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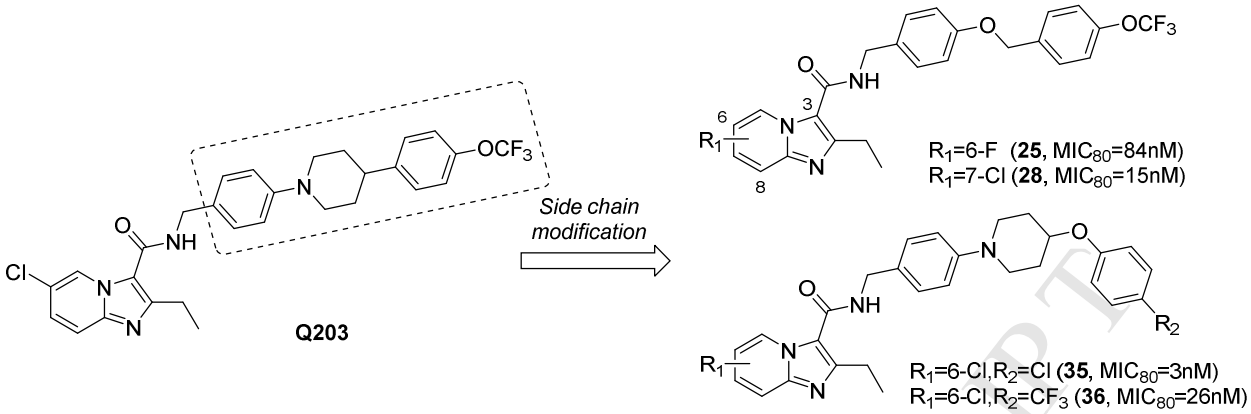

Synthesis and structure-activity studies of side chain analogues of the anti-tubercular agent, Q203

Sunhee Kang[1, 4], Young Mi Kim[1], Ryang Yeo Kim[2], Min Jung Seo[1], Zaesung No[1], Kiyean Nam[3], Sanghee Kim[4, *], Jaeseung Kim[1, *]

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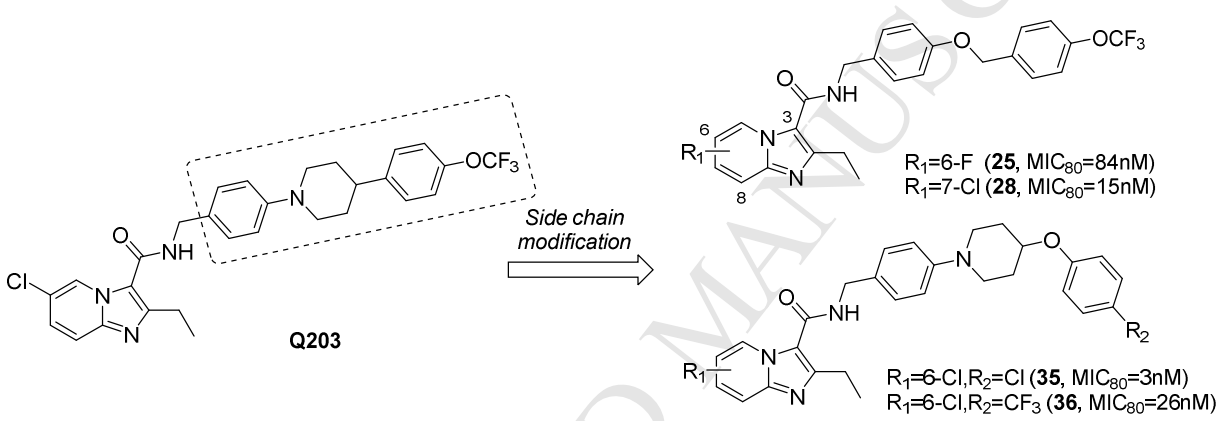
ABSTRACT

The anti-tubercular activity of 6-chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenyl)piperidin-1-yl)benzyl)imidazo[1,2-*a*]pyridine-3-carboxamide (**Q203**) is modified by varying its side chain. In this study, we synthesized **Q203** analogues with different side chains and studied their effects on anti-tubercular activity. Many analogues showed good potency against *M. tuberculosis* replicating in liquid broth culture medium (extracellular activity) regardless of chain length and conformational changes. However, a polar character in

the side chain region was unfavorable for anti-tubercular activity. The analogues, **25**, **28**, **35**, and **36**, displayed excellent activity against *M. tuberculosis* replicating inside macrophages (intracellular activity) and promising pharmacokinetic (PK) properties with high drug exposure level and long half-life.

Graphical abstract



Keywords

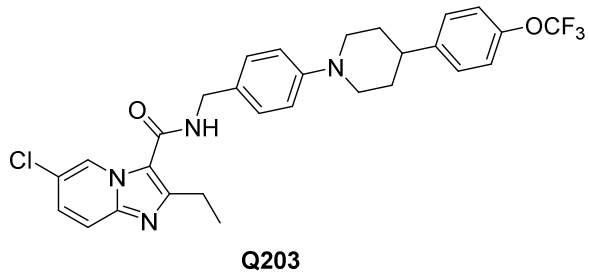
Tuberculosis, imidazo[1,2-*a*]pyridine-3-carboxamide (IPA), pharmacokinetics, structure-activity relationship (SAR), Q203

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. In 2014, the number of new TB cases was 9.6 million, and multidrug-resistant (MDR) and extensively drug-resistant TB occurred in over 100 countries[1]. In addition, 1.1 million TB deaths were reported, and 12% of these were caused by co-infection with human immunodeficiency virus[1]. Despite many efforts to develop diagnosis and treatment methods, the mortality rate of TB is unacceptably high. Fortunately, two drugs that have novel modes of action were recently approved for the treatment of MDR patients worldwide since effective drug regimen was first developed in the 1940s[1]. An ATP synthase inhibitor, bedaquiline, was approved by the United States Food and Drug Administration in 2012[2-3], and a mycolic acid synthesis inhibitor, delamanid, was approved by European Medicines Agency in 2013[4-5]. However, because of the continuing emergence of drug-resistant TB, the dearth of novel chemical entities in clinical trials is still a major threat to TB patients. Therefore, new drugs with novel targets should be prioritized in the drug development pipeline to respond to pandemic TB.

Q203 (Figure 1), which has an imidazo[1,2-*a*]pyridine-3-carboxamide (IPA) scaffold, was developed by our group as a novel TB drug and is currently in clinical trials[6,8-9]. Its novel mode of action is through blocking ATP production by targeting the b subunit of cytochrome bc1 complex, which is involved in the energy metabolism of *M. tuberculosis*[6-7]. Our previous studies revealed that the analogues possess a linear lipophilic side chain display superior anti-tubercular activity; thus, we suggested that linearity and lipophilicity of side chain on the right side of the molecule are important in drug potency[8]. In this study, we designed and synthesized new analogues of IPA with various chain lengths and flexibility to understand how linearity and

lipophilicity of the side chain region affects anti-tubercular activity. The study suggests a small set of analogues which could be alternative drug candidates based on the comparable *in vitro* potency as well as promising pharmacokinetic values with **Q203**.



Q203

Figure 1. Structure of Q203.

