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Synthesis and structure activity relationship of imidazo[1,2-*a*]pyridine-8-carboxamides as a novel antimycobacterial lead series

Sreekanth Ramachandran [a,*], Manoranjan Panda [a], Kakoli Mukherjee [b], Nilanjana Roy Choudhury [a], Subramanyam J. Tantry [a], Chaitanya Kumar Kedari [a], Vasanthi Ramachandran [b], Sreevalli Sharma [b], V. K. Ramya [b], Supreeth Guptha [b], Vasan K. Sambandamurthy [b]

[a] Department of Medicinal Chemistry, AstraZeneca India Pvt. Ltd, Avishkar, Bellary Road, Hebbal, Bangalore 560024, India
[b] Department of Biosciences, AstraZeneca India Pvt. Ltd, Avishkar, Bellary Road, Hebbal, Bangalore 560024, India

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ABSTRACT

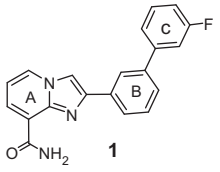
Imidazo[1,2-*a*]pyridine-8-carboxamides as a novel antimycobacterial lead were generated by whole cell screening of a focused library against *Mycobacterium tuberculosis*. Herein, we describe the synthesis and structure activity relationship evaluation of this class of inhibitors and the optimization of physicochemical properties. These are selective inhibitors of *Mycobacterium tuberculosis* with no activity on either gram positive or gram negative pathogens.

Tuberculosis (TB) remains the major cause of death from an infectious disease worldwide.[1a,b] The emergence of multidrug resistant (MDR) and extremely drug resistant (XDR) TB has rekindled a significant investment in identifying new drugs to address this huge unmet medical need.[2a,b] Hence there is an urgent focus to bring in new drugs with a novel mechanism of action to combat both drug-sensitive as well as drug resistant TB.

Various approaches in identifying novel inhibitors of *Mycobacterium tuberculosis* (Mtb) include both target based as well as whole cell screening against Mtb or surrogate species such as *M. smegmatis* (Msm) or *M. bovis* (BCG).[3] Despite the availability of the genome sequence of Mtb,[4] numerous efforts to identify novel chemical starting points against potential targets so far has resulted in limited success.[5] The target based lead generation approaches have often faced challenges in converting enzymatic activity (IC_{50}) to cellular potency (MIC) possibly due to the poor bacterial cell wall permeation. The recent successful identification and development of agents such as Sirturo™ for MDR-TB,[6a,6b] PA824[6c] and other candidates in different stages of clinical development can be attributed to the success of whole cell based screening.

In order to compliment our ongoing efforts to discover new TB drugs including AZD5847[7] and other compounds,[8] we embarked on a focused library screening against Mtb. Using a set of pharmacophores based on structural features derived from known antimycobacterial cell-division inhibitors[9] a focused library of 500 compounds was assembled. Whole cell screening of this library using *M. tuberculosis* H37Rv led to the identification of several hits. One of the actives, was a novel chemical class imidazo[1,2-*a*]pyridine-8-carboxamides with promising antimycobacterial activity. In this communication, we present our initial efforts towards the synthesis and structure activity relationship of this class of inhibitors including optimization of physicochemical properties.

The original hit (**1**) showed a modest minimum inhibitory concentration (MIC) of 16 μg/mL against *M. tuberculosis* H37Rv.



1

The synthesis of these class of compounds is shown in the Scheme 1. Coupling of commercially available 2-aminopyridines **2** with phenacyl bromides **3** in ethanol furnished moderate to good yields of imidazo[1,2-*a*]pyridines **4**. Hydrolysis of the nitrile using concentrated sulphuric acid yielded the corresponding amide **5** in quantitative yield. Suzuki reaction with various boronic acids

* Corresponding author. Tel.: +91 080 2362 1212; fax: +91 080 2362 1214.
E-mail address: sreekanth.ramachandran@astrazeneca.com (S. Ramachandran).

