Supplementary Information

Potent Inhibitors of Mycobacterium Tuberculosis Growth Identified by Using In-Cell NMR-based screening

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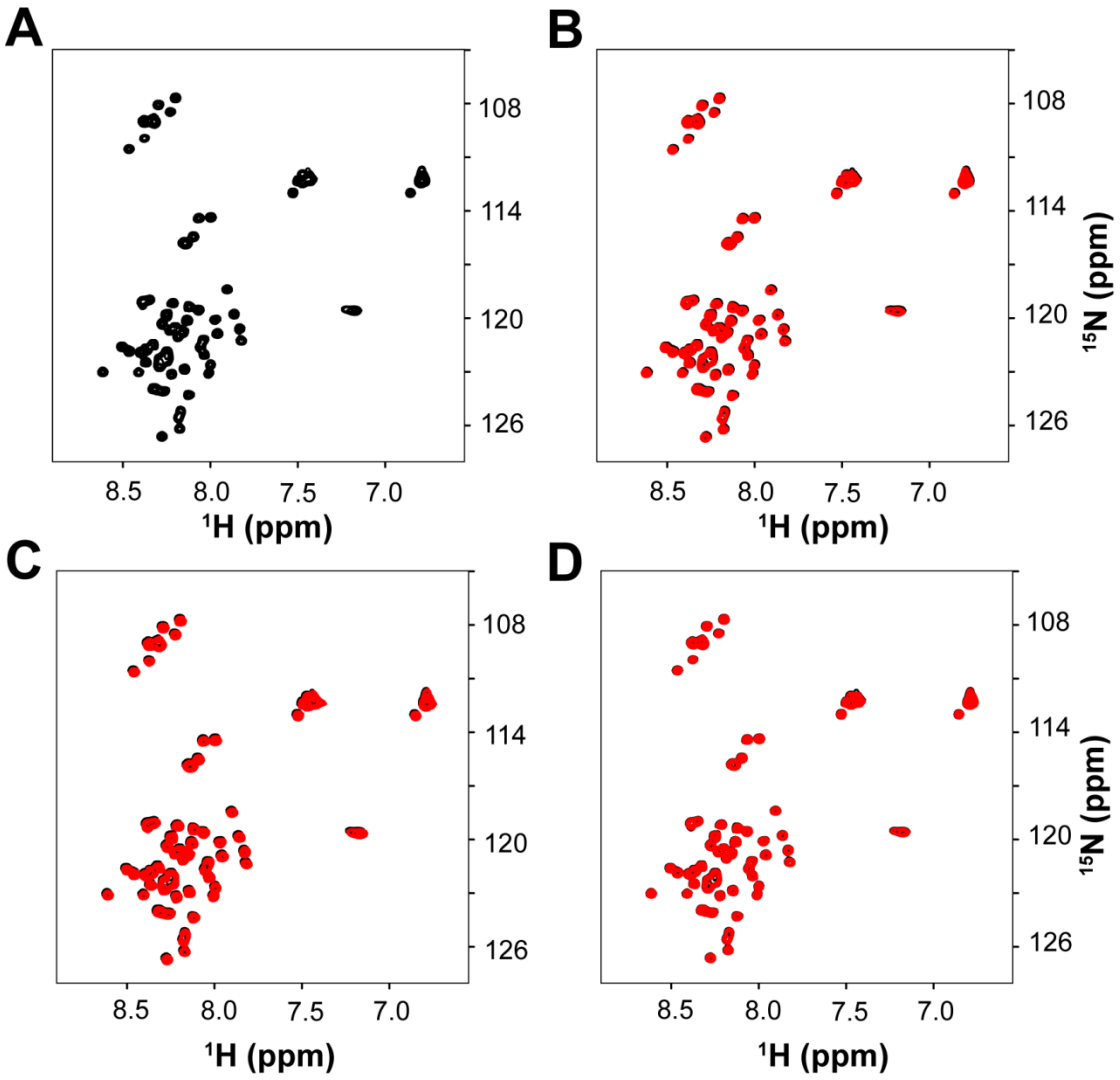
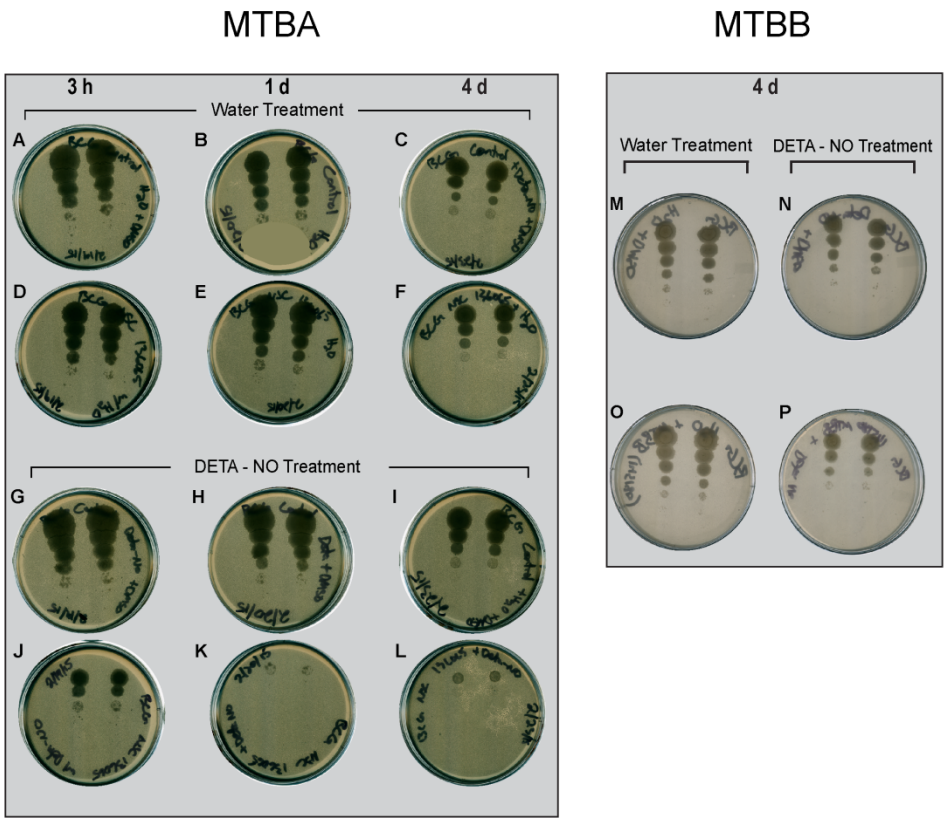


