Identification of Pyrazolo[1,5-*a*]pyridine-3-carboxamide Diaryl Derivatives as Drug Resistant Antituberculosis AgentsXianglong Hu,^{†,‡} Baojie Wan,^{‡,§} Yang Liu,^{§,‡} Jiayi Shen,[†] Scott G. Franzblau,^{‡,§} Tianyu Zhang,^{*,§} Ke Ding,^{*,†} and Xiaoyun Lu^{*,†}[†]International Cooperative Laboratory of Traditional Chinese Medicine Modernization and Innovative Drug Development of Chinese Ministry of Education (MOE), School of Pharmacy, Jinan University, 601 Huangpu Avenue West, Guangzhou 510632, China[‡]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States[§]State Key Laboratory of Respiratory Disease, Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, 190, Kai Yuan Avenue, Guangzhou 510530, China

Supporting Information

ABSTRACT: A series of pyrazolo[1,5-*a*]pyridine-3-carboxamide (PPA) derivatives bearing diaryl side chain was designed and synthesized as new antituberculosis agents, aiming to improve the efficacy toward drug resistant *Mycobacterium tuberculosis* (*Mtb*) strains. Most of the substituted diphenyl and heterodiaryl PPAs exhibited excellent *in vitro* potency against the drug susceptible H37Rv strain (MIC < 0.002–0.381 $\mu\text{g/mL}$) and drug resistant *Mtb* strains (INH-resistant (rINH), MIC < 0.002–0.465 $\mu\text{g/mL}$; RMP-resistant (rRMP), MIC < 0.002–0.004 $\mu\text{g/mL}$). Noticeably, some compounds also showed very low cytotoxicity against Vero cells. Further, compound 6j displayed good pharmacokinetic profiles with oral bioavailability (F) of 41% and significantly reduced the bacterial burden in an autoluminescent H37Ra infected mouse model.

KEYWORDS: Antituberculosis agents, INH-resistant *Mtb* strain, RMP-resistant *Mtb* strains, structure–activity relationship

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), remains the leading infectious cause of death worldwide. It was estimated that nearly 10.4 million people fell ill with TB and 1.7 million died of TB in 2016.¹ A standard 6–9 months regimen recommended by World Health Organization (WHO) results in multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) due to lack of patient adherence.^{2,3} At present, MDR-TB usually has to be treated with a combination of five to seven drugs lasting up to 18–24 months.⁴ Therefore, new drugs with the potential to shorten the duration of treatment or overcome drug-resistant TB are urgently needed. Encouragingly, bedaquiline (TMC207, 1)^{5,6} and delamanid (OPC67683, 2)⁷ were approved for the treatment of MDR-TB by FDA in 2012 and by EMA in 2014, respectively (Figure 1), even though the wide-scale use of 1 is restricted for safety risks.⁸ It is still imperative to identify new molecules with alternative scaffolds as effective agents to banish the scourge of TB.

The other two nitroimidazole drugs PA-824 (3) and TBA-354 (4) are currently in clinical studies for drug-sensitive and drug-resistant TB (Figure 1).^{9,10} TBA-354, only with a 4-trifluoromethoxyphenyl-pyridine group to replace the 4-trifluoromethoxyphenyl in PA-824, is promising to shorten TB treatment as part of a regimen superior to that of PA-824 in

Figure 1. Chemical structures of antitubercular drugs bedaquiline, delamanid, PA-824, and TBA-354.

terms of anti-TB potency and improved pharmacokinetic properties.¹¹ Our previous studies had identified a series of pyrazolo[1,5-*a*]pyridine-3-carboxamides (PPAs) as antituberculosis agents (Figure 2), which exhibited good activities with low nanomolar MIC values against H37Rv strain (Figure 2).^{12,13} Considering PPAs linked with the *N*-benzyl moiety

Received: September 6, 2018

Accepted: February 21, 2019

Published: February 21, 2019

Figure 2. Design of pyrazolo[1,5-*a*]pyridine-3-carboxamide diaryl derivatives.

were crucial for the antitubercular activity, we first envisioned to make structural modifications on the side chain of lead **5** by introducing substituted diaryl (diphenyl or heterodienyl) groups, which was similar to the optimization strategy of TBA354 and would possibly target drug resistant *Mtb* strains (Figure 2). In addition, our previous SAR study had suggested that the 2-methyl-5-methoxy-pyrazolo[1,5-*a*]pyridine scaffold-based compound displayed better potency than the related 2-methyl-5-methyl-pyrazolo[1,5-*a*]pyridine against drug resistant clinical *Mtb* isolates.¹² Thus, a series of 2-methyl-5-methoxy-pyrazolo[1,5-*a*]pyridine-3-carboxamide diaryl derivatives were designed and synthesized.