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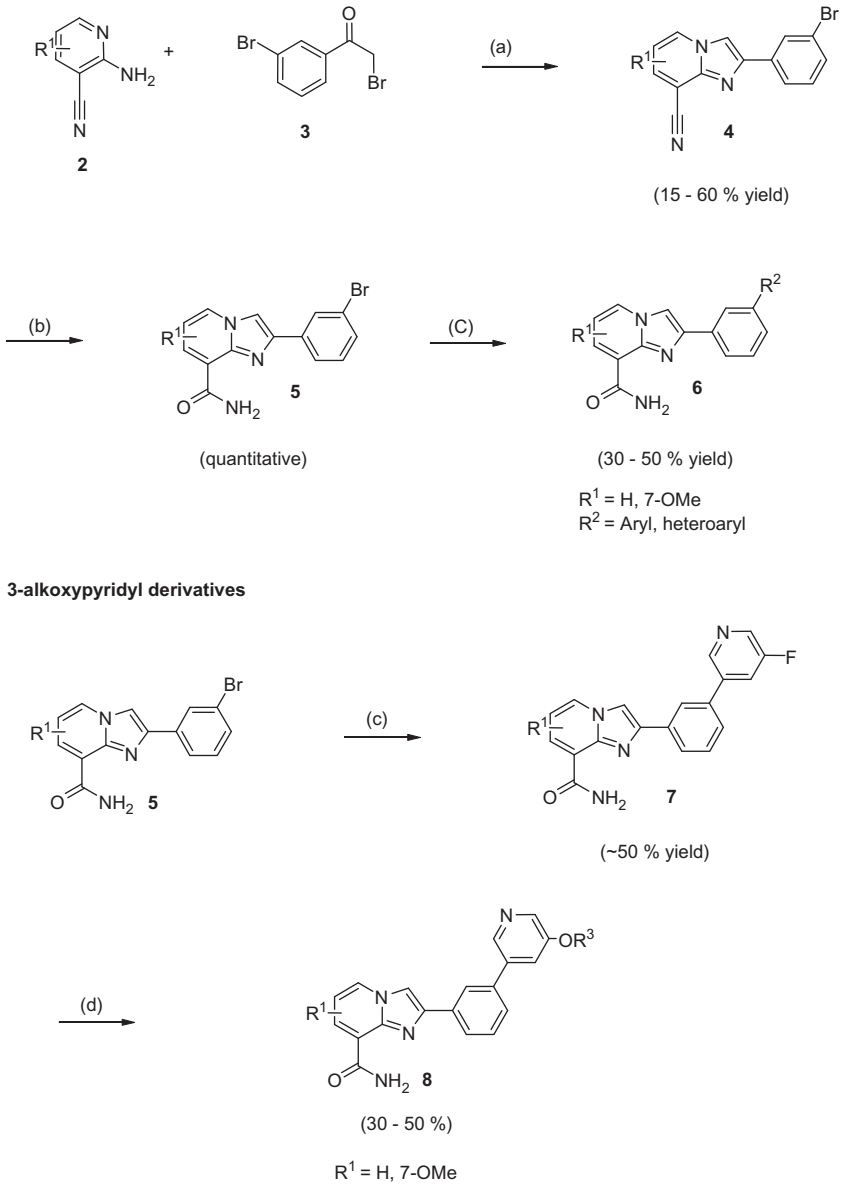


Scheme 1. Reagents and conditions: (a) EtOH, reflux, 10 h; (b) Con. H_2SO_4/H_2O, 50 °C, 3 h; (c) R^2-$B(OH)_2$, $PdCl_2(dppf)_2$, CsF, MeOH, 120 °C; (d) R^3-OH, NaH, DMF, 6 h.

under standard conditions provided the required imidazo[1,2-*a*]pyridines **6** in moderate yields. Similarly, 3-alkoxy pyridine derivatives **8** were synthesized starting from **5** by Suzuki using 3-fluoropyridine-5-boronic acid followed by SNAr reaction of **7** with various alcohols using sodium hydride as base.

Systematic medicinal chemistry exploration of **1** resulted in establishing MIC based SAR for the series with improved Mtb potency and physiochemical properties (Table 1).

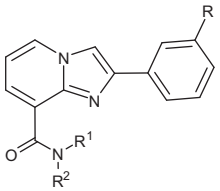
Initial SAR exploration was focused on C ring modifications in **1**. A small library synthesis using intermediate **5** allowed the exploration of SAR around the C ring using various aryl and heteroaryl substituents. This enabled the identification of compound **6a** with eightfold improvement in MIC and thereby fixing the 3-substituted aryl ring as important for driving MIC. Furthermore, SAR exploration of **6a** resulted in 3-methoxy pyridine **8a** which improved the MIC to 0.5 μg/ml range. The improvement in MIC with 3-methoxypyridine substituent in the ring C may be due to additional interactions at the active site of the target protein or to an improved cell permeation across the bacterial cell wall. Converting the primary amide in to secondary amide **9** retained the MIC of 2 μg/mL, while the tertiary amide **10** was inactive. These results led us to hypothesize that at least one H-bond donor at the A-ring is required for the activity.

