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Article

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Lead Optimization of a Novel series of Imidazo[1,2-*a*]pyridine Amides Leading to a Clinical Candidate (Q203) as a Multi- and Extensively-Drug Resistant Antituberculosis Agent

Sunhee Kang[1], Ryang Yeo Kim[2], Min Jung Seo[1], Saeyeon Lee[1], Young Mi Kim[1], Mooyoung Seo[1], Jeong Jea Seo[1], Yoonae Ko[1], Inhee Choi[1], Jichan Jang[2], Jiyoun Nam[3], Seijin Park[3], Hwankyu Kang[3], Hyung Jun Kim[2], Jungjun Kim[4], Sujin Ahn[3], Kevin Pethe[2], Kiyean Nam[4], Zaesung No[1,#,]*, Jaeseung Kim[1]*

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ABSTRACT

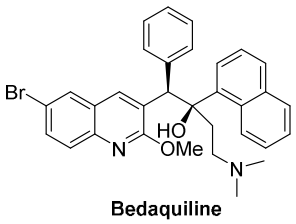
A critical unmet clinical need to combat the global tuberculosis epidemic is the development of potent agents capable of reducing the time of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis therapy. In this paper, we report on the optimization of the imidazo[1,2-*a*]pyridine amide (IPA) lead compound **1**, which led to the design and synthesis of Q203 (**50**). We found that the amide linker with IPA core is very important for activity against *Mycobacterium tubercu*losis H37Rv. Linearity and lipophilicity of the amine part in the IPA series play a critical role in improving *in vitro* and *in vivo* efficacy and pharmacokinetic profile. The optimized IPAs, **49** and **50** showed not only excellent oral bioavailability (80.2 and 90.7%, respectively) with high exposure of the area under curve (AUC) but also displayed significant colony-forming unit (CFU) reduction (1.52 and 3.13 $\log_{10}$ reduction at 10 mg/kg dosing level, respectively) in the lung of mice.

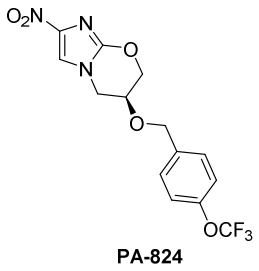
INTRODUCTION

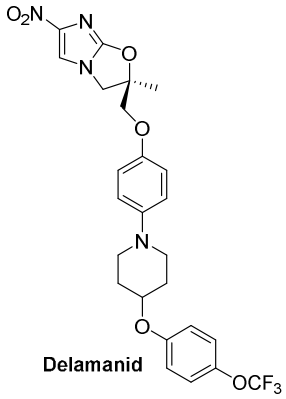
Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. TB remains a serious global health problem positioned as the second leading cause of death from a single infectious agent worldwide with an incidence rate of almost 9 million cases and a fatality rate of 1.4 million in 2011[1]. Notwithstanding the successful implementation of Directly Observed Treatment Short course (DOTS) in many countries, high prevalence of MDR and XDR tuberculosis has intensified the urgent need for new anti-tubercular drugs. Several new classes of compounds have been discovered for treatment of tuberculosis in the last decade[2-4]. A few of them are currently in clinical trials[5-7] and one of them, bedaquiline, was recently approved for the treatment of MDR tuberculosis (Figure 1). However, given the emergence of drug resistance and the low success rate encountered in clinical development, there is still a need to develop additional drug candidates for TB treatment.

We recently reported a novel chemical entity named Q203 (**50**) as a promising anti-TB drug candidate[8] using phenotypic high-content screening (HCS) technology inside infected macrophages[9]. The IPA series targets QcrB[8,10], which encodes the b subunit of the electron transport ubiquinol cytochrome C reductase. The IPA series was reported by others as an attractive anti-TB lead series[11-13]. Here, we report on the lead optimization that led to the design the potential clinical candidate **50**.

Figure 1. Structures of bedaquiline, PA-824[5] and delamanid.







Chemistry. General amide coupling of appropriately substituted imidazo[1,2-*a*]pyridine-3-carboxylic acid (**4a-4g**) with corresponding amines R3 afforded target compound in over 60 % yield (Scheme 1). One of the precursor, the imidazo[1,2-*a*]pyridine-3-carboxylic acid (**4a-4g**) was prepared starting from bromination of various β-keto esters except for commercially available ethyl 2-chloro-3-oxobutanoate (**2a**). Unsubstituted β-keto esters were treated with *N*-bromosuccinimide and over two equivalent of NH_4OAc in Et_2O to afford the 2-monobrominated product (**2b**)[14]. Alternatively, β-keto esters could be transformed to 2-bromo products by bromine (**2c-2d**) which resulted in comparable yields. The adequate monobrominated β-keto esters were condensed with the substituted 2-aminopyridines *via* imine-enamine formation in absolute ethanol at reflux temperature[15] and the resulting esters (**3a-3g**) were hydrolyzed to acids (**4a-4g**).

The counter parts, methanamine derivatives, can be classified into three functional groups; i) bi-aryl, ii) 2-ring system having a saturated cyclic amine, iii) 3-ring system having a saturated cyclic amine between two aryl rings. The synthetic route of the methanamine is shown in Scheme 2. Suzuki coupling of 4-chlorobenzonitrile and substituted phenylboronic acid using $PdCl_2$(dppf) and aqueous Na_2CO_3[16] followed by reduction with

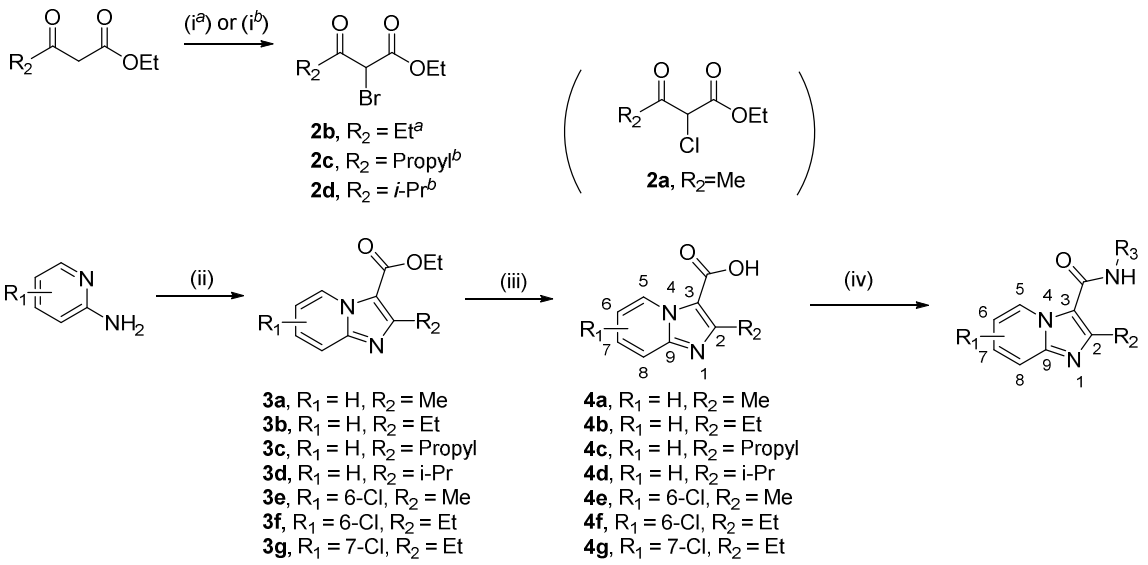
lithium aluminum hydride gave biphenyl methanamines **7a-7f**. Exceptionally, biphenyl methanamines having a cyano group and *tert*-butyl group, **7f** and **7g** were synthesized by direct Suzuki coupling with 4-bromobenzylamine. The benzylamines which have saturated ring contained bis- and tris-ring were prepared in a straightforward manner as shown in Scheme 3. Commercially available 4-fluorobenzonitrile was reacted with appropriate cyclic amine and K_2CO_3 by heating in dimethyl sulfoxide and then reduced with lithium aluminum hydride to produce benzylamines **8a-8n**[17].

The synthetic route for the linker modification is shown in Scheme 4. Compound **9,** which has no benzylic carbon next to amide was prepared *via* acid chloride activation of **4f** and *N*-methylated compound **10** was synthesized by treating sodium hydride and iodomethane from compound **1**. Amide reduction of **1** in a mild condition using boron trifluoride etherate and $NaBH_4$ provided **11** with moderate yield.

In the case of **14** and **15**, which have one more carbon between the imidazo[1,2-*a*]pyridine ring and the carbonyl group of amide bond, 4-brominated β-keto ester is required. Interestingly, the desired 4-brominated β-keto ester **12** was regioselectively synthesized by treating *N*-bromosuccinimide with 0.1 equivalent of neutral catalyst, NH_4OAc compared to the presence of over 2 equivalent of NH_4OAc for the synthesis of 2-brominated β-keto ester **2b**. In this reaction, 2-brominated β-keto ester, **2b** was generated initially and then the bromo group was migrated to 4-position after overnight reaction. However, the migration of the bromo group was not occurred in the presence of excess amount of NH_4OAc. **12** was condensed with 2-amino-5-chloropyridine and saponified to afford the intermediate acid **13** followed by general amide coupling afforded **14** and **15**. The reverse-amide compound **17** was synthesized *via* curtius rearrangement using diphenylphosporyl azide and

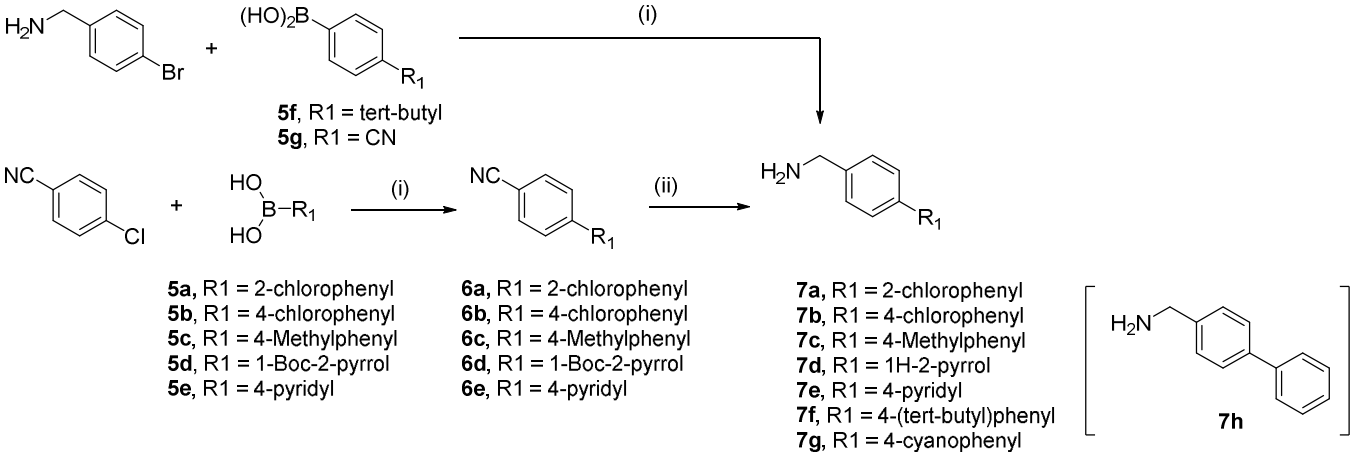
triethylamine in *t*-BuOH. Subsequent de-protection of Boc group by trifluoroacetic acid and amide coupling *via* acid chloride activation afforded the target compound.

