Supplementary Tables and Figures

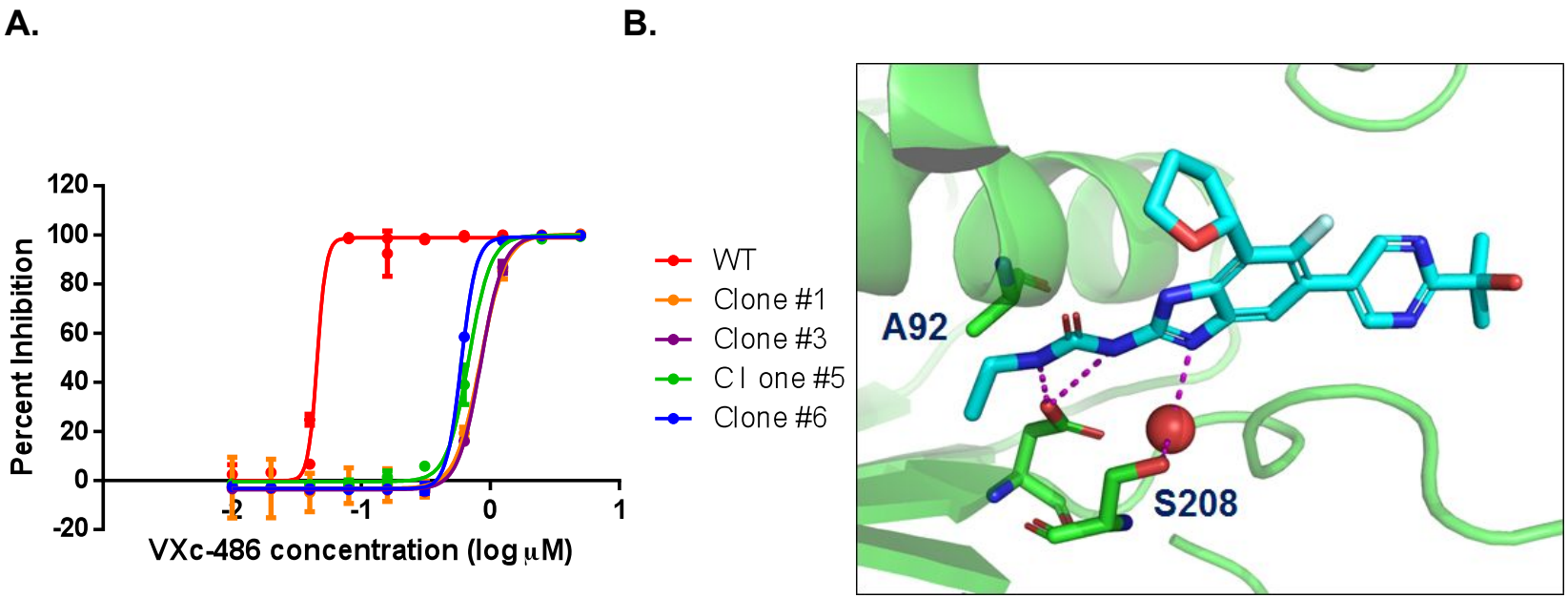

Figure S1. Mtb (H37Ra isolate) genetic variants generated after selection with VXc-486 at 2x and 4x minimal inhibitory concentration (MIC). A. Four *gyrB* variant clones were selected, three of which contained an A92S substitution (GCG – TCG) and one (clone 6) contained the S208A substitution, (TCA-GCA). The MIC potencies were evaluated in triplicate and the mean values with standard error are shown. B. A model of VXc-486 in complex with Mtb gyrase B illustrating the

location of the two sites of mutation relative to the bound inhibitor. Ser 208 is located in proximity of the urea of VXc-486. The S208A substitution alters the hydrogen bonding network in the complex and destabilizes the catalytic water (represented by the red sphere). The model was generated by docking of VXc-486 into the published crystal structure of Mtb gyrase B (PDB code: 3zkb) followed by energy minimization with rigid protein and flexible ligand. Docking was performed with ICM v. 3.7 (Molsoft, LLC, San Diego, CA, USA). Energy minimization was performed with Macromodel v. 10.0 (Schrodinger, Inc., New York, NY).

