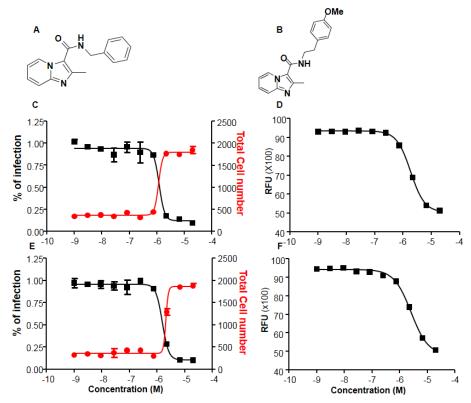
SUPPLEMENTARY INFORMATION

Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis.

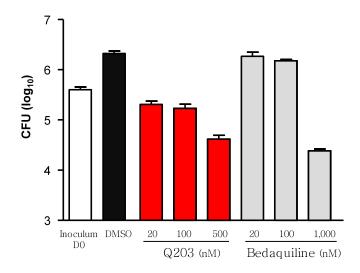
Kevin Pethe, Pablo Bifani, Jichan Jang, Sunhee Kang, Seijin Park, Sujin Ahn, Jan Jiricek, Juyoung Jung, Hee Kyoung Jeon, Jonathan Cechetto, Thierry Christophe, Honggun Lee, Marie Kempf, Mary Jackson, Anne J. Lenaerts, Ha Pham, Victoria Jones, Min Jung Seo, Young Mi Kim, Mooyoung Seo, Jeong Jea Seo, Dongsik Park, Yoonae Ko, Inhee Choi, Ryangyeo Kim, Se Yeon Kim, SeungBin Lim, SeungAe Yim, Jiyoun Nam, Hwankyu Kang, Haejin Kwon, Chun-Taek Oh, Yoojin Cho, Yunhee Jang, Junghwan Kim, Adeline Chua, Bee Huat Tan, Mahesh B. Nanjundappa, Srinivasa P.S. Rao, Whitney S. Barnes, René Wintjens, John R. Walker, Sylvie Alonso, Saeyeon Lee, Jungjun Kim, Soohyun Oh, Taegwon Oh, Ulf Nehrbass, Sung-Jun Han, Zaesung No, Jinhwa Lee, Priscille Brodin, Sang-Nae Cho, Kiyean Nam and Jaeseung Kim

Supplementary Figures 1-8

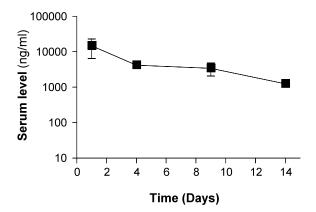
Supplementary Tables 1-7



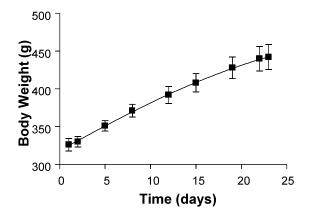
Supplementary Figure 1. Activity of IPA01 and IPA02 against *M. tuberculosis* H37Rv. (A) Structure of IPA01 and (B) structure of IPA02. The inhibitory activity of IPA01 (C and D) and IPA02 (E and F) were tested in dose-response against *M. tuberculosis* replicating inside macrophages (C and E) and in culture broth medium (D and F). Each concentration was tested in duplicate, the assays were repeated at least two times. The MIC₅₀ of IPA01 and IPA02 replicating inside macrophages was 1.25 μ M and 1.57 μ M, respectively. The MIC₅₀ of IPA01 and IPA02 replicating in culture broth medium was 1.86 μ M and 2.63 μ M, respectively.



Supplementary Figure 2. Activity of Q203 against *M. tuberculosis* by CFU determination. *M. tuberculosis* was exposed to DMSO (untreated control), Q203 or bedaquiline for 5 days in liquid broth medium (7H9-ADS-tween 80 0.05%). Cultures were serial-diluted and plated on 7H11-agar plates. Colony Forming Unit were determined after 3 weeks of incubation at 37°C. Initial inoculum size at day 0 (inoculum D0) is shown. Each concentration was tested in triplicate.

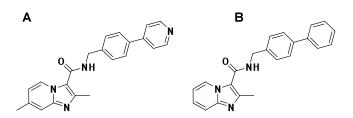


Supplementary Figure 3. Serum level after a single administration of 1,000 mg kg⁻¹ in the mouse model.