Scheme 1. Synthesis of imidazo[1,2-*a*]pyridine-3-carboxylic acids 4a-g[a]



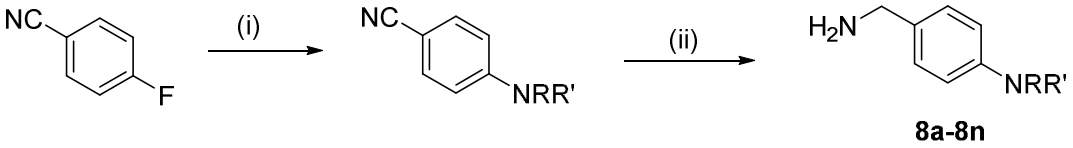
[a]Reagents and conditions: (i[a]) NBS, NH_4OAc (over 2eq.), ether, rt, 6h; (i[b]) Br_2, $CHCl_3$, 0℃ – rt, 20min; (ii) **2a-2d**, EtOH, reflux, overnight; (iii) LiOH, EtOH/H_2O (3:1, *v/v*), rt, overnight; (iv) corresponding amine, EDC, HOBt, TEA, DMF, 80℃, 2-4h

Scheme 2. Synthesis of [1,1'-biphenyl]-4-ylmethanamine analogues 7a-g[a]



[a]Reagents and conditions: (i) $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O (3:1, *v/v*), 150℃, 1h – 3h; (ii) LAH, THF, 0℃ - reflux, 1h

Scheme 3. Synthetic scheme for benzylamine tails 8a-8n[a]



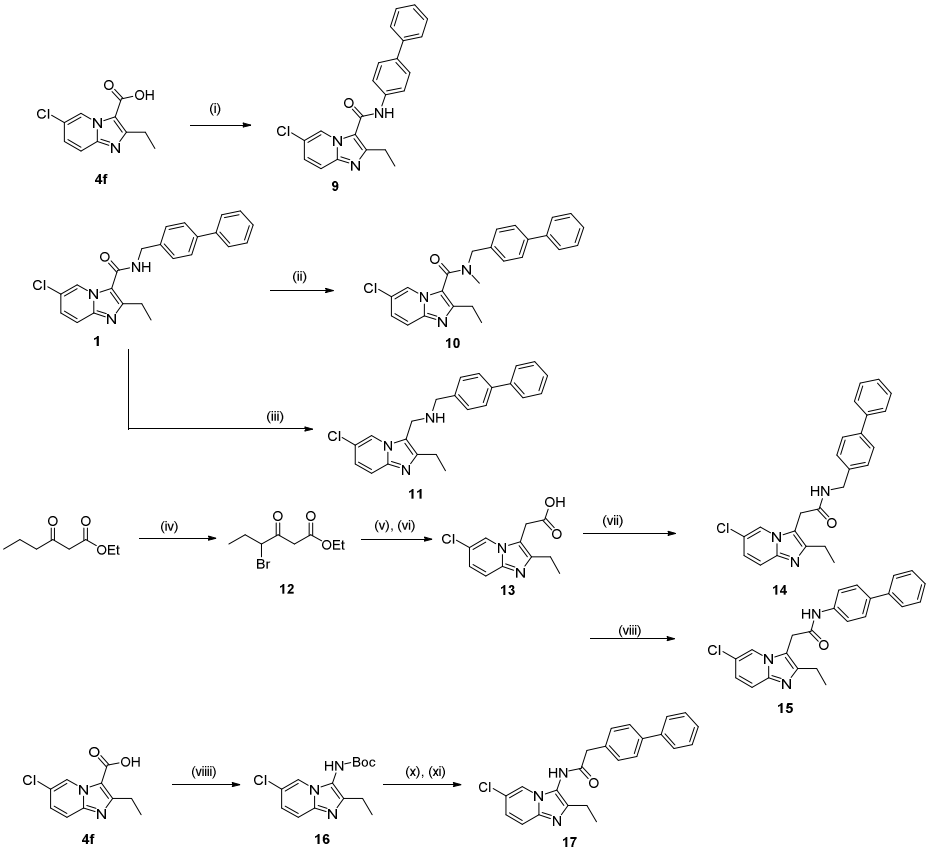
8a-8n

NRR' =

8a, piperidine
8b, azepane
8c, octahydro-1*H*-isoindole
8d, 4,5,6,7-tetrahydro-2*H*-isoindole
8e, 4-chloropiperidine
8f, 4-(trifluoromethyl)piperidine
8g,1-methylpiperazine
8h, 1-isopropylpiperazine
8i, 1-(4-fluorophenyl)piperazine
8j, 1-(4-(trifluoromethoxy)phenyl)piperazine
8k, 4-(4-fluorophenyl)piperidine
8l, 4-(4-chlorophenyl)piperidine
8m, 4-(4-(trifluoromethoxy)phenyl)piperidine
8n, 4-(4-fluorophenyl)piperidin-4-ol

[a]Reagents and conditions: (i) K_2CO_3, corresponding amine, 90-120℃, 3h; (ii) LAH, THF, reflux, 1h

Scheme 4. Synthetic scheme for linker modification[a]



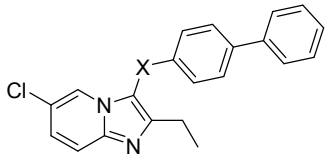
[a]Reagents and conditions: (i) $SOCl_2$, 100 ℃, 1h, then [1,1'-biphenyl]-4-amine, TEA, MC, rt, 1h; (ii) NaH, CH_3I, DMF, 0℃ - rt, 1h; (iii) $NaBH_4$, BF_3·etherate, THF, reflux, 2h; (iv) NBS, NH_4OAc (0.1 eq.), Et_2O, rt, overnight; (v) 2-amino-5-chloropyridine, ethanol, reflux, overnight; (vi) LiOH, MeOH/H_2O (3:1, *v/v*), rt, overnight; (vii) EDC, [1,1'-biphenyl]-4-ylmethanamine, HOBt, TEA, DMF, 80℃, 3h; (viii) [1,1'-biphenyl]-4-amine, EDC, HOBt, TEA, DMF, 80℃, 3h; (viiii) DPPA, TEA, *t*-BuOH, reflux, overnight; (x) TFA, MC, rt, 1h; (xi) 2-([1,1'-biphenyl]-4-yl)acetic acid, $SOCl_2$, TEA, MC, rt, 1h

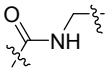
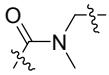
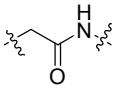
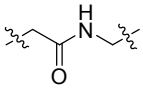
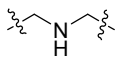
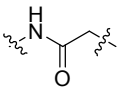
RESULTS AND DICUSSION

The activity of IPA derivatives was tested against *M. tuberculosis* replicating inside macrophages (intracellular MIC_{80}) and in liquid broth culture medium (extracellular MIC_{80}) (Tables 1-4), as previously described[8]. In addition, the metabolic stability of the compounds was evaluated in mouse and human liver microsomal preparations to study structure-property relationships (SPR) in order to prioritize compounds for *in vivo* pharmacokinetic evaluation. The initial structure-activity relationship (SAR) studies evaluated a set of analogues that contained replacement of the 3-carboxamide linker in an attempt to affect potency of compound **1** (Table 1). Replacement of hydrogen on NH with methyl (**10)** significantly decreased the activity against *M. tuberculosis* H37Rv replicating outside and inside macrophages by approximately 190 and 690-fold. Surprisingly, the modification of the length of the amide linker with one carbon (**14** and **15**) between the imidazo[1,2-*a*]pyridine ring and the carbonyl group of amide bond abolished the potency against *M. tuberculosis*. Introduction of *N*-phenyl group (**9**) at the amine position did not give any activity as well. We then

investigated the contribution of the H-bonding acceptor of the carbonyl group. Modification by a reversed amide (**17**) or removal of the oxygen on carbonyl group (**11**) reduced the activity to sub-micromolar range (intracellular MIC_{80} = 200 nM and 690 nM, respectively) compared to the parent compound **1**. Accordingly, this set of modifications revealed that the carboxamide linker with the *N*-benzylic group is critical for anti-mycobacterial activity.

Table 1. Activity of linker modified IPA analogues against *M. tuberculosis* H37Rv



		Antimycobacterial activity against *M. tuberculosis* H37Rv	
Compound	X	[a]extracellular MIC_{80} (nM)	[b]intracellular MIC_{80} (nM)
1		45	1.39
10		8690	970
9		>10000	>10000
15		>10000	>10000
14		>10000	>10000
11		810	200
17		1670	690

[a]extracellular MIC_{80}: the inhibitory activity against *M. tuberculosis* H37Rv replicating in culture broth medium;

[b]intracellular MIC_{80}: the inhibitory activity against *M. tuberculosis* H37Rv replicating inside macrophages;

MIC_{80} is the minimum concentration required to inhibit growth of 80 %; MIC_{80} indicates average value of two independent measurement.

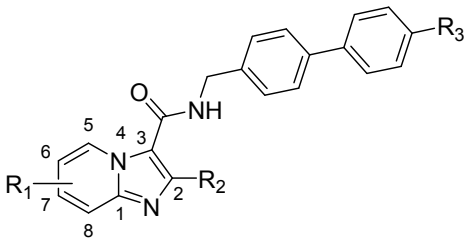
Next, the optimization was focused on R1, R2 and R3 modifications (Table 2). During the exploration of SAR at R2 and R3 positions, the substituent's at R1 was limited to H, 6-Cl, and 7-Cl. This is because we found early on that 6 or 7-Cl substitutions improved antibacterial activity and increased the metabolic stability compared to other substituent groups (data not shown). To investigate the hydrophobic interaction of R2 alkyl groups, the four compounds **18-21** were designed to alter the size of R2 position that might disturb positioning of the 3-carboxamide. As expected, the smaller size groups, methyl and ethyl (**18 and 19**) showed better activity (intracellular MIC_{80}= 20 nM and 97 nM, respectively) than longer and bulky groups, propyl and iso-propyl (**20 and 21**). Furthermore the sterically hindered compound **21** (intracellular MIC_{80}= 3130 nM) was much less potent than the linear compound **20** (intracellular MIC_{80}= 50 nM). To confirm the improved potency of methyl and ethyl group at the R2 position, the compound **1** and **22** were prepared and evaluated. Compound **1,** which possesses an ethyl group, showed approximately 30-fold greater potency against intracellular mycobacteria compared to methyl substituted compound **22.** However, the methyl substitution had a better metabolic stability compared to the ethyl substitution.

Further exploration of the SAR at the R3 position of the phenyl group having 6- or 7-Cl of imidazo[1,2-*a*]pyridine core was conducted by substitution of various functional groups such as donating, withdrawing and carboxylic acid. For the effect of the position on the benzene ring, a *para*-chloro group (**24**) offered much more potency and metabolic stability than the *ortho*-chloro group (**23**). Encouraged by the positive effect of the *para*-

substitution on antibacterial effect, we focused on screening several analogues at this position to investigate the activity and metabolic stability. Intracellular activity of all substituents such as donating and withdrawing groups (**24-28**), except a hydrophilic acid, were very similar within a 2-fold range of MIC_{80}= 0.4 ~1.3 nM. Even the more lipophilic and sterically hindered compound **30** showed similar intracellular activity comparable to compound **27**. On the other hand, compound **29** that has a hydrophilic carboxylic acid group, gave a deleterious effect on the activity. In terms of metabolic stability, the alkyl group such as methyl (**26** and **27**) and *tert*-butyl (**30**) showed much lower microsomal stability than the other groups Cl, CN and acid (**24, 25, 28** and **29**) in human and mouse liver microsomes. This again suggested that the position of R3 on the benzene ring may play a role in potency and microsomal stability.

From our initial SAR study, compound **24**, **25** and **28** showed desirable extra- and intracellular potency as well as metabolic stability in human and mouse liver microsomes to perform *in vivo* pharmacokinetic (PK) experiments. However, compound **25** was too insoluble in aqueous and organic solutions caused by extension of aromatic character to conduct *in vivo* experiment. Thus, compound **24** was selected and *in vivo* PK profile was evaluated in Spraque-Dawley rat after administration by oral (p.o.) and intravenous (i.v.) routes. However, the half-life and AUC after oral administration could not be calculated because the concentration of compound detected in the plasma remained constant (or even slightly increased) up to 16 hours after dosing (supporting information, Table S1). This result suggested that compound **24** was rebounded in absorption after 4 h, could be subject to compound precipitation in gut and extended absorption due to the highly lipophilic nature of the compound **24**. Therefore, our next optimization strategy was focused on replacement of the second phenyl group with various ring systems to improve solubility and reduce lipophilicity (Table 3).

Table 2. Activity and metabolic stability of bis-phenyl IPA analogues against *M. tuberculosis* H37Rv



Compound	R1	R2	R3	Antimycobacterial activity against *M. tuberculosis* H37Rv: extracellular MIC_{80} (nM)	Antimycobacterial activity against *M. tuberculosis* H37Rv: intracellular MIC_{80} (nM)	Microsomal stability ($t_{1/2}$, min): Human	Microsomal stability ($t_{1/2}$, min): Mouse
18	H	Me	H	250	20	[a]ND	[a]ND
19	H	Et	H	63	97	27.1	[a]ND
20	H	Pr	H	1180	50	22.4	8.7
21	H	^{i}Pr	H	3130	3130	31.6	[a]ND
22	6-Cl	Me	H	175	42	>120	>60
1	6-Cl	Et	H	45	1.39	22.8	19.3
23	6-Cl	Et	2-Cl	43	9.3	38.9	13.1
24	6-Cl	Et	4-Cl	0.9	0.45	83	>60
25	6-Cl	Et	4-CN	<0.5	0.68	>120	>60
26	6-Cl	Et	4-Me	0.7	0.43	30.5	40.6
27	7-Cl	Et	4-Me	<0.5	1.35	25.4	23.1
28	7-Cl	Et	4-Cl	1.3	1.01	>120	>60
29	7-Cl	Et	4-CO_2H	250	217	>120	>120
30	7-Cl	Et	4-^{t}Bu	12	0.46	18.5	50.7
Isoniazid(INH)				449	617		
Rifampicin(RIF)				26.6	180		

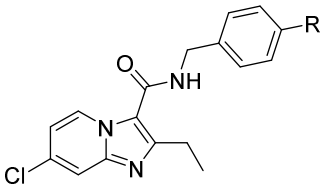
[a]ND, Not Determined.