Supplemental Figure 1. Pup-Mpa complex is required to bind candidate compounds. **(A)** *In vitro* ^{1}H-^{15}N HSQC spectrum of Pup. **(B, C, D)** *In vitro* ^{1}H-^{15}N HSQC spectra of Pup with compounds MTBA (B), MTBB (C), and MTBC (D). Pup concentration was 50 µM in 10 mM potassium phosphate buffer, pH 7.2; MTBA, MTBB and MTBC were at 100 µM. All baseline spectra contained 1.0% DMSO.



Supplemental Figure 2. Compounds MTBA and MTBB require nitric oxide stress to kill BCG cells. Control cells at 3 h **(A)**, 1 d **(B)**, 4 d **(C)** post-treatment. Control +100 μM MTBA at 3 h **(D)**, 1 d **(E)**, 4 d **(F)**. DETA-NO Control at 3 h **(G)**, 1 d **(H)**, 4 d **(I)**. DETA-NO +100 μM MTBA at 3 h **(J)**, 1 d **(K)**, 4 d **(L)**. Control cells at 4 d **(M)**. DETA-NO Control at 4 d **(N)**. Control +100 μM MTBB at 4 d **(O)** DETA-NO +100 μM MTBB at 4 d **(P)**. DETA-NO was at 250 μM; MTBA and MTBB were at 100 μM.

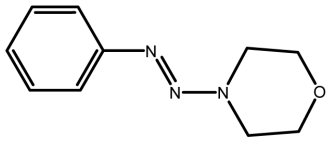
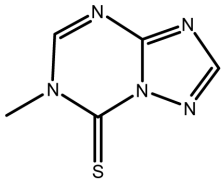
A B

Supplemental Figure 3. MTBC maintains BCG growth inhibition 8 days post-treatment. **(A)** Control treated with 250 μM DETA-NO, and DMSO (0.5% v/v). **(B)** BCG treated with 100 μM MTBC.

Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	In-cell NMR
	Target	Pup and Mpa interaction
	Primary measurement	^{1}H-^{15}N HSQC
	Key reagents	*E. coli* with 2 h [*U*-^{15}N]-Pup and 8 h Mpa over-expression, matrix of compounds in DMSO (neat), NMR buffer (pH 6.5), 99.8% D_2O
	Assay protocol	DOI:10.1021/jm9000743 10.1002/0471140864.ps1711s61
	Additional comments	
Library	Library size	1597
	Library composition	NIH/NCI Diversity Set III
	Source	NCI Developmental Therapeutics Program (DTP)
	Additional comments	Library is not *Mtb* specific
Screen	Format	96-well format
	Concentration(s) tested	50 μM
	Plate controls	4% and 5% DMSO
	Detection instrument and software	500 MHz Bruker NMR, Topspin 3.1 (Bruker), CARA
	Assay validation/QC	Inspection of ^{15}N-Pup chemical shift changes due to overexpression of Mpa
	Normalization	Singular Value Decomposition (SVD)
	Additional comments	
Post-HTS analysis	Hit criteria	Viability of BCG under NO-treatment
	Hit rate	0.20%
	Additional assay(s)	*In vitro* and *in vivo* functional studies
	Confirmation of hit purity and structure	NMR spectroscopy
	Additional comments	

Supplemental Table 2. Compounds that advanced to *in vivo* screening.

Compound Name	NSC Number	Shothand Name	Chemical Structure
(E)-4-(phenyldiazenyl)morpholine	136065	MTBA	
6-methyl-[1,2,4]triazolo[1,5-a][1,3,5] triazine-7(6H)-thione	145180	MTBB	
4-(benzo[d]thiazol-2-yl)benzene-1,3-diol	33005	MTBC