2. Result and discussion

The synthetic route for the preparation of IPA derivatives is outlined in Schemes 1-4. First, ethyl 2-bromo-3-oxopentanoate (**2)** was prepared from commercially available ethyl-3-oxopentanoate (**1)** by bromination[8,10]. Compound **2** was condensed with adequate aminopyridines, **3a-e**[11], to give key intermediate esters, **4a-e**, which were saponified using lithium hydroxide to give acids **5a-e**.

The coupling counterpart benzylamines were prepared in various ways, as described in Schemes 2-3. The benzylamines, **7a-b**, **9**, **11a-b**, and **13**, that possess a linker between two aryl moieties were synthesized as shown in Scheme 2. The benzyloxy analogues, **6a** and **6b**, were prepared from 4-hydroxybenzonitrile and

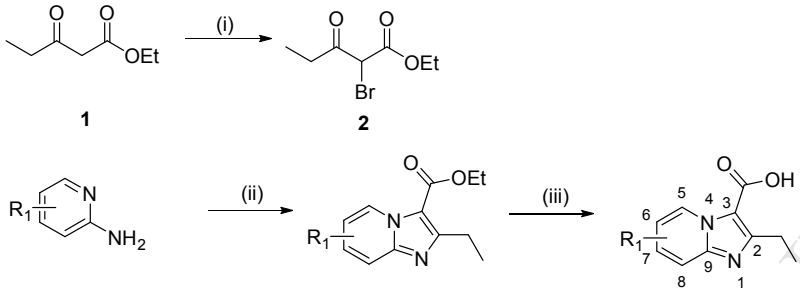
adequate benzyl bromide by S_N2 reaction. Reductive amination of 4-aminobenzonitrile and 4-trifluoromethoxybenzaldehyde using sodium triacetoxyborohydride was carried out to yield the aminomethyl compound **8**. Compounds **10a** and **10b**, which have an oxygen or a nitrogen atom between two aryl rings, were conveniently synthesized from 4-fluorobenzonitrile and appropriated aniline or phenol by heating in the presence of potassium carbonate. Methylation of **10b** was accomplished using NaH to yield **12**. The resulting benzonitrile intermediates, **6a-b**, **8**, **10a-b**, and **12**, were easily reduced to benzylamines, **7a-b**, **9**, **11a-b**, and **13**, using lithium aluminum hydride (LAH) in tetrahydrofuran[12].

The synthetic route to obtain benzylamines that possess a saturated ring with a linker between two aryl moieties is shown in Scheme 3. A mixture of 4-fluorobenzonitrile, adequate cyclic amine, and potassium carbonate in dimethylsulfoxide was heated to 120°C to obtain **14a-f**. Compounds **16a-f**, which have an oxygen linker, were synthesized from **14a**, **14c**, and **14d** *via* Mitsunobu reaction. For the preparation of **18**, the Mitsunobu reaction was also applied to **14e** and 4-trifluoromethoxyphenol. Compound **21**, which has a piperazine ring, was synthesized from **14f** through deprotection of a *t*-Boc group and reductive amination with 4-trifluoromethoxybenzaldehyde in the presence of sodium triacetoxyborohydride. Most of the benzonitrile intermediates were subsequently converted to benzylamines, **15**, **17a-f**, **19**, and **22**, by employing the same methodology as that used for converting **6** to **7**, whereas compound **23** was synthesized using Raney-Ni at H_2 atmosphere.

The synthetic route for the preparation of target IPA derivatives is shown in Scheme 4. General EDC coupling was accomplished with precursor acids and corresponding amines in the final step of synthesis to

obtain **24**-**43**. On the other hand, in the case of carbonyl analogues, **45** and **46**, acid **5c** was first coupled with amine **23**, the *tert*-butyloxy carbonyl group was removed in acidic conditions, and then the reaction with the corresponding acyl chloride followed.

Scheme 1. General synthetic scheme of 2-ethylimidazo[1,2-*a*]pyridine-3-carboxylic acids[a]



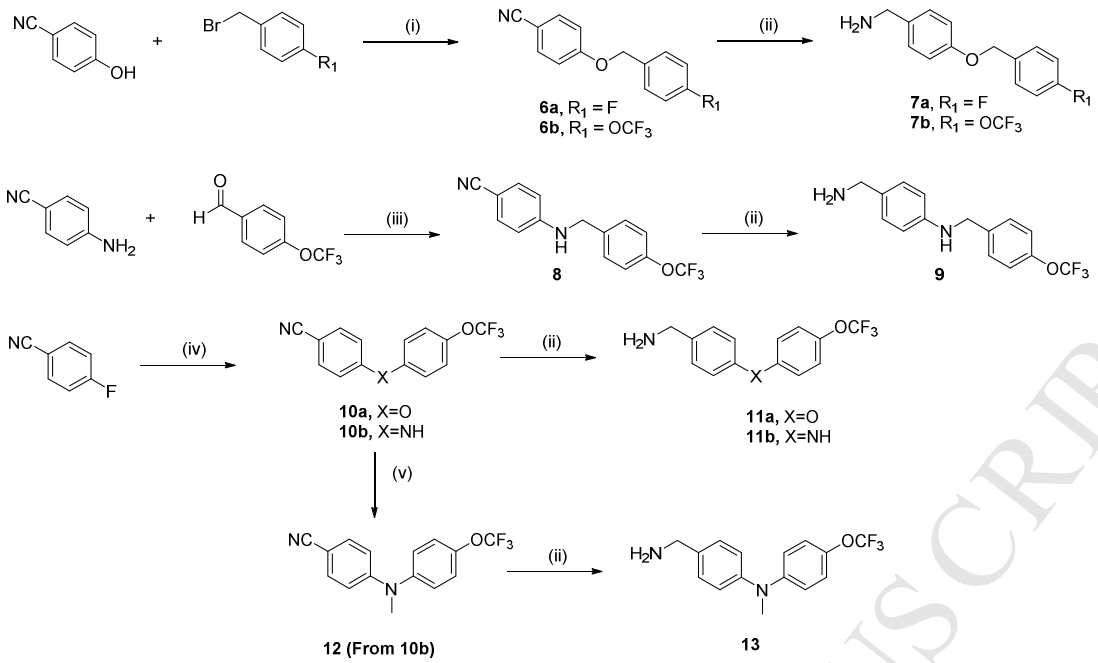
3a, pyridin-2-amine
3b, 5-fluoropyridin-2-amine
3c, 5-chloropyridin-2-amine
3d, 5-bromopyridin-2-amine
3e, 4-chloropyridin-2-amine