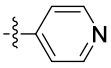
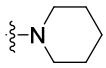
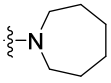
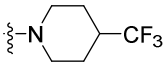
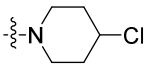
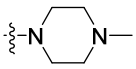
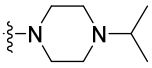
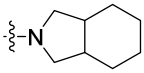
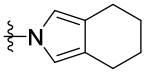
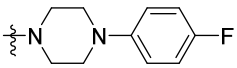
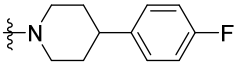
The changes were focused on the second phenyl ring on right-hand side while keeping the 7-chloro-2-ethylimidazo[1,2-*a*]pyridine-3-carboxamide at the left-hand side. Firstly, we introduced hetero aromatic groups that were favorable to make a salt formation to improve solubility. However, introduction of heteroaromatic

rings led to a dramatic reduction in potency (**31** and **32**, intracellular MIC_{80} of 140 and 740 nM, respectively). Another strategy involved the introducing of nitrogen containing saturated ring next to the first phenyl ring, such as a piperidine, azepane and piperazine. The nitrogen atom, if appropriately acidic, could potentially provide an ionizable site. When the piperidine and azepane moieties (**33** and **34**) were placed at the end, the cellular activity was maintained compared to heteroaromatic rings, **31** and **32**. However, not only the compounds were heavily metabolized in human and mouse liver microsomes, they also had decreased antibacterial activity in macrophages compared to the lead compound **1**. Furthermore we did not find any sign of improvement of microsomal stability by substitution at the 4-position on the piperidine ring (**35** and **36**).

A series of analogues containing piperazine at the end of the phenyl ring were also synthesized and evaluated. From the previous SAR studies, it was shown that a hydrophilic character on the second ring led to decreased potency. Thus, we applied small alkyl group such as methyl and *iso*-propyl at 4-position of piperazine to protect activity loss. Unlike piperidine compounds **35** and **36**, compounds **37** and **38** lost their potency against *M. tuberculosis* H37Rv replicating inside and outside macrophage (intracellular MIC_{80} > 140 nM). On the other hand, compounds **39** and **40** with the fused ring showed a potency comparable to compound **1,** whether or not the fused ring had an aromaticity. The excellent potency of the lipophilic and bulky analogues **39** and **40** suggested that there is more lipophilic space and that the two-ring system could be extended to a three-ring system on the right-hand side. In addition, compound **25** having a nitrile group that is representative linearity or aromaticity next to the biphenyl ring, showed a superior potency with MIC_{80} value of less than 1 nM against *M. tuberculosis* H37Rv replicating outside and inside macrophages, as well as good metabolic stability in human and mouse liver microsomes (Table 2).

Table 3. Activity of IPA analogues containing two or three rings against *M. tuberculosis* H37Rv



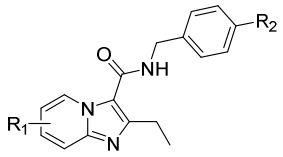
Compound	R	Antimycobacterial activity against *M. tuberculosis* H37Rv: extracellular MIC_{80} (nM)	Antimycobacterial activity against *M. tuberculosis* H37Rv: intracellular MIC_{80} (nM)	Metabolic stability ($t_{1/2}$, min): Human	Metabolic stability ($t_{1/2}$, min): Mouse
31		2000	140	>120	10.8
32		2220	740	28.9	[a]ND
33		16	8.2	13.2	2.3
34		35	10	2.9	1.9
35		0.8	0.47	6.5	5.1
36		19	2	11.0	5.2
37		4390	360	116.6	12.1
38		3000	140	>120	8.9
39		25	9.4	14.9	6.3
40		34	3.74	27.3	62.3
41		5.7	0.3	>120	33.3
42		540	0.66	6.8	18.4

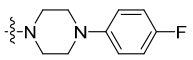
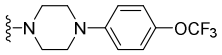
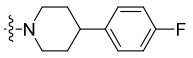
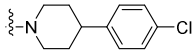
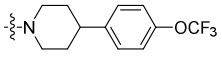
[a]ND, Not Determined.

With this structure-activity relationship (SAR) analysis, two analogues **41** and **42** were designed and evaluated against *M. tuberculosis* H37Rv replicating outside and inside macrophage. Compound **41** with a 4-

fluorophenyl piperazine showed not only dramatically increased with MIC_{80} values of 0.3 nM compared to analogue **38** (MIC_{80}=140 nM) against bacteria replicating inside macrophages but also approximately 3-fold improved stability in mouse liver microsomes. In the same manner, the 4-fluorophenyl piperidine analogue **42** showed over 10-fold better potency by incorporation of phenyl ring than analogue **33** (intracellular MIC_{80}= 8.2 nM) with better microsomal stability in mouse. However, the microsomal stability still required to be more improved.

Table 4. Activity of IPA analogues containing three ring system against *M. tuberculosis* H37Rv



			Antimycobacterial activity against *M. tuberculosis* H37Rv		Metabolic stability ($t_{1/2}$, min)		CYP inhibition (IC_{50}, uM)				
Compound	R1	R2	extracellular MIC_{80} (nM)	intracellular MIC_{80} (nM)	Human	Mouse	3A4	2D6	1A2	2C9	2C19
41	7-Cl		5.7	0.3	>120	33.3	>40	>40	>40	>40	>40
43	6-Cl		<0.5	0.3	>120	33.3	0.49	>40	>40	1.33	1.32
44	7-Cl		1.8	0.11	>120	>60	>40	>40	>40	0.17	>40
45	6-Cl		<0.5	0.23	>120	>60	11.54	>40	>40	0.57	>40
42	7-Cl		0.54	0.66	6.8	18.4	2.07	>40	>40	0.5	0.6
46	6-Cl		<0.5	0.36	62.6	20.3	>40	>40	>40	>40	>40
47	7-Cl		1	0.46	67.3	112.0	>40	>40	>40	0.19	0.38
48	6-Cl		4.1	1.3	57.9	116.0	>40	>40	>40	0.26	6.77
49	7-Cl		4.0	3.7	>120	>120	>100[a]	>100	>100	0.14	0.29
50 (Q203)	6-Cl		4.0	1.43	>120	>120	>100[b]	>100[b]	>100[b]	>100[b]	>100[b]

[a]Values were determined by LC/MS method; [b]The assay was performed using recombinant CYP enzymes and analyzed by LC/MS/MS

To improve the microsomal stability, substituents on the third aromatic ring were investigated with 7-chloro- or 6-chloro-2-ethylimidazo[1,2-*a*]pyridine-3-carboxamide as a left-hand side. As shown in Table 4, the analogues **47** and **48**, in which the fluorine was replaced by chlorine, had a retained potency (intracellular MIC_{80}= 0.46 nM and 1.3 nM, respectively) and improved microsomal stability ($t_{1/2}$ > 60 min, mouse microsomes). Other analogues (**44-45** and **49-50**) bearing a trifluoromethoxy substituent showed a similar activity and good stability in human and mouse microsome. All analogues in Table 4 displayed unprecedented MIC_{80} values in single-digit nanomolar or sub-nanomolar range against *M. tuberculosis* H37Rv replicating both outside and inside macrophage. In terms of metabolic stability, the stability-substituent correlation was followed in order of trifluoromethoxy > chlorine > fluorine and most compounds had good metabolic stability to perform *in vivo* experiment except 4-fluorophenyl piperidine analogues **42** and **46**. In addition to the stability, the incorporation of ionizable saturated ring between two aryl-rings compared to bis-phenyl IPA analogues (**24-26**) resulted in improved solubility under acidic condition without activity loss (supporting information, Table S2).

All analogues were also evaluated for cytochrome P450 inhibition with 5-different isozymes in order to prioritize compounds for *in vivo* PK experiments. Drug-drug interaction is a critical factor to develop an anti-TB agent due to combination therapy with other TB drugs or HIV drugs for co-infected treatment. Based on their overall properties, compounds **41, 49** and **50** were shortlisted for *in vivo* PK experiments.

Table 5. *In vivo* pharmacokinetic values in mice of selected compounds, 41, 49 and 50

Compd	Pharmacokinetics (i.v.)			Pharmacokinetics (p.o.)				
	$t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (mL/kg)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{0\text{-}inf}$ (ng·h/mL)	F (%)
41	6.15	1.9	877	4450	9.4	2.0	59576	69.9
49	62.3	3.15	14300	1987	21.3	2.0	27349	80.2
50[a]	16.5	4.0	5270	1490	23.4	2.0	44100	90.7

[a]PK values for compound **50** were adapted from reference 8 and presented for the sake of comparison.

The *in vivo* PK properties of compounds **41, 49** and **50** were evaluated in mice after intravenous (i.v.) and oral (p.o.) administration of 2 and 10 mg/kg, respectively. As shown in Table 5, those compounds displayed good PK properties with long half-life, low systemic clearance and moderate to high volume of distributions. After oral dosing, all compounds reached a maximum concentration in plasma within 2 hours, their elimination half-life was favorable (9.4, 21.3 and 23.4 hours, respectively) and the area under curve (AUC) was 59,576, 27,349 and 44,100 ng·h/mL, respectively. Overall, they achieved good oral exposure in systemic circulation that resulted in superior oral bioavailability (69.9, 80.2 and 90.7%, respectively).

On the basis of the promising *in vivo* PK profile, we conducted *in vivo* efficacy studies for compound **49** in an established mouse model under the same condition as previously described[8]. BALB/c mice were infected with 2 x10^2 to 2 x 10^3 CFU of *M. tuberculosis* H37Rv by the intranasal route. Compound treatment was initiated three weeks after infection. Compound **49** or the reference drug isoniazid (INH) was administered by oral gavage for 28 days, five times per week. Bacterial load in the lungs of infected mice was determined by CFU enumeration as shown in Table 6. Both compounds displayed potential efficacy results in a dose-

dependent manner. The compound **49** was active and promoted a significant reduction of the bacterial burden in the lungs of infected animals. The reduction in CFU was proportional to the administered dose, but was less pronounced than for isoniazid despite better pharmacodynamic indices. The compound **49** (1.52 $\log_{10}$ CFU reduction at 10 mg/kg) was also less efficacious than **50** (3.13 $\log_{10}$ CFU reduction at 10 mg/kg)[8], the drug candidate that was selected for clinical evaluation.

Table 6. *In vivo* efficacy of compound 49 against *M. tuberculosis* in an established mouse model

Compd.	Dose (mg/kg)	CFU (Log_{10})/lung
49	2	6.23 ± 0.30
	10	5.72 ± 0.40
	50	5.65 ± 0.40
INH	15	5.01 ± 0.14
Untreated		7.24 ± 0.17

CONCLUSIONS

In this study, we report on the optimization of the lead compound **1** that led to **50** including SAR studies to improve an activity against *M. tuberculosis* H37Rv replicating inside and outside macrophage assay and SPR studies for development potential. We found that 3-carboxamide linker of this series played a critical role in anti-tuberculosis activity. The issue of accumulation in plasma of bi-phenyl analogue **24** from *in vivo* PK study was investigated by replacement of the last phenyl ring with heterocyclic groups. Further SAR investigations with three ring systems, which had a linear and long hydrophobic groups revealed that the incorporation of a piperidine or piperazine group in middle of two phenyl rings showed enhanced potency (intracellular $MIC_{80} < 1$

nM), improved metabolic stability ($t_{1/2}$ > 60 min) and no inhibitory profile against 5 cytochrome P450 isozymes (IC_{50} > 40 μM). Ultimately, the optimization process led to compounds **49** and **50** that were performed *in vivo* PK and efficacy in a mouse model and **50** was selected as a final candidate for further evaluation as a clinical candidate based on its overall properties and high potency in the mouse model of tuberculosis. Preclinical study and target engagement study of **50** will be reported in due course.

EXPERIMENTAL SECTION

Chemistry. All reactions were carried out under an argon atmosphere in oven-dried glassware with magnetic stirring and the reaction solvents were purified by passage through a bed of activated alumina. Purification of reaction products was carried out by flash chromatography using silica gel 60 (Merck, 230-400 mesh). Analytical thin layer chromatography was performed on 0.25 mm silica gel 60-F_{254} plates (Merck). Visualization was accomplished with 254 nm of UV light and PMA or potassium permanganate staining followed by heating. ^{1}H-NMR (at 400 MHz), ^{13}C-NMR (at 100 MHz) and ^{19}F-NMR (at 376 MHz) spectra were reported on a Varian 400 MHz spectrometer. ^{1}H-NMR spectra ($CDCl_3$ at 7.26 ppm) and ^{13}C-NMR spectra ($CDCl_3$ at 77.2 ppm) were recorded in ppm using solvent as an internal standard. ^{19}F-NMR spectra were recorded in ppm using α,α,α-trifluorotoluene as an external standard (at -64.72 ppm). Data are reported as (ap = apparent, s = singlet, d= doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constant(s) in Hz; integration). LC/MS data were obtained using a Waters 2695 LC and Micromass ZQ spectrometer. The purity of all biologically tested compounds was ≥95 % by HPLC. Yields refer to purified products and are not optimized.

Minimum inhibitory concentration determination, metabolic stability, CYP inhibition assay (Fluorescence method), in vivo pharmacokinetics and in vivo efficacy were performed as previously descibed[8].

Compound **50** has been tested using recombinant CYP enzymes to confirm its inhibitory activities.

Recombinant CYP inhibition assay:

2 μL test compound/positive controls working solution are incubated with 100 μL substrate and recombinant CYP enzyme mixture working solution in the absence and presence of 98 μL cofactor solutions for 10 minutes for CYP1A2 and CYP2C9, 20 min for CYP2C19 and CYP2D6 and 3 min for CYP3A4. The final incubations are terminated by 200 μL cold IS-fortified (100 ng/mL tolbutamide) stop solution and the samples analyzed by LC-MS/MS. The reaction mixtures (200 μL final volume) contained approximately 100 mM potassium phosphate buffer (pH 7.4), 3.3 mM $MgCl_2$, 1 mM NADPH and 5 pmol/mL recombinant CYP enzymes.

