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Advancement of Imidazo[1,2-*a*]pyridines with Improved Pharmacokinetics and nM Activity vs. *Mycobacterium tuberculosis*

Garrett C. Moraski,[†] Lowell D. Markley,[†] Jeffrey Cramer,[‡] Philip A. Hipskind,[‡] Helena Boshoff,[§] Mai A. Bailey,[∥] Torey Alling,[∥] Juliane Ollinger,[∥] Tanya Parish,[∥] and Marvin J. Miller*[,†]

[†]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, United States

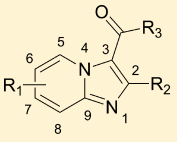
[‡]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, United States

[§]Tuberculosis Research Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, United States

[∥]Infectious Disease Research Institute, 1616 Eastlake Ave E, Suite 400, Seattle, Washington 98102, United States

Supporting Information

ABSTRACT: A set of 14 imidazo[1,2-*a*]pyridine-3-carboxamides was synthesized and screened against *Mycobacterium tuberculosis* $H_{37}Rv$. The minimum inhibitory concentrations of 12 of these agents were ≤1 μM against replicating bacteria and 5 compounds (**9**, **12**, **16**, **17**, and **18**) had MIC values ≤0.006 μM. Compounds **13** and **18** were screened against a panel of MDR and XDR drug resistant clinical *Mtb* strains with the potency of **18** surpassing that of clinical candidate **PA-824** by nearly 10-fold. The *in vivo* pharmacokinetics of compounds **13** and **18** were evaluated in male mice by oral (PO) and intravenous (IV) routes. These results indicate that readily synthesized imidazo[1,2-*a*]pyridine-3-carboxamides are an exciting new class of potent, selective anti-TB agents that merit additional development opportunities.



9, R_1 = 6-CH_3, R_2 = CH_3, R_3 = benzylamino
16, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-chlorophenoxy)benzyl)amino
17, R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
18, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
MIC *Mtb* $H_{37}Rv$ ≤ 0.006 μM for compounds 9, 16 - 18
MIC MDR- and XDR-*Mtb* from ≤ 0.03 to 0.8 μM for 18

KEYWORDS: *Mycobacterium tuberculosis, imidazo[1,2-a]pyridine-3-carboxamides, MDR-TB, XDR-TB, pharmacokinetics*

Tuberculosis (TB) is a serious global health problem caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). More than one-third of the world's population is infected with TB and that resulted in an estimated 1 400 000 deaths worldwide in 2010.[1] With someone dying of TB nearly every 20 s, and an increase in cases of drug resistant TB, there is an urgent need for discovery and development of new treatments. Herein we report on the additional structure activity relationship (SAR) studies on the imidazo[1,2-*a*]pyridine-3-carboxylate scaffold that we disclosed[2−4] and has since been identified and profiled by other groups.[5−7] Our new studies described herein have produced compounds with dramatically enhanced (low nanomolar) potency and significantly enhanced pharmacokinetics (PK).

We prepared five different imidazo[1,2-*a*]pyridine-3-carboxylic acid intermediates (**3a**−**3e**), which were elaborated into a focused set of antitubercular agents (**5**−**19**) with generalized structure **4**. These compounds were easily made in straightforward syntheses in good overall yields (Scheme 1). First, reaction of the appropriately substituted 2-aminopyridine (**1**) with ethyl 2-chloroacetoacetate followed by saponification with lithium hydroxide and acidic work up gave the free acids **3a**−**3d**. Use of the same procedure but starting with 2-amino-5-methylpyridine (**1**) and ethyl 2-chloro-4,4,4-trifluoroacetoacetate (**2**, R_2=CF_3) followed by saponification and acidic work up gave free acid **3e** (87% yield). It should be noted that while carboxylic acids **3a**−**3d** can all be prepared by the general method described; they also are commercially available, while **3e** was not. Finally, these imidazo[1,2-*a*]pyridine-3-carboxylic acid intermediates were all readily converted to various amide analogues (**5**−**19**) through classical EDC-mediated coupling reactions (55% for **7**, unoptimized).

