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 Anti-tuberculosis Agent

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · MAY 2014

Impact Factor: 5.45 · DOI: 10.1021/jm5003606 · Source: PubMed

20 AUTHORS, INCLUDING:

Yoonae Ko
Institut Pasteur Korea, Republic of Korea
17 PUBLICATIONS **119** CITATIONS
SEE PROFILE

Inhee Choi
Institut Pasteur Korea, Republic of Korea
23 PUBLICATIONS **239** CITATIONS
SEE PROFILE

Hyung Jun Kim
Institut Pasteur Korea, Republic of Korea
10 PUBLICATIONS **175** CITATIONS
SEE PROFILE

Kiyean Nam
10 PUBLICATIONS **212** CITATIONS
SEE PROFILE

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

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 Anti-tuberculosis Agent

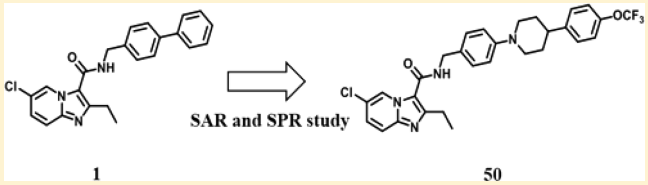
Sunhee Kang,[†] Ryang Yeo Kim,[‡] Min Jung Seo,[†] Saeyeon Lee,[†] Young Mi Kim,[†] Mooyoung Seo,[†] Jeong Jea Seo,[†] Yoonae Ko,[†] Inhee Choi,[†] Jichan Jang,[‡] Jiyoun Nam,[§] Seijin Park,[§] Hwankyu Kang,[§] Hyung Jun Kim,[‡] Jungjun Kim,[∥] Sujin Ahn,[§] Kevin Pethe,[‡] Kiyean Nam,[∥] Zaesung No,*[,†,⊥] and Jaeseung Kim*[,†]

[†]Medicinal and Bioorganic Chemistry Group, [‡]Antibacterial Drug Discovery Group, and [§]Drug Metabolism and Pharmacokinetics Group, Institut Pasteur Korea, 16 Daewnagpangyo-ro, 712 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea

[∥]Qurient Incorporated, 16 Daewnagpangyo-ro, 712 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Korea

Supporting Information

ABSTRACT: A critical unmet clinical need to combat the global tuberculosis epidemic is the development of potent agents capable of reducing the time of multi-drug-resistant (MDR) and extensively-drug-resistant (XDR) tuberculosis therapy. In this paper, we report on the optimization of 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 tuberculosis* 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 mouse lung.



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 multi-drug-resistant (MDR) and extensively-drug-resistant (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 past 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] from phenotypic high-content screening (HCS) technology inside infected macrophages.[9] The imidazo[1,2-*a*]pyridine amide (IPA) series targets *QcrB*,[8,10] which encodes the b subunit of the electron transport complex 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 design of the potential clinical candidate **50**.

Chemistry. General amide coupling of appropriately substituted imidazo[1,2-*a*]pyridine-3-carboxylic acid (**4a**−**4g**) with corresponding amines R_3 afforded target compounds in over 60% yield (Scheme 1). One of the precursors, 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 (NBS) and over 2 equiv 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 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 counterparts, methanamine derivatives, can be classified into three functional groups: (i) biaryl, (ii) 2-ring system having a saturated cyclic amine, and (iii) 3-ring system having a saturated cyclic amine between two aryl rings. The synthetic