General Procedure for the Preparation of 2b-d. Method A: *Ethyl 2-bromo-3-oxopentanoate **(2b).*** To a stirred solution of ethyl 3-oxopentanoate (13.8 mmol) in Et_2O (70 mL) were added ammonium acetate (41.4 mmol) and *N*-bromosuccinimide (13.8 mmol) and the reaction mixture was stirred at room temperature for 6 hours. The reaction mixture was diluted with Et_2O (20 mL) and washed with water (50 mL × 2). The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give a title a compound as a clear oil that was used for next reaction without further purification.

Method B: *Ethyl 2-bromo-3-oxohexanoate **(2c)** and ethyl 2-bromo-4-methyl-3-oxopentanoate **(2d)**.* To a stirred solution of ethyl 3-oxohexanoate (6.3 mmol) in chloroform (5 mL) was added a solution of bromine (6.3 mmol) in chloroform (30 mL) dropwise under ice-bath and the resulting solution was allowed to room temperature and stirred for 20 min. The reaction was quenched with saturated $NaHCO_3$ (aq. 10 mL) and extracted with chloroform. The resulting organic phase was washed with brine (20 mL), dried $MgSO_4$ and concentrated in vacuo to give a title compound **2c**. The resulting crude residue was used for next reaction without further purification.

In a similar manner, **2d** was synthesized according to method B.

General Procedure for the Preparation of 3a-3g. To a solution of ethyl 2-bromo-3-oxopentanoate (**2b**, 12.9 mmol) in EtOH (25 mL) was added 2-amino-5-chloropyridine (12.9 mmol). The mixture was stirred at reflux temperature for overnight. After cooling, the reaction mixture was concentrated. The resulting dark residue was dissolved in EtOAc (20 mL) and washed with water (20 mL). The organic phase was washed with brine (20 mL), dried $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (*n*-hexane: EtOAc = 4:1) to give **3f** as a pale yellow solid.

*Ethyl 2-methylimidazo[1,2-a]pyridine-3-carboxylate **(3a)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.29 (t, *J* = 7.2 Hz, 3H), 2.56 (s, 3H), 4.27 (q, *J* = 7.2 Hz, 2H), 6.77 – 6.81 (m, 1H), 7.17 – 7.22 (m, 1H), 7.42 – 7.45 (m, 1H), 9.11 – 9.13 (m, 1H).

*Ethyl 2-ethylimidazo[1,2-a]pyridine-3-carboxylate **(3b)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.36 (t, *J* = 7.6 Hz, 3H), 1.43 (t, *J* = 7.2 Hz, 3H), 3.12 (q, *J* = 7.6 Hz, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 6.95 – 6.98 (m, 1H), 7.35 – 7.39 (m, 1H), 7.63 – 7.65 (m, 1H), 9.31 – 9.33 (m, 1H).

Ethyl 2-propylimidazo[1,2-a]pyridine-3-carboxylate ***(3c)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.02 (t, *J* = 7.6 Hz, 3H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.76 – 1.85 (m, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 6.95 – 6.99 (m, 1H), 7.35 – 7.39 (m, 1H), 7.62 – 7.64 (m, 1H), 9.32 – 9.34 (m, 1H).

Ethyl 2-isopropylimidazo[1,2-a]pyridine-3-carboxylate ***(3d)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.38 (d, *J* = 6.8 Hz, 6H), 1.44 (t, *J* = 7.2 Hz, 3H), 3.80 – 3.87 (m, 1H), 4.43 (t, *J* = 7.2 Hz, 2H), 6.94 – 6.97 (m, 1H), 7.34 – 7.38 (m, 1H), 7.66 – 7.69 (m, 1H), 9.32 – 9.34 (m, 1H).

Ethyl 6-chloro-2-methylimidazo[1,2-a]pyridine-3-carboxylate ***(3e)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.43 (t, *J* = 7.2 Hz, 3H), 2.69 (s, 3H), 4.42 (q, *J* = 7.2 Hz, 2H), 7.34 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.54 (d, *J* = 9.2 Hz, 1H), 9.38 (d, *J* = 2.0 Hz, 1H).

Ethyl 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylate ***(3f)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.44 (t, *J* = 7.2 Hz, 3H), 3.11 (q, *J* = 7.6 Hz, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 7.35 (dd, *J* = 9.6, 2.0 Hz, 1H), 7.58 (d, *J* = 9.6 Hz, 1H), 9.42 (d, *J* = 2.0 Hz, 1H).

Ethyl 7-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylate ***(3g)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.34 (t, *J* = 7.6 Hz, 3H), 1.43 (t, *J* = 7.2 Hz, 3H), 3.09 (q, *J* = 7.6 Hz, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 6.95 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 9.26 (d, *J* = 7.6 Hz, 1H).

General Procedure for the Preparation of 4a-4g. To a solution of **3f** (4.3 mmol) in EtOH (30 mL) was added an aqueous solution of lithium hydroxide (13.0 mmol in 10 mL of water) and the mixture was stirred at room temperature for overnight. The organic solvent was evaporated and 1N HCl was added until pH was reached to 4. The residual pale solid was collected by filtration, washed with water and dried to give **4f** as a white solid.

In similar manner, **4a-4e** and **4g** were synthesized.

General Procedure for the Preparation of 7a-7g. Method A (7a-7e): To a solution of 4-chlorobenzonitrile (3.63 mmol) in dimethoxyethane (9 mL) were added 4-chlorophenylboronic acid (4.36 mmol), 1,1'-bis(diphenylphosphino)ferrocene)-dichloropalladium(II) (0.11 mmol), Na_2CO_3 (7.26 mmol in 3 mL of water) and the mixture was stirred at 150℃. After 1h, the mixture was cooled to room temperature, then the mixture was extracted with EtOAc (20 mL), washed with sat. $NaHCO_3$ (aq. 15 mL) and brine (15 mL) and dried over $MgSO_4$ and concentrated. The resulting residue was purified by flash column chromatography (*n*-hexane:EtOAc = 10:1) to give **6b** as a white solid (67% yield). To a solution of **6b** (2.38 mmol) in THF (24 mL) was added lithium aluminum hydride (7.14 mmol) under ice-bath and then the resulting mixture was refluxed for an hour. The reaction mixture was cooled to room temperature, quenched with water, added sat. Na_2CO_3 (aq. 15 mL) and extracted with EtOAc (30 mL × 2). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated in vacuo to give **7b** as a pale yellow solid (89%). The resulting residue was used for next reaction without further purification.

In a similar manner, **7a** and **7c-7e** were synthesized according to method A.

Method B (7f-7g): A solution of 4-bromobenzyl amine (0.32 mmol) in dimethoxyethane (1 mL) were added (4-(tert-butyl)phenyl)boronic acid (0.39 mmol), 1,1'-bis(diphenylphosphino)ferrocene)-dichloropalladium(II) (0.01 mmol), Na_2CO_3 (0.64 mmol in 350 uL of water) and the mixture was stirred at 150℃. After 1h, the mixture was cooled to room temperature, then the mixture was extracted with EtOAc (10 mL), washed with saturated $NaHCO_3$ (aq. 10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo to give crude **7f**. The resulting residue was used for next reaction without further purification.

In a similar manner, **7g** was synthesized according to method B.

General Procedure for the Preparation of 8a-8n. A mixture of 4-fluorobenzonitrile (3.0 mmol), 4-(4-trifluoromethoxy)piperidine (3.3 mmol) and K_2CO_3 (6.0 mmol) in DMSO (5 mL) was heated to 120℃ for 4 hours. After the cooling, the mixture was poured to the water and then generating solid was filtered, washed with water and dried. The resulting crude product (2.1 mmol) was dissolved in THF (10 mL) and then lithium aluminum hydride (6.2 mmol) was added slowly. After the refluxing for an hour, the reaction mixture was cooled to room temperature, quenched with water and filtered off the insoluble aggregates using of cellite. The filtrate was basified with saturated Na_2CO_3 (aq. 20 mL) and then extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ and concentrated in vacuo to give **8m** as a pale yellow solid (64%, 2 steps).

In a similar manner, **8a-8l** and **8n** were synthesized according to procedure above.

General Procedure for amide coupling. To a stirred solution of 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid (**4f**, 2.83 mmol) in anhydrous DMF (10 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.84 mmol), 1-hydroxybenzotriazole (1.54 mmol), triethylamine (5.12 mmol) and 4-(4-(4-(trifluoromethoxy)phenyl)piperidin-1-yl)phenyl)methanamine (**8n**, 2.56 mmol) at room temperature, then the resulting solution was heated to 70℃ with stirring. After 2 hours, the reaction mixture was cooled to room temperature and evaporated. Water (50 mL) was added into the crude residue, the resulting solid was collected by filtration, the filter cake was washed with water (50 mL) and dried to afford crude product. The resulting crude compound was purified by flash column chromatography (*n*-hexane:EtOAc:methylene chloride = 1:1:1), then recrystallized from EtOAc to give title compound, **50** as a white solid.

6-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(50)***. mp = 164.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.37 (t, *J* = 7.6 Hz, 3H), 1.82 – 1.97 (m, 4H), 2.64 – 2.70 (m, 1H), 2.80 – 2.87 (m, 2H), 2.93 (q, *J* = 7.6 Hz, 2H), 3.80 – 3.83 (m, 2H), 4.61 (d, *J* = 5.2 Hz, 2H), 6.00 (brt, *J* = 5.2 Hz, 1H), 6.96 – 6.99 (m, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.30 (m, 5H), 7.52 (dd, *J* = 9.6, 0.8 Hz, 1H), 9.53 (dd, *J* = 2.0, 0.8 Hz, 1H); 13C NMR (100 MHz, CDCl3) δ 13.3, 23.6, 33.4, 42.0, 43.3, 50.4, 115.4, 117.0, 121.2, 121.6, 121.9, 126.3, 128.2, 128.3, 128.7, 128.9, 144.5, 144.7, 147.7, 151.4, 151.5, 161.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ 58.31 (s, 3F); LCMS (ESI) m/z 557 $[M + H]^+$; HRESIMS calcd for $C_{29}H_{29}ClF_3N_4O_2$ $[M + H]^+$ 557.1926, found 557.1918.

N-([1,1'-Biphenyl]-4-ylmethyl)-6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(1).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (t, *J* = 7.6 Hz, 3H), 2.98 (q, *J* = 7.6 Hz, 2H), 4.74 (d, *J* = 5.6 Hz, 2H), 6.15 (brs, 1H), 7.29 (dd, *J* = 9.6, 2.0 Hz, 1H), 7.35 - 7.37 (m, 1H), 7.43 – 7.47 (m, 4H), 7.55 (d, *J* = 9.2 Hz, 1H), 7.58 – 7.62 (m, 4H), 9.56 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 390 $[M + H]^+$.

N-([1,1'-Biphenyl]-4-ylmethyl)-2-methylimidazo[1,2-a]pyridine-3-carboxamide ***(18).*** ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.62 (s, 3H), 4.57 (d, *J* = 6.0 Hz, 2H), 7.00 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.33 – 7.40 (m, 2H), 7.45 – 7.48 (m, 4H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.64 – 7.66 (m, 4H), 8.38 (brt, *J* = 6.0 Hz, 1H), 9.04 (d, *J* = 6.8 Hz, 1H); MS (ESI) m/z 342 $[M + H]^+$.

N-([1,1'-Biphenyl]-4-ylmethyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(19).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, *J* = 7.6 Hz, 3H), 3.02 (q, *J* = 7.6 Hz, 2H), 4.75 (d, *J* = 5.6 Hz, 2H), 6.19 (brs, 1H), 6.92 (dd, *J* = 6.4, 6.4 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 4H), 7.58 – 7.59 (m, 5H), 9.41 (d, *J* = 6.8 Hz, 1H); LCMS (ESI) m/z 356 $[M + H]^+$.

N-([1,1'-Biphenyl]-4-ylmethyl)-2-propylimidazo[1,2-a]pyridine-3-carboxamide ***(20).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.4 Hz, 3H), 1.80 – 1.89 (m, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 4.73 (d, *J* = 5.6 Hz, 2H), 6.29 (t, *J* = 5.6 Hz, 1H), 6.89 (dd, *J* = 6.8, 1.2 Hz, 1H), 7.27 – 7.37 (m, 2H), 7.42 – 7.46 (m, 4H), 7.56 – 7.61 (m, 5H), 9.35 (d, *J* = 6.8 Hz, 1H). ; LCMS (ESI) m/z 370 $[M + H]^+$.

N-([1,1'-Biphenyl]-4-ylmethyl)-2-isopropylimidazo[1,2-a]pyridine-3-carboxamide ***(21).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, *J* = 6.4 Hz, 6H), 3.34 – 3.41 (m, 1H), 4.76 (d, *J* = 5.6 Hz, 2H), 6.16 (m, 1H), 6.90 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.29 – 7.37 (m, 2H), 7.42 – 7.47 (m, 4H), 7.60 – 7.64 (m, 5H), 9.32 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 370 $[M + H]^+$.