Compounds **6a–6l** and **6o–6p** were readily synthesized using a straightforward amidation of pyrazolo[1,5-*a*]pyridine-3-carboxylic acid **10**¹² with different self-prepared primary amines **9**, **13**, **16**, and **19** (Scheme 1). Different strategies were

Scheme 1. Synthesis of Compounds **6a–6l** and **6o–6p**^a

^aReagents and conditions: (a) (4-(trifluoromethoxy)phenyl)boronic acid, Pd(PPh₃)₄, Na₂CO₃, toluene, 110°C, overnight, 83–92%; (b) LiAlH₄, THF, −40°C, 3 h, 48–53%; (c) HATU, DIPEA, DCM, rt, overnight, 49–53%; (d) i. NaHCO₃, Boc₂O, THF, rt, 3 h, 97%; ii. NBS, DMF, rt, 5 h, 82%; (e) i. 4-(trifluoromethoxy)phenylboronic acid, K₂CO₃, Pd(PPh₃)₄, DME, 80°C, 4 h, 87%; ii. TFA, DCM, rt, 5 h, 82%; (h) hydrochloric acid, NaNO₂, NaN₃, H₂O, 0 °C → rt, 2.5 h, 92%; (i) 2-propynylamine, CuI, THF, DIPEA, rt, overnight, 70%; (j) Boc-glycine, HATU, DIPEA, DMF, rt, overnight, 85%; (k) i. Et₃N, PPh₃, CCl₄, DMF, rt, overnight, 68%; ii. TFA, DCM, rt, 2 h, 98%.

used to synthesize these amines. Briefly, the amines **8** were synthesized by Suzuki coupling bromo-substituted materials **7** and (4-(trifluoromethoxy)phenyl)boronic acid followed by nitrile reduction with lithium aluminum hydride (Supporting Information). The synthesis of (5-(4-(trifluoromethoxy)phenyl)thiophen-2-yl)methanamine **13** was started by Boc protection and bromination of thiophen-2-ylmethanamine **11** to give bromothiophene **12**, which was followed by Suzuki coupling and deprotection reactions. The (1-(4-(trifluoromethoxy)phenyl)-1*H*-1,2,3-triazol-4-yl)methanamine

16 was obtained by diazo and click reactions starting from 4-(trifluoromethoxy)aniline **14**. The (5-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)methanamine **19** was synthesized starting from **17** that was condensed with BOC-glycine, then followed by cyclization and deprotection reactions based on the published procedures.¹⁴

The other two compounds **6m** and **6n** were synthesized in Scheme 2. The procedure was started by condensation of *tert*-

Scheme 2. Synthesis of Compounds **6m** and **6n**^a

^aReagents and conditions: (a) HATU, DIEA, DCM, rt, overnight, 70%; (b) TFA, DCM, rt, 2 h, 98%; (c) Cs₂CO₃, EtOH, DMF, rt, overnight, 46%; (d) ammonium acetate, xylene, 140°C, 6 h, 56%; (e) acetamide, xylene, 140°C, 3 h, 45%.

butyl glycinate **20** and pyrazolo[1,5-*a*]pyridine-3-carboxylic acid **10**, followed by hydrolysis in TFA to give intermediate **22**. The intermediate **22** was then reacted with 2-bromo-1-(4-(trifluoromethoxy)phenyl)ethan-1-one **23** to give the ester **24**, followed by cycloaddition with ammonium acetate and acetamide to produce compounds **6m** and **6n**, respectively.

The minimum inhibitory concentration (MIC) values of all the new compounds were preliminarily screened against *Mtb* H37Rv strain in microplate alamar blue assay (MABA) and low oxygen recovery assay (LORA) with the assay concentrations ranging from 0.02 to 5 μg/mL (Table 1).¹⁵ Compounds showing encouraging MICs (<0.02 μg/mL) in MABA were further tested against *Mtb* H37Rv, rRMP, and rINH with the assay concentrations ranging from 0.002 to 0.5 μg/mL and were also tested against Vero Cells to assess the compounds' potential cytotoxicity (Table 2). Rifampicin (RMP), isoniazid (INH), linezolid (LIZ), and TMC207 were used as positive control drugs to support the reliability of our screening results.