Received: March 7, 2014
Published: May 28, 2014

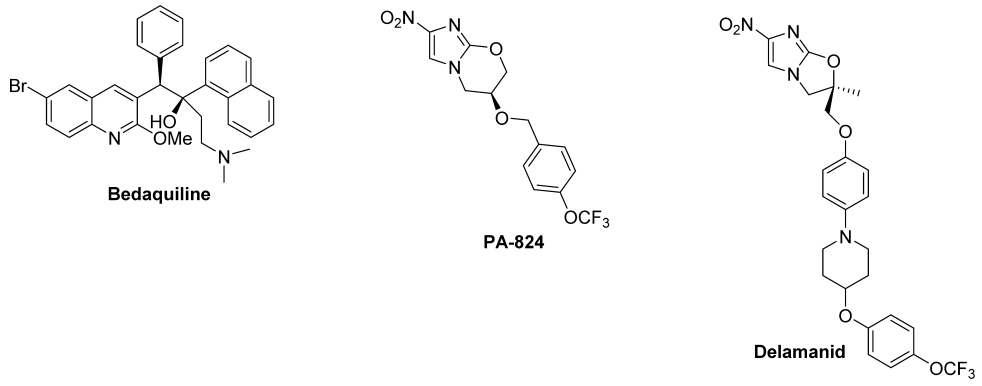
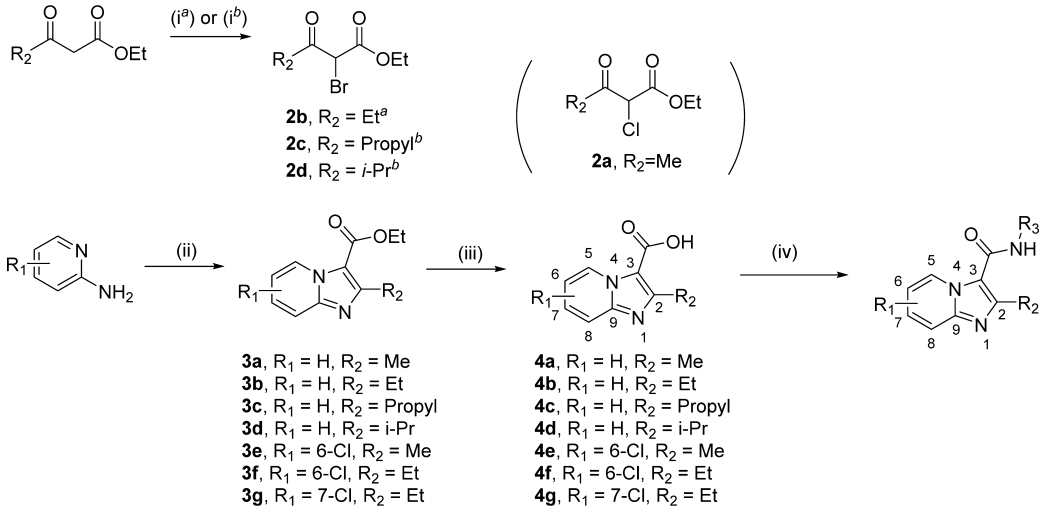


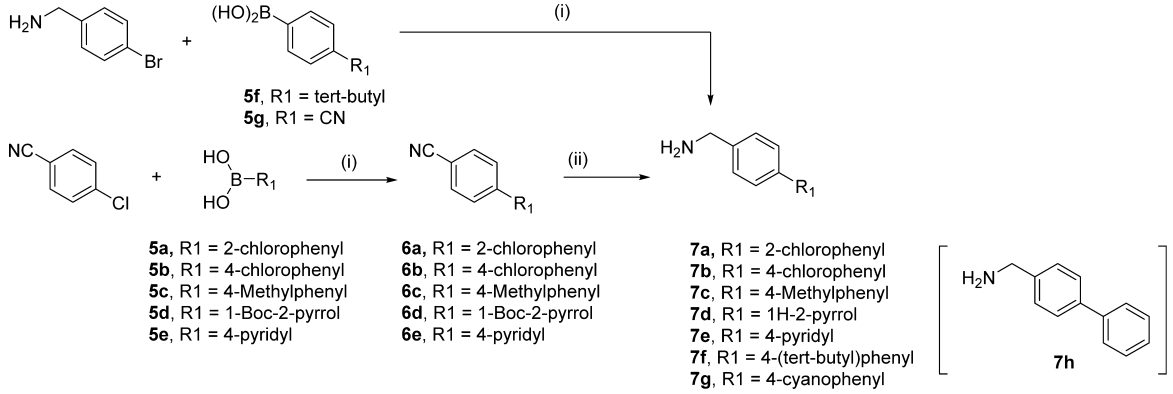
Figure 1. Structures of bedaquiline, PA-824,[5] and delamanid.

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 2 equiv), Et_2O, rt, 6 h, or (i[b]) Br_2, $CHCl_3$, 0 °C to rt, 20 min; (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 °C, 2−4 h.

Scheme 2. Synthesis of [1,1′-Biphenyl]-4-ylmethanamine Analogues 7a−7g[a]



[a]Reagents and conditions: (i) $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O (3:1 v/v), 150 °C, 1−3 h; (ii) LAH, THF, 0 °C to reflux, 1 h.

