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LETTER

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Advent of Imidazo[1,2-*a*]pyridine-3-carboxamides with Potent Multi- and Extended Drug Resistant Antituberculosis Activity

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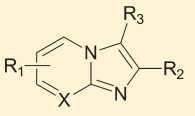
Supporting Information

ABSTRACT: A set of nine 2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxamides and one 2,6-dimethylimidazo[1,2-*a*]pyrimidine-3-carboxamide were synthesized. The compounds were evaluated for their in vitro antituberculosis activity versus replicating, nonreplicating, multi- and extensive drug resistant Mtb strains. The MIC_{90} values of seven of these agents were ≤ 1 μM against the various tuberculosis strains tested. A representative compound of this class (**1**) was screened against seven nontubercular strains as well as other nonmycobacteria organisms and demonstrated remarkable microbe selectivity. A transcriptional profiling experiment of Mtb treated with compound **1** was performed to give a preliminary indication of the mode of action. Lastly, the in vivo ADME properties of compounds **1**, **3**, **4**, and **6** were assessed. The 2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxamides are a druglike and synthetically accessible class of anti-TB agents that have excellent selective potency against multi- and extensive drug resistant TB and encouraging pharmacokinetics.



1, anti-TB activity
MIC H_{37}Rv TB = 0.4 - 1.9 μM
MIC MDR-TB = 0.07 - 2.2 μM
MIC XDR-TB = 0.07 - 0.14 μM
IC_{50} VERO toxicity > 128 μM

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

Tuberculosis (TB) is a serious global health risk. More than one-third of the human population is infected, resulting in an estimated 1 700 000 deaths in 2006 (1.5 million in HIV-negative people and 0.2 million in HIV-positive people).[1] Moreover, there were a staggering 14 400 000 cases estimated worldwide in 2006, with 83% of the total cases located in the African, South-East Asia, and Western Pacific regions.[1] *Mycobacterium tuberculosis*, the causative agent of TB, is an airborne pathogen that can be spread from one person to another by close contact. Because it can lie dormant in a latent state for many years, it is a silent killer among the poor, HIV-infected, immune-compromised, and the elderly. To make matters worse, multiple drug resistant TB [MDR-TB, strains that are resistant to first line drugs isoniazid (INH) and rifampin] and extensively drug resistant TB (XDR-TB, strains that are resistant to INH and rifampin, as well as any fluoroquinolone and at least one of three injectable second-line drugs, such as amikacin, kanamycin, or capreomycin) are on the rise.[2] Most alarming is the emergence of extremely drug resistant TB "XXDR-TB" (the proposed designation for TB that is resistant to all first- and second-line TB drugs), which is now documented.[3] In 2008, there were 12 898 cases of TB provisionally reported in the United States.[4]

A focus of our laboratories is to facilitate the decline of TB by the identification of therapeutically effective anti-TB agents to augment the long dosing regimen of first-line drugs.[5] Herein, we call attention to the in vitro potency of the imidazo[1,2-*a*]pyridine-3-carboxamide scaffold. To our knowledge, the anti-TB activity of the 2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide class is unprecedented. Imidazo[1,2-*a*]pyridine-3-nitroso derivatives were reported in 2004 to impart notable anti-TB activity (MIC = 3.1 μg/mL vs H_{37}Rv-TB) concurrent with notable toxicity to VERO cells (IC_{50} = 3.6 μg/mL).[6] Also in 2004, rationally designed imidazo[1,2-*a*]pyridine-3-hydrazones[7] were reported but were all inactive against H_{37}Rv-TB at 6.25 μg/mL. Most recently, in 2009, functionalized 3-amino-imidazo[1,2-*a*]pyridines were reported as in vitro Mtb glutamine synthetase inhibitors but without assessment of the in vitro activity versus H_{37}Rv-TB.[8] While reports on the syntheses of imidazo[1,2-*a*]-pyridine-3-carboxamides date to 1965,[9] the 2,7-dimethylimidazo[1,2-*a*]pyridine architecture is atypical within the cannon of medicinal chemistry literature and is unprecedented within the TB lexicon.