N-([1,1'-Biphenyl]-4-ylmethyl)-6-chloro-2-methylimidazo[1,2-a]pyridine-3-carboxamide ***(22).*** ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (s, 3H), 4.56 (d, *J* = 5.6 Hz, 2H), 7.32 – 7.34 (m, 1H), 7.41 – 7.46 (m, 5H), 7.62 – 7.64 (m, 5H), 8.47 (brt, *J* = 5.6 Hz, 1H), 9.13 – 9.14 (m, 1H); LCMS (ESI) m/z 376 $[M+H]^+$.

6-Chloro-N-((2'-chloro-[1,1'-biphenyl]-4-yl)methyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(23).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (t, *J* = 7.6 Hz, 3H), 3.02 (q, *J* = 7.6 Hz, 2H), 4.77 (d, *J* = 6.0 Hz, 2H), 6.18 (m, 1H), 7.27 – 7.35 (m, 4H), 7.43 – 7.48 (m, 5H), 7.56 (d, *J* = 9.6 Hz, 1H), 9.56 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 424 $[M + H]^+$.

6-Chloro-N-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(24).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (t, *J* = 7.6 Hz, 3H), 3.01 (q, *J* = 7.6 Hz, 2H), 4.71 (d, *J* = 6.0 Hz, 2H), 6.13 (m, 1H), 7.31 (dd, *J* = 9.6, 2.0 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.54 – 7.58 (m, 3H), 9.55 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 424 $[M+H]^+$; HRESIMS calcd for $C_{23}H_{20}Cl_2N_3O$ $[M + H]^+$ 424.0978, found 424.0989.

6-Chloro-N-((4'-cyano-[1,1'-biphenyl]-4-yl)methyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(25).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, *J* = 7.6 Hz, 3H), 2.992 (q, *J* = 7.2 Hz, 2H), 4.75 (d, *J* = 5.6 Hz, 2H), 6.18 (brt, *J* = 5.6 Hz, 1H), 7.30 (dd, *J* = 9.6, 2.0 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 9.6 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 9.55 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 415 $[M + H]^+$.

6-Chloro-2-ethyl-N-((4'-methyl-[1,1'-biphenyl]-4-yl)methyl)imidazo[1,2-a]pyridine-3-carboxamide ***(26).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, *J* = 7.6 Hz, 3H), 3.00 (q, *J* = 7.6 Hz, 2H), 2.40 (s, 3H), 4.74 (d, *J* = 5.6 Hz, 2H), 6.16 (m, 1H), 7.25 (d, *J* = 7.2 Hz, 2H), 7.30 (dd, *J* = 9.6, 2.0 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 9.6 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 9.55 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 404 $[M + H]^+$.

7-Chloro-2-ethyl-N-((4'-methyl-[1,1'-biphenyl]-4-yl)methyl)imidazo[1,2-a]pyridine-3-carboxamide ***(27).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (t, *J* = 7.6 Hz, 3H), 2.40 (s, 3H), 2.99 (q, *J* = 7.6 Hz, 2H), 4.73 (s, 2H), 6.91 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.58 – 7.60 (m, 3H), 9.38 (d, *J* = 7.2 Hz, 1H) ; LCMS (ESI) m/z 404 $[M + H]^+$.

7-Chloro-N-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(28).*** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, *J* = 7.6 Hz, 3H), 2.99 (q, *J* = 7.6 Hz, 2H), 4.73 (s, 2H), 6.15 (brs, 1H), 6.91 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 1.6 Hz, 1H), 9.38 (d, *J* = 7.2 Hz, 1H) ; LCMS (ESI) m/z 424 $[M + H]^+$; HRESIMS calcd for $C_{23}H_{20}Cl_2N_3O$ $[M + H]^+$ 424.0978, found 424.0983.

4'-((7-Chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamido)methyl)-[1,1'-biphenyl]-4-carboxylic acid ***(29)***. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26 (t, *J* = 7.6 Hz, 3H), 2.98 (q, *J* = 7.6 Hz, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 7.08 (dd, *J* = 7.2, 2.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.78 - 7.80 (m, 3H), 8.00 (d, *J* = 8.4 Hz, 2H), 8.51 (brt, *J* = 6.0 Hz, 1H), 8.97 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 434 $[M + H]^+$.

N-((4'-(tert-Butyl)-[1,1'-biphenyl]-4-yl)methyl)-7-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(30)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.41 (t, *J* = 7.6 Hz, 3H), 2.99 (q, *J* = 7.6 Hz, 2H), 4.73 (d, *J* = 5.6 Hz, 2H), 6.13 (brs, 1H), 6.91 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.59 – 7.61 (m, 3H), 9.38 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 446 $[M + H]^+$.

N-(4-(1H-Pyrrol-2-yl)benzyl)-7-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(31)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, *J* = 7.6 Hz, 3H), 2.94 (q, *J* = 7.6 Hz, 2H), 4.67 (d, *J* = 6.0 Hz, 2H), 6.10 (brs, 1H), 6.29 – 6.32 (m, 1H), 6.52 – 6.54 (m, 1H), 6.86 – 6.88 (m, 1H), 6.89 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 2.0 Hz, 1H), 8.51 (brs, 1H), 9.35 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 379 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(pyridin-4-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(32)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, *J* = 7.6 Hz, 3H), 3.01 (q, *J* = 7.6 Hz, 2H), 4.76 (d, *J* = 5.6 Hz, 2H), 6.20 (brs, 1H), 6.91 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.26 – 7.51 (m, 4H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 8.65 (brs, 2H), 9.37 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 391 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(33)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.55 – 1.57 (m, 2H), 1.66 – 1.70 (m, 4H), 2.91 (q, *J* = 7.6 Hz, 2H), 3.12 – 3.15 (m, 4H), 4.56 (d, *J* = 5.6 Hz, 2H), 6.07 (brs, 1H), 6.86 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.90 (d, *J* = 8.4 Hz,

2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 2.0 Hz, 1H), 9.30 (d, *J* = 7.6 Hz, 1H) ; LCMS (ESI) m/z 397 [M + H]$^+$.

N-(4-(Azepan-1-yl)benzyl)-7-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(34)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.6 Hz, 3H), 1.52-1.55 (m, 4H), 1.78 (m, 4H), 2.94 (q, *J* = 7.6 Hz, 2H), 3.45 (t, *J* = 6.0 Hz, 4H), 4.56 (d, *J* = 5.2 Hz, 2H), 5.95 (brs, 1H), 6.67 (d, *J* = 8.8 Hz, 2H), 6.89 (dd, *J* = 7.6, 2.4 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 2.4 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 411 [M+H]$^+$.

7-Chloro-2-ethyl-N-(4-(4-(trifluoromethyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(35)***. mp = 209.4 ℃; ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.34 (t, *J* = 7.6 Hz, 3H), 1.68 – 1.78 (m, 2H), 1.94 – 1.98 (m, 2H), 2.11 – 2.20 (m, 1H), 2.66 – 2.73 (m, 2H), 2.90 (q, *J* = 7.6 Hz, 2H), 3.73 – 3.77 (m, 2H), 4.58 (d, *J* = 5.2 Hz, 2H), 6.03 (brt, *J* = 5.2 Hz, 1H), 6.86 (dd, *J* = 7.6, 2.4 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 2.4 Hz, 1H), 9.32 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 465 [M + H]$^+$.

7-Chloro-N-(4-(4-chloropiperidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(36).*** ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.36 (t, *J* = 7.2 Hz, 3H), 1.99 – 2.04 (m, 2H), 2.17 – 2.20 (m, 2H), 2.92 (q, *J* = 7.2 Hz, 2H), 3.05 – 3.10 (m, 2H), 3.50 – 3.52 (m, 2H), 4.20 – 4.23 (m, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 5.99 (brs, 1H), 6.89 – 6.94 (m, 3H), 7.26 – 7.27 (m, 2H), 7.58 (s, 1H), 9.35 (d, *J* = 6.8 Hz, 1H); LCMS (ESI) m/z 431 [M + H]$^+$.

7-Chloro-2-ethyl-N-(4-(4-methylpiperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(37)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, *J* = 7.6 Hz, 3H), 2.35 (s, 3H), 2.57 – 2.59 (m, 4H), 2.94 (q, *J* = 7.6 Hz, 2H), 3.20 – 3.23 (m, 4H), 4.59 (d, *J* = 5.2 Hz, 2H), 6.00 (brs, 1H), 6.88 – 6.94 (m, 3H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 2.0 Hz, 1H), 9.35 (d, *J* = 7.6 Hz, 1H) ; LCMS (ESI) m/z 412 [M + H]$^+$.

7-Chloro-2-ethyl-N-(4-(4-isopropylpiperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(38)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (d, *J* = 6.0 Hz, 6H), 1.35 (t, *J* = 7.6 Hz, 3H), 2.65 – 2.75 (m, 4H), 2.91 (q, *J* = 7.6 Hz, 2H), 3.18 – 3.27 (m, 4H), 4.59 (d, *J* = 5.6 Hz, 2H), 5.99 (brs, 1H), 6.88 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.28 (m, 2H), 7.58 (d, *J* = 2.0 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 440 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(octahydro-2H-isoindol-2-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(39)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.36 (t, *J* = 7.6 Hz, 3H), 1.40 – 2.03 (m, 8H), 2.29 – 2.34 (m, 2H), 2.92 (q, *J* = 7.2 Hz, 2H), 3.16 (dd, *J* = 9.2, 5.2 Hz, 2H), 3.29 (dd, *J* = 8.8, 6.8 Hz, 2H), 4.55 (d, *J* = 5.2 Hz, 2H), 5.97 (brs, 1H), 6.49 (d, *J* = 8.4 Hz, 2H), 6.88 (dd, *J* = 7.6, 2.4 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 2.4 Hz, 1H), 9.33 (d, *J* = 7.6 Hz, 1H) ; LCMS (ESI) m/z 437 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(4,5,6,7-tetrahydro-2H-isoindol-2-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(40)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.39 (t, *J* = 7.6 Hz, 3H), 1.74 – 1.77 (m, 4H), 2.63 (m, 4H), 2.97 (q, *J* = 7.6 Hz, 2H), 4.68 (d, *J* = 6.0 Hz, 2H), 6.14 (brs, 1H), 6.78 (s, 2H), 6.91 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 2.0 Hz, 1H), 9.36 (d, *J* = 7.2 Hz, 1H) ; LCMS (ESI) m/z 433 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(4-(4-fluorophenyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(41)***. mp = 212 - 213 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ1.39 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.24 – 3.26 (m, 4H), 3.33 – 3.36 (m, 4H), 4.62 (d, *J* = 5.6 Hz, 2H), 6.01 (brs, 1H), 6.89 – 7.02 (m, 7H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 2.0 Hz, 1H), 9.37 (d, *J* = 7.2 Hz, 1H) ; LCMS (ESI) m/z 492 $[M + H]^+$; HRESIMS calcd for $C_{27}H_{28}ClFN_5O$ $[M + H]^+$ 492.1961, found 492.1964.

7-Chloro-2-ethyl-N-(4-(4-(4-fluorophenyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(42)***. mp = 182.7 ℃; ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.79 – 1.95 (m, 4H), 2.59 – 2.67 (m, 1H), 2.78 – 2.85 (m, 2H), 2.91 (q, *J* = 7.6 Hz, 2H), 3.79 – 3.82 (m, 2H), 4.59 (d, *J* = 5.6 Hz, 2H), 6.03 (brt, *J* = 5.6 Hz, 1H), 6.87 (dd, *J* = 7.6, 2.4 Hz, 1H), 6.96 – 7.01 (m, 4H), 7.17 – 7.21 (m, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 2.4 Hz, 1H), 9.33 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 491 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{29}ClFN_4O$ $[M + H]^+$ 491.2008, found 491.2003.

6-Chloro-2-ethyl-N-(4-(4-(4-fluorophenyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(43)***. mp = 212 - 213 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.24 – 3.27 (m, 4H), 3.29 – 3.63 (m, 4H), 4.62 (d, *J* = 5.6 Hz, 2H), 6.03 (brs, 1H), 6.89 – 7.02 (m, 7H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 2.0 Hz, 1H), 9.37 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 492 $[M + H]^+$; HRESIMS calcd for $C_{27}H_{28}ClFN_5O$ $[M + H]^+$ 492.1961, found 492.1958.

7-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(44)***. mp = 216.3 – 217.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.30 – 3.40 (m, 8H), 4.62 (d, *J* = 5.6 Hz, 2H), 6.01 – 6.02 (m, 1H), 6.90 (dd, *J* = 7.2, 2.0 Hz, 1H), 6.94 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 1.6 Hz, 1H), 9.37 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 558 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{28}ClF_3N_5O_2$ $[M + H]^+$ 558.1878, found 558.1885.

6-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(45)***. mp = 206.5 – 207.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.6 Hz, 3H), 2.96 (q, *J* = 7.6 Hz, 2H), 3.30 – 3.40 (m, 8H), 4.63 (d, *J* = 5.2 Hz, 2H), 6.03 – 6.04 (m, 1H), 6.95 (d, *J* = 9.2 Hz, 2H), 6.98

(d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 7.27 – 7.32 (m, 3H), 7.54 (d, *J* = 9.6 Hz, 1H), 9.53 – 9.34 (m, 1H); LCMS (ESI) m/z 558 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{28}ClF_3N_5O_2$ $[M + H]^+$ 558.1878, found 558.1881.