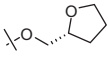
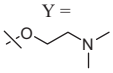
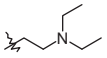
The 3-alkoxypyridine derivatives at ring C gave us a diversity handle to further optimize the MIC. This led to compounds **8c** and **8d** with MICs of 0.25 μg/mL having a tetrahydrofuran and fluoroethyl side chains, respectively, but with only marginal improvement in solubility.

We hypothesized that the series displayed poor aqueous solubility mainly due to high lipophilicity (as measured by Log*D*) and the planarity of the scaffold. To improve solubility, we employed two strategies either using a solubilizing group or breaking the planarity associated with the linking of 3 aromatic rings. The first strategy that led to compounds such as 3-alkoxypyridine **8e**, resulted in less potent compound, while introduction of similar side chain at secondary amide of imidazopyridine ring **11**, retained the potency with >2000 fold improved solubility compared to **8a**.

During the SAR exploration, we identified an alternative approach to optimize physicochemical properties without compromising the antimycobacterial potency (Fig. 1). This was achieved

Table 1
Antimycobacterial activity of compounds



Compound no.	R	R^1	R^2	Mtb MIC (μg/ml)	Aq. solubility (μM)	AZLog*D*[a]
						
1	X = F	H	H	16	<1	3.9
6a	X = OCH_3	H	H	2	<1	3.5
						
8a	Y = OCH_3	H	H	0.5	<1	3.1
8b	Y = 	H	H	0.5	<1	3.7
8c	Y = 	H	H	0.25	5.3	3.4
8d	Y = 	H	H	0.25	16	3.4
8e	Y = 	H	H	16	320	2.3
9	Y = OCH_3	Me	H	2	5.5	4.1
10	Y = OCH_3	Me	Me	>32	10	3.0
11	Y = OCH_3		H	4	>2460	2.9

[a] Predicted Log*D*.

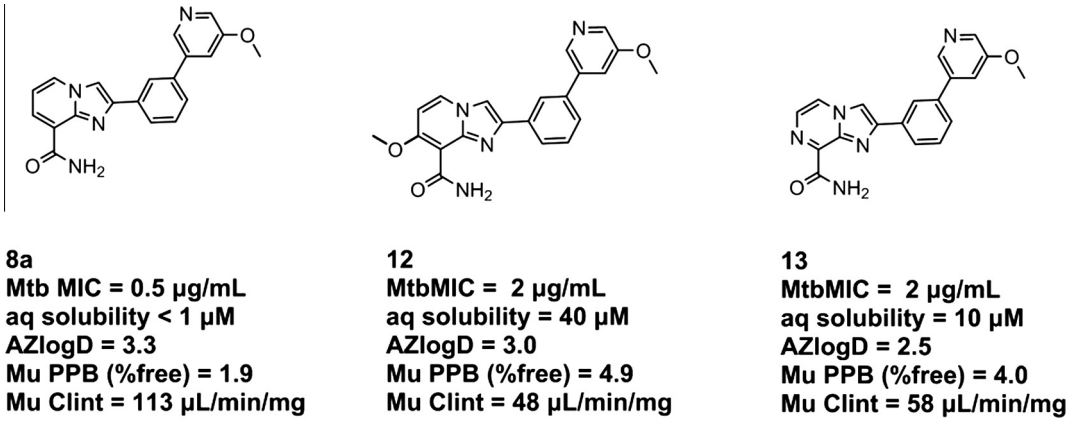


Figure 1. Matched pairs of **8a** with **12** and **13** with improved physico-chemical properties.