Since 2007, we have had a collaborative agreement with Dow AgroScience to screen their compound inventory for inhibitors of Mtb. This effort, coupled with a program to elaborate novel heterocylic anti-TB agents from fragment-based studies of mycobacterial siderophores,[10] led to the identification of an ethyl 2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxylate. This compound

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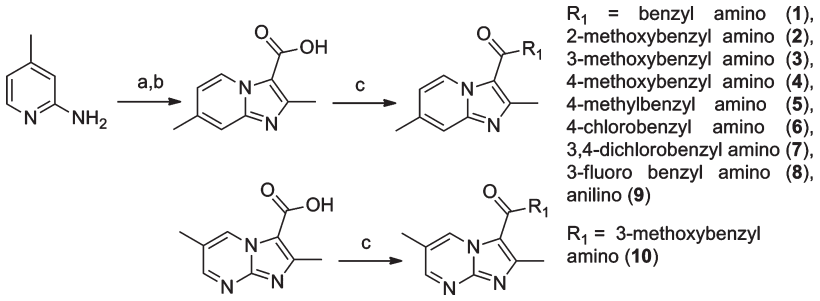
Scheme 1. Synthesis of Imidazo[1,2-*a*]pyridines[a]



[a] Reagents: (a) Ethyl 2-chloroacetoacetate, DME, reflux, 48 h. (b) (1) LiOH, EtOH; (2) HCl, 56 h. (c) EDC, DMAP, R_1, ACN, 16 h.

had weak activity against H_{37}Rv TB (MIC ~ 65 μM, average) but was nonetheless an attractive heterocyclic scaffold to optimize as we did previously with related heterocyclic classes.[11]

The simplest synthesis of the imidazo[1,2-*a*]pyridine-3-carboxylate ring system[12] is the straightforward reaction of 2-amino-4-picoline with ethyl 2-chloroacetoacetate to give the desired heterocyclic scaffold in 78% yield (Scheme 1). Saponification with lithium hydroxide followed by acidic work up gave the free acid, which was then easily converted to various amide analogues through classical EDC-mediated couplings in good yields (70% for **1**).

Our initial structure−activity relationship (SAR) strategy evaluated a representative panel of nine imidazo[1,2-*a*]pyridine-3-carboxamide analogues. The chosen imidazopyridine analogues included the classical Topliss[13] set of benzyl, 4-methoxyphenyl, 4-methylphenyl, 4-chlorophenyl, and 3,4-dichlorophenyl amides. This set was augmented by *ortho*- and *meta*-methoxyphenyl analogues to probe possible steric effects. Next, because of potential metabolic issues associated with a benzylic methylene group, the corresponding aniline was prepared as well as a fluorine replacement for the chlorine. Finally, we explored the influence on potency by changing the imidazo[1,2-*a*]pyridine to an imidazo[1,2-*a*]pyrimidine core (as in **10**).

Table 1 summarizes the in vitro anti-TB activity of these 10 analogues in three different media (GAS,[14] GAST,[15] and 7H12[14]), their potency against nonreplicating "latent" TB (LORA[16]), and an assessment of their toxicity by the VERO[17] assay. All compounds were potent (MIC < 10 μM in the GAS assay media) with the exception of the aniline derived analogue (**9**, MIC > 128 μM), suggesting that in the imidazo[1,2-*a*]pyridine series the benzylic position is important for activity. Additionally, by running the TB assay in three different media (GAS, GAST, and 7H12), we eliminated concern that the activity of these compounds might be carbon source dependent, a flaw discovered in the pyrimidine-imidazoles reported by Pethe and colleagues at Novartis[18] as the GAS and GAST assays use glycerol-alanine salts as the carbon source, while the 7H12 media use palmitic acid.