6-Chloro-2-ethyl-N-(4-(4-(4-fluorophenyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(46)***.

mp = 164.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.76 – 1.95 (m, 4H), 2.60 – 2.66 (m, 1H), 2.78 – 2.85 (m, 2H), 2.92 (q, *J* = 7.6 Hz, 2H), 3.79 – 3.82 (m, 2H), 4.60 (d, *J* = 5.2 Hz, 2H), 6.03 (brt, *J* = 5.2 Hz, 1H), 6.96 – 7.01 (m, 4H), 7.17 – 7.21 (m, 2H), 7.26 – 7.29 (m, 3H), 7.51 (d, *J* = 9.6 Hz, 1H), 9.52 (d, *J* = 1.6 Hz, 1H); LCMS (ESI) m/z 491 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{29}ClFN_4O$ $[M + H]^+$ 491.2008, found 491.1996.

7-Chloro-N-(4-(4-(4-chlorophenyl)piperidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(47)***.

mp = 177.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.4 Hz, 3H), 1.83 – 1.95 (m, 4H), 2.60 – 2.67 (m, 1H), 2.79 - 2.86 (m, 2H), 2.96 (q, *J* = 7.4 Hz, 2H), 3.80 – 3.83 (m, 2H), 4.62 (q, *J* = 5.2 Hz, 2H), 6.02 (brs, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.31 (m, 5H), 7.54 (d, *J* = 9.6 Hz, 1H), 9.30 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 507 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{29}Cl_2N_4O$ $[M + H]^+$ 507.1713, found 507.1709.

6-Chloro-N-(4-(4-(4-chlorophenyl)piperidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(48)***.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 1.80 – 1.96 (m, 4H), 2.60 – 2.66 (m, 1H), 2.79 – 2.86 (m, 2H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.79 – 3.83 (m, 2H), 4.61 (q, *J* = 5.2 Hz, 2H), 6.00 (brs, 1H), 6.90 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.29 (m, 4H), 7.59 (d, *J* = 2.0 Hz, 1H), 9.30 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 507 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{29}Cl_2N_4O$ $[M + H]^+$ 507.1713, found 507.1711.

7-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(49)***. ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.36 (t, *J* = 7.6 Hz, 3H), 1.82 – 1.96 (m, 4H), 2.64 – 2.70 (m, 1H), 2.79 – 2.86 (m, 2H), 2.91 (q, *J* = 7.6 Hz, 2H), 3.80 – 3.83 (m, 2H), 4.59 (d, *J* = 5.6 Hz, 2H), 6.04 (brs, 1H), 6.87 (dd, *J* = 1.6, 7.2 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.24 – 7.28 (m, 4H), 7.57 (d, *J* = 1.6 Hz, 1H), 9.34 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 558 $[M + H]^+$; HRESIMS calcd for $C_{29}H_{29}ClF_3N_4O_2$ $[M + H]^+$ 557.1926, found 557.1912.

Synthesis of N-([1,1'-biphenyl]-4-yl)-6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(9)***. A mixture of **4f** (0.13 mmol) and thionyl chloride (2 mL) was heated to 100℃ for an hour. The reaction mixture was concentrated and dried in vacuo. The resulting residue was dissolved in methylene chloride (5 mL) and then [1,1'-biphenyl]-4-amine (0.16 mmol) and triethylamine (0.40 mmol) were added. The reaction mixture was stirred for 2 hours, diluted with methylene chloride (10 mL) and washed with water (10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The resulting crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 1:1 to methylene chloride:MeOH = 20:1) to give a target compound as a white solid (60%). ^{1}H NMR (400MHz, DMSO-d_6) δ 1.25 – 1.29 (m, 3H), 3.01 – 3.05 (m, 2H), 7.31 – 7.35 (m, 1H), 7.42 – 7.46 (m, 2H), 7.46 – 7.51 (m, 1H), 7.65 – 7.71 (m, 5H), 7.80 (d, *J* = 8.8 Hz, 2H), 8.96 (d, *J* = 2.0 Hz, 1H), 10.17 (s, 1H); LCMS (ESI) m/z 376 $[M + H]^+$.

Synthesis of N-([1,1'-biphenyl]-4-ylmethyl)-6-chloro-2-ethyl-N-methylimidazo[1,2-a]pyridine-3-carboxamide ***(10)***. To a stirred solution of **1** (0.26 mmol) in DMF (5 mL) was added NaH (60% dispersion in paraffin, 0.38 mmol) under ice-bath. After 20 min, iodomethane (0.32 mmol) was added and the reaction mixture was allowed to ambient temperature and further stirred for an hour. The mixture was diluted with EtOAc (20 mL), washed

with water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by flash column chromatography (*n*-hexane:EtOAc = 1:2) to give a target compound as a white solid (81%). ^{1}H NMR (400MHz, DMSO-d_6) δ 1.26 (t, *J* = 7.6 Hz, 3H), 2.73 (q, *J* = 7.6 Hz, 2H), 2.96 (s, 3H), 4.72 (s, 2H), 7.36 – 7.48 (m, 6H), 7.63 – 7.67 (m, 5H), 8.51 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 404 $[M + H]^+$.

*Synthesis of 1-([1,1'-biphenyl]-4-yl)-N-((6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)methyl)methanamine **(11)***. To a stirred solution of **1** (0.050 mmol) in THF (10 mL) under ice-bath was added sodium borohydride (0.25 mmol) and boron trifluoride etherate (0.25 mmol) was then added dropwise. The mixture was heated to 70℃ for 2 hours. After cooling, the mixture was diluted with EtOAc (10 mL), washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The resulting crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 1:1 to 1:2) to give a title compound as a pale yellow solid (22%). ^{1}H NMR (400MHz, acetone-d_6) δ 1.26 (t, *J* = 7.6 Hz, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 3.85 (s, 2H), 4.17 (s, 2H), 7.17 (dd, *J* = 9.6, 1.2 Hz, 1H), 7.34 – 7.36 (m, 1H), 7.42 – 7.47 (m, 5H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 8.53 (d, *J* = 1.6 Hz, 1H); LCMS (ESI) m/z 376 $[M + H]^+$.

*Synthesis of ethyl 4-bromo-3-oxohexanoate **(12)***. To a flask containing ethyl 3-oxohexanoate (9.48 mmol) in diethyl ether (50 mL) was added ammonium acetate (0.95 mmol). Here, 0.1 equivalent of ammonium acetate is essential to give 4-bromo product. After 2 hours of pre-stirring, *N*-bromosuccinimide (10.43 mmol) was then added and the resulting mixture was stirred for overnight. The reaction mixture was diluted with diethyl ether (20 mL), washed with brine (50 mL), dried over $MgSO_4$ and concentrated in vacuo to give a title compound as a pale yellow oil. The resulting crude compound was used for next reaction without further purification.

*Synthesis of 2-(6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)acetic acid **(13)***. A mixture of **12** (9.48 mmol) and 2-amino-5-chloroaniline (9.48 mmol) in EtOH (20 mL) was heated to reflux temperature. After overnight, the solution was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (50 mL), washed with sat. $NaHCO_3$ (aq. 40 mL) and brine (40 mL), dried over $MgSO_4$ and concentrated in *vacuo*. The crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 5:1 to 2:1). The obtained ester (2.63 mmol) was dissolved in MeOH (9 mL) and aqueous lithium hydroxide (7.89 mmol in 3mL H_2O) was then treated. The resulting mixture was stirred at ambient temperature for overnight. The organic solvent was removed and remained aqueous solution was acidified with 1N HCl until pH reach around 5. The generated pale yellow solid was filtered and dried under reduced pressure to give a title compound as a pale yellow solid. ^{1}H NMR (400MHz, MeOH-d_4) δ 1.30 (t, J = 7.6 Hz, 3H), 3.08 (q, J = 7.6 Hz, 2H), 4.03 (s, 2H), 7.90 – 7.98 (m, 2H), 9.00 (s, 1H).

The target compounds (**14-15**) were synthesized according to general amide coupling procedure which was described above.

*N-([1,1'-biphenyl]-4-ylmethyl)-2-(6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)acetamide **(14)***. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.22 (t, J = 7.6 Hz, 3H), 2.91 (q, J = 7.6 Hz, 2H), 3.78 (s, 2H), 4.50 (d, J = 5.6 Hz, 2H), 7.12 – 7.14 (m, 1H), 7.28 – 7.53 (m, 10H), 7.55 (brs, 1H), 7.93 – 7.94 (m, 1H); LCMS (ESI) m/z 404 $[M + H]^+$.

*N-([1,1'-biphenyl]-4-yl)-2-(6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)acetamide **(15)***. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.26 (t, J = 7.6 Hz, 3H), 2.94 (q, J = 7.6 Hz, 2H), 3.86 (s, 2H), 7.19 – 7.20 (m, 1H), 7.38 – 7.39 (m, 1H), 7.40 – 7.41 (m, 2H), 7.51 – 7.62 (m, 7H), 7.97 – 7.98 (m, 1H), 9.70 (s, 1H); LCMS (ESI) m/z 390 $[M + H]^+$.

*Synthesis of tert-butyl (6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)carbamate **(16)***. To a stirred solution of **4f** (1.33 mmol) in *t*-BuOH (8 mL) were added diphenylphosphoryl azide (1.60 mmol) and triethyl amine (2.00 mmol). The resulting mixture was heated to 95℃ with stirring for overnight. The organic solvent was removed and the resulting residue was dissolved in methylene chloride (20 mL). The organic solution was washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 2:1 to 1:1) to give a title compound as a white solid (51%). ^{1}H NMR (400MHz, acetone-d_6) δ1.25 (t, *J* = 7.6 Hz, 3H), 1.41 (s, 9H), 2.63 (q, *J* = 7.6 Hz, 2H), 7.21 (dd, *J* = 2.0, 9.2 Hz, 1H), 7.46 (d, *J* = 9.2 Hz, 1H), 8.09 (d, *J* = 2.0 Hz, 1H), 8.15 (brs, 1H, NH).

*Synthesis of 2-([1,1'-biphenyl]-4-yl)-N-(6-chloro-2-ethylimidazo[1,2-a]pyridin-3-yl)acetamide **(17)***. To a stirred solution of **16** (0.64 mmol) in methylene chloride (2 mL) was treated trifluroacetic acid (2 mL) and the reaction mixture was stirred for an hour at room temperature. The mixture was evaporated and then sticky residue was dissolved in methylene chloride (15 mL) again. The organic solution was washed with saturated Na_2CO_3 (aq. 10 mL), dried over $MgSO_4$ and concentrated in *vacuo* to give the amine intermediate as a yellow solid (88%). A mixture of 2-([1,1'-biphenyl]-4-yl)acetic acid (0.76 mmol) and thionyl chloride (6 mL) was heated to 100℃ for an hour. The reaction mixture was concentrated and dried in *vacuo*. The resulting residue was dissolved in methylene chloride (5 mL) and then amine intermediate (0.64 mmol) and triethylamine (1.92 mmol) were added. The reaction mixture was stirred for 2 hours, diluted with methylene chloride (10 mL) and washed with water (10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$ and concentrated in *vacuo*. The resulting crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 1:1 to methylene chloride:MeOH = 20:1) to give a title compound (12%) as a white solid. ^{1}H NMR (400MHz,

acetone-d_6) δ 1.18 (t, J = 7.6 Hz, 3H), 2.60 (q, J = 7.6 Hz, 2H), 3.93 (s, 2H), 7.16 – 7.20 (dd, J = 9.2, 2.0 Hz, 1H), 7.32 – 7.38 (m, 1H), 7.40 – 7.49 (m, 3H), 7.57 – 7.68 (m, 2H), 7.65 – 7.69 (m, 4H), 7.98 (d, J = 2.0 Hz, 1H), 9.14 (s, 1H); LCMS (ESI) m/z 390 $[M + H]^+$.

ASSOCIATED CONTENT

Supporting Information

In vivo PK results of compound **24**; kinetic solubility values of compound **24-26**, **43**, **45-46** and **48-50**; HPLC purity of compounds **41 – 50**; ^{1}H NMR spectra of compounds **3a – 3g, 1, 9-11** and **13-50**; ^{13}C and ^{19}F NMR spectra of compound **50**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

K.P., J.J., H.J.K. and R.K., designed and performed growth inhibition experiments, S.K., M.J.S, S.L., Y.M.K., M.S., J.J.S., Y.K., I.C., and Jaeseung K., designed and synthesized the compounds, S.A., S.P., J.N., Jung. K., H.K. and K.N. performed and designed *in vivo* pharmacokinetic and efficacy experiments, S.K. and Jaeseung K. wrote the manuscript with contributions from other authors. Z. N. and Jaeseung K. supervised the project.

The authors declare no competing financial interest.

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Abbreviations

AUC, area under curve; Boc, *tert*-butyloxycarbonyl; CFU, colony-forming unit; EDC, *N*-(3-dimethylamino-propyl)-*N* ´-ethylcarbodiimide hydrochloride; HCS, high-content screening; HOBt, 1-hydroxybenzotriazole; INH, isoniazid; IPA, imidazo[1,2-*a*]pyridine amide; MDR, multidrug-resistant; XDR, extensively drug-resistant;

REFERENCES

1. WHO. *Global Tuberculosis Control WHO Report 2012*; WHO/ HTM/TB/2012.6, **2012**.

2. Stover, C.K.; Warrener, P.; VanDevanter, D.R.; Sherman, D.R.; Arain, T.M.; Langhorne, M.H.; Anderson, S.W.; Towell, J.A.; Yuan, Y.; McMurray, D.N.; Kreiswirth, B.N.; Barry, C.E.; Baker, W.R. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* **2000**, *405*, 962–966.