The first round of modifications was to investigate the role of the substituents at the 3-position of the phenyl group (Figure 2). Therefore, keeping intact the diphenyl chain, small functional groups (CH₃, OCH₃, CF₃, F) were introduced at the 3-position of the phenyl group. The resulting compounds **6a–6d** exhibited strong antitubercular activity against *Mtb* H37Rv with all MICs values of <0.02 μg/mL at the first round assay with concentrations ranging from 0.02 to 5 μg/mL (Table 1). This prompted us to gain further insights into the exact MICs at lower concentrations. Under the second screening concentrations (0.002–0.5 μg/mL), the MICs values of compounds **6a–6d** were 0.013, 0.007, 0.029, and <0.002 μg/mL, respectively, which were comparable to that of RMP, TMC207, and the lead compound **5** (MIC = 0.006 μg/mL) (Table 2).¹² These results suggested that this position is tolerated for substitution, especially for the OCH₃ and F

Table 1. *In Vitro* Antitubercular Activity of Compounds 6a–6p against the *Mtb* Strains H37Rv in MABA and LORA with the Assay Concentrations Ranging from 0.02 to 5 $\mu\text{g/mL}$

Compds	Ar Or Het	H37Rv MIC ($\mu\text{g/mL}$)		ClogP ^a
		MABA	LORA	
6a		<0.02	2.28	5.87
6b		<0.02	>5	5.20
6c		<0.02	>5	6.23
6d		<0.02	>5	5.50
6e		0.57	>5	5.87
6f		0.28	2.25	5.20
6g		>5	>5	6.23
6h		<0.02	>5	5.50
6i		<0.02	4.36	4.52
6j		<0.02	>5	4.22
6k		0.53	>5	4.52
6l		<0.02	>5	5.26
6m		>5	>5	3.23
6n		1.96	2.84	3.29
6o		>5	>5	2.79
6p		>5	>5	3.49
RMP	-	0.02	2.95	-
INH	-	0.48	>128	-
LIZ	-	3.27	5.98	-
TMC207	-	0.03	-	-

^aCalculated by MarvinSketch 5.4.1.1 version.

groups. Switching these substituents from the 3 to 2 position of the phenyl group, an almost 43- and 40-fold reduction in activity was noticed for compounds 6e (MIC = 0.57 $\mu\text{g/mL}$) and 6f (MIC = 0.28 $\mu\text{g/mL}$), whereas the antitubercular activity of compound 6g was completely lost when the CF_3 was introduced at the 2 position. Interestingly, introduction of F atom in the 2 position led to compound 6h (MIC = 0.007 $\mu\text{g/mL}$), which kept potent antitubercular activity compared to compound 6d. A plausible explanation of this result may be that F atom does not affect the activity due to its relatively small size (similar to that of H atom).

Table 2. *In Vitro* Antitubercular Activity of Compounds 6a–6d, 6h–6j, and 6l against the *Mtb* Strains H37Rv, rRMP, and rINH with the Assay Concentrations Ranging from 0.002 to 0.5 $\mu\text{g/mL}$, and Vero Cellular Toxicity

compds	MIC ($\mu\text{g/mL}$)			VERO cell IC ₅₀ ($\mu\text{g/mL}$)	selectivity index (SI) ^a
	H37Rv	rRMP	rINH		
6a	0.013	<0.002	<0.002	3.15	242
6b	0.007	<0.002	0.003	11.8	1685
6c	0.029	<0.002	0.003	11.6	402
6d	<0.002	<0.002	0.004	>50	>25000
6h	0.007	<0.002	0.004	>50	>7142
6i	0.053	0.458	0.004	28.4	535
6j	<0.002	<0.002	0.002	>50	>25000
6l	<0.002	0.465	0.002	>50	>25000
RMP	<0.008	>2	<0.008	>100	>12500
INH	0.239	0.438	>8		
LIZ	1.254	1.870	0.953		
TMC207	0.020	<0.016	<0.016	>10	>500

^aSI: IC₅₀/MIC for H37Rv.