SAR analysis based on the whole cell assay readout indicated that the 3,4-dichloro analogue (**7**) had diminished activity (MICs of 9−14 μM) when compared to the 4-chloro (**6**, 0.5 μM) and 3-fluoro analogues (**8**, ~0.3 μM). There appeared to be a slight preference for *para*-substitution in terms of potency (MIC = 2.8 μM for *ortho*- vs 1.2 μM for *meta*- vs 0.5 μM for *para*-methoxy analogue in the GAS assay media). Comparison of the imidazo-[1,2-*a*]pyrimidine analogue (**10**) to the corresponding imidazo-[1,2-*a*]pyridine analogue (**3**) indicated that the additional nitrogen in the heterocyclic core was well tolerated (submicomolar potency) although VERO toxicity (IC_{50} = 89 μM) was noted. Compounds **1** and **10** were rescreened in the presence of 4% BSA (bovine serum albumin) and 10% FBS (fetal equine serum), and their MICs were found to shift less than 2-fold by ATP and MABA readouts (see the Supporting Information), indicating that protein binding is not a problem.

Encouraged that six of the 10 analogues tested had submicromolar MIC values against the H_{37}Rv Mtb strain, we next screened compounds **1** and **10** against a panel of single drug resistant strains (Table 2) against controls rifampicin (RIF) and isoniazid (INH) and then three of the more promising compounds (**1**, **3**, and **8**) against a panel of MDR and XDR clinical strains (Table 2). The difference in potencies of these compounds against the clinical strains may be due to the difference in growth media where growth inhibition for clinical strains was tested in media containing glucose as well as glycerol as the carbon source, as well as the fact that many clinical strains exhibit poor growth in vitro since they are not adapted to laboratory conditions and media, which may affect their apparent susceptibility to certain inhibitors.

The excellent activity found when these imidazo[1,2-*a*]-pyridine agents were tested against the drug resistant strains compared favorably to the published MIC values of the nitroimidazole clinical candidate PA-824[19] (MICs against MDR-TB from 0.03 to 0.25 μg/mL or 0.08 to 0.7 μM, comparatively). Furthermore, the improved, and indeed outstanding, potency of these agents against the various drug resistant strains suggests that they inhibit a novel target. Selectivity screening against various nontubercular mycobacteria revealed that compounds **1** and **10** are also inhibitors of *Mycobacterium avium*, *Mycobacterium bovis* BCG, and *Mycobacterium kansasii* but not inhibitors of *Mycobacterium smegmatis*, *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium marinum* (Table 3). This unusual selectivity prompted us to further screen compounds **1**, **3**, **4**, **6**, **8**, and **10** against a panel of representative nonmycobacterial organisms. Compounds **1**, **3**, **4**, **6**, **8**, and **10** were all found to be inactive against the Gram-positive strain of *Staphylococcus aureus* (MIC > 128 μM), the Gram-negative strain of *Escherichia coli* (MIC > 128 μM), and the fungus *Candida albicans* (MIC > 128 μM), further suggesting a mycobacterium specific target of these agents.

The in vivo pharmacokinetics (PK) of compounds **1**, **3**, **4**, and **6** were evaluated in Sprague−Dawley rats by oral (po) and intravenous (iv) routes of administration at 10 and 1 mg/kg dosing levels, respectively (Table 4). Compound **4** had moderate in vitro rat microsomal stability (69% metabolized, $t_{1/2}$ = 19 min) and also displayed promising PK by having the lowest in vivo clearance (28 mL/min/kg, $t_{1/2}$ = 0.28 hours by iv). The aqueous