4a, R_1 = H
4b, R_1 = 6-F
4c, R_1 = 6-Cl
4d, R_1 = 6-Br
4e, R_1 = 7-Cl

5a, R_1 = H
5b, R_1 = 6-F
5c, R_1 = 6-Cl
5d, R_1 = 6-Br
5e, R_1 = 7-Cl

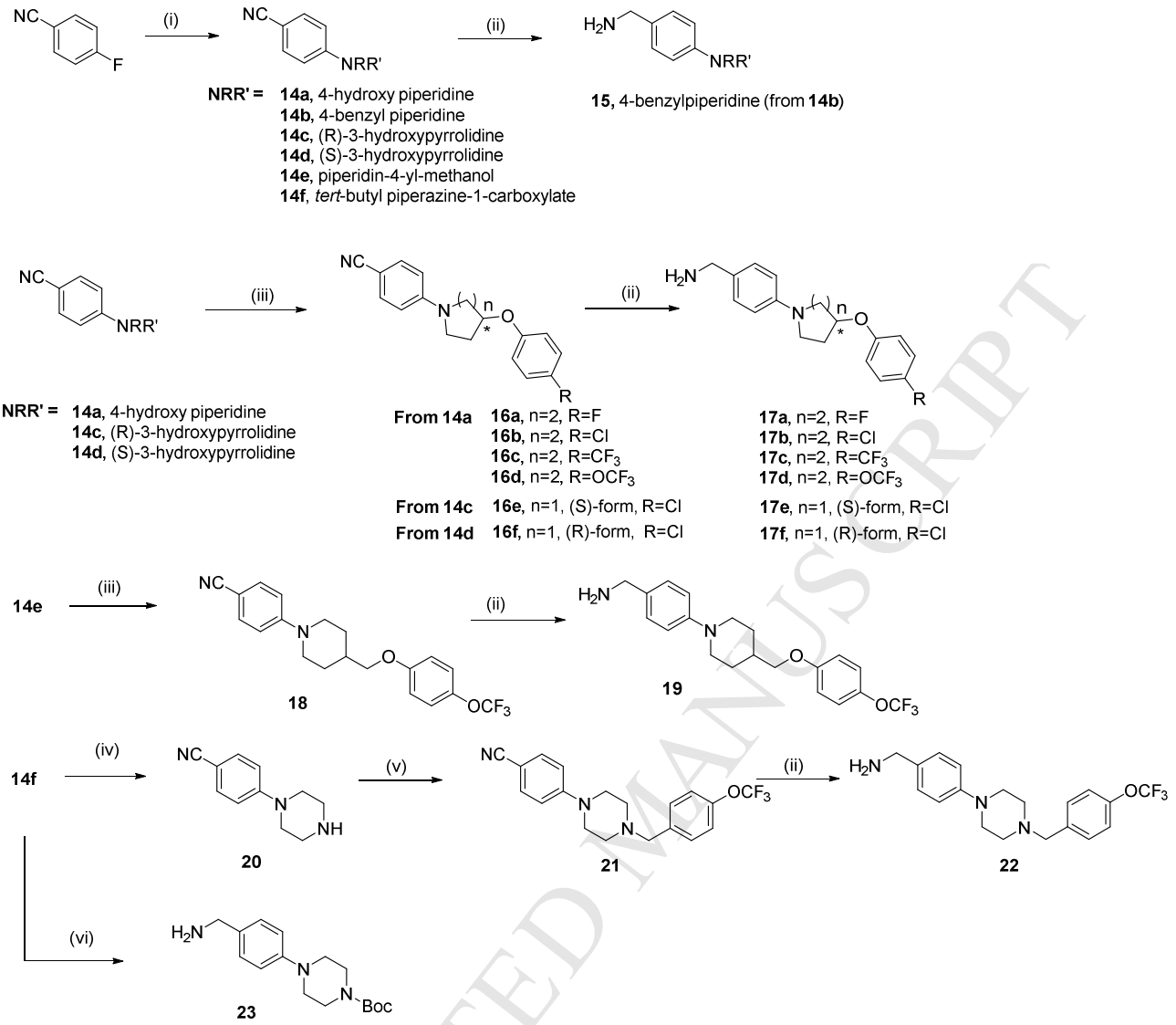
[a]Reagents and conditions: (i) NBS, NH_4OAc (over 2.0 eq.), Et_2O, 6h; (ii) **2**, EtOH, reflux, overnight; (iii) LiOH, EtOH/H_2O (3:1, *v/v*), overnight

Scheme 2. Synthesis of benzylamine counterparts for IPA derivatives as form I[a].



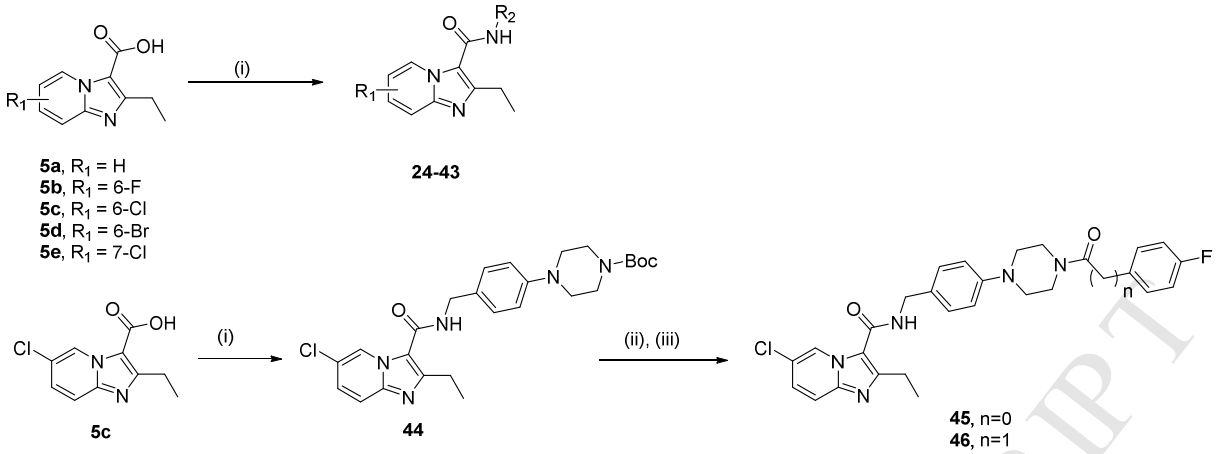
[a]Reagents and conditions: (i) NaH, DMF, 0°C - room temperature, 4 h; (ii) LAH, THF, 0°C – reflux, 2 h; (iii) $NaBH(OAc)_3$, MC, overnight; (iv) 4-trifluoromethoxy phenol (for **10a**) or 4-trifluoromethoxy aniline (for **10b**), K_2CO_3, DMF or DMSO, 120–150°C, 6 h; (v) NaH, iodomethane, 0°C - room temperature, 2h

Scheme 3. Synthesis of benzylamine analogues for IPA derivatives as form II[a].



