Supplemental Material for

Characterization of DprE1-mediated benzothiazinone resistance in *Mycobacterium tuberculosis*

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SUPPLEMENTARY METHODS

Determination of PBTZ169 and BDQ interaction in H37Rv and C387N mutant. Drug interactions between PBTZ169 and BDQ were determined using the checkerboard assay [1, 2]. *M. tuberculosis* strains were grown in 7H9 medium to log- phase (OD_{600} 0.4 - 0.8) and diluted to an OD_{600} of 0.0001. 75 µl of bacterial suspension (2.25 x 10^3 cells) was added per well of a 96-well plate. The first compound was added to the first column and two-fold serial dilutions were made column-wise. The second compound was prepared in 7H9 at a concentration 8x MIC and serial dilutions were made to 0.125x MIC. 25 µl of diluted compound at each concentration was added to a row of the 96-well plate. Various combinations of PBTZ169 and BDQ were thus obtained in a final volume of 100 µl per well. Plates were incubated for 6 days at 37°C, 10 µl of 0.025% w/v resazurin was added to each well. The fluorescence intensity was read after 24 h incubation using an Infinite F200 Tecan plate reader.

For rows where an MIC value could be determined, the fractional inhibitory concentration index (ΣFICI) was calculated using the equation ΣFIC index = FIC_{PBTZ} + FIC_{BDQ} = (MIC of PBTZ, tested in combination)/(MIC of PBTZ, alone) + (MIC of BDQ, tested in combination)/(MIC of BDQ, alone). $\Sigma FIC \leq 0.5$ is considered as synergism, $0.5 < \Sigma FIC \leq 4$ additivity, and $\Sigma FIC > 4$ antagonism.

SUPPLEMENTARY FIGURES

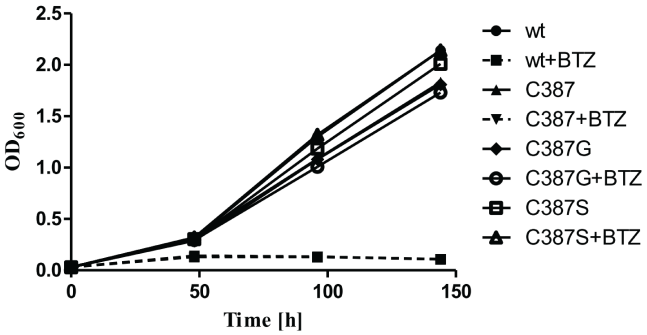

FIG S1 Growth comparison of H37Rv and mutant strains in liquid medium with or without BTZ043. M. tuberculosis H37Rv (wt) was grown with (+BTZ) or without 400 ng/ml BTZ043. Wild-type growth was compared to the merodiploid strain harbouring the cysteine codon (C387), which behaves like the wild-type strain, and the glycine or serine mutants (C387G and C387S), which are both fully resistant to BTZ. The C387A, C387N and C387T mutants behaved like the other mutants (not shown in the graph).

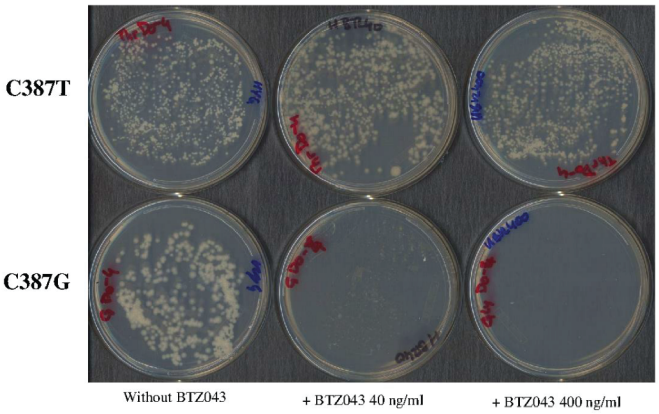

FIG S2 Growth comparison between C387T and C387G mutant strains on solid media with or without BTZ043. The mutants DprE1^{C387G} and DprE1^{C387T} were plated with or without 40 or 400 ng/ml BTZ on the same day. The picture was taken after 21 days of incubation at 37°C. The C387G mutant CFUs on BTZ-containing plates were hardly detectable after 3 weeks of incubation but were countable after 6 weeks.

