Identification of *N*-(2-Phenoxyethyl)imidazo[1,2-*a*]pyridine-3-carboxamides as New Antituberculosis AgentsZhaoyang Wu,^{†,‡} Yu Lu,^{†,‡} Linhu Li,[†] Rui Zhao,[†] Bin Wang,[‡] Kai Lv,^{†,§} Mingliang Liu,^{*,†} and Xuefu You^{*,†}[†]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China[‡]Beijing Key Laboratory of Drug Resistance Tuberculosis Research, Department of Pharmacology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China[§]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

Supporting Information

ABSTRACT: A series of imidazo[1,2-*a*]pyridine carboxamides (IPAs) bearing an *N*-(2-phenoxyethyl) moiety was designed and synthesized as new antitubercular agents. Seven 2,6-dimethyl IPAs demonstrated excellent *in vitro* activity (MIC: 0.025–0.054 $\mu\text{g/mL}$) against the drug susceptible H37Rv strain and two clinically isolated multidrug-resistant *Mycobacterium tuberculosis* strains. Compound **10j** displayed acceptable safety and pharmacokinetic properties, opening a new direction for further development.

KEYWORDS: Antitubercular agents, MTB H37Rv, multidrug resistant-MTB, *N*-(2-phenoxyethyl)imidazo[1,2-*a*]pyridine carboxamides, structure–activity relationship

10a: X = H; **10b:** X = 2-Me; **10c:** X = 3-Me; **10f:** X = 3-OMe; **10g:** X = 4-OMe; **10i:** X = Cl; **10j:** X = 4-Br
MIC MTB H37Rv: 0.027–0.054 $\mu\text{g/mL}$
MIC MDR-MTB: 0.025–0.051 $\mu\text{g/mL}$

Tuberculosis (TB) remains one of the world's deadliest pandemic diseases with over 9 million new cases and 1.5 million deaths estimated by the World Health Organization (WHO) in 2014.¹ The spread of multidrug-resistant tuberculosis (MDR-TB) and the emergence of extensively drug-resistant tuberculosis (XDR-TB) have reinvigorated drug discovery efforts in search of novel drugs.^{2–4} Encouragingly, bedaquiline (an ATP synthase inhibitor) was, for the first time in at least 40 years, approved by the US FDA for clinical management of MDR-TB in 2012.⁵ However, considering the high attrition rate in clinical trials, it is still highly valuable to identify new molecules with alternative scaffolds as effective anti-TB drug candidates.

Imidazo[1,2-*a*]pyridine-3-carboxamides (IPAs) as TB antibiotics have garnered great interest recently. Structure–activity relationship (SAR) studies of IPAs have demonstrated that the carboxamide linker with the *N*-benzylic group is critical for antimycobacterial activity.⁶ Two candidates, Q203 (Figure 1)^{6,7} and ND09759 (Figure 1),^{8,9} targeting the *Mycobacterium tuberculosis* (MTB) QcrB^{7,10} were found to have excellent inhibitory potency against drug-sensitive, MDR and XDR strains. DNB1 (Figure 1), a dinitrobenzamide targeting DprE1/DprE2, was reported to be highly active against MTB, including XDR strains.¹¹ Given that all three candidates belong to aromatic carboxamides, we intended to make structural modifications on Q203 and ND09759 by peripheral modification. Thus, a series of 2,6-/2,7-dimethyl IPAs (**10a–m**, **11a–n**) were designed as new anti-TB agents in which *N*-(2-phenoxy)ethyl groups were introduced at first in this study (Figure 1). Our primary objective was to identify alternative

Figure 1. Design of the new molecules.

linkers with potent antimycobacterial activity. A preliminary SAR study was also explored to facilitate the further development of IPAs.

The synthetic route for target compounds **10a–m** and **11a–n** is outlined in Scheme 1. Commercially unavailable 2-phenoxyethanamines **3a–n** were first prepared from different phenols **1a–n** via the reaction with *N*-(2-hydroxyethyl)-phthalimide in the presence of azodicarboxylate (DEAD) and PPh₃ in THF at 0–5 °C, and then the resulting condensates **2a–n** were treated with hydrazine hydrate in ethanol at 50 °C.¹²

Condensations of 2-aminopyridines **4** and **5** with ethyl 2-chloroacetoacetate in the presence of Et₃N provided ethyl

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Scheme 1. Synthesis of target compounds 10a–n, 11a–m, and 14^a

^aReagents and conditions: (i) *N*-(2-hydroxyethyl) phthalimide, DEAD, PPh₃, THF, 0–5 °C; (ii) hydrazine hydrate, EtOH, 50 °C; (iii) ethyl 2-chloroacetoacetate, Et₃N, MeCN, reflux; (iv) LiOH, H₂O, EtOH, rt, and then HCl; (v) EDCI, HOBT, CH₂Cl₂, rt; (vi) toluene, reflux, then NaOH, rt.

imidazo[1,2-*a*]pyridine-3-carboxylates **6** and **7**.¹³ Hydrolysis of the esters **6** and **7** in a LiOH–H₂O–C₂H₅OH system yielded compounds **8** and **9**. The target compounds **10a–m** and **11a–n** were obtained by amidation of the acids **8** and **9** with the amines **3a–n** in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBT). In addition, compound **14** with the aminoethyl linker was also synthesized from the amine **13**, which can be easily prepared through the nucleophilic substitution of 4-bromoaniline **12** and 2-bromoethan-1-amine hydrobromide (Scheme 1).