[a]Reagents and conditions: (i) K_2CO_3, DMSO, 120°C, 4–6 h; (ii) LAH, THF, 0°C - reflux, 2 h; (iii) corresponding phenol, DIAD, PPh_3, MC, overnight; (iv) TFA, MC, 4 h; (v) 4-trifluoromethoxybenzaldedyde, $NaBH(OAc)_3$, MC, overnight; (vi) Raney-Ni, H_2, MeOH, 2 h

Scheme 4. Synthesis of IPA derivatives (24-43, 45, and 46)[a]



[a]Reagents and conditions: (i) corresponding amine, EDC, HOBt, TEA, DMF, 80°C, 2–4 h; (ii) TFA, MC, 6 h; (iii) corresponding acyl chloride, TEA, MC, 1.5 h

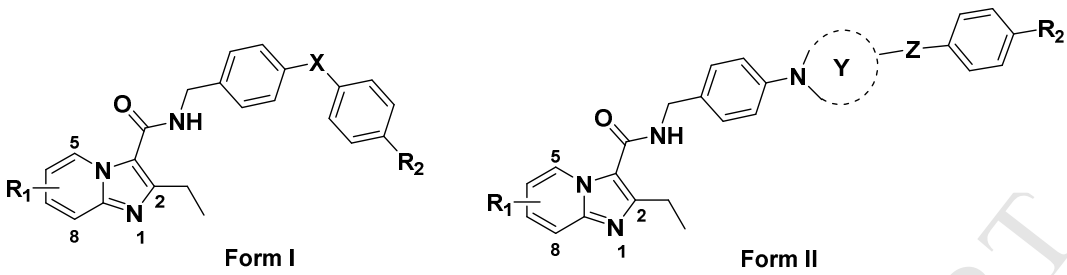
2.2. Structure-activity relationship (SAR) study

Structure-activity relationship (SAR) was explored in two types of compounds designated as form I and form II. Form I compounds have a short linking unit between a cyclic amine and the last phenyl ring, whereas form II compounds possess an additional cyclic amine moiety. All synthesized compounds were evaluated for their anti-tubercular activity against H37Rv-GFP in liquid broth culture medium (extracellular MIC_{80}), and results are summarized in Table 1. Our initial SAR study focused on the molecules that have flexible linkers, such as ether, methyl ether, amine, and methyl amine between two aryl moieties (**24-33**, described as form I). Compounds **24-29** have a methyl ether linker (X = OCH_2); thus, they are conformationally more flexible than **Q203**. First, a halogen atom was introduced at position 6 or 7 as R1 because our previous experiment showed that a substitution on position 5 or 8 was less potent (data not

shown). The assay results revealed that the small and highly electronegative fluorine group (**25**) showed a 3-fold decrease in activity and that the sterically more hindered bromo group (**26**) led to a 16-fold decrease in activity (extracellular MIC_{80} = 84 nM and 432 nM, respectively) compared to compound **24**. However, the chlorine atom, whether it was placed at position 6 or 7, showed increased potency over a hydrogen atom (**27** and **28**, extracellular MIC_{80} = 15 nM for both). For R1 substituents, all compounds showed good potency except for the 6-bromo compound. It suggested that the steric factor seems to be more important than the electronic factor. Furthermore, changing the R2 group from a trifluoromethoxy group to a fluorine group had no influence on activity (**29**, extracellular MIC_{80} = 20 nM). On the other hand, compound **30**, which has an alkyl amine linker (X = NCH_2), showed an approximately 16-fold decrease in MIC values compared to compound **28**, although both have similar linker lengths. In addition, even with a shorter oxygen linker (X = O), compound **31** showed a 4-fold reduction in potency (extracellular MIC_{80} = 64 nM) compared to compound **28**. Also, the presence of a hydrogen bond donor (such as X = NH) led to significant activity loss (**32**, extracellular MIC_{80} = 839 nM). Interestingly, for compound **33**, in which a hydrogen atom on the nitrogen of compound **32** was replaced with a methyl group, the activity strikingly recovered to 40 nM in contrast to 839 nM for compound **32**. We explored additional SAR for a set of compounds which have more extended linker length by the cyclic amine moiety described as form II. For this study, flexible and rotatable linkers were placed between the second cyclic amine and the last phenyl ring. The cyclic amine was varied to piperidine, pyrrolidine, and piperazine (**34-43**, **45**, and **46**). As shown in Table 1, analogues that have an ether linker (Z = O) next to the piperidine ring displayed excellent potency, with MIC_{80} range of 3 to 26 nM, regardless of the R2 substituent at the end (**34-38**). 7-Chloro on R1 did not show a big difference in potency

(**38**, extracellular MIC_{80} = 21 nM) compared with the 6-chloro analogue (**37)**. Potency was retained by analogue **39**, which possesses an alkyl linker (Z = CH_2) instead of an ether bridge (extracellular MIC_{80} = 22 nM). The even smaller cyclic amine (pyrrolidine) showed comparable anti-tubercular activity, regardless of the stereochemistry at its 3-position (**40** and **41**, extracellular MIC_{80} = 6 and 14nM, respectively). A flexibly extended linker (Z = CH_2O) showed tolerable potency as well (**42**, extracellular MIC_{80} = 37nM), despite its difference in side chain conformational change compared to compound **37**. A series of analogues containing a piperazine as the cyclic amine in the middle of the side chain to give more polarity was synthesized and evaluated for anti-tubercular activity (**43**, **45**, and **46**). Compound **43**, which has a benzyl group next to the piperazine ring, showed good potency, with a MIC_{80} value of 15 nM. On the other hand, the carbonyl-containing analogues, **45** and **46**, displayed over a 10-fold decrease in activity compared to compounds **42** and **43**, despite having similar chain lengths. These results suggest that a rigid linker would restrict side chain reorientation for target binding, and that, more importantly, the decreased lipophilicity in the linker region may adversely affect anti-tubercular activity.

Table 1. Extracellular activity of IPA analogues against *M. tuberculosis* H37Rv[a,b]



Compound	Form	R_1	X	Y	Z	R_2	[a]Extracellular activity MIC_{80} (H37Rv-GFP, nM)
24	I	H	OCH_2	–		OCF_3	27
25	I	6-F	OCH_2	–		OCF_3	84
26	I	6-Br	OCH_2	–		OCF_3	432
27	I	6-Cl	OCH_2	–		OCF_3	15
28	I	7-Cl	OCH_2	–		OCF_3	15
29	I	7-Cl	OCH_2	–		F	20
30	I	7-Cl	NCH_2	–		OCF_3	250
31	I	7-Cl	O	–		OCF_3	64
32	I	7-Cl	NH	–		OCF_3	839
33	I	7-Cl	NMe	–		OCF_3	40
34	II	6-Cl	–	4-piperidyl	O	F	10
35	II	6-Cl	–	4-piperidyl	O	Cl	3
36	II	6-Cl	–	4-piperidyl	O	CF_3	26
37	II	6-Cl	–	4-piperidyl	O	OCF_3	7
38	II	7-Cl	–	4-piperidyl	O	OCF_3	21
39	II	7-Cl	–	4-piperidyl	CH_2	H	22
40	II	6-Cl	–	(R)-3-pyrrolidyl	O	Cl	6
41	II	6-Cl	–	(S)-3-pyrrolidyl	O	Cl	14
42	II	6-Cl	–	4-piperidyl	CH_2O	OCF_3	37
43	II	6-Cl	–	4-piperazinyl	CH2	OCF_3	15
45	II	6-Cl	–	4-piperazinyl	$C(O)CH_2$	F	373
46	II	6-Cl	–	4-piperazinyl	C(O)	F	534
INH							490
[b]**Q203**							4

[a]Extracellular MIC_{80} = inhibitory activity against *M. tuberculosis* H37Rv replicating in liquid broth culture medium. MIC_{80} is the minimum concentration required to inhibit growth by 80% and indicates an average value of two independent measurements. [b]Anti-tubercular activity of Q203 were adapted from ref 8.

