SUPPLEMENTARY INFORMATION

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

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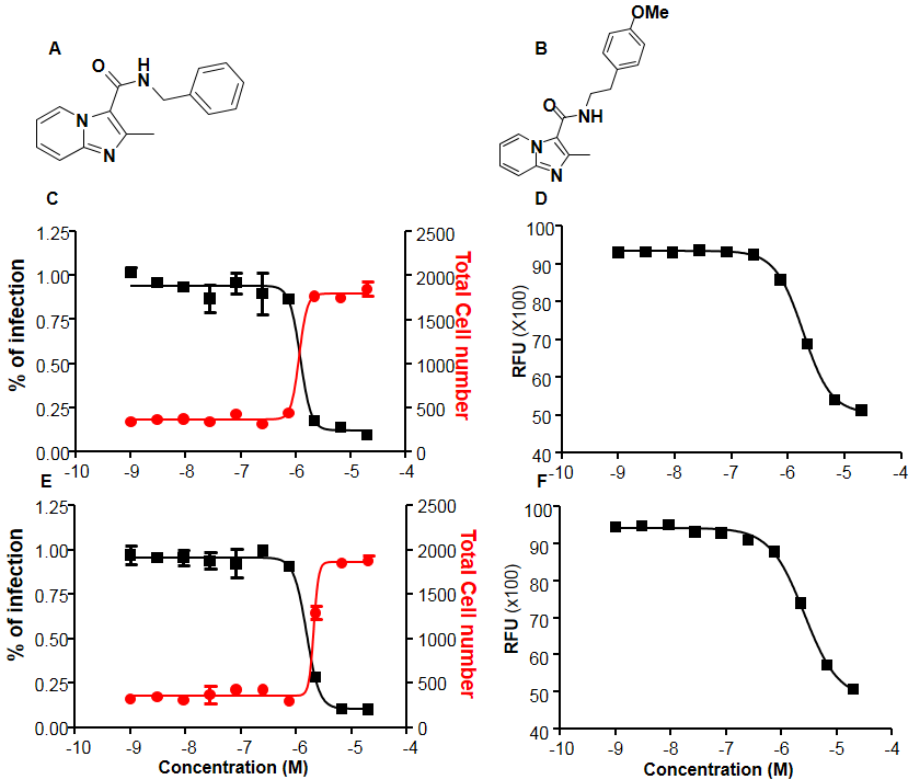
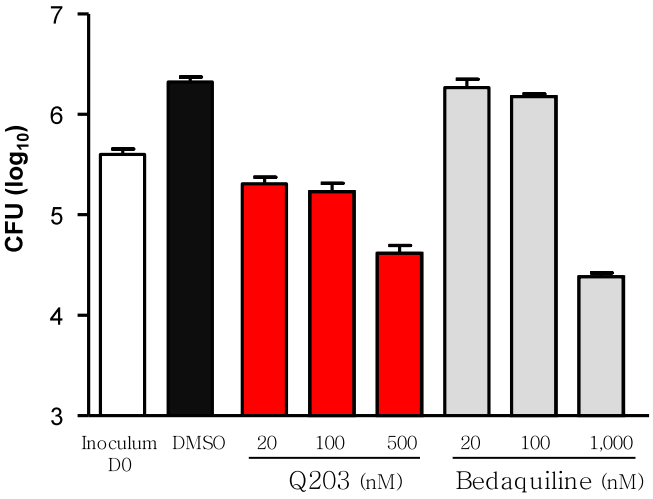
Supplementary Figures 1-8

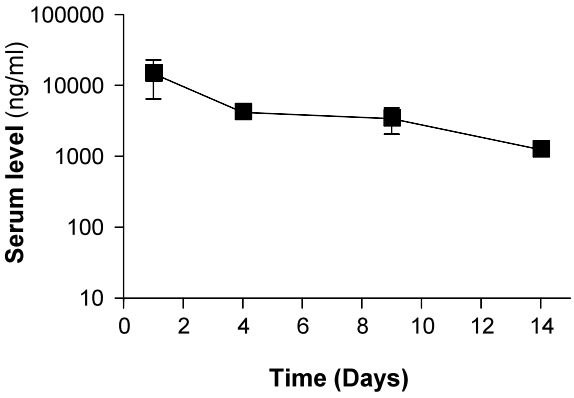
Supplementary Tables 1-7



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 MIC_{50} of IPA01 and IPA02 replicating inside macrophages was 1.25 μM and 1.57 μM, respectively. The MIC_{50} of IPA01 and IPA02 replicating in culture broth medium was 1.86 μM and 2.63 μM, 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.