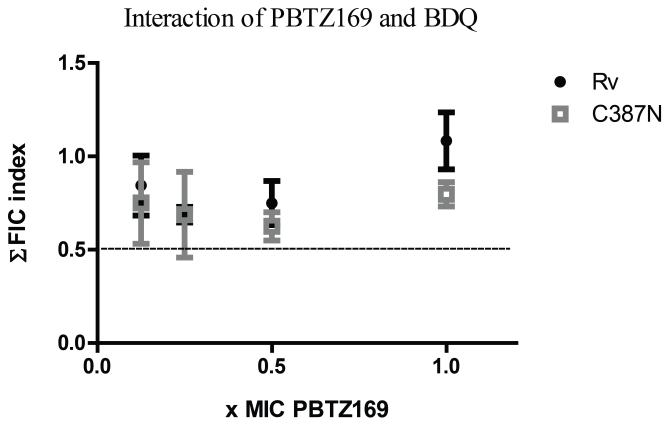

FIG S3 Interactions of PBTZ169 and BDQ in H37Rv and C387N mutant. The sum of fractional inhibitory concentration (FIC) of PBTZ169 and of BDQ were calculated with the equation ΣFIC index = $FIC_{PBTZ169}$ + FIC_{BDQ} = (MIC of PBTZ169, tested in combination)/(MIC of PBTZ169, alone) + (MIC of BDQ, tested in combination)/(MIC of BDQ, alone). ΣFIC indices were calculated for drug concentrations where an MIC could be determined using a checkerboard assay with REMA as a viability marker. Black circles represent H37Rv, grey squares represent C387N mutant strain. The dotted line indicates the threshold for synergism. Data from two independent experiments are presented as mean ± SD.

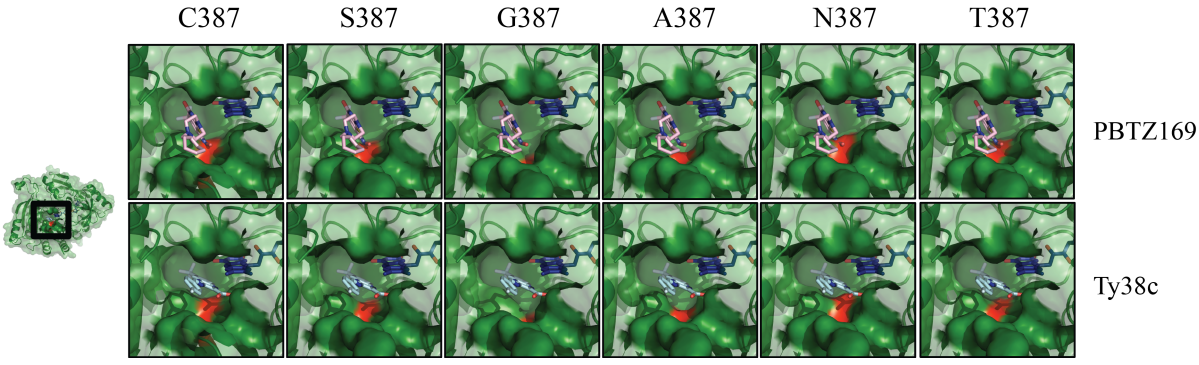

FIG S4 Models of binding of PBTZ169 and Ty38c in the binding pocket of the five DprE1 mutants. The 387 position of DprE1 is represented in red, FAD in blue. PBTZ169 and Ty38c are shown docked into the binding pocket of DprE1 WT and mutant crystal structures. Both compounds are able to bind to the 5 mutants.