SAR studies resulted in a panel of 14 new imidazo[1,2-*a*]pyridine-3-carboxamide analogues (**6**−**19**) for evaluation. A schematic grouping of the analogues produced is provided in Scheme 2. The initial "hit" compound **5** was previously evaluated as active using the MABA for *Mtb*;[2] we rescreened this compound using an alternative minimal inhibitory concentration (MIC, defined as the concentration that prevents >99% of bacterial growth at 5 days post inhibitor exposure) assay to confirm activity.[4] We found that **5** had a slightly lower MIC (0.2 μM) in this assay compared to the MIC range using MABA in various media (0.8−2.3 μM).[2] It is possible this is due, in part, to the use of an alternative medium as this assay uses glucose as a carbon source which is different from that used in the MABA assays. Initially, the SAR focus was to make compounds with lower cLog *P* values than compound **5** (clogP of 3.6) in the hope that the more polar compounds would be substantially more potent through favorable interactions and also be more soluble.[8] Therefore, sulfonamide (**6**), sulfonyl (**7** and **8**), and 2-pyridyl (**13**) compounds were made and screened. Compound **9** is a positional isomer of compound **5** while compound **12**

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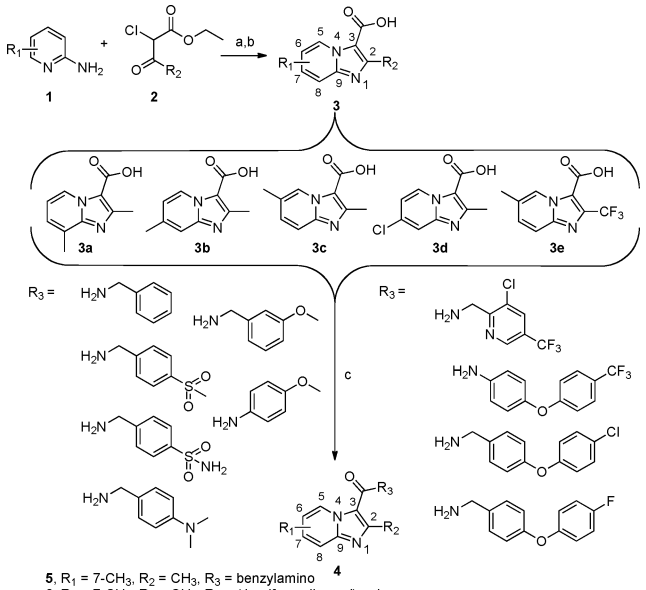
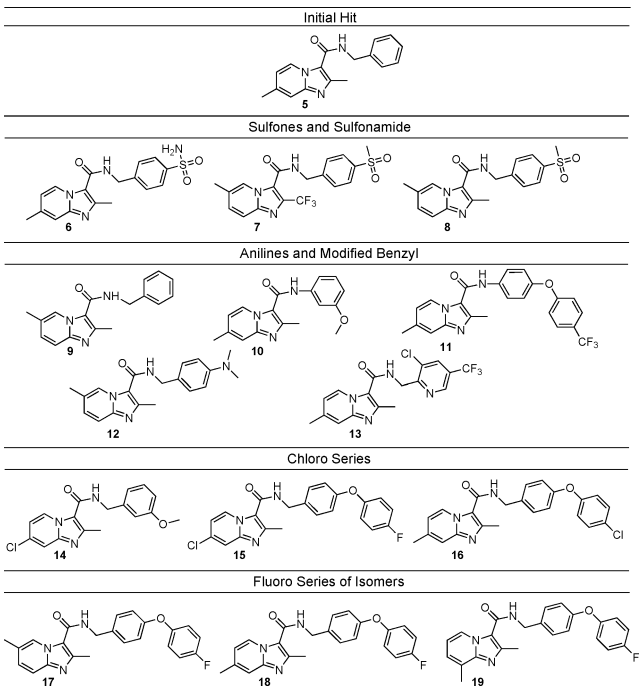
Scheme 1. General Synthesis of Imidazo[1,2-a]pyridine-3-carboxamides[a]



