrile **5** were treated with various nitrogen heterocyclic amines ZH in DMSO in the presence of $K_2CO_3$ at 80 °C, and the resulting condensates were subsequently reduced with $LiAlH_4$ in THF to produce the desired compounds **6**, **7** and **8**, respectively.

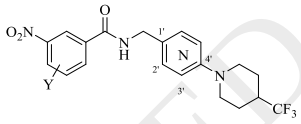
Leads **1**, **2** and targets **A1-11, B1-21**, **C1-4** were easily obtained by coupling 3-nitrobenzoic acids **9–13** and 5-nitronicotinic acid **14** with the above side chain compounds **6–8** or commercially available benzylamines **15a–d** in the presence of triethylamine and condensation agent bis(2-oxo-3-oxazolidinyl) phosphonic chloride (BOP-Cl) (Scheme S2).

**Table 1**

Structures and activity of compounds **A–C** against MTB H37Rv.



**A1-4**



**B1-4** (pyrin-3-yl) **C1, C2** (pydin-2-yl)

| Compd. | Y | MIC (μg/mL) | Compd. | Y | MIC (μg/mL) |
|---|---|---|---|---|---|
| **1** | 5-$NO_2$ | <0.016 | **B3** | 4,6-di-Cl | >16 |
| **2** | 5-$CF_3$ | 0.016 | **B4** | H | 15.354 |
| **A1** | 5-F | 1.357 | **B5** | | 15.176 |
| **A2** | 5-Br | 0.459 | **C1** | 5-$NO_2$ | <0.016 |
| **A3** | 4,6-di-Cl | >16 | **C2** | H | 31.088 |
| **A4** | H | >16 | **C3** | | 15.732 |
| **A5** | | 14.735 | PBTZ169 | | <0.016 |
| **B1** | 5-$NO_2$ | 0.059 | INH | | 0.0781 |
| **B2** | 5-Br | 0.944 | RFP | | 0.0781 |

INH: isoniazid; RFP: rifampicin.

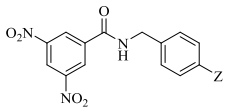
The target compounds **A1–5**, **B1–5** and **C1–3** bearing different kinds of substituents to ensure A and B rings flexibility and structure diversity, were first synthesized. They were preliminarily screened for *in vitro* activity against MTB H37Rv ATCC27294 strain, using the Microplate Alamar Blue Assay (MABA) [31,32]. 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 the leads **1** and **2**, PBTZ169, isoniazid (INH), and rifampicin (RFP) for comparison were obtained from three independent experiments and presented in μg/mL in Table 1.

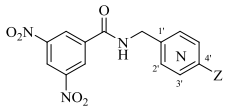
Effect of the substituents on A ring was first investigated. The nature and position of the substituents greatly influence activity. Replacement of one nitro group of **1** or the trifluoromethyl of **2** with halogen in compounds **A1**（F） and **A2** (Br) leads to decreased activity (MIC: 1.357 and 0.459 μg/mL, respectively). Introduction of 4,6-dichloro (**A3**) or reservation of one nitro (**A4**) destroys activity. Moreover, *N*-benzyl nicotinamide analogue (**A5**) displays very poor potency. Overall, these results reveal that the presence of a strong electron-withdrawing group ($CF_3$, $NO_2$) at C-5 position of nitrobenzamide core is essential for excellent activity (Table 1).

**Table 2**

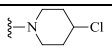
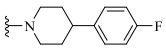
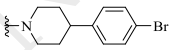
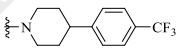
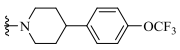
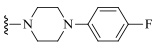
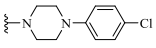
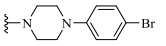
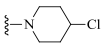
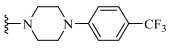
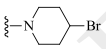
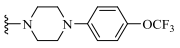
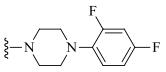
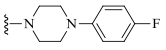
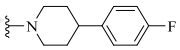
Structures and activity of 3,5-dinitrobenzamides **A–C** against MTB H37Rv.