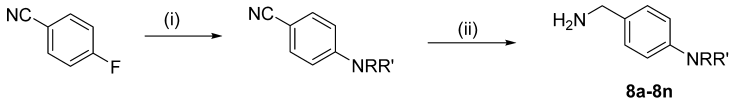
route to methanamines is shown in Scheme 2. Suzuki coupling of 4-chlorobenzonitrile and substituted phenylboronic acid using $PdCl_2(dppf)$ and aqueous Na_2CO_3[16], followed by reduction with lithium aluminum hydride, gave biphenyl methanamines **7a−7e**. Exceptionally, biphenyl methanamines having a *tert*-butyl or cyano group, **7f** and **7g**, were synthesized by direct Suzuki coupling with 4-bromobenzylamine.

Benzylamines with saturated rings containing bis- and tris-rings 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 (DMSO) and then reduced with lithium aluminum hydride (LAH) to produce benzylamines **8a−8n**.[17]

The synthetic route for linker modification is shown in Scheme 4. Compound **9,** which has no benzylic carbon next to

dx.doi.org/10.1021/jm5003606 | *J. Med. Chem.* 2014, 57, 5293−5305

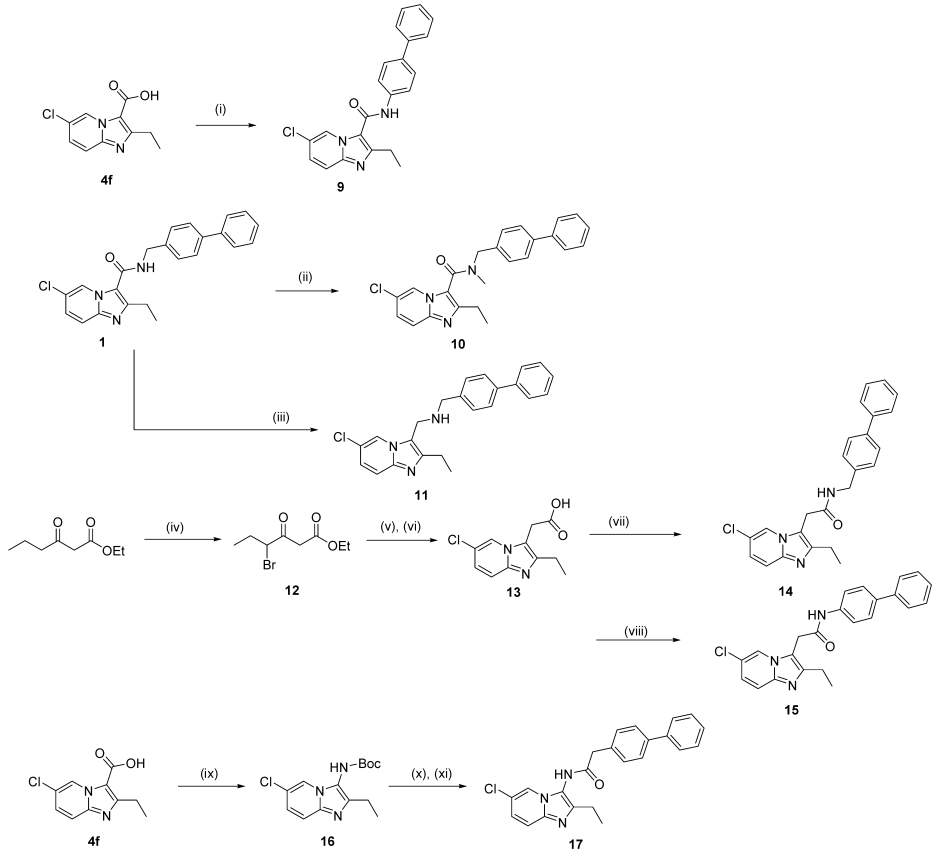
Scheme 3. Synthetic Scheme for Benzylamine Tails 8a−8n[a]



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

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

Scheme 4. Synthetic Scheme for Linker Modification[a]