5, R_1 = 7-CH_3, R_2 = CH_3, R_3 = benzylamino
6, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-sulfamoylbenzyl)amino
7, R_1 = 6-CH_3, R_2 = CF_3, R_3 = (4-methylsulfonylbenzyl)amino
8, R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-methylsulfonylbenzyl)amino
9, R_1 = 6-CH_3, R_2 = CH_3, R_3 = benzylamino
10, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (3-methyloxybenzyl)anilino
11, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-trifluoromethyl)phenoxy)anilino
12, R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-(dimethylamino)benzyl)amino
13, R_1 = 7-CH_3, R_2 = CH_3, R_3 = ((3-chloro-5-(trifluoromethyl)pyridin-2-yl)methyl)amino
14, R_1 = 7-Cl, R_2 = CH_3, R_3 = (3-methyloxybenzyl)amino
15, R_1 = 7-Cl, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
16, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-chlorophenoxy)benzyl)amino
17, R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
18, R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
19, R_1 = 8-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino

[a]Reagents: (a) ethyl 2-chloroacetoacetate (for **3a–3d**) and ethyl 2-chloro-4,4,4-trifluoroacetoacetate (for **3e**), DME, reflux, 48 h; (b) (1) LiOH, EtOH, (2) HCl, 56 h; (c) EDC, DMAP, R_3, CH_3CN, 16 h.

Scheme 2. Grouping of Imidazo[1,2-a]pyridine Analogues



incorporates the basic dimethylaniline moiety. Neither compound **9** nor **13** lowered the cLog *P* but they did add chemical diversity to this series. Compounds **11**, **16–19** were made to explore possible size limitations of having biaryl ether amides and further expanded upon the chemical diversity screened within this class.

Table 1 summarizes the *in vitro* antitubercular activity of these 15 analogues against *Mtb* H_{37}Rv grown using glucose as a carbon source. All compounds evaluated were very potent (MIC < 2 μM) with the exception of the sulfonamide (**6**, MIC > 20 μM) and the aniline-derivative (**10**, >20 μM). Previous work with pyrimidine-imidazoles had suggested that similar types of compounds might have a glycerol-dependent effect.[9] We eliminated any concern that the activity of these compounds might be glycerol-dependent by evaluating compounds **5** and **18** against *Mtb* grown with and without glycerol as an additional carbon source. In fact, the MICs of both compounds **5** and **18** were lower (3 and 4-fold, respectively), in the absence of glycerol (see the Supporting Information). This confirmed that glycerol metabolism was not responsible for the potency of these compounds. We can also rule out concerns of high protein binding as a set of compounds was run with and without the addition of bovine serum albumin (BSA) and no shift in MIC was observed (see the Supporting Information). Additionally, the toxicity against the Hep2G cell line was determined for compounds **11**, **12**, **16**, and **15** and a large therapeutic window was observed (IC_{50} > 50 μM, respectively; see the Supporting Information).

Table 1. *In Vitro* Evaluation of Compounds 5–19, Two Controls, and Two Clinical Candidates against Replicating *Mtb* H_{37}Rv (μM)

			MIC (μM)	
cmpd	MW	ClogP[a]	avg	SD
5	279.34	3.60	0.2	0.2
6	358.41	1.76	>20	ND
7	411.40	1.75	0.9	0.3
8	357.43	1.95	0.4	0.1
9	279.34	3.60	0.006	0.003
10	295.34	3.34	>20	ND
11	425.40	6.40	0.7	0.4
12	322.18	3.76	0.05	0.008
13	382.77	3.13	0.7	0.1
14	329.78	3.74	1.1	0.4
15	409.84	6.06	0.02	0.009
16	405.88	6.41	0.006	0.004
17	389.42	5.84	0.005	0.002
18	389.42	5.84	0.004	0.002
19	389.42	5.84	0.1	0.02
rifampicin	822.94	6.04	0.003	0.006
isoniazid	137.14	−0.67	0.3	0.02
BTZ043[b]	431.39	2.45	0.003	0.001
PA-824[c]	359.26	2.80	0.6	0.1