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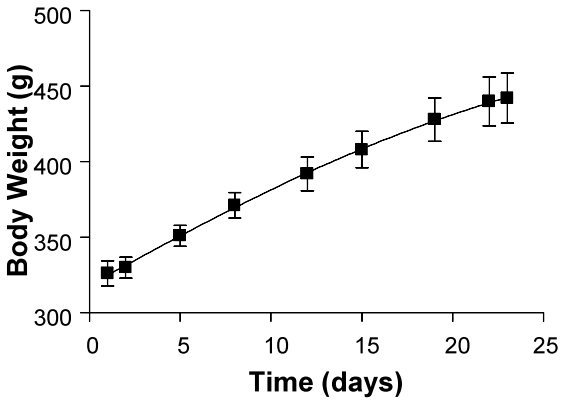
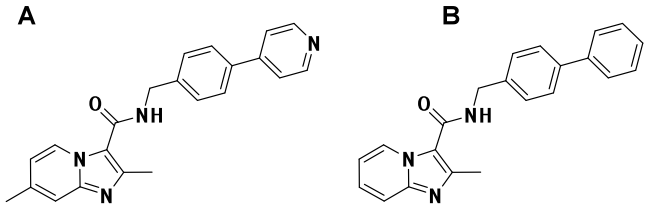
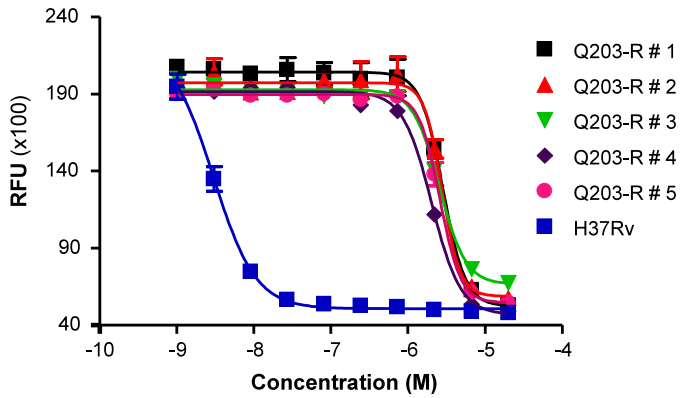


Supplementary Figure 4. Progression of the body weight of rats during a long-term administration study.
10 mg/kg of Q203 was administered to male SD rats for 20 days. Weight variation was monitored throughout the study and up to 3 days after the last administration. Each point represents the mean and standard variation of the body weight of five animals.