2.3. Anti-tubercular activity against M. tuberculosis inside macrophages (Intracellular activity)

The *in vitro* activity against *M. tuberculosis* replicating inside macrophages (intracellular MIC_{80})[13] was also investigated for a small set of compounds (**25**, **28**, **31**, **33**, **35**, **36**, and **43**) that displayed good extracellular potency. As shown in Table 2, most of the compounds showed also good potency against *M. tuberculosis* replicating inside and in the liquid broth culture medium as well. Compounds **28**, **35**, and **36**, in particular, showed excellent intracellular potency with a single-digit nanomolar range (intracellular MIC_{80} = less than 1 nM–9 nM). However, compound **43** exhibited quite decreased intracellular potency despite having the same extracellular activity as compound **28**. It seems that decreased lipophilicity due to the presence of a piperazine group might have influenced cell permeability in our macrophage infection assay system. Overall, all analogues, except for compound **43**, showed better potency against *M. tuberculosis* replicating inside macrophages than against *M. tuberculosis* in liquid broth culture medium and showed good correlation between extracellular and intracellular activity, with R^2 value of 0.93.

Table 2. Anti-tubercular activity against *M. tuberculosis* H37Rv-GFP replicating inside macrophages (Intracellular activity)[a,b,c]

Compd	[a]Extracellular MIC_{80} (nM)	[b]Intracellular MIC_{80} (nM)
25	84	28
28	15	<1
31	64	27
33	40	23
35	3	<1
36	26	9
43	15	82
INH	490	200
[c]Q203	4	1.43

[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 measurements. [c]Anti-tubercular activity of Q203 were adapted from ref 8.

2.4. Pharmacokinetic evaluation

The *in vivo* pharmacokinetic properties of compounds **25**, **28**, **35**, and **36** were evaluated in mice after intravenous (i.v.) and oral (p.o.) administration of 2 and 10 mg/kg, respectively. As shown in Table 3, all compounds reached maximum concentration in plasma within 2 h after oral dosing. In addition, they showed not only high drug exposure level in plasma, from 11500 ng·h/mL to 48900 ng·h/mL with long half-life (11–20 h), but also low systemic clearance (2.23–5.77 mL/min/kg). Further they had good oral bioavailability (51.4 %–80.5 %). These pharmacokinetic values indicate that they are potential anti-tubercular candidates that can show *in vivo* efficacy comparable to **Q203**[6,8].

Table 3. ***In vivo*** **pharmacokinetic values in mice of compound 25, 28, 35, and 36**[a]

Compd	Pharmacokinetics (i.v.) 2mpk			Pharmacokinetics (p.o.) 10mpk				
	$t_{1/2}$ (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 (%)
25	11.0	4.76	3850	2620	11.4	1.00	28200	80.5
28	13.9	5.77	4680	1610	11.9	0.50	11500	53.4
35	14.1	2.83	1340	3540	18.9	2.00	30300	51.4
36	19.6	2.23	2390	4100	20.0	2.00	48900	65.6
[a]**Q203**	16.5	4.0	5270	1490	23.4	2.0	44100	90.7

[a]PK values of Q203 were adapted from ref 6 and 8 for the sake of comparison.

3. Conclusion

In summary, we have synthesized and evaluated analogues of **Q203** to understand the role of the right hand side chain moiety in anti-tubercular activity. Most of the analogues retained activity regardless of chain length and showed comparable potency. However, the analogues with an amine linker reduced activity. Based on these results, we postulate that lipophilicity is more important than linearity of side chain in retaining activity and that the decreased lipophilic character of the linker region negatively affects anti-tubercular activity.

In addition, we found that a set of analogues, **25**, **28**, **35**, and **36**, exhibited better potency against *M. tuberculosis* inside macrophages than against *M. tuberculosis* replicating in liquid broth culture medium. Based on their promising pharmacokinetic properties with potent intracellular activities, these newly

synthesized analogues are promising anti-tubercular candidates, which could show efficacy comparable with **Q203**. Studies on the *in vivo* efficacy of these analogues will be reported in due course.

4. Experimental

4.1.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 254nm of UV light and PMA or potassium permanganate stain followed by heating. Melting points (mp) were measured on an electro thermal melting point apparatus, M-565 (BÜCHI). ^{1}H-NMR (at 400 MHz) and ^{13}C NMR (at 100 MHz) spectra were recorded on a Varian 400 MHz spectrometer and are reported in ppm using solvent as an internal standard ($CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.50 for ^{1}H NMR and $CDCl_3$ at 77.2 ppm for ^{13}C NMR). 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. Yields refer to purified products and are not optimized and the ^{13}C NMR data of representative compounds **25**, **28**, **35** and **36** were reported. The purity of all biologically tested compounds was >95% and minimum inhibitory

concentration determination (extracellular & intracellular), *in vivo* pharmacokinetics were performed as previously described[6, 13, 14].

*4.1.1. General Procedure for Preparation of acid counterpart **5a-5e**.*

The synthetic procedure for preparation of imidazopyridine[1,2-*a*] carboxylic acids was previously reported[8] and ^{1}H NMR data of carboxylic acid **4d** and intermediate ester **4a**-**4c** and **4e** are as below.

*Ethyl 2-ethylimidazo[1,2-a]pyridine-3-carboxylate (**4a**).* ^{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-ethyl-6-fluoroimidazo[1,2-a]pyridine-3-carboxylate (**4b**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.44 (t, *J* = 7.6 Hz, 3H), 3.12 (q, *J* = 7.6 Hz, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 7.28 -7.31 (m, 1H), 7.58 -7.62 (m, 1H), 9.31 – 9.33 (m, 1H).

*Ethyl 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylate (**4c**).* ^{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 (**4e**).* ^{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).

*Ethyl 6-bromo-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid (**5d**).* ^{1}H NMR (400 MHz, DMSO-

d_6) δ 1.24 (t, J = 7.6 Hz, 3H), 3.02 (q, *J*= 7.6 Hz, 2H), 7.63-7.69 (m, 2H), 9.41 (s, 1H), 13.3 (brs, 1H).

*4.1.2. General Procedure for Preparation of **6a-6b**.*

To a stirred solution of 4-hydroxybenzonitrile (1.68 mmol) in DMF (5 mL) was added NaH (60% dispersion in paraffin, 2.01 mmol) under ice-bath. After 10min, 4-fluorobenzyl bromide (2.01 mmol) was added then the resulting solution was further stirred for 4 hours at room temperature. The mixture was quenched with water (10 mL) and extracted with EtOAc (10 mL × 2). The organic phase was washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo to give **6a** as a white solid. In a similar manner, **6b** was synthesized according to procedure above.

*4.1.3. General procedure for preparation of **8** and **21**.*

To a stirred solution of 4-aminobenzonitrile (4.23 mmol) and 4-(trifluoromehtoxy)benzaldehyde (4.65 mmol) in methylene chloride (10 mL) was added sodium triacetoxyborohydride (6.34 mmol) and the resulting mixture was stirred for overnight. The mixture was diluted with methylene chloride (10 mL), washed with saturated Na_2CO_3 (aq. 10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 7:1) to give **8** as a pale yellow solid (85%). In a similar manner, compound **21** was synthesized from **20** according to this procedure.