The synthesized compounds **10a–n**, **11a–m**, and **14** were initially screened for *in vitro* activity against MTB H37Rv ATCC 27294 strain (MTB-1) using the Microplate Alamar Blue Assay (MABA).^{14,15} 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 isoniazid (INH) and rifampicin (RFP) for comparison are presented in Table 1.

The data revealed that most of the target compounds had potent activity against this strain (MIC: <1 μg/mL). 2,6-Dimethyl IPAs were found to be much more active than the corresponding 2,7-dimethyl IPAs (**10a–m** vs **11a–m**). Among 2,6-dimethyl IPAs, **10a–c**, **10f**, **10g**, **10i**, and **10j** showed excellent activity (MIC: 0.027–0.054 μg/mL). The nature and position of the substituent (X) on the benzene ring of compounds **10a–m** influenced activity. With a few exceptions (**10d**, **10e**, **10h**), the presence of an electron-donating group (methyl, methoxyl) or a halogen atom on the ring was found to be favorable, but introduction of an electron-withdrawing group (nitro) or a bulkier (phenyl) group resulted in decreased activity.

In further modifications, the oxyethyl linker was replaced by the isostereaminoethyl moiety. Compound **14** demonstrated weaker activity (MIC: 0.25 μg/mL) than **10j** (MIC: 0.027 μg/mL) against MTB H37Rv strain. The result revealed that the oxyethyl moiety seems to be preferred over the aminoethyl one.

Encouraged by their strong potency against the drug-sensitive MTB H37Rv strain, compounds **10a–c**, **10f**, **10g**, **10i**, and **10j** were further evaluated against two clinical isolated 11168 (MTB-2) and 9160 (MTB-3) MDR strains resistant to both of INH and RFP. It was shown that all of the compounds

Table 1. Structures and *in Vitro* Activity of 10, 11, and 14 against MTB H37Rv

compd	X	MIC (μg/mL)
10a	H	0.054
10b	2-CH ₃	0.038
10c	3-CH ₃	0.029
10d	4-CH ₃	0.204
10e	2-OCH ₃	0.401
10f	3-OCH ₃	0.030
10g	4-OCH ₃	0.031
10h	4-F	0.146
10i	4-Cl	0.030
10j	4-Br	0.027
10k	2-NO ₂	0.303
10l	4-NO ₂	0.245
10m	4-C ₆ H ₅	0.390
11a	H	0.935
11b	2-CH ₃	0.231
11c	3-CH ₃	0.329
11d	4-CH ₃	0.673
11e	2-OCH ₃	2
11f	3-OCH ₃	0.254
11g	4-OCH ₃	2
11h	4-F	2
11i	4-Cl	2
11j	4-Br	0.463
11k	2-NO ₂	0.5
11l	4-NO ₂	4
11m	4-C ₆ H ₅	0.518
11n	3-NO ₂	0.5
14		0.25
INH		0.049
RFP		0.05

displayed excellent activity against these two strains with similar MIC values (0.025–0.051 μg/mL) to that of the wild-type MTB H37Rv, suggesting their promising potential for both drug-sensitive and resistant MTB strains (Table 2).

Moreover, the compounds **10a–m**, **11a–n**, and **14** were evaluated for their *in vitro* antibacterial activity against representative strains using standard techniques.¹⁶ MIC is defined as the concentration of the compound required to give complete inhibition of bacterial growth. It was found that none

Table 2. *In Vitro* Activity of 10a–c, 10f, 10g, 10i, and 10j against MDR Strains Resistant to Both INH and RFP

compd	MIC (μg/mL)	
	MDR-MTB 11168	MDR-MTB 9160
10a	0.030	0.047
10b	0.036	0.050
10c	0.026	0.033
10f	0.029	0.035
10g	0.026	0.034
10i	0.025	0.028
10j	0.036	0.051
INH	>40	>40
RFP	36.889	38.647

of the compounds had activity (MIC: >32 $\mu\text{g/mL}$) against any of the tested Gram-negative (*E. coli* ATCC 25922, *K. pneumoniae* 12–1, *P. aeruginosa* 12–12) or Gram-positive strains (*S. aureus* ATCC25923, methicillin-sensitive *S. aureus* 12–1, methicillin-resistant *S. aureus* 12–2, methicillin-sensitive *S. epidermidis* 12–1, methicillin-resistant *S. epidermidis* 12–6, *S. pneumoniae* ATCC 49619). This indicated that the target compounds are remarkably selective since they had potent activity against MTB. Pethe and colleagues have also confirmed that this class of compounds is not active against other Gram-negative and Gram-positive strains.⁷