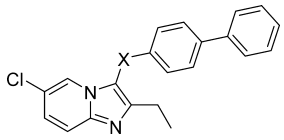
[a]Reagents and conditions: (i) $SOCl_2$, 100 °C, 1 h, then [1,1′-biphenyl]-4-amine, TEA, MC, rt, 1 h; (ii) NaH, CH_3I, DMF, 0 °C to rt, 1h; (iii) $NaBH_4$, BF_3·etherate, THF, reflux, 2 h; (iv) NBS, NH_4OAc (0.1 equiv), Et_2O, rt, overnight; (v) 2-amino-5-chloropyridine, ethanol, reflux, overnight; (vi) LiOH, MeOH/H_2O (3:1 v/v), rt, overnight; (vii) EDC, [1,1′-biphenyl]-4-ylmethanamine, HOBt, TEA, DMF, 80 °C, 3 h; (viii) [1,1′-biphenyl]-4-amine, EDC, HOBt, TEA, DMF, 80 °C, 3 h; (ix) DPPA, TEA, *t*-BuOH, reflux, overnight; (x) TFA, MC, rt, 1 h; (xi) 2-([1,1′-biphenyl]-4-yl)acetic acid, $SOCl_2$, TEA, MC, rt, 1 h.

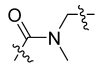
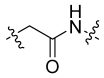
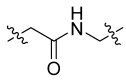
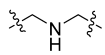
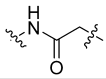
amide, was prepared via acid chloride activation of **4f**, and N-methylated compound **10** was synthesized by treatment of **1** with sodium hydride and iodomethane. Amide reduction of **1** under mild conditions with boron trifluoride etherate and $NaBH_4$ provided **11** in moderate yield.

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 the 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 equiv of neutral catalyst NH_4OAc, compared to the presence of over 2 equiv 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 migrated to the 4-position after overnight reaction. However, migration of the bromo group did not occur in the presence of excess NH_4OAc. Compound **12** was condensed with 2-amino-5-chloropyridine and saponified to afford the intermediate acid **13**, followed by general amide coupling to give **14** and **15**. The reverse-amide compound **17** was synthesized via Curtius rearrangement with diphenylphosphoryl azide and triethylamine in *t*-BuOH. Subsequent deprotection of Boc group by trifluoroacetic acid and amide coupling via acid chloride activation afforded the target compound.

dx.doi.org/10.1021/jm5003606 | *J. Med. Chem.* 2014, 57, 5293−5305

Table 1. Activity of Linker-Modified IPA Analogues against *M. tuberculosis* H37Rv[a,b]



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

[a]Extracellular MIC_{80} = inhibitory activity against *M. tuberculosis* H37Rv replicating in culture broth medium. [b]Intracellular MIC_{80} = inhibitory activity against *M. tuberculosis* H37Rv replicating inside macrophages. MIC_{80} is the minimum concentration required to inhibit growth by 80% and indicates an average value of two independent measurement.

RESULTS AND DISCUSSION

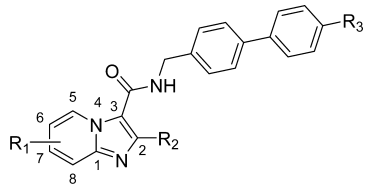
The activity of IPA derivatives was tested against *M. tuberculosis* replicating inside macrophages (intracellular MIC_{80}) and in liquid broth culture medium (extracellular MIC_{80}) (Tables 1−4), as previously described.[8] In addition, metabolic stability of the compounds was evaluated in mouse and 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 **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, modification of the length of the amide linker with one carbon between the imidazo[1,2-*a*]pyridine ring and the carbonyl group of amide bond (**14** and **15**) 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 submicromolar 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 antimycobacterial activity.

Next, the optimization was focused on R_1, R_2, and R_3 modifications (Table 2). During the exploration of SAR at R_2 and R_3 positions, the substituent at R_1 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 R_2 alkyl groups, the four compounds **18**−**21** were designed to alter the size of R_2 position that might disturb positioning of the 3-carboxamide. As expected, the smaller 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 isopropyl (**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 groups at the R_2 position, **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 showed better metabolic stability compared to the ethyl substitution.

Further exploration of SAR at the R_3 position of the phenyl group with 6- or 7-Cl of imidazo[1,2-*a*]pyridine core was conducted by substitution of various functional groups such as electron-donating and -withdrawing groups 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

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293−5305

Table 2. Activity and Metabolic Stability of Bis(phenyl) IPA Analogues against *M. tuberculosis* H37Rv



				antimycobacterial activity against *M. tuberculosis* H37Rv		microsomal stability ($t_{1/2}$, min)	
compd	R_1	R_2	R_3	extracellular MIC_{80}(nM)	intracellular MIC_{80}(nM)	human	mouse
18	H	Me	H	250	20	ND[a]	ND
19	H	Et	H	63	97	27.1	ND
20	H	Pr	H	1180	50	22.4	8.7
21	H	iPr	H	3130	3130	31.6	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
INH[a]				449	617		
RIF[a]				26.6	180		