by disturbing the intra-molecular hydrogen bonding nature of primary amide. We hypothesized that a pseudo ring is formed by intramolecular hydrogen bonding between the primary amide and the nitrogen of imidazopyridine core (Fig. 2). The placement of a methoxy group next to the carboxamide resulted in **12** with improved solubility, reduced intrinsic microsomal clearance and decreased plasma protein binding. Replacement of an imidazole[1,2-*a*]pyridine ring carbon with nitrogen (imidazolopiperazine) **13** led to similar improvements in the molecule. This could be attributed to the perturbation of the amide group away from the ring planarity. To explore this further, we performed conformational analysis using Omega[10] followed by geometry optimization

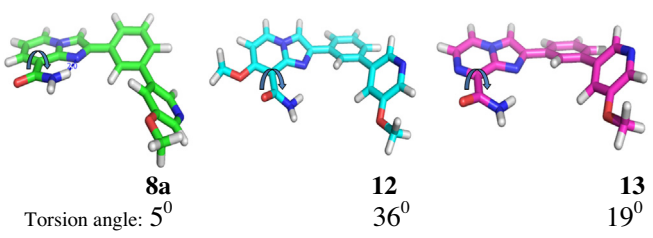


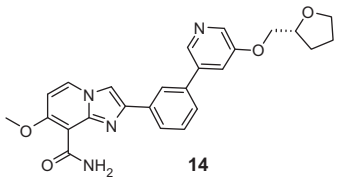
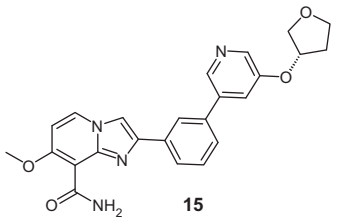
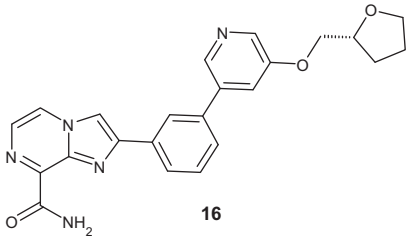
Figure 2. MP2/6-31G(d) optimized geometries of **8a**, **12** and **13**.

of the most preferred conformers using quantum mechanical electronic structure calculation.[11] We used the MP2/6-31G(d) method for the electronic calculations.

In the most preferred conformer, the amide group in compound **8a** is involved in an intramolecular hydrogen bond with the imidazopyridine nitrogen atom. This locks the core ring into a pseudo-planar tricyclic system which is rigid. This is reflected in its poor solubility and other physicochemical properties such as high plasma protein binding. Introduction of a methoxy group at the 7-position resulted in compound **12**, for which the most preferred conformer has the amide substituent twisted away from the core imidazolopyridine ring (torsional angle obtained from MP2 geometry optimization is 36°). Similarly, a ring nitrogen at the 7-position resulted in **13** causing a smaller perturbation. We hypothesized that the breaking of planarity and hence crystal packing[12] plays an important role in improving solubility and other physicochemical properties for these compounds. Theobserved high metabolic clearance for compound **8a** might be due to the hydroxylation occurring at 7-position, whereas in compounds **12** and **13** the 7-position is blocked by methoxy and nitrogen, respectively.

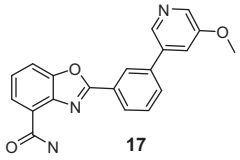
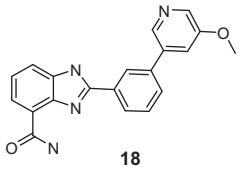
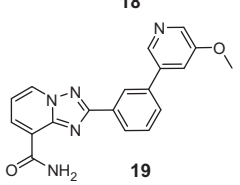
Based on these results we have designed and synthesized derivatives **14**–**16** (Table 2), which further support this hypothesis.

Table 2
Improving the aqueous solubility

Compound	Mtb MIC (μg/mL)	Aq. solubility (μM)	AZLog*D*[a]
 **14**	1	120	3.6
 **15**	4	600	3.3
 **16**	2	40	2.7

[a] predicted log D.

Table 3
Ring A variations

Compound	Mtb MIC (μg/mL)	Aq. solubility (μM)	AZLog*D*[a]
 **17**	2	2	3.0
 **18**	4	3	2.8
 **19**	2	18	2.5

[a] Predicted Log*D*.