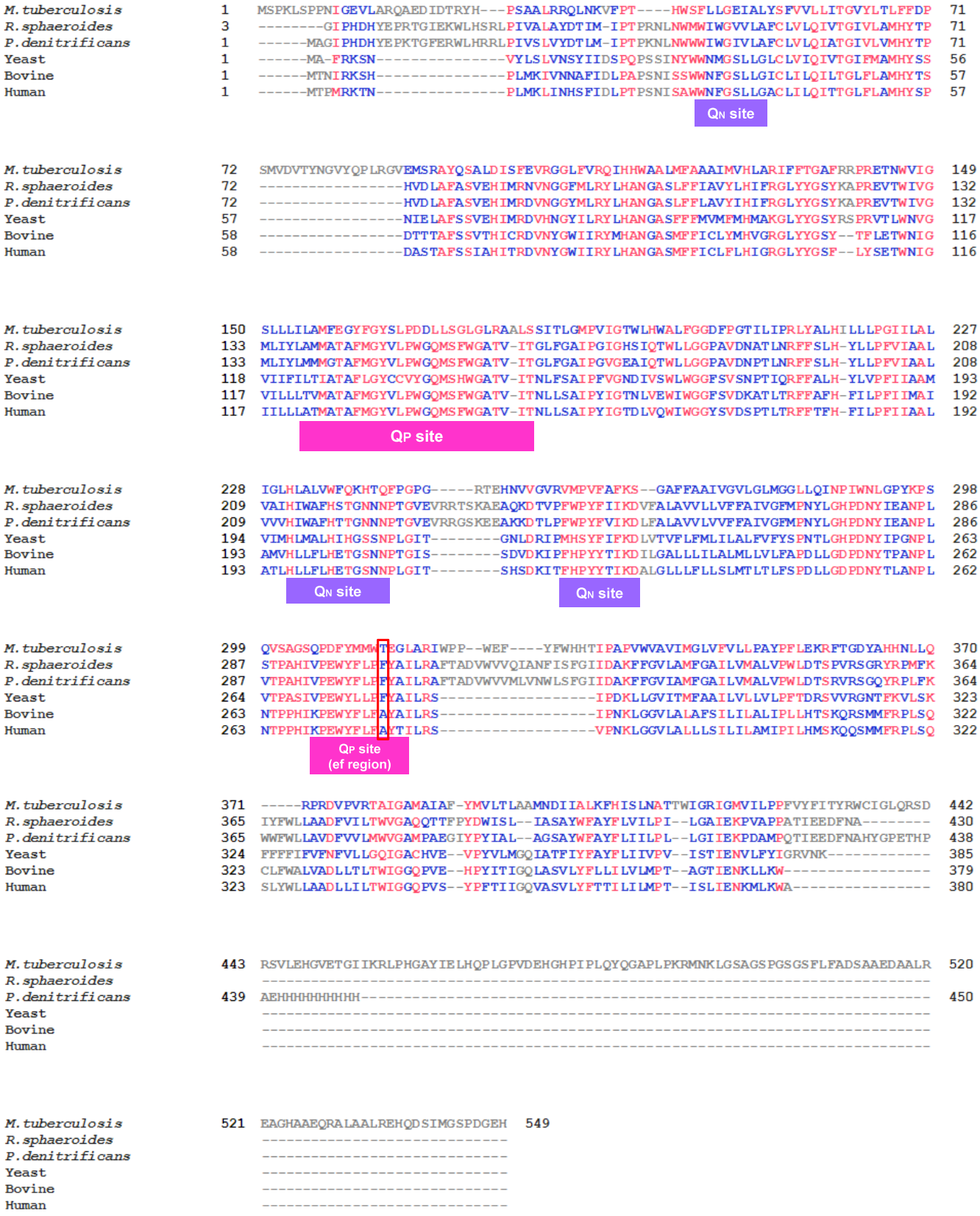


Supplementary Figure 5. Structure of IPA04 and IPA05
The MIC_{50} of IPA04 (A) and IPA05 (B) against *M. tuberculosis* replicating in culture broth medium was of 10 nM and 5 nM, respectively.



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 μM. In this representative experiment, the MIC_{50} of Q203 against H37rv was 2.9 nM. Each concentration was tested in triplicates, the experiment was performed two times.

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.



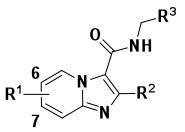
Supplementary Figure 8. Multiple sequence alignment of cytochrome b subunit. The wild type sequence of *Mycobacterium tuberculosis* Rv2196 was aligned to four cytochrome b proteins whose crystal structures have been solved. *Rhodobacter sphaeroides* (*R. sphaeroides*), *Paracoccus denitrificans* (*P. 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.

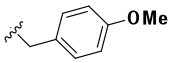
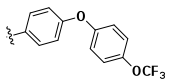
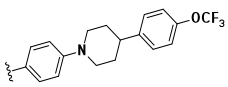
Supplementary Table 1. Activity of IPA01 against 9 clinical isolates of *M. tuberculosis.* The inhibitory activity of IPA01 was tested in dose-response against *M. tuberculosis* replicating in culture broth medium. Each concentration was tested in duplicate, the assays were repeated at least once.

#	INH	Rif	Strept	EMB	MIC_{90} (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	H	Me		2.03
IPA02	H	Me		2.63
IPA03	H	Et		0.013
Q-203	6-Cl	Et		0.0027

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

Bacteria	MIC_{90} (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 (-).

Type	#	Family	INH	Rif	Strept	Oflox	MIC_{90} (nM)
MDR	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
	9	Beijing	R	R	R	S	<0.43
	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
XDR	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
	23	Beijing	R	R	R	R	<0.43
	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

Supplementary Table 5. ***In vitro*** **pharmacokinetic and toxicity of Q203**

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

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
$AUC_{0\text{-last}}$	ng.h ml^{-1}	7,280	33,000
$AUC_{0\text{-}\infty}$ ¥	ng.h ml^{-1}	8,280	44,100
$MRT_{0\text{-last}}$	h	15.0	17.8
$MRT_{0\text{-}\infty}$	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	±	0.39
12.00	2.11	±	0.70
24.00	3.11	±	0.55
48.00	2.61	±	1.12