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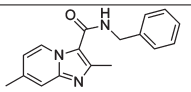
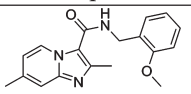
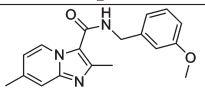
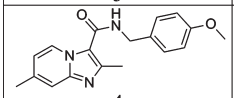
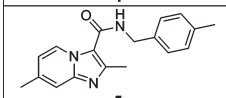
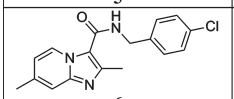
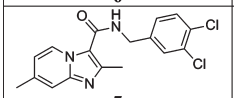
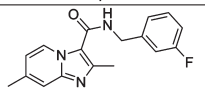
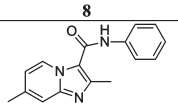
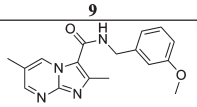
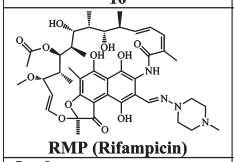
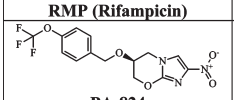
Table 1. In Vitro Evaluation of Compounds 1−10 against H_{37}Rv-TB in Various Assays and Media (MIC_{90} in μM), Stability to Rat Liver (RLM) and Human Liver (HLM) Microsomes and VERO Cellular Toxicity (IC_{50} in μM)

Compound ID	Mol Wt	Calc. Clog P*	GAS	GAST	7H12	LORA	VERO	RLM % metab. (30 min)	HLM % metab. (30 min)
 **1**	279.34	3.60	0.37	0.69	1.9	53.6	>128	71	59
 **2**	309.36	3.51	2.8	1.9			>128		
 **3**	309.36	3.51	1.2	1.0	5.9	31.4	>128	80	47
 **4**	309.36	3.51	0.51	0.50	1.35	10.5	>128	69	30
 **5**	293.36	4.09	0.51	0.80			54.7		
 **6**	313.78	4.31	0.50	0.51	1.94	28	>128	79	82
 **7**	348.23	4.90	9.3	14.4					
 **8**	297.33	3.74	0.29	0.38	1.98	40.1	>128		
 **9**	265.31	3.42	>128	>128					
 **10**	310.35	2.51		0.10	0.48	>10	88.7		
 **RMP (Rifampicin)**	822.95	6.04	0.8	0.2	0.07	2.6	113		
 **PA-824 (nitroimidazole)**	359.26	2.62	0.31	0.21	0.47	4.9	>128		

*Calculated ClogP by ChemDraw version 12.0. GAS, glycerol-alanine-salts media; GAST, iron deficient glycerol-alanine-salts with Tween 80 media; 7H12, 7H9 broth base media with BSA, casein hydrolysate, catalase, palmitic acid; LORA, low oxygen recover assay; VERO, African green monkey kidney cell line; RLM, rat liver microsomes; and HLM, human liver microsomes. Values reported are the average of three individual measurements.

solubility for compounds **1**, **3**, **4**, and **6** was measured at 181, 149, 148, and 25 μM, respectively, in phosphate-buffered saline (PBS) at pH 7.4. Additional in vivo ADME properties including terminal half-life ($t_{1/2ss}$), the area under the curve (AUC), the volume of distribution (V_d) and volume of distribution at steady state (V_{dss}) for compounds **1**, **3**, **4**, and **6** can be found in the Supporting Information. Encouraged by the potency, PK, and favorable oral bioavailability of these imdazo[1,2-*a*]pyridine agents, we intend to evaluate various analogues in vivo by the murine gamma knockout (GKO) infection model, and the results will be reported in due course.

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Finally, curious as to the mechanism of action of these agents, we performed transcriptional profiling experiments of *M. tuberculosis* treated with compound **1**, and comparison to the existing database of drug-induced transcriptional profiles indicated that this compound inhibited an aspect of energy generation in the cell (see the Supporting Information). Thus, compound **1** resulted in up-regulation of the cytochrome bd oxidase, which is the high oxygen-affinity respiratory enzyme[21] observed to be up-regulated during oxygen restriction as well as inhibition of respiration by agents such as cyanide, sodium azide, the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and the nitric oxide-releasing pro-drug PA-824.[22] In addition, this compound up-regulated the phosphoenolpyruvate carboxykinase, which plays an important role in modulating carbon flow during cellular energy restriction[23] and has previously been observed to be up-regulated by stresses such as hypoxia, sodium azide, valinomycin, nigericin, carbonyl cyanide rn-chlorophenylhydrazone, cyanide, PA-824, and the ATP inhibitor dicyclohexylcarboxydiimide, that limit energy generation through respiration.[22]