[a]ND, not determined; INH, isoniazid; RIF, rifampicin.

than the ortho-chloro group (**23**). Encouraged by the positive effect of para substitution on antibacterial activity, we focused on screening several analogues at this position to investigate activity and metabolic stability. Intracellular activity of all substituents such as electron-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 **30** showed similar intracellular activity comparable to **27**. On the other hand, **29**, which has a hydrophilic carboxylic acid group, had a deleterious effect on activity. In terms of metabolic stability, alkyl groups such as methyl (**26** and **27**) and *tert*-butyl (**30**) showed much lower microsomal stability than Cl, CN, and acid (**24**, **25**, **28**, and **29**) in human and mouse liver microsomes. This again suggested that the position of R_3 on the benzene ring may play a role in potency and microsomal stability.

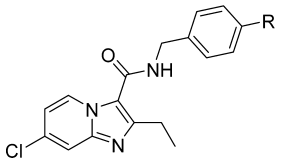
From our initial SAR study, **24**, **25**, and **28** showed desirable extra- and intracellular potency as well as metabolic stability in human and mouse liver microsomes to perform in vivo pharmacokinetic (PK) experiments. However, **25** was too insoluble in aqueous and organic solutions, caused by extension of aromatic character, to conduct in vivo experiments. Thus, **24** was selected and its in vivo PK profile was evaluated in Sprague-Dawley rats after administration by oral (po) and intravenous (iv) routes. However, the half-life and area under curve (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 h after dosing (Supporting Information, Table S1). This result suggested that **24** rebounded in absorption after 4 h and could be subject to compound precipitation in the gut and extended absorption due to its highly lipophilic nature. 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).

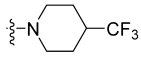
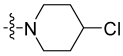
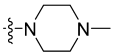
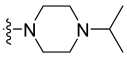
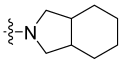
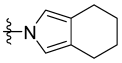
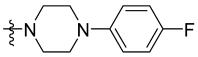
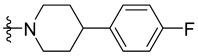
Changes were focused on the second phenyl ring (shown on the right-hand side in Table 3) while the 7-chloro-2-ethylimidazo[1,2-*a*]pyridine-3-carboxamide was maintained (shown on the left-hand side). First, we introduced heteroaromatic groups that were favorable for salt formation to improve solubility. 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 introduction of a nitrogen-containing saturated ring, such as piperidine, azepane, or piperazine, next to the first phenyl ring. 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 were the compounds heavily metabolized in human and mouse liver microsomes but also they 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 previous SAR studies, it was shown that hydrophilic character on the second ring led to decreased potency. Thus, we applied small alkyl groups such as methyl and isopropyl at the 4-position of piperazine to protect activity loss. Unlike piperidine compounds **35** and **36**, **37** and **38** lost their potency against *M. tuberculosis* H37Rv replicating inside and outside macrophage (intracellular MIC_{80} > 140 nM). On the other hand, **39** and **40**,

dx.doi.org/10.1021/jm5003606 | *J. Med. Chem.* 2014, 57, 5293−5305

Table 3. Activity of IPA Analogues Containing Two or Three Rings against *M. tuberculosis* H37Rv[a]



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.

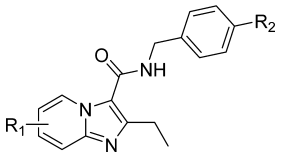
with the fused ring, showed potency comparable to that of **1**, whether or not the fused ring had an aromaticity. The excellent potency of the lipophilic and bulky analogues **39** and **40** suggested that there is more lipophilic space and that the two-ring system could be extended to a three-ring system on the right-hand side. In addition, **25**, with a nitrile group that is representative of linearity or aromaticity next to the biphenyl ring, showed 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).

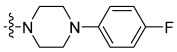
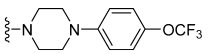
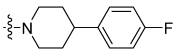
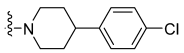
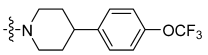
With this structure−activity relationship (SAR) analysis, two analogues **41** and **42** were designed and evaluated against *M. tuberculosis* H37Rv replicating outside and inside macrophage. Compound **41** with a 4-fluorophenylpiperazine showed not only dramatically increased MIC_{80} values of 0.3 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-fluorophenylpiperidine 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 improvement.