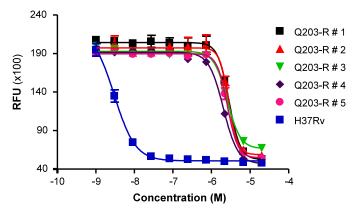


Supplementary Figure 4. Progression of the body weight of rats during a long-term administration study.

10 mg/kg of Q203 was administered to male SD rats for 20 days. Weight variation was monitored throughout the study and up to 3 days after the last administration. Each point represents the mean and standard variation of the body weight of five animals.



Supplementary Figure 5. Structure of IPA04 and IPA05 The MIC₅₀ of IPA04 (A) and IPA05 (B) against *M. tuberculosis* replicating in culture broth medium was of 10 nM and 5 nM, respectively.



Supplementary Figure 6. Activity of Q203 against 5 spontaneous-resistant clones selected on Q203. Dose-response of Q203 against Q203-R clones #1-5 using the resazurin-based assay. The activity of Q203 against the 5 spontaneous-resistant clones was between 3.0 and 3.7 μ M. In this representative experiment, the MIC₅₀ of Q203 against H37rv was 2.9 nM. Each concentration was tested in triplicates, the experiment was performed two times.

H37Rv	301	SAGSQPDFYMMWTEGLARI 319
CDC1551	301	SAGSQPDFYMMWTEGLARI 319
W4	301	SAGSQPDFYMMWTEGLARI 319
XDR #27	301	SAGSQPDFYMMWTEGLARI 319
XDR #29	301	SAGSQPDFYMMWTEGLARI 319
XDR #31	301	SAGSQPDFYMMWTEGLARI 319
Q203-R #1	301	SAGSQPDFYMMWAEGLARI 319
Q203-R #2	301	SAGSQPDFYMMWAEGLARI 319
Q203-R #3	301	SAGSQPDFYMMWAEGLARI 319
Q203-R #4	301	SAGSQPDFYMMWAEGLARI 319
Q203-R #5	301	SAGSQPDFYMMWAEGLARI 319

Supplementary Figure 7. Polymorphism in *qcrB* **identified by sequencing.** *QcrB* was amplified from various strains of *M. tuberculosis* and sequenced. The polymorphism T313A was identified in 5/5 strains resistant to Q203 (Q203-R #1-5). No polymorphism was identified in the pan-susceptible clinical isolates CDC1551, W4 or in the XDR strains #27, #29 and #31.