[a]ClogP calculated by ChemDraw version 12.0; MICs are the minimum inhibitory concentration required to inhibit growth by >99%. *Mtb* was grown in Middlebrook 7H9 medium supplemented with OADC (oleic acid, BSA, glucose, catalase) and 0.05% Tween 80 using two readouts of growth (optical density and fluorescence) as described.[4] SD = standard deviation; ND = not determined values reported are the average of MICs generated with both readouts from a minimum of two independent runs. [b]A gift from Alere Technologies GmbH and Alere Inc. [c]Obtained from PracticaChem LLC.

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Table 2. MDR- and XDR-*Mtb* Activity of Compounds 13 and 16 and Control PA-824

	compound MIC μM (μg/mL)			
strain[a]	**9**	**13**	**18**	**PA-824**
DS- *Mtb*1	0.04−0.07 (0.01−0.02)	0.8 (0.3)	<0.03 (<0.01)	0.5−0.9 (0.2−0.3)
DS- *Mtb*2	<0.04 (<0.01)	0.8 (0.3)	<0.03 (<0.01)	>13.9 (>5)
MDR-*Mtb* HREZSKP	<0.04 (<0.01)	6.5 (2.5)	0.03−0.8[b] (0.01−0.3[b])	0.5−0.9 (0.2−0.3)
MDR-*Mtb* HREKP	<0.04 (<0.01)	0.4 (0.2)	<0.03 (<0.01)	0.5−0.9 (0.2 −0.3)
MDR-*Mtb* HRERb	2.3 (0.63)	13−26 (5−10)	0.80 (0.31)	0.5−0.9 (0.2−0.3)
XDR-*Mtb* HRESKO	<0.04 (<0.01)	0.8 (0.3)	<0.03 (<0.01)	0.9 (0.3)
XDR-*Mtb* HREKO	<0.04 (<0.01)	0.8 (0.3)	<0.03 (<0.01)	0.2 (0.08)

[a]DS-*Mtb*1 = drug sensitive clinical isolate no. 1; DS-*Mtb*2 = drug sensitive clinical isolate no. 2; MDR-*Mtb* = multidrug resistant *Mtb*; XDR-*Mtb* = extensively drug resistant *Mtb*; drugs abbreviated as H = isoniazid, R = rifampin, E = ethambutol, Z = pyrazinamide, S = streptomycin, K = kanamycin, P = p-aminosalicylic acid, Rb = rifabutin, O = ofloxacin. [b]Growth inhibited >90% up to 0.8 μM (0.3 μg/mL). MICs are the minimum concentration required to inhibit growth by >99%; MICs were done in 7H9/glucose/glycerol/BSA/0.05% Tween 80 and the average of three individual measurements.

The SAR analysis based on the whole cell assay readouts indicated that while some polar compounds (**7** and **8**, MIC = 0.9 and 0.4 μM; clog *P* 1.8 and 2, respectively) did retain good activity, others did not (**6**, MIC > 20 μM; clog *P* 1.8). This keeps the question of whether increased polarity will yield increased potency open for further SAR studies. The substitution of the 7-methyl with the 7-chloro group on the imidazo[1,2-*a*]pyridine core appeared to diminish activity by 5-fold when **18** is compared to **15** (MIC = 0.004 and 0.02 μM, respectively).