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 on the left-hand side. As shown in Table 4, analogues **47** and **48**, in which the fluorine was replaced by chlorine, retained potency (intracellular MIC_{80} = 0.46 nM and 1.3 nM, respectively) and improved microsomal stability ($t_{1/2}$ > 60 min in mouse microsomes). Other analogues (**44**, **45**, **49**, and **50**) bearing a trifluoromethoxy substituent showed similar activity and good stability in human and mouse microsomes. All analogues in Table 4 displayed unprecedented MIC_{80} values in the single-digit nanomolar or subnanomolar range against *M. tuberculosis* H37Rv replicating both outside and inside macrophage. In terms of metabolic stability, the stability−substituent correla-

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293−5305

Table 4. Activity of IPA Analogues Containing Three-Ring Systems against *M. tuberculosis* H37Rv[a,b]



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 with recombinant CYP enzymes and analyzed by LC/MS/MS.

Table 5. In Vivo Pharmacokinetic Values in Mice of 41, 49, and 50

compd	pharmacokinetics (iv): $t_{1/2}$ (h)	CI ($mL \cdot min^{-1} \cdot kg^{-1}$)	Vd_{ss} ($mL \cdot kg^{-1}$)	pharmacokinetics (po): C_{max} ($ng \cdot mL^{-1}$)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{0,inf}$ ($ng \cdot h \cdot mL^{-1}$)	*F* (%)
41	6.15	1.9	877	4450	9.4	2.0	59 576	69.9
49	62.3	3.15	14 300	1987	21.3	2.0	27 349	80.2
50[a]	16.5	4.0	5270	1490	23.4	2.0	44 100	90.7

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

tion followed the order trifluoromethoxy > chlorine > fluorine, and most compounds had good metabolic stability to perform in vivo experiments except 4-fluorophenylpiperidine analogues **42** and **46**. In addition to stability, the incorporation of an 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 five 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 coinfected treatment. On the basis of their overall properties, **41**, **49**, and **50** were shortlisted for in vivo PK experiments.

The in vivo PK properties of **41**, **49**, and **50** were evaluated in mice after intravenous (iv) and oral (po) 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 h; their elimination half-life was favorable (9.4, 21.3, and 23.4 h, 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 **49** in an established mouse model under the same conditions previously described.[8] BALB/c mice were infected with 2×10^2 to 2×10^3 CFU of *M. tuberculosis* H37Rv by the intranasal route. Compound treatment was initiated 3 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

Table 6. In Vivo Efficacy of 49 against *M. tuberculosis* in an Established Mouse Model

compd	dose (mg/kg)	CFU (log_{10})/lung
49	2	6.23 ± 0.30
49	10	5.72 ± 0.40
49	50	5.65 ± 0.40
INH	15	5.01 ± 0.14
untreated		7.24 ± 0.17

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293–5305

potential efficacy results in a dose-dependent manner. 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. 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.

CONCLUSIONS

In this study, we report on optimization of lead compound **1** that led to **50**, including SAR studies to improve activity against *M. tuberculosis* H37Rv replicating inside and outside macrophage assay and SPR studies for development potential. We found that the 3-carboxamide linker of this series played a critical role in anti-tuberculosis activity. The issue of accumulation in plasma of biphenyl 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 linear and long hydrophobic groups, revealed that incorporation of a piperidine or piperazine group in the 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 five cytochrome P450 isozymes (IC_{50} > 40 μM). Ultimately, the optimization process led to **49** and **50**, which were evaluated for in vivo PK and efficacy in a mouse model, and **50** was selected as a final candidate for further evaluation as a clinical candidate on the basis of 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 reaction solvents were purified by passage through a bed of activated alumina. Purification of reaction products was carried out by flash chromatography on silica gel 60 (Merck, 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on 0.25 mm silica gel 60-F_{254} plates (Merck). Visualization was accomplished with 254 nm UV light and phosphomolybdic acid (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 recorded 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) are reported in parts per million (ppm) with solvent as an internal standard. ^{19}F NMR spectra are reported in ppm with 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) are given in hertz; with integration). LC/MS data were obtained by use of a Waters 2695 liquid chromatograph 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]

Recombinant CYP Inhibition Assay. Compound **50** has been tested with recombinant CYP enzymes to confirm its inhibitory activities. Test compound/positive controls working solution (2 μL) was incubated with 100 μL of substrate and recombinant CYP enzyme mixture working solution in the absence and presence of 98 μL of cofactor solutions for 10 min for CYP1A2 and CYP2C9, 20 min for CYP2C19 and CYP2D6, and 3 min for CYP3A4. The final incubations were terminated by 200 μL of cold IS-fortified (100 ng/mL tolbutamide) stop solution, and the samples were 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 Procedures for 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 h. 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 **2b** as a clear oil that was used for the 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 come 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 over $MgSO_4$, and concentrated in vacuo to give **2c**. The resulting crude residue was used for the next reaction without further purification. In a similar manner, **2d** was synthesized according to method B.