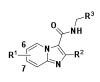
M.tuberculosis	1	MSPKLSPPNIGEVLARQAEDIDTRYHPSAALRRQLNKVFPTHWSFLLGEIALYSFVVLLITGVYLTLFFDP	71
R.sphaeroides	3	GIPHDHYEPRTGIEKWLHSRLPIVALAYDTIM-IPTPRNLNWMWIWGVVLAFCLVLQIVTGIVLAMHYTP	71
P.denitrificans	1	MAGIPHDHYEPKTGFERWLHRRLPIVSLVYDTLM-IPTPKNLNWWIWGIVLAFCLVLQIATGIVLVMHYTP	71
Yeast Bovine	1 1	MA-FRKSNVYLSLVNSYIIDSPQPSSINYWWMGSLLGLCLVIQIVTGIFMAMHYSS	56 57
Human	1	MTNIRKSHPLMKIVNNAFIDLPAPSNISSWWNFGSLLGICLILQILTGLFLAMHYTS MTPMRKTNPLMKLINHSFIDLPTPSNISAWWNFGSLLGACLILQITTGLFLAMHYSP	57
hundit	1	Q _N site	57
M.tuberculosis	72	SMVDVTYNGVYQPLRGVEMSRAYQSALDISFEVRGGLFVRQIHHWAALMFAAAIMVHLARIFFTGAFRRERETNWVIG	149
R.sphaeroides P.denitrificans	72	HVD LAFA SVEH IMRNVNGGFMLRY LHANGA SLFFI AVYLHI FRGLYYG SYKAPREVTWI VG	132
Yeast	72 57	NVD LAFA SVEH IMRD VNGGYMLRY LHAN GA SLFFLAVYI HI FRGLYYG SYKAPREV TWI VG NIE LAFS SVEH IMRD VHNGY I LRY LHAN GA SFFFMVMFMHMAKGLYYG SYRS PRVT LWNVG	132 117
Bovine	58	DTTTAFSSVENIMADVINGTIERTEIMAGASFFFMVMFMMARGETIGSTRSFRVTEMVG	116
Human	58	DASTAFSSIAHITRDVNYGWIIRYLHANGASMFFICLFLHIGRGLYYGSFLYSETWNIG	116
M.tuberculosis R.sphaeroides P.denitrificans Yeast Bovine Human		SLLLILAMFEGYFGYSLPDDLLSGLGLRAALSSITLGMPVIGTWLHWALFGGDFPGTILIPRLYALHILLLPGIILAL MLIYLAMMATAFMGYVLPWGQMSFWGATV-ITGLFGAIPGIGHSIQTWLLGGPAVDNATLNRFFSLH-YLLPFVIAAL MLIYLMMMGTAFMGYVLPWGQMSFWGATV-ITGLFGAIPGVGEAIQTWLLGGPAVDNPTLNRFFSLH-YLLPFVIAAL VIIFILTIATAFLGYCCVYGQMSHWGATV-ITNLFSAIPFVGNDIVSWLWGGFSVSNPTIQRFFALH-YLVPFIIAAM VILLLTVMATAFMGYVLPWGQMSFWGATV-ITNLLSAIPYIGTNLVEWIWGGFSVDKATLTRFFAFH-FILPFIIMAI IILLLATMATAFMGYVLPWGQMSFWGATV-ITNLLSAIPYIGTDLVQWIWGGYSVDSPTLTRFFTFH-FILPFIIAAL QP site	227 208 208 193 192 192
M.tuberculosis R.sphaeroides P.denitrificans Yeast Bovine Human	228 209 209 194 193 193	IGLHLALVWFQKHTQFPGPGRTEHNVVGVRVMPVFAFKSGAFFAAIVGVLGLMGGLLQINPIWNLGPYKPS VAIHIWAFHSTGNNNPTGVEVRRTSKAEAQKD TVPFWPYFIIKDVFALAVVLLVFFAIVGFMPNYLGHPDNYIEANPL VVVHIWAFHTTGNNNETGVEVRRGSKEEAKKDTLPFWPYFVIKDLFALAVVLVVFFAIVGFMPNYLGHPDNYIEANPL VIMHLMALHIHGSSNPLGITGNLDRIPMHSYFIFKDLVVFVFAIVGFMPNYLGHPDNYIPGNPL AMVHLLFLHETGSNNPTGISSDVDKIPFHPYYTIKDILGALLLILALFVFYSPNTLGHPDNYTPANPL ATLHLLFLHETGSNNPLGITSNSDKITFHPYYTIKDILGALLLILALMULVFAPDLLGDPDNYTPANPL QN site	298 286 286 263 262 262
M.tuberculosis R.sphaeroides P.denitrificans Yeast Bovine Human		QVSAGSQPDFYMMNTEGLARIWPPWEFYFWHHTIPAPVWVAVIMGLVFVLLPAYPFLEKRFTGDYAHHNLLQ STPAHIVPEWYFLEFYAILRAFTADVWVVQIANFISFGIIDAKFFGVLAMFGAILVMALVPWLDTSPVRSGRYRPFK VTPAHIVPEWYFLEFYAILRAFTADVWVVMLVNWLSFGIIDAKFFGVLAMFGAILVMALVPWLDTSRVRSGQYRPLFK VTPASIVPEWYLLFFYAILRS	370 364 364 323 322 322
	071		
M.tuberculosis R.sphaeroides	371 365	RPRDVFVRTAIGAMAIAF-YMVLTLAAMNDIIALKFHISLNATTWIGRIGMVILPPFVYFITYRWCIGLQRSD IYFWLLAADFVILTWVGAQQTTFPYDWISLIASAYWFAYFLVILPILGAIEKPVAPPATIEEDFNA	442 430
P.denitrificans	365	WWFWLLAVDFVVLMWVGAMPAEGIYPYIALAGSAYWFAYFLIILPLLGIIEKPDAMPQTIEEDFNAHYGPETHP	438
Yeast	324	FFFFIFVFNFVLLGQIGACHVEVPYVLMGQIATFIYFAYFLIIVPVISTIENVLFYIGRVNK	385
Bovine	323	CLFWALVADLLTLTWIGGQPVEHPYITIGQLASVLYFLLILVLMPTAGTIENKLLKW	379
Human	323	SLYWLLAADLLILTWIGGQPVSYPFTIIGQVASVLYFTTILILMPTISLIENKMLKWA	380
M.tuberculosis P.smbaercides	443	RSVLEHGVETGIIKRLPHGAYIELHQPLGPVDEHGHPIPLQYQGAPLPKRMNKLGSAGSPGSGSFLFADSAAEDAALR	520
R.sphaeroides P.denitrificans	439	AEHHHHHHHH	450
Yeast			
Bovine			
Human			
M. tuberculosis	521	EAGHAAEQRALAALREHQDSIMGSPDGEH 549	
R.sphaeroides P.denitrificans			
Yeast			
Bovine			
Human			