*4.1.4. General procedure for preparation of **10a-10b and 14a-14f**.*

A mixture of 4-fluorobenzonitrile (2.48 mmol), 4-(trifluoromethoxy)phenol (2.73 mmol) and K_2CO_3 (7.44 mmol) in DMF or DMSO (3 mL) was heated to 150℃ for 6 hours. After the cooling, the mixture was poured to the water. The resulting solid was filtered, washed with water and dried to give **10a**.

In a similar manner, **10b and 14a-14f** were synthesized according to this procedure.

*4.1.5. General procedure for preparation of compound **12**.*

To a stirred solution of **10b** (1.27 mmol) in anhydrous DMF (5 mL) was added NaH (60% dispersion in paraffin, 1.91 mmol) under ice-bath. After 20 min, iodomethane (1.91 mmol) was added and the reaction mixture was allowed to ambient temperature. After 2 hours of stirring, the mixture was quenched with water (5 mL) and concentrated. The resulting residue was diluted with EtOAc (20 mL) and washed with water (15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 2:1) to give **12**.

*4.1.6. General procedure for preparation of compound **7a-7b, 9, 11a-11b, 13, 15, 17a-17f, 19 and 22**.*

To a solution of **6a** (1.71 mmol) in THF (10 mL) was added lithium aluminum hydride (5.12 mmol) under ice-bath and then the resulting mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and quenched with water (5 mL). The insoluble solid was filtered off using cellite and the filtrate was diluted with sat. Na_2CO_3 (aq. 15 mL) and extracted with EtOAc (20 mL × 2). The organic phase was washed with brine (20 mL), dried over $MgSO_4$ and concentrated in vacuo to give **7a**.

The crude residue was used for next reaction without further purification. In a similar manner, compound **7b, 9, 11a-11b, 13, 15, 17a-17f, 19 and 22**were synthesized according to this procedure.

*4.1.7. General procedure for preparation of **16a-16f and18**.*

To a stirred solution of **14a** (1.38 mmol), triphenylphosphine (1.94 mmol) and 4-chlorophenol (1.38 mmol) in methylene chloride (10 mL) was added diisopropyl azodicarboxylate (1.66 mmol) slowly and the resulting mixture was stirred for overnight. The reaction mixture was diluted with methylene chloride (10 mL) and washed with water (10 mL) and brine (10 mL). The resulting organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (*n*-hexane:EtOAc = 7:1) to give **16b** as a white solid. In a similar manner, compound **16a, 16c-16f and 18** were synthesized according to this procedure.

4.1.8. Synthesis of *compound **45 and 46**.*

To a stirred solution of **44** (14.6 mmol) in methylene chloride (30 mL) was added trifluoroacetic acid (10 mL) and the resulting mixture was stirred at room temperature for 4h. The reaction mixture was diluted with water (40 mL) and basified with aqueous 1N NaOH. Then the mixture was extracted with EtOAc (100 mL × 3) and the combined extract was washed with water (100 mL) and brine (100 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated to give intermediate amine with 98% yield. The intermediate amine (2.48 mmol) was dissolved with tetrahydrofuran (5 mL) and triethylamine (7.4 mmol) was added. The reaction mixture was cooled under ice-bath and benzoyl chloride (2.98 mmol) was added slowly. The resulting solution was allowed to room temperature and

further stirred for 30min. The mixture was diluted with water (100 mL) and extracted with EtOAc (50 mL × 3). The resulting organic phase was washed with brine (20 mL), dried over Na_2SO_4 and concentrated. The crude residue was purified by flash column chromatography (PE:EA = 4:1 to 1:2) to give **45**. In a similar manner, compound **46 was** synthesized according to this procedure.

4.1.9. General procedure for preparation of **23**.

To a stirred solution of **14f** (1.55 mmol) in methanol (10 mL) was added a portion of Raney-Ni and the mixture was stirred for 4h under H_2 atmosphere. The reaction mixture was filtered by cellite and the filtrate was concentrated. The crude residue was purified by flash column chromatography (methylene chloride:MeOH = 20:1) to give **23**.

4-((4-(Trifluoromethoxy)benzyl)oxy)benzonitrile (**6b**). White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (s, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H).

4-(4-(4-Chlorophenoxy)piperidin-1-yl)benzonitrile (**16b**). White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60 – 1.68 (m, 2H), 1.98 – 2.02 (m, 2H), 3.24 – 3.40 (m, 2H), 3.68 – 3.74 (m, 2H), 4.61 – 4.66 (m, 1H), 7.01 – 7.06 (m, 4H), 7.31 – 7.34 (m, 2H), 7.57 (d, *J* = 8.8 Hz, 2H).

4-(4-(4-(Trifluoromethyl)phenoxy)piperidin-1-yl)benzonitrile (**16c**). White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 – 1.98 (m, 2H), 2.04 – 2.12 (m, 2H), 3.32 – 3.38 (m, 2H), 3.58 – 3.64 (m, 2H), 4.61 – 4.64 (m, 1H), 6.87 – 6.90 (m, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.48 – 7.51 (m, 2H), 7.55 (d, *J* = 8.8 Hz, 2H).

4.1.10.General procedure for amide coupling.

The target imidazopyridine[1,2-*a*]-3-carboxamide derivatives were synthesized using EDC and HOBt and synthetic procedure was previously reported[8].

2-Ethyl-N-(4-((4-(trifluoromethoxy)benzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(24)***. White so lid; mp = 138.7 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 4.61 (d, *J* = 5.2 Hz, 2H), 5.03 (s, 2H), 6.15 (brt, *J* = 5.6 Hz, 1H), 6.86 (ddd, *J* = 1.2, 7.2, 7. 2 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.26 – 7.30 (m, 3H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 9.2 Hz, 1H), 9.34 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 470 $[M + H]^+$.

2-Ethyl-6-fluoro-N-(4-((4-(trifluoromethoxy)benzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(25).*** White solid; mp = 157.4 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.2 Hz, 3H), 2.96 (q, *J* = 7 .2 Hz, 2H), 4.63 (d, *J* = 5.6 Hz, 2H), 5.06 (s, 2H), 6.08 (brt, *J* = 5.2 Hz, 1H), 6.96 (d, *J* = 8.8 H z, 2H), 7.22 – 7.26 (m, 3H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.56 (dd, *J* = 5.2 , 9.6 Hz, 1H), 9.43 – 9.45 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 23.8, 43.2, 69.3, 115.3, 1 15.8, 115.9, 1163.9, 117.0, 118.6, 118.8, 119.3, 121.3, 121.9, 124.4, 129.0, 129.3, 130.8, 135.7, 143.8 , 149.0, 151.8, 152.4, 154.8, 158.2, 161.3; LCMS (ESI) m/z 488 $[M + H]^+$.