General Procedure for 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 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 over $MgSO_4$, 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 (q, 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 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 overnight. The organic solvent was

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293−5305

evaporated and 1 N HCl was added until the pH reached 4. The residual pale solid was collected by filtration, washed with water, and dried to give **4f** as a white solid. In a similar manner, **4a**−**4e** and **4g** were synthesized.

General Procedure for 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), and Na_2CO_3 (7.26 mmol in 3 mL of water), and the mixture was stirred at 150 °C. After 1 h, the mixture was cooled to room temperature, and then the mixture was extracted with EtOAc (20 mL), washed with saturated $NaHCO_3$ (aq, 15 mL) and brine (15 mL), 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 tetrahydrofuran (THF) (24 mL) was added lithium aluminum hydride (7.14 mmol) under ice bath, and then the resulting mixture was refluxed for 1 h. The reaction mixture was cooled to room temperature and quenched with water; saturated Na_2CO_3 (aq, 15 mL) was added, and the mixture was 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 the next reaction without further purification. In a similar manner, **7a** and **7c**−**7e** were synthesized according to method A.

*Method B (**7f** and **7g**).* To 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), and Na_2CO_3 (0.64 mmol in 350 μL of water), and the mixture was stirred at 150 °C. After 1 h, the mixture was cooled to room temperature and then 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 the next reaction without further purification. In a similar manner, **7g** was synthesized according to method B.

General Procedure for 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 dimethyl sulfoxide (DMSO, 5 mL) was heated to 120 °C for 4 h. After cooling, the mixture was poured into water and the generated 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 refluxing for 1 h, the reaction mixture was cooled to room temperature and quenched with water, and the insoluble aggregates were filtered off through Celite. 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%, two steps). In a similar manner, **8a**−**8l** and **8n** were synthesized according to this procedure.

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 *N*,*N*-dimethylfoirmamide (DMF, 10 mL) were added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 3.84 mmol), 1-hydroxybenzotriazole (HOBt, 1.54 mmol), triethylamine (TEA, 5.12 mmol) and (4-{4-[4-(trifluoromethoxy)-phenyl]piperidin-1-yl}phenyl)methanamine (**8n**, 2.56 mmol) at room temperature, and the resulting solution was heated to 70 °C with stirring. After 2 h, 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, and 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) and then recrystallized from EtOAc to give **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 °C; ^{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 (br t, 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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); LC/MS (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 (br s, 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); LC/MS (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 (br t, 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 (br s, 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); LC/MS (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); LC/MS (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); LC/MS (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 (br t, J = 5.6 Hz, 1H), 9.13−9.14 (m, 1H); LC/MS (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); LC/MS (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); LC/MS (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), 2.99 (q, J = 7.2 Hz, 2H), 4.75 (d, J = 5.6 Hz, 2H), 6.18 (br t, 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); LC/MS (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); LC/MS (ESI) m/z 404 $[M + H]^+$.

dx.doi.org/10.1021/jm5003606 | *J. Med. Chem.* 2014, 57, 5293−5305

*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) ; LC/MS (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 (br s, 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) ; LC/MS (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 (br t, J = 6.0 Hz, 1H), 8.97 (d, J = 7.2 Hz, 1H); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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 (br s, 1H), 9.35 (d, J = 7.2 Hz, 1H); LC/MS (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 (br s, 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 (br s, 2H), 9.37 (d, J = 7.6 Hz, 1H); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 °C; ^{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 (br t, 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); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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) ; LC/MS (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 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, J = 7.6 Hz, 3H), 2.95 (q, J = 7.6 Hz, 2H), 3.24−3.26 (m, 4H), 3.33−3.36 (m, 4H), 4.62 (d, J = 5.6 Hz, 2H), 6.01 (br s, 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) ; LC/MS (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 °C; ^{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 (br t, 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); LC/MS (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 °C; ^{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 (br s, 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); LC/MS (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 °C; ^{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); LC/MS (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 °C; ^{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); LC/MS (ESI) m/z 558 $[M + H]^+$; HRESIMS calcd for $C_{28}H_{28}ClF_3N_5O_2$ $[M + H]^+$ 558.1878, found 558.1881.