Supplementary Figure 8. Multiple sequence alignment of cytochrome b subunit. The wild type sequence of *Mycobacterium tuberculosis* Rv2196 was aligned to four cytochrome b proteins whose crystal structures have been solved. *Rhodobacter sphaeroides* (*R. sphaeroides*), *Paracoccus denitrificans* (*P. denitrificans*), *Saccharomyces cerevisiae* (Yeast), and *Bos taurus* (Bovine). Human cytochrome b protein sequence (NCBI accession code: P00156) was also aligned. Q_N and Q_P sites are annotated by boxes colored in light purple and pink, respectively. Residues aligned to Thr313 are depicted in a red box. Red residues are conserved and blue indicates less conserved ones.

Supplementary Table 1. Activity of IPA01 against 9 clinical isolates of *M. tuberculosis*. The inhibitory activity of IPA01 was tested in dose-response against *M. tuberculosis* replicating in culture broth medium. Each concentration was tested in duplicate, the assays were repeated at least once.

assa	assays were repeated at least once.					
#	INH	Rif	Strept	EMB	MIC₉₀ (μM)	
33	R	R	S	S	2.0	
48	R	R	R	R	3.1	
61	R	R	R	R	1.9	
80	R	R	R	R	1.6	
125	R	R	R	S	1.3	
137	R	R	R	R	6.9	
143	R	R	R	R	1.3	
146	R	R	R	R	9.2	
171	S	S	S	S	1.2	

INH : isoniazid, Rif : rifampin, Strept : streptomycin, EMB : ethambutol Supplementary Table 2. Activity of IPA derivatives against *M. tuberculosis* H37Rv. The inhibitory activity of the IPA derivatives was tested in dose-response against *M. tuberculosis* replicating in culture broth medium. Each concentration was tested in duplicate, the assay was repeated at least two times.



#	R ¹	R ²	R ³	MIC ₅₀ (μM)
IPA01	н	Me	2	2.03
IPA02	н	Me	, is the second se	2.63
IPA03	н	Et		0.013
Q-203	6-CI	Et	A CONSTRUCTION OF	0.0027

Supplementary Table 3. Activity of Q203 against a bacterial and micro-organisms panel.

Bacteria	MIC
	(μM)
Mycobacterium bovis BCG	0.0035
Mycobacterium smegmatis	>20
Mycobacterium marinum	3.5
Mycobacterium avium	>28
Mycobacterium terrae	7.1
Mycobacterium intracellulare	>28
Mycobacterium nonchromogenicum	14
Mycobacterium xenopi	>28
Acinetobacter baumannii CIP107292	>100
Acinetobacter baumannii CIP5377	>100
Acinetobacter baumannii SAN008	>100
MR Staphylococcus aureus 0706C0025	>100
MR Staphylococcus aureus clinical isolate	>100
Escherichia coli ATCC25922	>100
Enterobacter aerogenes 0705A0867	>100
Enterobacter cloacae clinical strain	>100
Klebsiella oxytoca clinical strain	>100
Salmonella enteridis clinical strain	>100
Enterococcus faecium clinical strain	>100
Enterococcus faecalis clinical strain	>100
Pseudomonas aeruginosa ATCC27853	>100
Pseudomonas aeruginosa clinical strain	>100
Pseudomonas aeruginosa clinical strain	>100
Corynebacterium striatum clinical strain	>100
Candida albicans ATCC66396	>100
Bacillus subtilis CIP 5262	>100
Saccharomyces cerevisiae clinical strain	>100

Supplementary Table 4. Activity of Q203 against MDR and XDR *M. tuberculosis* clinical isolates. The strains were classified as belonging to the Beijing family (Beijing) or non-Beijing family (-).