6-Bomo-2-ethyl-N-(4-((4-(trifluoromethoxy)benzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(26)***. Pale yellow solid; mp = 189.7 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 4.63 (d, *J* = 5.6 Hz, 2H), 5.05 (s, 2H), 6.08 (brt, *J* = 5.2 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.38 (dd, *J* = 1.6, 9.2 Hz, 1H), 7.44 – 7.49 (m, 3H), 9.61 (d, *J* = 1.6 Hz, 1H); LCMS (ESI) m/z 548 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-((4-(trifluoromethoxy)benzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(27)***. White solid; mp = 168.5 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 2.96 (q, *J* = 7.6 Hz, 2H), 4.64 (d, *J* = 5.6 Hz, 2H), 5.06 (s, 2H), 6.05 (brs, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 7.20 - 7.33 (m, 5H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 9.2 Hz, 1H), 9.53 (d, *J* = 1.2 Hz, 1H); LCMS (ESI) m/z 504 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-((4-(trifluoromethoxy)benzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(28)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 4.63 (d, *J* = 5.6 Hz, 2H), 5.06 (s, 2H), 6.03 (brs, 1H), 6.90 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 2.0 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.5, 22.2, 42.3, 68.6, 114.2, 115.0, 115.5, 116.0, 121.4, 128.4, 129.1, 129.8, 131.8, 132.2, 137.1, 145.1, 148.2, 151.4, 157.5, 160.9; LCMS (ESI) m/z 504 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-((4-fluorobenzyl)oxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(29)***. Pale yellow solid; mp = 181.0 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.6 Hz, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 4.62 (d, *J* = 5.6 Hz, 2H), 5.02 (s, 2H), 6.02 (brs, 1H), 6.90 (dd, *J* = 7.6, 2.4 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 2H), 7.07 (dd, *J* = 8.8, 8.8 Hz, 2H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.40 (dd, *J* = 5.6, 8.8 Hz, 2H), 7.58 (d, *J* = 1.6 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 438 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-((4-(trifluoromethoxy)benzyl)amino)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(30)***. White solid; mp = 169.6 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, *J* = 7.6 Hz, 3H), 2.93 (q, *J* = 7.6 Hz, 2H), 4.18 (brs, 1H), 4.34 (s, 2H), 4.55 (d, *J* = 5.2 Hz, 2H), 6.00 (brs, 1H), 6.60 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 4H), 7.38 (d, J = 8.0 Hz, 2H), 7.56 (s, 1H), 9.33 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 503 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(4-(trifluoromethoxy)phenoxy)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(31)***. White solid; mp = 141 - 142 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (t, *J* = 7.6 Hz, 3H), 2.98 (q, *J* = 7.6 Hz, 2H), 4.68 (d, *J* = 5.6 Hz, 2H), 6.10 (brs, 1H), 6.91 (dd, *J* = 2.0, 7.6 Hz, 1H), 6.98 – 7.02 (m, 4H), 7.18 (d, *J* =

8.8 Hz, 2H), 7.37 (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 490 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-((4-(trifluoromethoxy)phenyl)amino)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(32)***. Pale yellow solid; mp = 200 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.6 Hz, 3H), 2.98 (q, *J* = 7.6 Hz, 2H), 4.63 (d, *J* = 5.6 Hz, 2H), 5.73 (s, 1H), 6.05 (brs, 1H), 6.91 (dd, *J* = 2.0, 7.6 Hz, 1H), 7.03 - 7.07 (m, 4H), 7.12 (d, *J* = 9.2 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 1.6 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); LCMS (ESI) m/z 489 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(methyl(4-(trifluoromethoxy)phenyl)amino)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(33)***. White solid; mp = 134 ℃; ^{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), 3.24 (s, 3H), 4.50 (d, *J* = 6.0 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 7.08 - 7.10 (m, 3H), 7.19 (d, *J* = 8.8 Hz, 2H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.78 (s, 1H), 8.44 (brs, 1H), 8.97 (d, *J* = 7.2 Hz, 1H); LCMS (ESI) m/z 503 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-(4-fluorophenoxy)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(34)***. Pale yellow solid; mp = 148.4 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 1.91 - 1.94 (m, 2H), 2.06 - 2.11 (m, 2H), 2.96 (q, *J* = 7.6 Hz, 2H), 3.08 - 3.14 (m, 2H), 3.47 - 3.54 (m, 2H), 4.37 - 4.39 (m, 1H), 4.61 (d, *J* = 5.6 Hz, 2H), 6.01 (brs, 1H), 6.86 - 6.89 (m, 2H), 6.95 - 7.00 (m, 4H), 7.26 - 7.30 (m, 3H), 7.53 (d, *J* = 8.8 Hz, 1H), 9.53 (d, *J* = 1.6 Hz, 1H); LCMS (ESI) m/z 507 $[M + H]^+$.

6-Chloro-N-(4-(4-(4-chlorophenoxy)piperidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(35)***. Pale yellow solid; mp = 165.3 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.6 Hz, 3H), 1.19 – 1.28 (m, 2H), 1.54 – 1.57 (m, 2H), 2.53 (q, *J* = 7.6 Hz, 2H), 2.55 – 2.59 (m, 2H), 3.00 – 3.06 (m, 2H), 3.98 (d,

J = 6.0 Hz, 2H), 4.05 – 4.11 (m, 1H), 6.48 (d, *J* = 8.8 Hz, 2H), 6.53 – 6.56 (m, 2H), 6.78 (d, *J* = 8.4 Hz, 2H), 6.83 – 6.87 (m, 2H), 6.98 (dd, *J* = 9.6, 2.4 Hz, 1H), 7.20 (d, *J* = 9.6 Hz, 1H), 7.93 (brt, *J* = 6.0 Hz, 1H), 8.62 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.5, 22.3, 30.3, 42.3, 46.4, 72.9, 116.1, 116.3, 117.6, 118.0, 120.0, 124.6, 125.2, 127.4, 128.7, 129.7, 129.8, 143.7, 150.2, 151.4, 156.3, 160.8; LCMS (ESI) m/z 523 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethyl)phenoxy)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(36)***. White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 1.94 - 1.99 (m, 2H), 2.10 - 2.15 (m, 2H), 2.96 (q, *J* = 7.6 Hz, 2H), 3.12 - 3.18 (m, 2H), 3.47 - 3.53 (m, 2H), 4.53 - 4.57 (m, 1H), 4.61 (d, *J* = 5.2 Hz, 2H), 6.02 (brs, 1H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.27 - 7.31 (m, 3H), 7.51 - 7.55 (m, 3H), 9.53 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.5, 22.3, 30.2, 42.3, 46.4, 72.8, 116.2, 116.3, 116.4, 117.5, 120.0, 120.9, 121.2, 121.5, 121.9, 123.6, 125.2, 126.3, 127.3, 127.4, 127.5, 128.7, 129.8, 143.7, 150.1, 151.4, 160.4, 160.9; LCMS (electrospray) m/z 557 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(37)***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.6 Hz, 3H), 1.89 - 1.97 (m, 2H), 2.07 - 2.12 (m, 2H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.09 - 3.15 (m, 2H), 3.48 - 3.53 (m, 2H), 4.42 - 4.47 (m, 1H), 4.60 (d, *J* = 5.6 Hz, 2H), 6.00 (brs, 1H), 6.88 – 6.93 (m, 3H), 6.96 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.8 Hz, 1H), 7.26 - 7.31 (m, 2H), 7.58 (d, *J* = 2.0 Hz, 1H), 9.36 (d, *J* = 7.6 Hz, 1H); LCMS (electrospray) m/z 573 $[M + H]^+$.