Most apparent was that the larger, more lipophilic biaryl ethers had nanomolar potency. Indeed, independent of whether the substitution on the biaryl ether was a chlorine (**16**) or fluorine (**18**), potency was outstanding (MIC = 0.006 and 0.004 μM, respectively). Interestingly, the position of the methyl group might play a role in potency as the 8-methyl analogue (**19**) was much less potent (MIC = 0.1 μM) than the other isomeric 6- and 7-methyl analogues (**17** and **18**, respectively) which were nearly equipotent (MIC = 0.005 and 0.004 μM, respectively). The possible effect of position of the methyl group is seen more dramatically by comparing compounds **5** and **9**, where compound **9** (MIC = 0.004 μM) was much more potent than its positional isomer, compound **5** (MIC = 0.2 μM). This again suggests that the position of the methyl group may play a role in potency as the 6-methyl was considerably more active than the 7-methyl analogue. The 2-pyridyl compound, **13**, had good potency (MIC = 0.7 μM) and a lower cLog *P* value of 3.1. Moreover, compound **13** was not as susceptible to *in vitro* and *in vivo* metabolism compared to the published values[2] for compound **5**, presumably due to the steric and electronic factors imparted by the pyridine substituents (19% metabolized for **13** compared to 71% for **5** in rat liver microsomes).

Encouraged that 5 of the 14 new analogues tested had low nanomolar MIC values against the replicating *Mtb* $H_{37}Rv$ strain, we screened compounds **9**, **13**, and **18** against a panel of sensitive, multidrug resistant (MDR) and extensively drug resistant (XDR) clinical strains (Table 2) with the nitroimidazole clinical candidate PA-824[10] as the control.

Compound **9** had excellent potency against these clinical strains (MIC of 0.04−2.3 μM) that was comparable to or slightly lower than that reported for the isomer (**5**) which had MICs of 0.07−2.3 μM (against a panel of 12 MDR- and XDR-*Mtb* strains).[2] Compound **13** had the widest range of activity against these clinical strains (MICs of 0.4−26 μM) and in general was not quite as potent as PA-824 (MICs of 0.2 to >14 μM) against the majority of the drug resistant strains screened.

It did, however, show a different "fingerprint" of inhibition than PA-824 and was potent (MIC of 0.8 μM) against the drug sensitive strain that was resistant to PA-824 (MIC > 14 μM), suggesting a different mechanism of action than PA-824,[11] as would be expected for these structurally different molecules. Compound **18** was most potent against all the drug resistant strains screened (MICs of <0.03 to 0.8 μM) and was much more active than PA-824 in nearly every strain screened with the exception of the MDR strain "HRERb", a strain that is resistant to isoniazid, rifampin, ethambutol, and rifabutin. Against that strain, **18** was about equipotent to PA-824. This outstanding potency against the various drug resistant strains suggests that this class is most likely inhibiting a novel and essential target in these clinical strains. This hypothesis appears to be supported by recent publications wherein various imidazo[1,2-*a*]pyridines were found to be inhibitors of ATP homeoestasis[5] by targeting *QcrB*, which encodes the b subunit of the electron transport ubiquinol cytochrome C reductase.[6] This single mutation generated in *M. bovis BCG* resulted in cross-resistance of three compounds evaluated by the GSK group and is therefore a proposed target of this class.[6] We intend to cross screen our imidazopyridines against *QcrB* and results will be reported in due course.

The *in vivo* pharmacokinetics (PK) of compounds **13** and **18** were evaluated in male mice by oral (PO) and intravenous (IV) routes of administration at 3 and 1 mg/kg dosing levels, respectively (Table 3). The *in vitro* microsomal stability of compound **13** was evaluated in various species (rat, mouse, dog, and human) and a range of metabolism was observed (19.3% metabolized in rat, 79.9% metabolized in mouse, 41.2%

Table 3. *In Vivo* Mouse PK Evaluation of Imidazo[1,2-*a*]pyridines

compd	PO AUC (ng h/mL)	PO Cmax (ng/mL)	PO Tmax (h)	PO $t_{1/2}$ (h)	IV clearance (mL/min/kg)	% *F*
13[a]	411	181	0.25	5	43.1	35.8
18[a]	3 850	337	0.5	ND[c]	ND[c]	31.1
13[b]	54 200	15 600	2.0	1.93		
18[b]	11 000	1 160	0.5	13.2		

[a]**13** and **18** at 3 mg/kg PO and 1 mg/kg IV. [b]**13** at 100 mg/kg PO and **18** at 10 mg/kg PO. [c]ND = not determined value because compound remained after the 24 h time point; extrapolation from raw data would suggest $t_{1/2}$ >12 h and clearance would be estimated at moderate to slow (see the Supporting Information).