Туре	#	Family	INH	Rif	Strept	Oflox	MIC ₉₀
							(nM)
	4	Beijing	R	R	R	S	0.43
	5	Beijing	R	R	S	R	< 0.43
	6	Beijing	R	R	S	R	< 0.43
	7	Beijing	R	R	S	R	< 0.43
	8	-	R	R	S	R	< 0.43
MDR	9	Beijing	R	R	R	S	< 0.43
MDK	10	Beijing	R	R	S	S	< 0.43
	11	Beijing	R	R	S	R	< 0.43
	12	Beijing	R	R	S	R	0.98
	13	Beijing	R	R	S	S	< 0.43
	14	Beijing	R	R	S	S	0.88
	15	Beijing	R	R	S	R	3.51
	16	Beijing	R	R	R	S	< 0.43
	17	Beijing	R	R	R	R	0.43
	18	Beijing	R	R	R	R	< 0.43
	19	-	R	R	R	R	< 0.43
	20	-	R	R	R	R	< 0.43
	21	Beijing	R	R	R	R	< 0.43
	22	-	R	R	R	R	< 0.43
XDR	23	Beijing	R	R	R	R	< 0.43
ADK	24	Beijing	R	R	R	R	< 0.43
	25	Beijing	R	R	R	R	< 0.43
-	26	-	R	R	R	R	< 0.43
	27	Beijing	R	R	R	R	7.02
	28	-	R	R	R	R	< 0.43
	29	-	R	R	R	R	7.02
	30	Beijing	R	R	R	R	< 0.43
	31	Beijing	R	R	R	R	28

INH: isoniazid, Rif: rifampin; Strep: streptomycin, Oflox: ofloxacin

		+S9 fraction	>60 μM (Cyclophosphamide active at 5 μg ml ⁻¹)
O	Micronucleus formation		>60 uM
		-S9 fraction	1
Genotoxicity			(mitomycin C active at 0.3 µg ml ⁻¹)
	Mini-Ames assay		>50 µM
	(TA98 and TA100 bacteria)		(2-Aminoanthracene active at 0.4 mg ml ⁻¹ ,
	,		sodium azide active at 0.2 μg ml ⁻¹)
		Human	10.3
	Microsomal stability	Rat	3.07
	(Clint, μL min ⁻¹ mg ⁻¹)	Mouse	4.96
Metabolic stability		Dog	2.35
		Human	95.5 %
	Cryopreserved hepatocytes	Monkey	89.9 %
	(% recovery after 4h)	Dog	90.9 %
		Rat	96.7 %
	P- glycoprotein substrate/inhi	ibitor	>25 μM
	(IC ₅₀)		(verapamil: 3.22 μM)
	hPXR activation		Negative at 0.1,1 and 10 μ M
			(Rifampin: 12.8 fold induction at 10 µM)
	CYP inhibition (IC ₅₀)	1A2	>10 μM
			(a-naphtoflavone: 0.02 μM)
Drever Drever instance stiller		2C9	>10 µM
Drug-Drug interaction			(sulfaphenazone: 0.50 μM)
			>10 µM
		2C19	((+)-N-benzynirvanol: 0.46 μM)
			>10 µM
		2D6	(quinidine: 0.05 μM)
			>10 µM
	3A4		(ketoconazole: 0.02 μM)
Cardiotoxicity	hERG patch clamp (IC ₅₀)		>30 μM
Gardiotoxicity			(Amitriptyline: 1.90 μM)

Supplementary Table 5. In vitro pharmacokinetic and toxicity of Q203

Supplementary Table 6. Pharmacokinetic parameters of Q203 in mice after intravenous (IV) and oral (PO) administration

		IV	PO
Dose	mg kg ⁻¹	2	10
Cmax	ng ml ⁻¹	387	1,490
Tmax	h	-	2.0
Vd	L Kg ⁻¹	5.27	-
Cl	mL min ⁻¹ kg ⁻¹	4.03	-
T _{1/2}	h	16.5	23.4
AUC _{0-last}	ng.h ml ⁻¹	7,280	33,000
AUC_{0-*} ¥	ng.h ml ⁻¹	8,280	44,100
MRT _{0-last}	h	15.0	17.8
MRT _{0-*}	h	21.8	33.9
F	%	-	90.7

Supplementary Table 7. Mean Lung/Plasma ratio after oral dosing in mice

Sampling time (h)	Mean		S.D.
6.00	2.67	Ħ	0.39
12.00	2.11	H	0.70
24.00	3.11	Ħ	0.55
48.00	2.61	Ħ	1.12