Like TBA-354, the pyridine ring was used to replace the phenyl group. The yielded compound 6i showed an MIC value of 0.053 $\mu\text{g/mL}$, which was better than that of the control drug INH and LIZ, and comparable to TMC207 (Table 2). Moving the N atom from the 3 to 2 position led to compound 6j, with improved antitubercular activity against H37Rv to <0.002 $\mu\text{g/mL}$. Inspired by the activities of compounds 6i and 6j, we further investigated the five-membered heterocyclic ring instead of the phenyl group. However, only the thiophene derivative 6l (MIC < 0.002 $\mu\text{g/mL}$) had the MIC value comparable to 6j. The thiazole 6k (MIC = 0.53 $\mu\text{g/mL}$) and oxazole 6n (MIC = 1.96 $\mu\text{g/mL}$) had almost 265- and 980-fold reduction in activities compared to 6j (Table 1). The imidazole 6m, oxadiazole 6o, and triazole 6p completely lost the activities with all MICs values >5 $\mu\text{g/mL}$. Briefly, it was suggested that a five-membered heterocyclic ring such as thiophene can substitute the phenyl, but others led to loss of activity in varying degrees.

The compounds were also tested in LORA, which is an *in vitro* model for the assessment of activity against persistent *Mtb*. Among the current antitubercular drugs, only RMP and PZA have been reported to possess activity against dormant strain. From the results (Table 1), it was very remarkable that compounds 6a (MIC = 2.28 $\mu\text{g/mL}$), 6f (MIC = 2.25 $\mu\text{g/mL}$), and 6n (MIC = 2.84 $\mu\text{g/mL}$) exhibited good LORA MICs values comparable to that of RMP (MIC = 2.95 $\mu\text{g/mL}$). However, most of the other compounds were not very active in LORA with MIC values of >5 $\mu\text{g/mL}$.

Encouraged by their strong potencies against the drug sensitive H37Rv strain, compounds 6a–6d, 6h–6j, and 6l were further evaluated against rRMP and rINH strains. It was shown that all of the compounds displayed excellent potencies against rRMP and rINH strains with MIC values <0.002–0.465 and <0.002–0.004 $\mu\text{g/mL}$ (Table 2). Moreover, the *in vitro* Vero cell toxicity of these compounds was further determined. As it was shown in Table 2, the compounds had low or no cytotoxicity against VERO cells with IC₅₀ values from 3.15 to >50 $\mu\text{g/mL}$. Particularly, compounds 6d, 6j, and 6l had no cytotoxicity against VERO cells, with all of the IC₅₀ values of >50 $\mu\text{g/mL}$ and the selectivity index (SI) values of >25000.

Combined with other factors such as ClogP, we selected compound **6j** for further study. The pharmacokinetic (PK) properties of compound **6j** were evaluated in rats by single intravenous injection (i.v. 2.5 mg/kg) and oral administration (p.o. 10 mg/kg). As shown in Table 3, **6j** exhibited a half-life of

Table 3. Pharmacokinetic Profile of Compound **6j** in Rats

route	dose (mg/kg)	AUC _{0-∞} (h·μg/L)	C _{max} (μg/L)	T _{max} (h)	T _{1/2} (h)	MRT _{0-∞} (h)	F (%)
oral	10	15638	1428	4.67	5.10	8.21	41
iv	2.5	9002	4152	0.08	3.49	4.02	

5.1 h and a relative high optimal plasma exposure with AUC value of 15638 h·μg/L. It also displayed a good oral bioavailability of 41%, suggesting its good absorption in rats.

Given the promising antitubercular activity against H37Rv and good PK properties in rats, **6j** was evaluated for its *in vivo* antitubercular efficacy by using the mouse model infected with the selectable marker-free autoluminescent *Mtb* strain H37Ra.^{12,16} The animals were infected with log-phase autoluminescent *Mtb* H37Ra via intravenous injection (2×10^6 CFU per mouse) and then were repeatedly administered with **6j** once daily via oral gavage for 5 consecutive days. The bacterial burden was measured by monitoring the bioluminescence intensity (relative light unit, RLU) from the same batch of live mice every day. As shown in Figure 3, with