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metabolized in dog, and 0% metabolized in human). The lack of human (microsomal) metabolism observed prompted evaluation of **13** against a panel of human cytochrome P-450 enzymes. It was found that compound **13** only inhibited 2.8% of CYP2D6, 11.5% of CYP2C9, and 0% of CYP 3A4 at 10 μM. Thus, **13** is not an inhibitor of these CYP isoforms. However, compounds **13** and **18** were found to be time dependent inhibitors of human CYP3A4 at 10 μM, a concentration well above their respective MICs. Additionally, compounds **13** and **18** were profiled to identify metabolites in mouse, rat, and human microsomes. The major metabolites observed for both compounds were monohydroxylations on the imidazopyridine. The exact site of hydroxylation could not be determined by the fragmentation pattern and will need to be determined by additional efforts. As the mouse microsomal assays predicted, compound **13** displayed only moderate *in vivo* mouse PK (Table 3). However, when compound **13** was re-evaluated at a 100 mg/kg PO dose, the PK parameters were much more promising as the area under the curve (AUC) increased nonlinearly from 411 to 54 200 ng h/mL. Also, as predicted by the rat microsomes, the *in vivo* rat PK showed slower clearance (25 mL/min/kg) than that observed in mouse (43 mL/min/kg) following a 1 mg/kg IV dose, along with greater exposure with an AUC >2000 ng h/mL at a 3 mg/kg oral dose (see the Supporting Information). Compound **18** had more promising mouse PK than compound **13** with an AUC of 3850 ng h/mL (compared to AUC of 411 ng h/mL for **13**) and a half-life greater than 12 h (compared to 5 h for **13**), Table 3. When compound **18** was re-evaluated at 10 mg/kg PO, the AUC increased roughly linearly from 3850 to 11 000 ng h/mL and the half-life was determined to be 13.2 h. Additional *in vivo* ADME properties for compounds **13** and **18** can be found in the Supporting Information. The free fractions (fu) for compounds **13** and **18** were measured using an equilibrium dialysis method.[12] Compound **13** had 8.9% free drug *in vivo* while **18** had only 0.4% free drug. Moreover, the Cmax of unbound drug for compound **13** would be 657 nM (near its MIC) at 100 mg/kg PO dose and 18.6 nM at 10 mg/kg PO dose for compound **18** (>4-fold higher than its MIC). Given the free unbound fraction and duration of drug exposure (extrapolated AUC of 15 400 ng h/mL at 10 mg/kg PO) and a very low MIC (<5 nM) against replicating *Mtb* for compound **18**, we are seeking to evaluate this compound for efficacy in an *in vivo* TB infection model and the results will be reported in due course.

Herein we report on our additional efforts to advance synthetically accessible imidazo[1,2-*a*]pyridine-3-carboxamides as an emerging and exciting class of antitubercular agents that will hopefully attract greater attention for development within the scientific and medical communities.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details for compounds synthesized, descriptions of assays, PK data, as well as copies of relevant NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 574-631-7571. Fax: +1 574-631-6652. E-mail: mmiller1@nd.edu.

Author Contributions

G.C.M. participated in the design, performed the syntheses, drafted the manuscript, and facilitated interactions. L.D.M. participated in the design and coordinated interactions through Dow AgroSciences. P.A.H. and J.C. (from Eli Lilly and Company) facilitated microsome and PK assessment. H.B. (NIH) performed MDR- and XDR-*Mtb* assays. M.B., T.A., J.O., and T.P. conducted MICs against replicating *Mtb*. J.O. and T.P. provided scientific input and assisted with the manuscript. M.J.M. drafted the manuscript and participated in the design and direction of the project.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DME, 1,2-dimethoxyethane; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; HCl, hydrochloric acid; ADME, absorption distribution metabolism and excretion

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