TABLE S1. Partial sequencing of drug resistant *Mycobacterium tuberculosis* clinical isolates revealed specific genetic mutations associated with drug resistance.

Sample	rpoB1	katG1	tlyA	embB	rrs	rrs500	inhA-promoter	gyrA	pncA1
TT135	531TTG	315ACC	WT	306ATC	1401G	513CAC	-15	90GTG	14CGC
TT149	516TAC	315ACC	WT	WT	1401G	WT	WT	94GCC	WT
X_3	531TTG	315ACC	11CTG	36ATC	141G	WT	-15	94GGC	14CGC
X_27	531TTG	WT	11CTG	306GTC	1400 region	WT	-15	90GTG	Del8G
X_60	531TTG	WT	11CTG	36GTC	141G	WT	WT	94GGC	13TAG
X_61	531TTG	WT	11CTG	WT	141G	WT	-15	94GGC	97GAT
X_131	531TT	315ACC	11CTG	497CGG	1400 region	WT	WT	94GGC	130GCG, 85CCG

WT: wild type.

Table S2. Synergistic and additive interactions of anti-mycobacterial compounds tested in combination with VXc-486 in *Mycobacterium tuberculosis* (H37Ra) broth cultures or in cultures of a *M. tuberculosis*-infected human macrophage-like cell line (THP-1 cells).

Compound	MIC (µg/ml)	FIC	Mtb THP-1 Cells (IC_{50})	FIC
Isoniazid	0.21	0.75	0.01	0.75
Rifampin	0.03	0.63	0.01	2.00
Ethambutol	2.40	0.63	1.30	2.00
Amikacin	0.94	2.00	0.26	2.00
Moxifloxacin	0.16	1.00	0.12	1.00
Linezolid	0.53	0.50	0.36	2.00
Bedaquiline	0.05	1.00	0.03	0.50
PA-824	0.14	2.00	0.05	1.00
Clofazimine	0.73	2.00	0.18	0.50

Minimal inhibitory concentrations (MICs) were determined at 90% inhibition by two-fold serial dilution of compounds after 11 days of bacterial culture. A reporter strain of Mtb expressing firefly luciferase was used to infect THP-1 cells and

measure bacterial growth a 50% inhibitory concentration at five days post-infection. VXc-486 had an MIC of 0.07 µg/ml and an IC_{50} of 0.01 in Mtb-infected THP-1 cells. The fractional inhibitory concentration (FIC) was determined for combinations of two compounds as a synergistic interaction (< 0.5), additive interaction (0.5-2.0) or antagonistic interaction (>2.0).

Table S3. Minimal inhibitory concentrations (MIC, μg/ml) of VXc-486, Clarithromycin (CLR), and moxifloxacin (MXF) against clinical isolates of *Mycobacterium abscessus.*

Organism	**VXc-486**	**CLR**	**MXF**
BB2	1	0.25	8
6153	1	0.5	4
6025	0.5	0.5	8
6005	1	0.125	8
5908	0.5	0.5	4
6142	8	0.25	8
5931	0.03	0.25	0.06
5605	2	0.125	8
5901	0.5	0.015	1
5812	1	0.25	4
5960	4	>8	0.5
BB1	4	0.125	>8
BB3	0.5	0.03	0.5
5922	2	0.5	2
BB4	1	>8	4
BB5	1	1	8
BB6	1	>8	2
BB7	2	0.25	2
BB8	1	0.06	4
6111	2	0.5	8
6126	1	0.25	>8
LT949	2	0.125	4
MIC_{50}	**1**	**0.25**	**4**
MIC_{90}	**4**	**>8**	**>8**