**A6-11**



**B6-21** (pyrin-3-yl) **C4** (pydin-2-yl)

| Compd. | Z | MIC (μg/mL) | Compd. | Z | MIC (μg/mL) |
|---|---|---|---|---|---|
| **A6** |  | <0.016 | **B12** |  | 0.452 |
| **A7** |  | 0.060 | **B13** |  | 0.235 |
| **A8** | F | 0.120 | **B14** |  | 0.480 |
| **A9** | $CF_3$ | 0.059 | **B15** |  | 1.255 |
| **A10** | $OCF_3$ | 0.033 | **B16** |  | 0.210 |
| **A11** | $OCH_3$ | <0.016 | **B17** |  | 0.178 |
| **B6** |  | 0.094 | **B18** |  | 0.233 |
| **B7** |  | 0.030 | **B19** |  | 0.491 |
| **B8** |  | 0.030 | **B20** |  | 0.973 |
| **B9** |  | 0.108 | **B21** |  | 0.143 |
| **B10** |  | 0.059 | **C4** |  | <0.016 |
| **B11** |  | 0.119 | PBTZ169 | | <0.016 |

In further modifications, the benzene ring (B ring) was replaced by a pyridine ring. As shown in Table 1, in accordance with SAR of *N*-benzyl analogues (**A1**–**5**), *N*-(pyridin-3-yl)methyl and *N*-(pyridin-2-yl)methyl 3,5-dinitrobenzamides (**B1**, **C1**) demonstrate potent MIC values of 0.059 and <0.016 μg/mL against this strain, respectively, indicating that *N*-pyridinylmethyl on the amide is also acceptable.

Based on the above SAR, and better activity of lead compound **1** than **2**, *N*-benzyl and *N*-pyridinylmethyl 3,5-dinitrobenzamides with various groups at *para*-position of B ring were further designed and synthesized. As shown in Table 2, all of them show good to excellent activity against MTB H37Rv strain (MIC: <0.016–0.973 μg/mL), with

one exception **B15**. Among them, nine compounds **A6, 7, 9**–**11**, **B7, 8, 10** and **C4** (MIC: <0.016–0.060 μg/mL) are more active than INH/RFP (MIC: 0.0781 μg/mL), and roughly comparable to PBTZ169.

[a]MDR-TB 16833 and MDR-TB 16995 were isolated from patients in Beijing Chest Hospital; [b]the 50% cytotoxic concentration; [c]SI: selectivity index for MTB H37Rv, $CC_{50}$ / MIC

For *N*-benzyl 3,5-dinitrobenzamides, the presence of a halogen atom instead of trifluoromethyl at *para*-position of the piperidine ring (C ring) was found to be also favorable. For example, compound **A6** shows the same MIC value of <0.016 μg/mL as the lead **1**. Introduction of an additional aromatic moiety on C ring, such as 4-fluorophenyl (**A7**, MIC: 0.059 μg/mL), is also acceptable. More interestingly, removal of C ring and direct attachment of a simple group to B ring remain considerable activity (**A8**–**11**), and an electron-donating group ($OCH_3$) is preferred over an electron-withdrawing one ($CF_3$, $OCF_3$) or a halogen atom (F).

For *N*-(pyridin-3-yl)methyl 3,5-dinitrobenzamides, the presence of a halogen atom (Cl, Br) instead of trifluoromethyl on C ring is more beneficial to activity (**B1** *vs.* **B7** and **B8**), and replacement of C ring in **B1** with thiomorpholine in compound **B10** maintains the same potent activity (MIC: 0.059 μg/mL). However, introduction of 4-substituented phenyls on C ring, or replacement of the piperidine with piperazines bearing a substituted phenyl moiety leads decreased activity (**B1** *vs* **B11**–**21**). Conversely, *N*-(pyridin-2-yl)methyl compound **C4** with a 4-(fluorophenyl)piperazine as C ring, displays the same potent MIC value of <0.016 μg/mL as **C1**, much more active than the corresponding *N*-(pyridin-3-yl)methyl analogue **B16** (MIC: 0.210 μg/mL) (Table 2).

Encouraged by their strong potency against the drug sensitive MTB H37Rv strain (MIC: <0.016–0.060 μg/mL), eleven 3,5-dinitrobenzamide derivatives **A6**, **7**, **9**–**11**, **B1**, **7**, **8**, **10** and **C1**, **4** were further evaluated against two clinical isolated MTB-MDR (16833 and 16995) strains resistant to both INH and RFP. The cytotoxic potential of these compounds was also investigated in a mammalian Vero cell line by MTS assay. As shown in Table 3, all of them exhibit potent MIC values of <0.016–0.071 μg/mL, similar to that against MTB H37Rv. Among of them, compounds **A6**, **A11, C1** and **C4** have the same excellent activity (MIC: <0.016 μg/mL) as PBTZ169 and the lead **1**. With a few exceptions, these compounds ($CC_{50}$: 22.63–34.57 μg/mL) are less cytotoxic than the lead **1**, although generally more cytotoxic than PBTZ169.