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293−5305

*6-Chloro-2-ethyl-N-{4-[4-(4-fluorophenyl)piperidin-1-yl]benzyl}-imidazo[1,2-a]pyridine-3-carboxamide (**46**).* Mp 164.0 °C; ^{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 (br t, 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); LC/MS (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 °C; ^{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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 (br s, 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); LC/MS (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 °C for 1 h. 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 h, 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 **9** as a white solid (60%). ^{1}H NMR (400 MHz, 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); LC/MS (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 come to ambient temperature and further stirred for 1 h. 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 **10** as a white solid (81%). ^{1}H NMR (400 MHz, 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); LC/MS (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 °C for 2 h. 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 = 1:1−1:2) to give **11** as a pale yellow solid (22%). ^{1}H NMR (400 MHz, 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); LC/MS (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 equiv of ammonium acetate is essential to give the 4-bromo product. After 2 h of prestirring, *N*-bromosuccinimide (10.43 mmol) was then added and the resulting mixture was stirred 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 **12** as a pale yellow oil. The resulting crude compound was used for the 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 refluxing overnight, the solution was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (50 mL), washed with saturated $NaHCO_3$ (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 = 5:1−2:1). The obtained ester (2.63 mmol) was dissolved in MeOH (9 mL) and then treated with aqueous lithium hydroxide (7.89 mmol in 3 mL of H_2O). The resulting mixture was stirred at ambient temperature overnight. The organic solvent was removed, and the remaining aqueous solution was acidified with 1 N HCl until the pH reached around 5. The generated pale yellow solid was filtered and dried under reduced pressure to give **13** as a pale yellow solid. ^{1}H NMR (400 MHz, 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).

Synthesis of Target Compounds 14 and 15. Target compounds **14** and **15** were synthesized according to the general amide coupling procedure described in a previous section.

*N-([1,1′-Biphenyl]-4-ylmethyl)-2-(6-chloro-2-ethylimidazo[1,2-a]-pyridin-3-yl)acetamide (**14**).* ^{1}H NMR (400 MHz, $CDCl_3$) 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 (br s, 1H), 7.93−7.94 (m, 1H); LC/MS (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 (400 MHz, $CDCl_3$) 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); LC/MS (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 triethylamine (2.00 mmol). The resulting mixture was heated to 95 °C with stirring 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 = 2:1−1:1) to give **16** as a white solid (51%). ^{1}H NMR (400 MHz, acetone-d_6) δ 1.25 (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 (br s, 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 added trifluoroacetic acid (2 mL), and the reaction mixture was stirred for 1 h at room temperature. The mixture was evaporated and then the sticky residue was dissolved in methylene chloride (15 mL) again. The

dx.doi.org/10.1021/jm5003606 | J. Med. Chem. 2014, 57, 5293−5305

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 °C for 1 h. 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 h, 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 **17** (12%) as a white solid. ^{1}H NMR (400 MHz, acetone-d_6) δ 1.18 (t, J = 7.6 Hz, 3H), 2.60 (q, J = 7.6 Hz, 2H), 3.93 (s, 2H), 7.18 (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); LC/MS (ESI) m/z 390 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Authors

*Phone +82-31-888-6006; e-mail jsnoh@gstep.re.kr.

*Phone +82-10-4262-3528; fax +82-31-8018-8015; e-mail silanediol@gmail.com.

Present Address

⊥Gyeonggi Biocenter, 147 Gwanggyo-ro, Youngtong-gu, Suwon-si, Gyeonggi-do, 443-270. Korea.

Author Contributions

K.P., J.J., H.J.K. and R.Y.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., Jungjun 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.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (2007-00559), Gyeonggi-do, Qurient Inc., and KISTI.

■ ABBREVIATIONS USED

AUC, area under curve; Boc, *tert*-butyloxycarbonyl; CFU, colony-forming unit; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HCS, high-content screening; HOBt, 1-hydroxybenzotriazole; HRESIMS, high-resolution electrospray ionization mass spectrometry; INH, isoniazid; IPA, imidazo[1,2-*a*]pyridine amide; MC, methylene chloride; MDR, multi-drug-resistant; XDR, extensively-drug-resistant

■ REFERENCES

(1) World Health Organization. *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, J.. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat. Med.* **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*, No. 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*, No. 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*, No. 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.