Communication

Design, synthesis and antimycobacterial activity of novel nitrobenzamide derivatives

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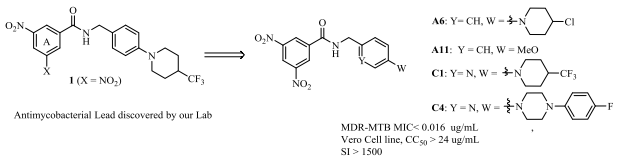
Graphical abstract



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.

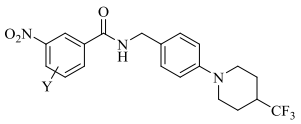
Inspired by the above research results, compounds **1** and **2** were employed as lead compounds, and the three moieties (A, B and C ring) were all explored in this study. We started with the modification of A ring and B ring. Replacement of X group on ring A with various substituents (Y) leaded to 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.

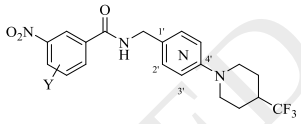
Leads **1**, **2** and targets **A1-11, B1-21**, **C1-4** were easily obtained by coupling 3-nitrobenzoic acids **9–13** and 5-nitronicotinic acid **14** with the above side chain compounds **6–8** or commercially available benzylamines **15a–d** in the presence of triethylamine and condensation agent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

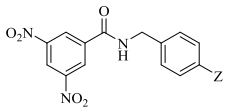
The target compounds **A1–5**, **B1–5** and **C1–3** bearing different kinds of substituents to ensure A and B rings flexibility and structure diversity, were first synthesized. They were preliminarily screened for *in vitro* activity against MTB H37Rv ATCC27294 strain, using the Microplate Alamar Blue 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.

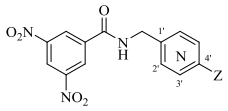
Effect of the substituents on A ring was first investigated. The nature and position of the substituents greatly influence activity. Replacement of one nitro group of **1** or the trifluoromethyl of **2** with halogen in compounds **A1**（F） and **A2** (Br) leads to decreased activity (MIC: 1.357 and 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

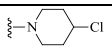
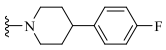
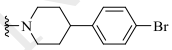
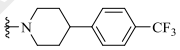
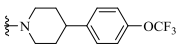
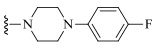
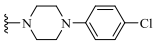
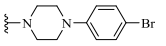
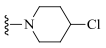
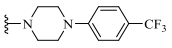
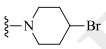
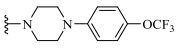
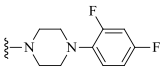
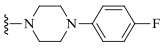
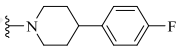
Structures and activity of 3,5-dinitrobenzamides **A–C** against MTB H37Rv.



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.

Acknowledgments

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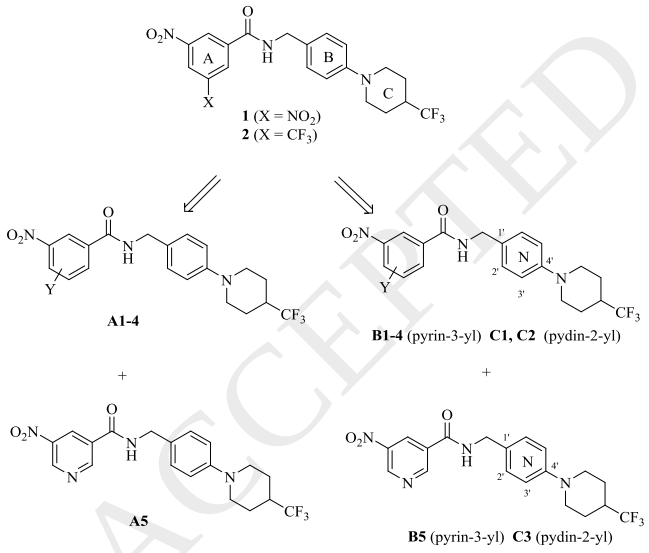

Fig. 1. Design of the new molecules.

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	