Figure 3. Mean RLU count ($\pm$ SD) assessed at day 0 and the day after treatment completion (A) for lung and (B) for spleen. y-axis, corresponding Log₁₀RLU/lung; x-axis, different treatment groups (ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

the two tested doses of 10 and 50 mg/kg/day, compound **6j** exhibited dose-dependent *in vivo* antitubercular activity. The **6j** 50 mg/kg/day group showed a significant reduction in lung RLU by ~ 1.0 log₁₀ compared with the untreated CMC-Na group (Figure 3A). The **6j** 50 mg/kg/day group also reduced the spleen bacterial burdens by ~ 0.61 log₁₀ RLU relative to that of CMC-Na group. The RMP-treated group showed decreases by ~ 1.42 log₁₀ RLU and ~ 1.34 log₁₀ RLU in lung and spleen, respectively. These results strongly suggest the promising potential of compound **6j** to serve as a lead compound for further antitubercular drug discovery.

A series of PPA diaryl derivatives has been synthesized and evaluated for the antitubercular activity. Most of the derivatives exhibited excellent *in vitro* potency against the drug susceptible H37Rv strain (MIC < 0.002 – 0.381 μg/mL) and two rINH (MIC < 0.002 – 0.465 μg/mL) and rRMP (MIC < 0.002 – 0.004 μg/mL) *Mtb* strains. The representative compound **6j** exhibited promising *in vitro* activities against *Mtb* H37Rv, rRMP, and rINH with MIC values ≤ 0.002 μg/mL and no toxicity against Vero cells. Further *in vivo* studies indicated that compound **6j** displayed good pharmacokinetic profiles with an

oral bioavailability of 41% and significantly reduced the mycobacterial burden in an H37Ra infected mouse model.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00410.

Chemistry procedures and analytical data for title compounds, and biological assay (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: 020-85223259. Fax: 020-85224766. E-mail: zhang_tianyu@gibh.ac.cn.

*E-mail: dingke@jnu.edu.cn.

*E-mail: luxy2016@jnu.edu.cn.

ORCID

Scott G. Franzblau: 0000-0002-8698-0243

Ke Ding: 0000-0001-9016-812X

Xiaoyun Lu: 0000-0001-7931-6873

Author Contributions

#These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Guangdong Natural Science Funds (2016A030313106, 2015A030306042), National Natural Science Foundation of China (81673285), Guangzhou City Key Laboratory of Precision Chemical Drug and Chinese Academy of Sciences Grants (154144KYSB20150045, YJ-KYYQ20170036), and Guangzhou Municipal Industry and Research Collaborative Innovation Program (201508020248, 201604020019) for the financial support. We thank Jian Tang for establishing the synthetic method of intermediates 13 and 16 in Scheme 1.

■ ABBREVIATIONS

TB, tuberculosis; PPA, pyrazolo[1,5-a]pyridine-3-carboxamide; *Mtb*, *Mycobacterium tuberculosis*; WHO, World Health Organization; MDR-TB, multidrug-resistant TB; XDR-TB, extensively drug-resistant TB; MIC, minimum inhibitory concentration; MABA, microplate alamar blue assay; MRT, mean residence time; LORA, low oxygen recovery assay; RMP, rifampicin; INH, isoniazid; LIZ, linezolid; RLU, relative light unit; rINH, INH-resistant; rRMP, RMP-resistant; DIPEA, *N,N*-diisopropylethylamine; HATU, 2-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; EMA, European Medicines Agency; FDA, Food and Drug Administration

■ REFERENCES

- (1) Global Tuberculosis Report 2017. https://www.who.int/tb/publications/global_report/archive/en/ (accessed December 2017).
- (2) World Health Organization Treatment of Tuberculosis: Guidelines for National Programmes; WHO/HTM/TB/2009.420, 4th ed., 2009.
- (3) Zumla, A. I.; Gillespie, S. H.; Hoelscher, M.; Philips, P. P.; Cole, S. T.; Abubakar, I.; McHugh, T. D.; Schito, M.; Maeurer, M.; Nunn, A. J. New antituberculosis drugs, regimens, and adjunct therapies: needs, advances, and future prospects. *Lancet Infect. Dis.* **2014**, *14*, 327–340.

(4) Guidelines Approved by the Guidelines Review Committee. WHO Treatment Guidelines for Drug-Resistant Tuberculosis, 2016 Update; World Health Organization: Geneva, 2016.