Table S4. Minimal inhibitory concentrations (MIC, µg/ml) of VXc-486 and moxifloxacin (MXF) against *Mycobacterium kansasii*

Isolate	VXc-486	MXF
0008	0.03	0.06
262	0.03	0.06
2242	0.06	0.06
4302	0.03	0.125
5075	0.06	0.125
5983	2	2
1673	0.06	0.06
1701	0.06	0.06
5076	0.06	0.125
2610	0.06	0.06
ATCC 35755	0.03	0.06
1295	0.06	0.06
0009	0.015	0.06
ATCC 12478	0.015	0.03
0164	0.06	0.06
W5219	0.25	0.5
PIC	0.06	0.06
RSL	0.03	2
379	0.03	0.03
258	0.03	0.06
SHP	0.03	0.03
399	0.06	0.25
MIC_{50}	**0.06**	**0.06**
MIC_{90}	**0.06**	**0.5**

Table S5. Minimal inhibitory concentrations (MIC, μg/ml) of VXc-486 and clarithromycin (CLR) against *Mycobacterium avium* and *Nocardia* spp.

Organism	VXc-486	CLR
M. avium 103	0.23	120
M. avium Far	0.23	≤ 0.125
M. avium 3404.4	0.23	≤ 0.125
Nocardia caviae 2497	0.125	0.5, 0.5
N. asteroids 2039	1	≤ 0.008
N. nova 10	1	≤ 0.008

Table S6. Mean pharmacokinetic parameters in plasma from female BALB/c mice following single oral administration of pVXc-486 at nominal doses.

pVXc-486 (mg/kg)	Analyte	AUC $_{(0\text{-}t)}$ (µg*hr/ml)	C_{max} (µg/ml)	$t_{1/2}$ (hr)	$AUC_{(0\text{-}inf)}$ µg*hr/ml
3	VXc-486	0.508	1.01	1.10	0.517
	pVXc-486	BQL	BQL	BQL	BQL
10	VXc-486	4.16	4.01	2.12	4.18
	pVXc-486	BQL	BQL	BQL	BQL
30	VXc-486	20.8	13.3	6.53	20.8
	pVXc-486	BQL	BQL	BQL	BQL
100	VXc-486	72.4	26.7	7.80	72.5

The pVXc-486 was rapidly converted to VXc-486 and pVXc-486 was below the limit of detection in each plasma sample (BQL).

Table S7. pVXc-486 improves the potency of a second-line regimen in a mouse model of tuberculosis.

Regimen*	Mean lung log_{10} CFU count (± SD) after treatment for:				Proportion (%) of mice with positive lung cultures 3 months after treatment for:	
	2 months	4 months	5 months	6 months	5 months	7 months
2MEZA/5ME	3.68 ± 0.29	1.79 ± 0.22	1.51 ± 0.36	0.59 ± 0.31	15 of 15 (100%)	14 of 15 (93%)
2MPZA/5MP	2.33 ± 0.35	1.06 ± 0.21	1.35 ± 0.33	0.31 ± 0.20	15 of 15 (100%)	15 of 15 (100%)
2MEZP/5MEP	2.81 ± 0.15	1.48 ± 0.27	1.59 ± 0.91	0.92 ±0 .22	15 of 15 (100%)	15 of 15 (100%)

**Definition of abbreviations: M, moxifloxacin*; E, ethambutol; Z, pyrazinamide; A, amikacin; P, VXc-486 prodrug. Drugs were administered orally, 5 days per week, at the following doses: M 100 mg/kg; E 100 mg/kg; Z 150 mg/kg; P 100 mg/kg.

A was administered subcutaneously at 30 mg/kg. Z and A were administered for the first 2 months only. The early control mean lung log10 CFU count was 4.68±0.12, while the late control was 7.90±0.17.