All of the data suggest that we have discovered a class of compounds with promising attributes of synthetic accessibility, no redox active moieties,[19] impressive potency, and selectivity toward replicating MDR and XDR Mtb strains. This class has good in vivo ADME properties that potentially can be improved through further analogue generation. Additionally, compound **1** appears to act by a novel mechanism of action based on transcriptional profiles to known anti-TB agents. With new anti-TB agents desperately needed, we offer the imidazo[1,2-*a*]pyridine class as a potential therapeutic for further development.

Table 2. Potency of Imidazo[1,2-*a*]pyridines (1, 3, and 8) and Imidazo[1,2-*a*]pyrimidine (10) against Single Drug Resistant Strains, MDR-TB and XDR-TB Strains (MIC_{90} in μM)[a]

	control/compound ID					
strains resistant to drugs	RMP	INH	**1**	**3**	**8**	**10**
RMP	>1	0.23	0.28			1.49
INH	0.01	>8	0.33			5.84
kanamycin	0.02	0.43	1.07			1.02
streptomycin	0.02	0.23	1.02			5.84
MDR-HRESP			2.24	1.01	0.26	
MDR-HREZSP			1.12	0.06	0.06	
MDR-HCPTh			1.12	0.13	0.26	
MDR-HREKP			0.28	0.13	0.26	
MDR-HRERb[b]			0.14	≤0.03	0.13	
MDR-HRERb[b]			0.14	0.03	0.34	
MDR-HRERb[b]			0.28	0.06	0.26	
MDR-HREZSKPTh			0.07	≤0.03	0.06	
MDR-HREZRbTh			0.14	0.06	0.06	
XDR-HRESPOCTh			0.07	0.03	0.07	
XDR-HREPKOTh			0.07	0.02	0.03	
XDR-HRESPO			0.14	0.02	0.03	

[a] Abbreviations: H = isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide, S = streptomycin, C = cycloserine, Th = ethionamide, K = kanamycin, P = *p*-aminosalicylic acid, Rb = rifabutin, Th = thioacetazone, and O = ofloxacin. [b] Different clinical strains.[20] Values reported are the average of three individual measurements.

■ ASSOCIATED CONTENT

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

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Author Contributions

G.C.M. participated in the design, performed the syntheses, drafted the manuscript, and facilitated all interactions. L.D.M. participated in the design and coordinated interactions through Dow AgroSciences. P.A.H. facilitated microsome and PK assessment. H.B. performed MDR and XDR anti-TB assays and the transcriptional profiling. S.C. and S.G.F. provided anti-TB and selectivity assays. M.J.M. drafted the manuscript and participated in the design and direction of the project.

Table 3. Nontubercular Mycobacteria Activity and Selectivity of Imidazo[1,2-*a*]pyridine (1) and Imidazo[1,2-*a*]pyrimidine (10) (MIC_{90} in μM)[a]

compound ID	TB-H_{37}Rv	*M. abscessus*	*M. chelonae*	*M. marinum*	*M. avium*	*M. kansasii*	*M. bovis* BCG	*M. smegmatis*
1	1.07	>50	>50	>50	1.32	1.32	0.33	>50
10	5.94	>50	>50	>50	12.00	12.00	2.78	>50
RMP	0.05	162.3	150.00	<0.78	<0.78	<0.78	<0.78	162.3

[a] Values reported are the average of three individual measurements.

Table 4. In Vivo PK Evaluation of Imidazo[1,2-*a*]pyridines[a]

compound ID	po C_{max} (ng/mL)	po T_{max} (h)	iv $t_{1/2}$ (h)	iv clearance (mL/min/kg)	% *F*
1	3012	0.25	0.35	91	76
3	3140	0.25	0.33	43	43
4	5741	0.25	0.28	28	50
6	1995	0.31	0.4	51	49

[a] Values reported are the average of three individual measurements.

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