(5) Andries, K.; Verhasselt, P.; Guillemont, J.; Göhlmann, H. W.; Neefs, J. M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* **2005**, *307*, 223–227.

(6) Diacon, A. H.; Donald, P. R.; Pym, A.; Grobusch, M.; Patientia, R. F.; Mahanyele, R.; Bantubani, N.; Narasimooloo, R.; De Marez, T.; van Heeswijk, R.; Lounis, N.; Meyvisch, P.; Andries, K.; McNeeley, D. F. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob. Agents Chemother.* **2012**, *56*, 3271–3276.

(7) Thakare, R.; Soni, I.; Dasgupta, A.; Chopra, S. Delamanid for the treatment of pulmonary multidrug-resistant tuberculosis. *Drugs Today* **2015**, *51*, 117–123.

(8) Cox, E.; Laessig, K. FDA approval of bedaquiline—the benefit-risk balance for drug-resistant tuberculosis. *N. Engl. J. Med.* **2014**, *371*, 689–691.

(9) Singh, R.; Manjunatha, U.; Boshoff, H. I.; Ha, Y. H.; Niyomrattanakit, P.; Ledwidge, R.; Dowd, C. S.; Lee, I. Y.; Kim, P.; Zhang, L.; Kang, S.; Keller, T. H.; Jiricek, J.; Barry, C. E. PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO release. *Science* **2008**, *322*, 1392–1395.

(10) Kmentova, I.; Sutherland, H. S.; Palmer, B. D.; Blaser, A.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Denny, W. A.; Thompson, A. M. Synthesis and structure-activity relationships of aza- and diazabiphenyl analogues of the antitubercular drug (6S)-2-nitro-6-{{4-(trifluoromethoxy)benzyl}oxy}-6,7-dihydro-5H-imidazo[2,1-b]-[1,3]oxazine (PA-824). *J. Med. Chem.* **2010**, *53*, 8421–8439.

(11) Upton, A. M.; Cho, S.; Yang, T. J.; Kim, Y.; Wang, Y.; Lu, Y.; Wang, B.; Xu, J.; Mdluli, K.; Ma, Z.; Franzblau, S. G. In vitro and in vivo activities of the nitroimidazole TBA-354 against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **2015**, *59*, 136–144.

(12) Tang, J.; Wang, B. X.; Wu, T.; Wan, J. T.; Tu, Z. C.; Njire, M.; Wan, B. J.; Franzblau, S. G.; Zhang, T. Y.; Lu, X. Y.; Ding, K. Design, synthesis and biological evaluation of pyrazolo[1,5-a]pyridine-3-carboxamides as novel antitubercular agents. *ACS Med. Chem. Lett.* **2015**, *6*, 814–818.

(13) Lu, X. Y.; Tang, J.; Cui, S. Y.; Wan, B. J.; Franzblau, S. G.; Zhang, T. Y.; Zhang, X. T.; Ding, K. Pyrazolo[1,5-a]pyridine-3-carboxamide hybrids: Design, synthesis and evaluation of antitubercular activity. *Eur. J. Med. Chem.* **2017**, *125*, 41–48.

(14) van der Westhuyzen, R.; Winks, S.; Wilson, C. R.; Boyle, G. A.; Gessner, R. K.; Soares de Melo, C.; Taylor, D.; de Kock, C.; Njoroge, M.; Brunschwig, C.; Lawrence, N.; Rao, S. P. S.; Sirgel, F.; van Helden, P.; Seldon, R.; Moosa, A.; Warner, D. F.; Arista, L.; Manjunatha, U. H.; Smith, P. W.; Street, L. J.; Chibale, K. Pyrrolo[3,4-c]pyridine-1,3(2H)-diones: a novel antimycobacterial class targeting mycobacterial respiration. *J. Med. Chem.* **2015**, *58*, 9371–9381.

(15) Cho, S.; Lee, H. S.; Franzblau, S. Microplate alamar blue assay (MABA) and low oxygen recovery assay (LORA) for *mycobacterium tuberculosis*. *Methods Mol. Biol.* **2015**, *1285*, 281–292.

(16) Zhang, T.; Li, S. Y.; Nuermberger, E. L. Autoluminescent *mycobacterium tuberculosis* for rapid, real-time, non-invasive assessment of drug and vaccine efficacy. *PLoS One* **2012**, *7*, No. e29774.