3. 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.

4. Makarov, V.; Manina, G.; Mikusova, K.; Möllmann, U.; Ryabova, O.; Saint-Joanis, B.; Dhar, N.; Pasca, M.R.; Buroni, S.; Lucarelli, A.P.; Milano, A.; De Rossi, E.; Belanova, M.; Bobovska, A.; Dianiskova, P.; Kordulakova, J.; Sala, C.; Fullam, E.; Schneider, P.; McKinney, J.D.; Brodin, P.; Christophe, T.; Waddell, S.; Butcher, P.; Albrethsen, J.; Rosenkrands, I.; Brosch, R.; Nandi, V.; Bharath, S.; Gaonkar, S.; Shandil, R.K.; Balasubramanian, V.; Balganesh, T.; Tyagi, S.; Grosset, J.; Riccardi, G.; Cole, S.T. Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. Science **2009**, *324*, 801–804.

5. Diacon, A.H.; Dawson, R.; Hanekom, M.; Narunsky, K.; Maritz, S.J.; Venter, A.; Donald, P.R.; van Niekerk, C.; Whitney, K.; Rouse, D.J.; Laurenzi, M.W.; Ginsberg, A.M.; Spigelman, M.K. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrob. Agents Chemother.* **2010**, *54*, 3402–3407.

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. Gler, M.T.; Skripconoka, V.; Sanchez-Garavito, E.; Xiao, H.; Cabrera-Rivero, J.L.; Vargas-Vasquez, D.E.; Gao, M.; Awad, M.; Park, S.K.; Shim, T.S.; Suh, G.Y.; Danilovits, M.; Ogata, H.; Kurve, A.; Chang, J.; Suzuki, K.; Tupasi, T.; Koh, W.J.; Seaworth, B.; Geiter, L.J.; Wells, C.D. Delamanid for multidrug-resistant pulmonary tuberculosis. *N. Engl. J. Med.* **2012**, *366*, 2151–2160.

8. Pethe, K.; Bifani, P.; Jang, J.; Kang, S.; Park, S.; Ahn S.; Jiricek, J.; Jung, J.; Jeon, H.; Cechetto, J.; Christophe, T.; Lee, H.; Kempf, M.; Jackson, M.; Lenaerts, A.J.,; Pham, H.; Jones, V.; Seo, M.J.; Kim, Y.; Seo, M.; Seo, J.; Park, D.; Ko, Y.; Choi, I.; Kim, R.; Kim, S.; Lim, S.; Yim, S.; Nam, J.; Kang, H.; Kwon, H.; Oh, C.; Cho, Y.; Jang, Y.; Kim, J.; Chua, A.; Tan, B.H.; Nanjundappa, M.B.; Rao, S.P.; Barnes, W.S.; Wintjens, R.; Walker, J.R.; Alonso, S.; Lee, S.; Kim, J.; Oh, S.; Oh, T.; Nehrbass, U.; Han, S-J.; No, Z.; Lee, J.; Brodin, P.; Cho, S.; Nam, K.; Kim, Jaeseung. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nature Medicine* **2013,** *19(9)*, 1157-1160.

9. Christophe, T.; Jackson, M.; Jeon, H.K.; Fenistein, D.; Contreras-Dominguez, M.; Kim, J.; Genovesio, A.; Carralot, J.P.; Ewann, F.; Kim, E.H.; Lee, S.Y.; Kang, S.; Seo, M.J.; Park, E.J.; Skovierová, H.; Pham, H.; Riccardi, G.; Nam, J.Y.; Marsollier, L.; Kempf, M.; Joly-Guillou, M.L.; Oh, T.; Shin, W.K.; No, Z.; Nehrbass, U.; Brosch, R.; Cole, S.T.; Brodin, P. High content screening identifies decaprenyl-phosphoribose 2′ epimerase as a target for intracellular antimycobacterial inhibitors. *PLoS Pathog.* **2009**, *5*, e1000645

10. Abrahams, K. A.; Cox, J. A. G.; Spivey, V. L.; Loman, N. J.; Patten, M.J.; Constantinidoou,C.; Fernandex, R.; Alemparte,C.; Remuinan, M. J.; Barros, D.; Ballell, L.; Besra, G.S. Identification of novel imidazo[1,2-a]pyridine inhibitors targeting *M. tuberculosis* QcrB. *PLoS One* **2012**, *7*, e52951.

11. Moraski, G. C.; Markley, L. D.; Hipskind, P. A.; Boshoff, H.; Cho, S.; Franzblau, S. G.; Miller, M. J. Advent of imidazo[1,2-a]pyridine-3carboxamides with potent multi- and extended drug resistant antituberculosis activity. *ACS Med. Chem. Lett.* **2011**, *2*, 466−470.

12. Ollinger, J.; Bailey, M-A; Moraski, G. C.; Casey, A.; Florio, S.; Alling, T.; Miller, M. J.; Parish, T. A dual read-out assay to evaluate the potency of compounds active against *Mycobacterium tuberculosis*. *PLoS One,* **2013**, *8*, e60531.

13. Mak, P. M.; Rao, S. P. S.; Tan, M.-P.; Lin, X.; Chyba, J.; Tay, J.; Ng, S.-H.;Tan,B.-H.;Cherian,J.; Duraiswamy,J.; Bifani,P.;Vim,V.;Lee,B.H.; Ma, N.-L.; Beer, D.; Thayalan, P.; Kuhen, K.; Chatterjee, A.; Supek, F.; Glynne, R.; Zheng, J.; Boshoff, H. I.; Barr, C. E.; Dick, T.; Pethe, K.; Camacho, L. R. A high-throughput screen to identify inhibitors of ATP homeostasis in non replicating *Mycobacterium tuberculosis*. *ACS Chem. Biol.* **2012**, *7*, 1190−1197.

14. Tanemura, K.; Suzuki, T.; Nishida, Y.; Satsumabayashi, K.; Horaguchi, T. A mild and efficient procedure for α-brominatio of ketones using N-bromosuccinimide catalysed by ammonium acetate. *Chem. Commun*. **2004,** *4*, 470-471.

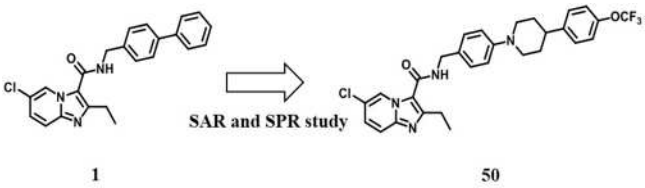
15. Ribeiro, I. G.; da Silva, K. C. M.; Parrini, S. C.; de Miranda, A. L. P.; Fraga, C. A. M.; Barreiro, E. J. Synthesis and antinociceptive properties of new structurally planned imidazo[1,2-a]pyridine 3-acylarylhydrazone derivatives. *Eur. J. Med. Chem.* **1998,** *33*, 225-235.

16. Michellys, P. Y.; D'Arrigo, J.; Grese, T. A.; Karanewsky, D. S.; Leibowitz, M. D.; Mais, D. A.; Mapes, C. M.; Reifel-Miller, A.; Rungta, D.; Boehm, M. F. Design, synthesis and structure-activity relationship of novel RXR-selective modulators. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1593-1598.

17. Perner, R. J.; DiDomenico, S.; Koenig, J. R.; Gomtsyan, A.; Bayburt, E. K.; Schmidt, R. G.; Drizin, I.; Zheng, G. Z.; Turner, S. C.; Jinkerson, T.; Brown, B. S.; Keddy, R. G.; Lukin, K.; McDonald, H. A.; Honore, P.; Mikusa, J.; Marsh, K. C.; Wetter, J. M.; George, K. S.; Jarvis, M. F.; Faltynek, C. R.; Lee, C. H. In vitro structure-activity relationship and in vivo characterization of 1-(aryl)-3-(4-(amino)benzyl)urea transient receptor potential vanilloid 1 antagonists. *J. Med. Chem.* **2007,** *50*, 3651-3660.

18. Koul, A., Arnoult, E., Lounis, N., Guillemont, J. & Andries, K. The challenge of new drug discovery for tuberculosis. *Nature* **2011**, *469*, 483–490.

Table of Contents graphic



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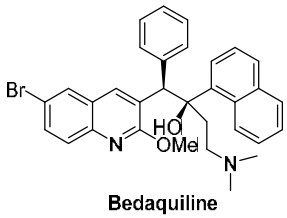

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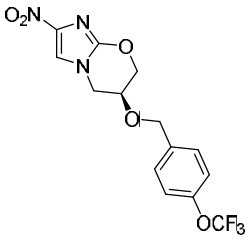

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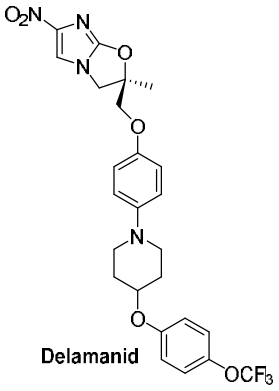
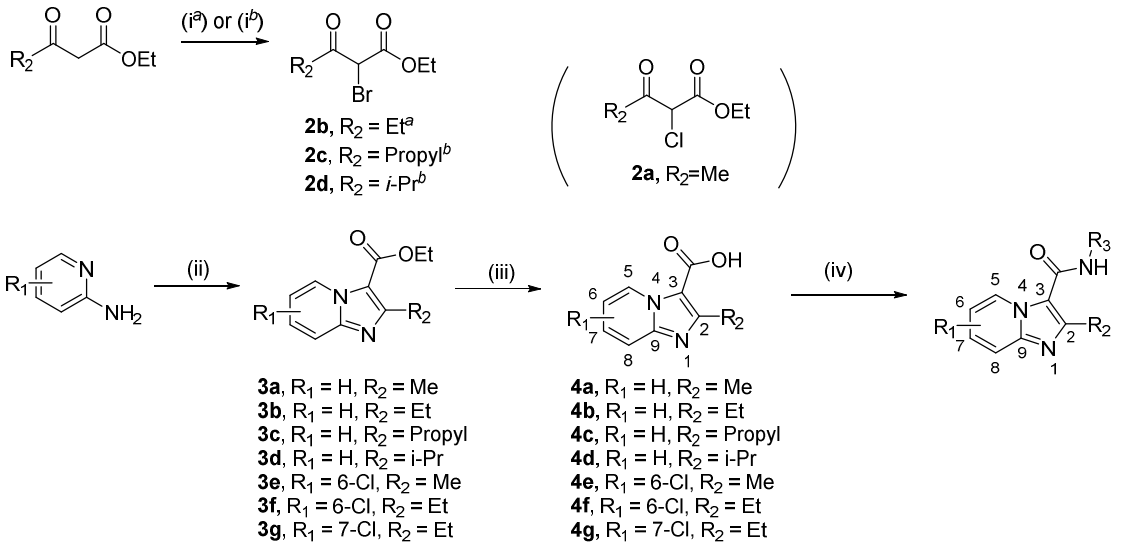


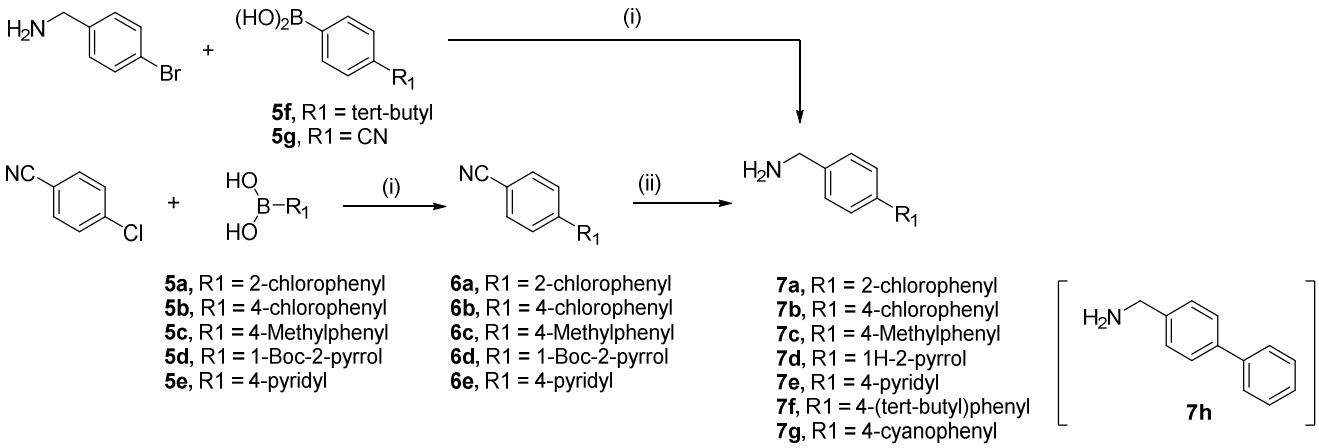
Delamanid

Scheme 1. Synthesis of imidazo[1,2-*a*]pyridine-3-carboxylic acids 4a-g[a]