Lipinski's rules are important guidelines for determining drug-likeness compounds [33]. The related values of most potent compounds **A6**, **A11**, **C1** and **C4** were calculated using the online chemo-informatics software molinspiration (http://www.molinspiration.com). As shown in Table S1 (Supporting information), none violation of Lipinski's rule-of-five was found among compounds **A6**, **A11**, and **C1**. The hydrogen bond acceptors of compound **C4** (HBA = 11) are more than the recommended number (HBA <10). However, compound **C4** is still incorporate with the Lipinski's rule-of-five (violations ≤1). Thus, these compounds display good drug like properties, are all deserved further development.

In conclusion, a series of nitrobenzamide derivatives containing *N*-benzyl or *N*-pyridinylmethyl moieties, based on lead compounds **1** and **2** discovered in our lab, were designed and synthesized as new anti-TB agents. Many of them exhibit potent *in vitro* antitubercular activity. Especially, *N*-benzyl 3,5-dinitrobenzamides **A6** and **A11**, and *N*-(pyridine-2-yl)methyl analogues **C1** and **C4** have not only the same excellent activity (MIC: <0.016 μg/mL) against both drug-sensitive MTB strain H37Rv and two drug-resistant clinical isolates as PBTZ169 and the lead **1**, but also have acceptable safety indices (SI: >1500). In addition, compounds **A6**, **A11**, **C1** and **C4** display good drug like properties, suggesting these compounds may serve as new and promising candidates for further antitubercular drug discovery. By the way, the further expansion of the 3,5-dinitrobenzamides is underway to find potent anti-TB agents.

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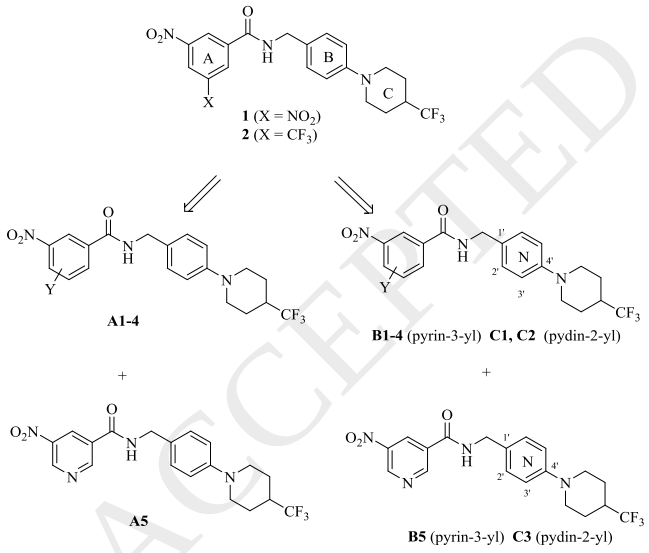


**Fig. 1**. Design of the new molecules.

**Table 3**

Activity against MDR-MTB, cytotoxicity and selectivity index (SI) values for selected compounds.

| Compd. | MIC (μg/mL) | | $CC_{50}$[b] (μg/mL) | SI[c] |
|---|---|---|---|---|
| | MDR-MTB 16833[a] | MDR-MTB 16995[a] | | |
| **1** | <0.016 | <0.016 | 20.15 | >1259 |
| **A6** | <0.016 | <0.016 | 24.74 | >1546 |
| **A7** | 0.071 | 0.056 | 10.51 | 175 |
| **A9** | 0.070 | 0.042 | 33.62 | 569 |
| **A10** | 0.029 | 0.056 | 31.21 | 945 |
| **A11** | <0.016 | <0.016 | 28.02 | >1751 |
| **B1** | 0.043 | 0.028 | 16.80 | 284 |
| **B7** | 0.030 | 0.056 | 23.17 | 772 |
| **B8** | 0.030 | 0.029 | 22.63 | 754 |
| **B10** | 0.063 | 0.060 | 17.60 | 298 |
| **C1** | <0.016 | <0.016 | 26.61 | >1663 |
| **C4** | <0.016 | <0.016 | 34.57 | >2160 |
| PBTZ169 | <0.016 | <0.016 | 36.68 | >2292 |
| INH | >40 | >40 | NT | |
| RFP | >40 | >40 | NT | |