TABLE S1 Primers used in this study

Primer name	Sequence
Mut90_fwd	CCATCCCGGGCTGGAACATCNNNGTCGACTTCCCCATCAAGGACG[§]
Mut90_rev	CGTCCTTGATGGGGAAGTCGACNNNGATGTTCCAGCCCGGGATGG
90C387G_fwd	CCATCCCGGGCTGGAACATCGGCGTCGACTTCCCCATCAAGGACG
90C387G_rev	CGTCCTTGATGGGGAAGTCGACGCCGATGTTCCAGCCCGGGATGG
Rv3790_fwd	AATACTCC*ATGG*CCATCCTGACGGATGGCCTTGCAGCCCACTAGGTCAGGC*
Rv3790_rev	AATGCA*AGTAC*TCTACAGCAGCTCCAAGCGTC*
JN C387S _fwd	GGCTGGAACATCAGCGTCGACTTCCCC
JN C387S _rev	CCGACCTTGTAGTCGCAGCTGAAGGGG
JN C387G _fwd	GGCTGGAACATCGGCGTCGACTTCCCC
JN C387G _rev	CCGACCTTGTAGCCGCAGCTGAAGGGG
Rv3790c387G_For	gccgctcagcttccccatcccgggctggaacatcggcgtcgacttccccatcaaggacgggctggggaag
Rv3790c387G_Rev	cttccccagcccgtccttgatggggaagtcgacgccgatgttccagcccgggatggggaagctgagcggc
Rv3790c387S_For	gccgctcagcttccccatcccgggctggaacatctccgtcgacttccccatcaaggacgggctggggaag
Rv3790c387S_Rev	cttccccagcccgtccttgatggggaagtcgacggagatgttccagcccgggatggggaagctgagcggc
Rv3790c387A_For	gccgctcagcttccccatcccgggctggaacatcgccgtcgacttccccatcaaggacgggctggggaag
Rv3790c387A_Rev	cttccccagcccgtccttgatggggaagtcgacggcgatgttccagcccgggatggggaagctgagcggc
Rv3790c387T_For	gccgctcagcttccccatcccgggctggaacatcaccgtcgacttccccatcaaggacgggctggggaag
Rv3790c387T_Rev	cttccccagcccgtccttgatggggaagtcgacggtgatgttccagcccgggatggggaagctgagcggc
Rv3790c387N_For	gccgctcagcttccccatcccgggctggaacatcaatgtcgacttccccatcaaggacgggctggggaag
Rv3790c387N_Rev	cttccccagcccgtccttgatggggaagtcgacattgatgttccagcccgggatggggaagctgagcggc
Rv3790C387N_F	CGGGCTGGAACATCAACGTCGACTTCCCCATC
Rv3790C387N_R	GATGGGGAAGTCGACGTTGATGTTCCAGCCCG
Rv3790C387A_F	CGGGCTGGAACATCGCCGTCGACTTCCCCATC
Rv3790C387A_R	GATGGGGAAGTCGACGGCGATGTTCCAGCCCG
Rv3790C387T_F	CGGGCTGGAACATCACCGTCGACTTCCCCATC
Rv3790C387T_R	GATGGGGAAGTCGACGGTGATGTTCCAGCCCG

[*] Restriction sites are indicated in italics in the primer sequence

[§] NNN: random bases used for C387 mutagenesis

TABLE S2 Plasmids used in this study

Name	Description and features	Resistance marker	References
pND255	L5-integrative vector	Hyg^R	N. Dhar, unpublished
pBL2	*wild-type promoter –rv3789-dprE1* cloned into pCR®– Blunt II – TOPO® Vector (Invitrogen)	Kan^R	this study
pET28a-dprE1	Expression vector for DprE1 protein production	Kan^R, Cam^R	[3]
pND255-derivative plasmid			
pBLX1-6	Pools of vectors carrying randomly mutated *dprE1* and expressed under the natural promoter located upstream of *rv3789*	Hyg^R	this study
pBLG	Mutated *dprE1(C387G)* and expressed under the natural promoter located upstream of *rv3789*	Hyg^R	this study

TABLE S3 Strains used in this study

Strain	**Plasmid**	**Genotype**	**Reference**
H37Rv	-	Wild-type	[4]
H37Rv::*dprE1* (C387X)	pBLX1-6*	wt promoter-rv3789- dprE1(C387X) at the L5-attB site	this study
H37Rv::*dprE1* (C387G)	pBLG	wt promoter-rv3789- dprE1(C387G) at the L5-attB site	this study
H37Rv/pJV53	pJV53	Expression of Che9c gene products 60 and 61 to facilitate double-stranded DNA recombination in mycobacteria	[5]
H37RvΔRD1	-	Deletion of RD1 region of H37Rv	[6]
dprE1 (C387S)	-	H37Rv strain carrying C387S mutation in *dprE1*	this study
dprE1 (C387A)	-	H37Rv strain carrying C387A mutation in *dprE1*	this study
dprE1 (C387T)	-	H37Rv strain carrying C387T mutation in *dprE1*	this study
dprE1 (C387G)	-	H37Rv strain carrying C387G mutation in *dprE1*	this study
dprE1 (C387N)	-	H37Rv strain carrying C387N mutation in *dprE1*	this study

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