7-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(38)***. Pale yellow solid; mp = 141.3 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.6 Hz, 3H), 1.89 -1.97 (m, 2H), 2.07 - 2.12 (m, 2H), 2.96 (q, *J* = 7.6 Hz, 2H), 3.09 - 3.16 (m, 2H), 3.47 - 3.53 (m, 2H),

4.42 - 4.47 (m, 1H), 4.61 (d, *J* = 5.6 Hz, 2H), 6.01 (brs, 1H), 6.91 (dd, *J* = 2.0, 6.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 8.8Hz, 2H), 7.26 - 7.30 (m, 3H), 7.53 (d, *J* = 9.6 Hz, 1H), 9.53 (s, 1H); LCMS (ESI) m/z 573 $[M + H]^+$.

N-(4-(4-benzylpiperidin-1-yl)benzyl)-7-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(39).*** White solid; mp = 103.4 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.6 Hz, 3H), 1.37 - 1.44 (m, 2H), 1.63 - 1.70 (m, 1H), 1.72 - 1.76 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 2.61 - 2.67 (m, 2H), 2.92 (q, *J* = 7.6 Hz, 2H), 3.63 - 3.66 (m, 2H), 4.57 (d, *J* = 5.2 Hz, 2H), 6.08 (brs, 1H), 6.84 - 6.87 (m, 1H), 6.90 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.2 Hz, 2H), 7.19 - 7.30 (m, 5H), 7.55 (d, *J* = 1.6 Hz, 1H), 9.29 - 9.32 (m, 1H); LCMS (ESI) m/z 487 $[M + H]^+$.

(S)-6-Chloro-N-(4-(3-(4-chlorophenoxy)pyrrolidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(40)***. Pale yellow solid; mp = 211.8 ℃; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.26 (t, *J* = 7.6 Hz, 3H), 2.20 - 2.25 (m, 2H), 2.85 (q, *J* = 7.6 Hz, 2H), 3.33 - 3.45 (m, 3H), 3.58 - 3.62 (m, 1H), 4.47 (d, *J* = 5.2 Hz, 2H), 4.93 - 4.94 (m, 1H), 6.34 (brs, 1H), 6.49 (d, *J* = 8.8 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 7.13 - 7.17 (m, 4H), 7.23 -7.26 (m, 1H), 7.43 (d, *J* = 9.2 Hz, 1H), 9.30 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 509 $[M + H]^+$.

(R)-6-Chloro-N-(4-(3-(4-chlorophenoxy)pyrrolidin-1-yl)benzyl)-2-ethylimidazo[1,2-a]pyridine-3-carboxamide ***(41)***. Pale yellow solid; mp = 218.8 ℃; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.30 (t, *J* = 7.6 Hz, 3H), 2.23 - 2.28 (m, 2H), 2.88 (q, *J* = 7.6 Hz, 2H), 3.40 - 3.49 (m, 3H), 3.61 - 3.65 (m, 1H), 4.51 (d, *J* = 5.2 Hz, 2H), 4.96 - 4.97 (m, 1H), 6.22 (brs, 1H), 6.53 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 9.2 Hz, 2H), 7.17

- 7.20 (m, 4H), 7.28 (d, *J* = 2.0 Hz, 1H), 7.47 (d, *J* = 9.6 Hz, 1H), 9.37 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 509 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-((4-(trifluoromethoxy)phenoxy)methyl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(42)***. Pale yellow solid; mp = 183.6 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, *J* = 7.6 Hz, 3H), 1.50 - 1.54 (m, 2H), 1.93 - 1.95 (m, 3H), 2.72 - 2.78 (m, 2H), 2.94 (q, *J* = 7.6 Hz, 2H), 3.71 - 3.74 (m, 2H), 3.82 (d, *J* = 6.0 Hz, 2H), 4.59 (d, *J* = 5.6 Hz, 2H), 6.06 (brt, *J* = 5.6 Hz, 1H), 6.86 (d, *J* = 9.2 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 7.24 - 7.28 (m, 3H), 7.51 (d, *J* = 9.6 Hz, 1H), 9.50 (d, *J* = 1.2 Hz, 1H); LCMS (ESI) m/z 587 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-(4-(trifluoromethoxy)benzyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(43)***. White solid; mp = 138.1 - 138.7 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.6 Hz, 3H), 2.58 - 2.61 (m, 4H), 2.95 (q, *J* = 7.6 Hz, 2H), 3.19 - 2.61 (m, 4H), 3.55 (s, 2H), 4.60 (d, *J* = 5.6 Hz, 2H), 6.00 (brs, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.26 - 7.30 (m, 3H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 9.2 Hz, 1H), 9.52 (d, *J* = 2.0 Hz, 1H); LCMS (ESI) m/z 572 $[M + H]^+$.

6-Chloro-2-ethyl-N-(4-(4-(2-(4-fluorophenyl)acetyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(45)***. White solid; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.24 (t, *J* = 7.5 Hz, 3H), 2.91 - 3.12 (m, 6H), 3.51 - 3.65 (m, 4H), 3.74 (s, 2H), 4.42 - 4.44 (m, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 7.10 (t, *J* = 8.8 Hz, 2H), 7.19 - 7.31 (m, 4H), 7.69 (dd, *J* = 9.6, 1.8 Hz, 1H), 7.78 (d, *J* = 9.6 Hz, 1H), 8.66 (t, *J* = 5.7 Hz, 1H), 9.10 (s, 1H); LCMS (ESI) m/z 534 $[M + H]^+$.

6-chloro-2-ethyl-N-(4-(4-(4-fluorobenzoyl)piperazin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide ***(46)***. White solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (t, *J* = 7.5 Hz, 3H), 3.00 (q, *J* = 7.5 Hz, 2H), 3.08 -

3.28 (m, 4H), 3.31 - 3.91 (m, 4H), 4.43 (d, *J* = 5.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 7.20 - 7.33 (m, 4H), 7.49 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.70 (dd, *J* = 9.2, 1.8 Hz, 1H), 7.80 (d, *J* = 9.2 Hz, 1H), 8.70 (t, *J* = 5.7 Hz, 1H), 9.10 (s, 1H); LCMS (ESI) m/z 520 $[M + H]^+$.

ASSOCIATED CONTENT

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://

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Author Contributions

R.Y.K. performed growth inhibition experiments, S.K., Y.M.K., M.J.S., Z.N., K.N. Sanghee K. and Jaeseung K. designed and synthesized the compounds, Y.M.K. analyzed purity of the compounds, K.N. designed and

performed *in vivo* PK evaluation, S.K. wrote the manuscript with contributions from other authors. Sanghee K. and Jaeseung K. supervised synthesis of molecules and Jaeseung K. supervised TB project.

The authors declare no competing financial interest.

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ABBREVIATION

TB, tuberculosis; MDR, multidrug-resistance; XDR, extensively drug-resistance; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NBS, N-bromosuccinimide; HOBt, 1-hydroxybenzotriazole; DMF, N,N-dimethylformamide; ADME, absortion distribution, metabolism and excretion

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Highlights

- SAR study for the novel imidazo[1,2-*a*]pyridine-3-carboxamides against *M. tb.*
- Evaluation of anti-tubercular activites inside/outside macrophage.
- *In vivo* pharmacokinetics evaluation for a set of analogues.
- Suggested promising TB drug candidates based on the activities and PK properties.