Based on the measured activity levels against all of the tested strains, the three most active compounds with structural diversity, **10a**, **10f**, and **10j**, were further tested for *in vivo* tolerability by recording the number of survivors after a single intravenous dose in mice of 50 and 100 mg/kg, or an oral dose in rats of 500 and 1000 mg/kg, followed by a 7-day observation. As shown in Table 3, all three compounds showed good

Table 3. Acute Toxicity Study of **10a**, **10f**, and **10j**

compd	dose (mg/kg)		no. of animals that survived/ total no. of animals	
	i.v. (mice)	p.o. (rats)	i.v. (mice)	p.o. (rats)
10a	50	500	5/5	5/5
	100	1000	5/5	2/5
10f	50	500	5/5	5/5
	100	1000	5/5	4/5
10j	50	500	5/5	5/5
	100	1000	5/5	5/5

tolerance in mice at 100 mg/kg (i.v.) or in rats at 500 mg/kg (p.o.). Compound **10j** displayed the lowest oral acute lethal toxicity, followed by **10f** and then **10a**.

The *in vivo* pharmacokinetic (PK) profiles of **10a**, **10f**, and **10j** were evaluated in rats after a single oral administration of 25 mg/kg. As shown in Table 4, compound **10j**, with a bromo atom at the para-position of the benzene ring, displayed relatively good PK properties, with C_{max} of 225.33 ± 200.71 ng/mL, $\text{AUC}_{0-\infty}$ of 288.22 ± 126.06 h·ng/mL, $T_{1/2}$ of 1.50 ± 0.43 h, Vd/F of 185975.1 ± 137163.90 mL/kg, and CL/F of 86284.81 ± 51685.89 mL/h/kg. Compound **10a** had a high C_{max} of 654.86 ± 311.87 ng/mL and an $\text{AUC}_{0-\infty}$ of 453.24 ± 254.13 h·ng/mL, but a short $T_{1/2}$ of 0.43 ± 0.09 h and a poor Vd/F of 33806.34 ± 13481.74 mL/kg. The results suggested that the presence of a substituent at the para-position of the benzene ring is favorable for the PK profiles of 2,6-dimethyl IPAs.

In conclusion, a series of novel IPA derivatives bearing an *N*-(2-phenoxy)ethyl moiety was designed as new antitubercular agents by using a scaffold hopping strategy. Many 2,6-dimethyl IPAs exhibited excellent *in vitro* inhibitory activity against both

drug-sensitive MTB strain H37Rv and drug-resistant clinical isolates. Compound **10j** displayed acceptable safety and PK properties, and it may serve as a new and promising lead compound for further antitubercular drug discovery. Also, compound **14**, with its considerable activity (MIC: 0.25 $\mu\text{g/mL}$) against MTB H37Rv strain, arouses our great interest in exploring SAR of *N*-(2-phenylamino)ethyl IPAs. Further modifications of *N*-(2-phenoxy) and *N*-(2-phenylamino)ethyl IPAs are in progress, and the study results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.6b00330.

Experimental procedures and analytical data for compounds **10a–m**, **11a–n**, and **14** (PDF)

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Notes

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■ ABBREVIATIONS

IPAs, imidazo [1,2-*a*] pyridine carboxamides; MTB, *Mycobacterium tuberculosis*; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; EDCI, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole; MIC, minimum inhibitory concentration; MABA, Microplate Alamar Blue Assay; C_{max} , maximum concentration; T_{max} , time to maximum concentration; $\text{AUC}_{0-\infty}$, area under curve from time zero to infinity;

Table 4. Pharmacokinetic Parameters of **10a**, **10f**, and **10j** Dosed Orally in Rats at 25 mg/kg (Mean $\pm$ SD, $n = 6$)

compd	10a	10f	10j
C_{max} (ng/mL)	654.86 $\pm$ 311.87	351.16 $\pm$ 127.23	225.33 $\pm$ 200.71
T_{max} (h)	0.33 $\pm$ 0.16	0.17 $\pm$ 0.09	0.50 $\pm$ 0.25
$\text{AUC}_{0-\infty}$ (h·ng/mL)	453.24 $\pm$ 254.13	214.46 $\pm$ 89.20	288.22 $\pm$ 126.06
$T_{1/2}$ (h)	0.43 $\pm$ 0.09	1.12 $\pm$ 0.38	1.50 $\pm$ 0.43
MRT (h)	0.61 $\pm$ 0.20	0.71 $\pm$ 0.15	1.60 $\pm$ 0.45
Vd/F (mL/kg)	33806.34 $\pm$ 13481.74	188349.28 $\pm$ 114024.43	185975.1 $\pm$ 137163.90
CL/F (mL/h/kg)	55158.14 $\pm$ 24763.45	116573.23 $\pm$ 67837.38	86284.81 $\pm$ 51685.89

$t_{1/2}$, plasma elimination half-life; MRT, mean residence time; Cl, clearance; V_z , volume of distribution; F, bioavailability

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