Table 4

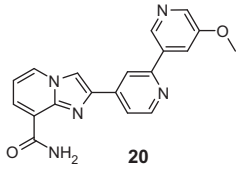
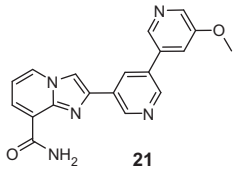
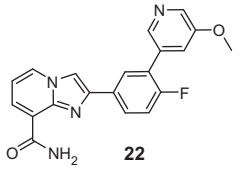
Compound	Mtb MIC (μg/mL)	Aq. solubility (μM)	AZLog*D*[a]
 **20**	16	22	2.1
 **21**	>32	3.5	2.0
 **22**	>32	<1	3.0

Table 5
Cytotoxicity index of representative compounds

Compound	Mtb MIC (μM)	A549 IC50 (μM)
16	6.25	>100
15	12.5	>100
8b	3.12	>100
13	12.5	>100
8d	0.78	>100

Table 6
Broad-spectrum activity of representative compounds

Broad spectrum panel (μM)	Compound **8b**	Compound **8c**	Compound **8d**
Mtb MIC	3.12	0.78	0.78
E. coli MIC	>200	>200	>200
S. aureus	>200	>200	>200
H. influenzae	>200	>200	>200
S. pnuemoniae	>200	>200	>200

Replacement of the ring A imidazopyridine with various heterocycles such as benzoxazole, benzimidazole and triazolopyridine resulted in compounds **17**–**19** (Table 3) which retained good MIC values thereby suggesting that the series has further potential for diversification.

A limited SAR exploration around the central phenyl linker (ring B) showed that unsubstituted phenyl remained the best in terms of potency and physicochemical properties (Table 4; **20–22**)

The series displayed an excellent selectivity index for cytotoxicity (Table 5), where all representative compounds demonstrated no effect on human A549 cell line at 100 μM.

Selected compounds which were profiled for broad spectrum antibacterial activity (Table 6) showed that these compounds were highly selective for Mtb with no MIC values observed against gram negative or gram positive pathogens. The narrow antimycobacterial spectrum of these inhibitors might be attributed to a *Mycobacteria* specific mode of action or due to selective cell permeability/efflux in other bacterial pathogens.

A few representative compounds were also profiled for *in-vitro* DMPK properties (Table 7). The series exhibited a medium to high clearance in human microsomal preparations despite having low protein binding in the presence of human plasma. In general for this series, the measured Log*D* correlates well with predicted Log*D* (AZ Log*D*), which could be helpful in further design and optimization of physicochemical properties of this series.

Table 7
Invitro DMPK properties of representative compounds

Compound	Hu Clint (μl/min/mg)	Hu PPB (% free)	Log*D*[a]
12	56	2	2.3
8e	47	7.3	ND
8d	75	1	3.7

[a] Measured Log*D* at pH 7.4, ND: not determined.

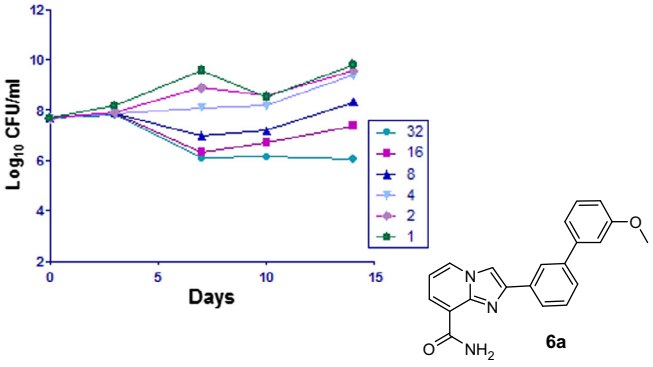


Figure 3. Killing kinetics for compound **6a(μg/mL)**.

Finally, cidality was measured for compound **6a** against replicating Mtb grown in Middlebrook 7H9 broth under aerobic conditions (Fig. 3). This compound displayed a concentration dependent bacterial kill. A 2 Log reduction in bacterial numbers were achieved at the highest tested concentration of **6a** (32 μg/mL).

In conclusion, we have discovered a novel chemotype, the imidazo[1,2-*a*]pyridine-8-carboxamides, as potent inhibitors of *M. tuberculosis*. Initial medicinal chemistry efforts resulted in improved potency and in vitro physicochemical properties with **8d** being the best compound identified as the lead for further progression. Current efforts are directed towards identifying the mode of

action and optimization of in vivo pharmacokinetic and pharmacodynamic properties to progress this series forward.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.043.

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