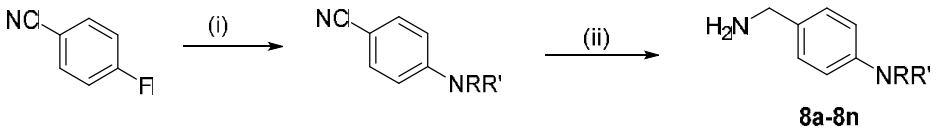


[a]Reagents and conditions: (i[a]) NBS, NH_4OAc (over 2eq.), ether, rt, 6h; (i[b]) Br_2, $CHCl_3$, 0℃ – rt, 20min; (ii) **2a-2d**, EtOH, reflux, overnight; (iii) LiOH, EtOH/H_2O (3:1, *v/v*), rt, overnight; (iv) corresponding amine, EDC, HOBt, TEA, DMF, 80℃, 2-4h

Scheme 2. Synthesis of [1,1'-biphenyl]-4-ylmethanamine analogues 7a-g[a]



[a]Reagents and conditions: (i) $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O (3:1, *v/v*), 150℃, 1h – 3h; (ii) LAH, THF, 0℃ - reflux, 1h

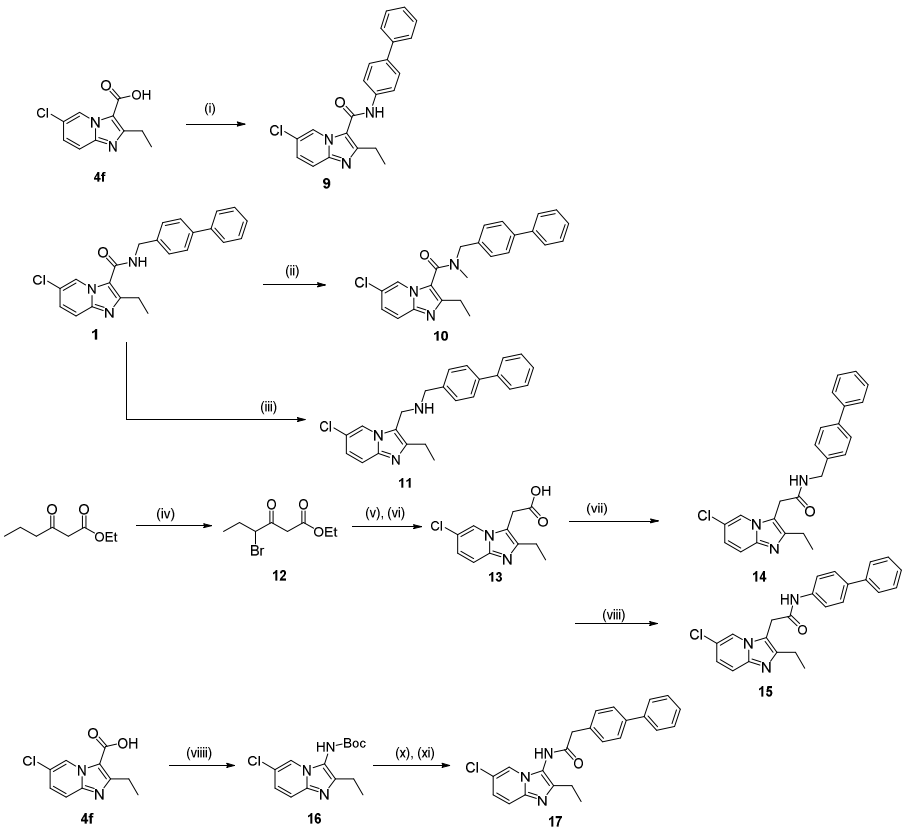


8a-8n

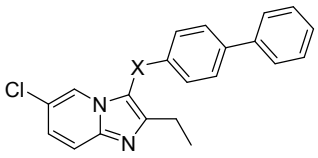
NRR' =

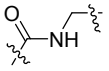
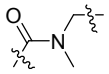
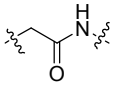
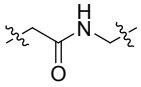
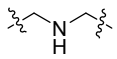
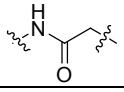
8a, piperidine
8b, azepane
8c, octahydro-1*H*-isoindole
8d, 4,5,6,7-tetrahydro-2*H*-isoindole
8e, 4-chloropiperidine
8f, 4-(trifluoromethyl)piperidine
8g,1-methylpiperazine
8h, 1-isopropylpiperazine
8i, 1-(4-fluorophenyl)piperazine
8j, 1-(4-(trifluoromethoxy)phenyl)piperazine
8k, 4-(4-fluorophenyl)piperidine
8l, 4-(4-chlorophenyl)piperidine
8m, 4-(4-(trifluoromethoxy)phenyl)piperidine
8n, 4-(4-fluorophenyl)piperidin-4-ol

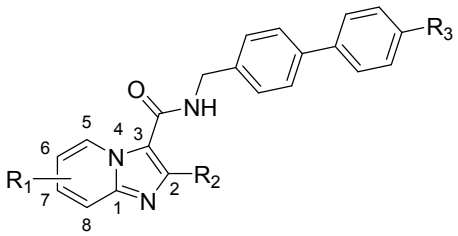
Scheme 4. Synthetic scheme for linker modification[a]



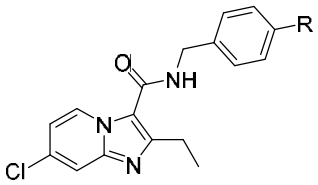
[a]Reagents and conditions: (i) $SOCl_2$, 100 ℃, 1h, then [1,1'-biphenyl]-4-amine, TEA, MC, rt, 1h; (ii) NaH, CH_3I, DMF, 0℃ - rt, 1h; (iii) $NaBH_4$, BF_3·etherate, THF, reflux, 2h; (iv) NBS, NH_4OAc (0.1 eq.), Et_2O, rt, overnight; (v) 2-amino-5-chloropyridine, ethanol, reflux, overnight; (vi) LiOH, MeOH/H_2O (3:1, *v/v*), rt, overnight; (vii) EDC, [1,1'-biphenyl]-4-ylmethanamine, HOBt, TEA, DMF, 80℃, 3h; (viii) [1,1'-biphenyl]-4-amine, EDC, HOBt, TEA, DMF, 80℃, 3h; (viiii) DPPA, TEA, *t*-BuOH, reflux, overnight; (x) TFA, MC, rt, 1h; (xi) 2-([1,1'-biphenyl]-4-yl)acetic acid, $SOCl_2$, TEA, MC, rt, 1h



Compound	X	Antimycobacterial activity against *M. tuberculosis* H37Rv	
		[a]extracellular MIC_{80} (nM)	[b]intracellular MIC_{80} (nM)
1		45	1.39
10		8690	970
9		>10000	>10000
15		>10000	>10000
14		>10000	>10000
11		810	200
17		1670	690



Compound	R1	R2	R3	Antimycobacterial activity against *M. tuberculosis* H37Rv: extracellular MIC_{80} (nM)	Antimycobacterial activity against *M. tuberculosis* H37Rv: intracellular MIC_{80} (nM)	Microsomal stability ($t_{1/2}$, min): Human	Microsomal stability ($t_{1/2}$, min): Mouse
18	H	Me	H	250	20	[a]ND	[a]ND
19	H	Et	H	63	97	27.1	[a]ND
20	H	Pr	H	1180	50	22.4	8.7
21	H	^{i}Pr	H	3130	3130	31.6	[a]ND
22	6-Cl	Me	H	175	42	>120	>60
1	6-Cl	Et	H	45	1.39	22.8	19.3
23	6-Cl	Et	2-Cl	43	9.3	38.9	13.1
24	6-Cl	Et	4-Cl	0.9	0.45	83	>60
25	6-Cl	Et	4-CN	<0.5	0.68	>120	>60
26	6-Cl	Et	4-Me	0.7	0.43	30.5	40.6
27	7-Cl	Et	4-Me	<0.5	1.35	25.4	23.1
28	7-Cl	Et	4-Cl	1.3	1.01	>120	>60
29	7-Cl	Et	4-CO_2H	250	217	>120	>120
30	7-Cl	Et	4-^{t}Bu	12	0.46	18.5	50.7
Isoniazid(INH)				449	617		
Rifampicin(RIF)				26.6	180		



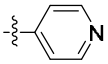
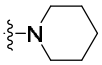
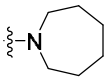
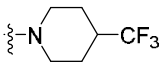
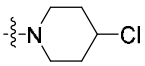
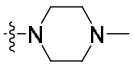
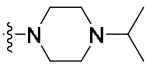
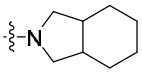
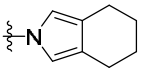
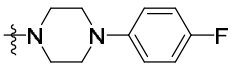
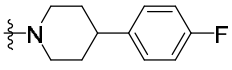
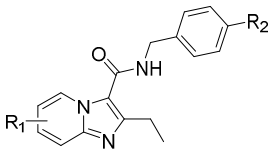
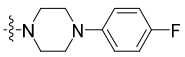
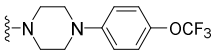
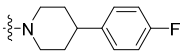
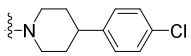
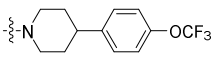
Compound	R	Antimycobacterial activity against *M. tuberculosis* H37Rv: extracellular MIC_{80} (nM)	Antimycobacterial activity against *M. tuberculosis* H37Rv: intracellular MIC_{80} (nM)	Metabolic stability ($t_{1/2}$, min): Human	Metabolic stability ($t_{1/2}$, min): Mouse
31		2000	140	>120	10.8
32		2220	740	28.9	[a]ND
33		16	8.2	13.2	2.3
34		35	10	2.9	1.9
35		0.8	0.47	6.5	5.1
36		19	2	11.0	5.2
37		4390	360	116.6	12.1
38		3000	140	>120	8.9
39		25	9.4	14.9	6.3
40		34	3.74	27.3	62.3
41		5.7	0.3	>120	33.3
42		540	0.66	6.8	18.4

Table 4. Activity of IPA analogues containing three ring system against *M. tuberculosis* H37Rv

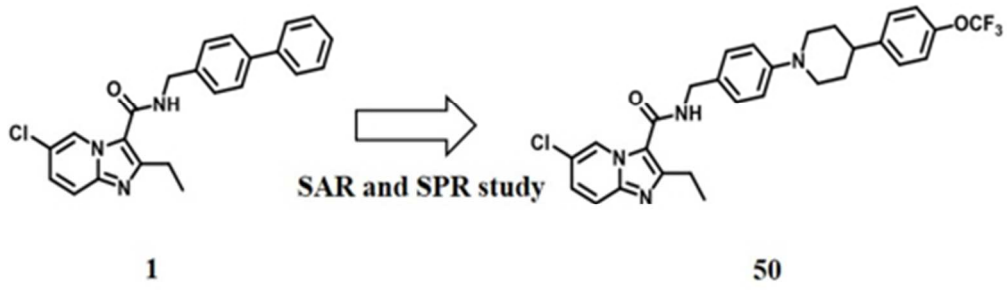


Compound	R1	R2	Antimycobacterial activity against *M. tuberculosis* H37Rv: extracellular MIC_{80} (nM)	Antimycobacterial activity against *M. tuberculosis* H37Rv: intracellular MIC_{80} (nM)	Metabolic stability ($t_{1/2}$, min): Human	Metabolic stability ($t_{1/2}$, min): Mouse	CYP inhibition (IC_{50}, uM): 3A4	2D6	1A2	2C9	2C19
41	7-Cl		5.7	0.3	>120	33.3	>40	>40	>40	>40	>40
43	6-Cl		<0.5	0.3	>120	33.3	0.49	>40	>40	1.33	1.32
44	7-Cl		1.8	0.11	>120	>60	>40	>40	>40	0.17	>40
45	6-Cl		<0.5	0.23	>120	>60	11.54	>40	>40	0.57	>40
42	7-Cl		0.54	0.66	6.8	18.4	2.07	>40	>40	0.5	0.6
46	6-Cl		<0.5	0.36	62.6	20.3	>40	>40	>40	>40	>40
47	7-Cl		1	0.46	67.3	112.0	>40	>40	>40	0.19	0.38
48	6-Cl		4.1	1.3	57.9	116.0	>40	>40	>40	0.26	6.77
49	7-Cl		4.0	3.7	>120	>120	>100[a]	>100	>100	0.14	0.29
50 (Q203)	6-Cl		4.0	1.43	>120	>120	>100[b]	>100[b]	>100[b]	>100[b]	>100[b]

[a]Values were determined by LC/MS method; [b]The assay was performed using recombinant CYP enzymes and analyzed by LC/MS/MS

Compd	Pharmacokinetics (i.v.)			Pharmacokinetics (p.o.)				
	$t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (mL/kg)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{0\text{-}inf}$ (ng·h/mL)	F (%)
41	6.15	1.9	877	4450	9.4	2.0	59576	69.9
49	62.3	3.15	14300	1987	21.3	2.0	27349	80.2
50[a]	16.5	4.0	5270	1490	23.4	2.0	44100	90.7

Compd.	Dose (mg/kg)	CFU (Log_{10})/lung
49	2	6.23 ± 0.30
	10	5.72 ± 0.40
	50	5.65 ± 0.40
INH	15	5.01 ± 0.14
Untreated		7.24 ± 0.17



1 50

85x25mm (150 x 150 DPI)