

# Supporting Information

## Inhibition of CorA dependent Magnesium Homeostasis is critical in *Mycobacterium tuberculosis*.

Yumi Park<sup>a\*</sup>, Yong-Mo Ahn<sup>a\*</sup>, Surendranadha Jonnala<sup>a</sup>, Sangmi Oh<sup>a</sup>, Julia M. Fisher<sup>a</sup>, Michael B. Goodwin<sup>a</sup>, Thomas R. Ioerger<sup>b</sup>, Laura E. Via<sup>a</sup>, Tracy Bayliss<sup>c</sup>, Simon R. Green<sup>c</sup>, Peter C. Ray<sup>c</sup>, Paul G. Wyatt<sup>c</sup>, Clifton E. Barry 3<sup>rd</sup><sup>a</sup>, Helena I. Boshoff<sup>a#</sup>

<sup>a</sup>Tuberculosis Research Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland, USA

<sup>b</sup>Department of Computer Science and Engineering, Texas A&M University, College Station, Texas, USA

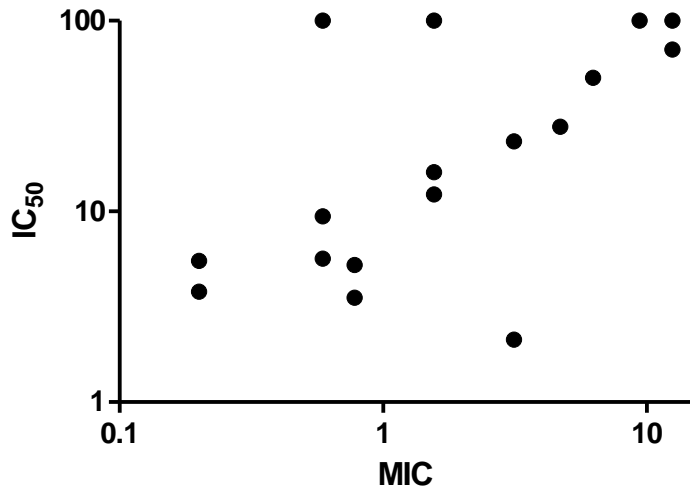
<sup>c</sup>Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK

\*Present addresses: Yumi Park, Center for Personalized Precision Medicine of TB, Inje University College of Medicine, Busan, Republic of Korea; Yong-Mo Ahn, Department of Pharmacology, Physiology & Neuroscience, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, New Jersey, USA

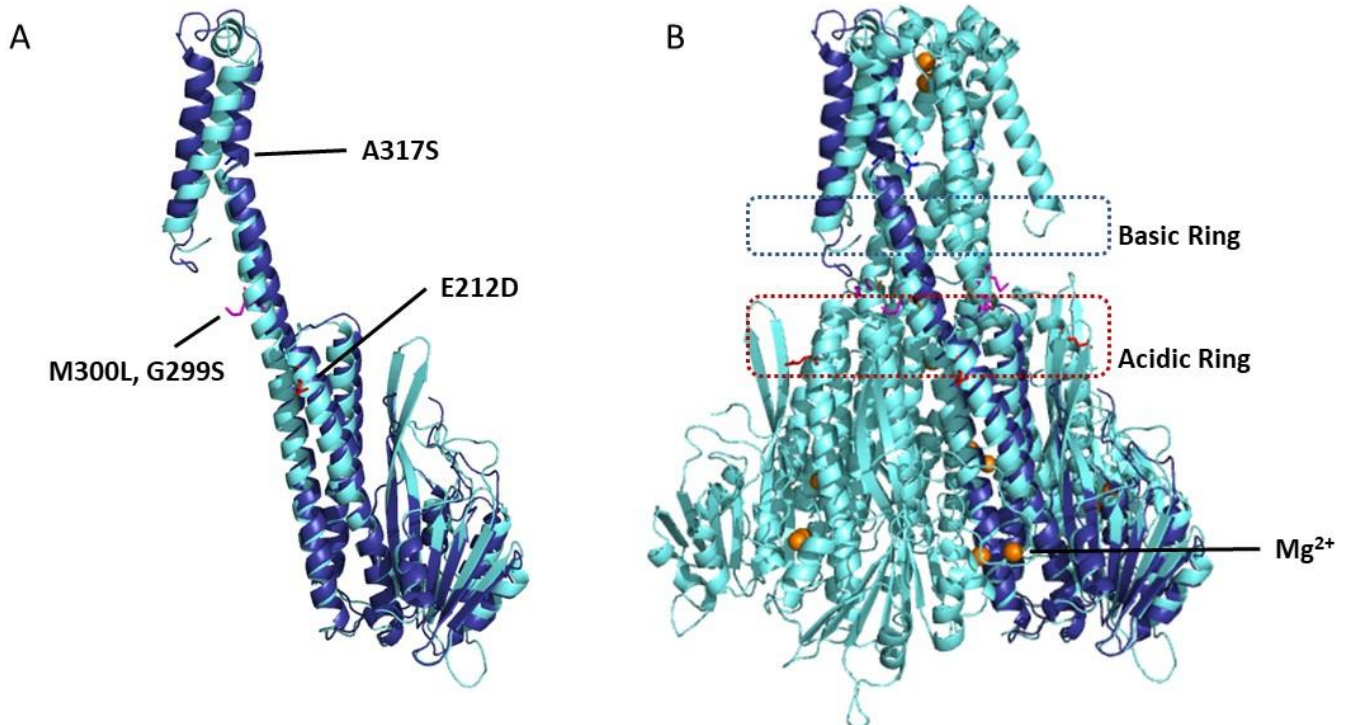
#Corresponding author, <sup>c</sup>contributed equally

[hboshoff@niaid.nih.gov](mailto:hboshoff@niaid.nih.gov)

## Supplementary Figures

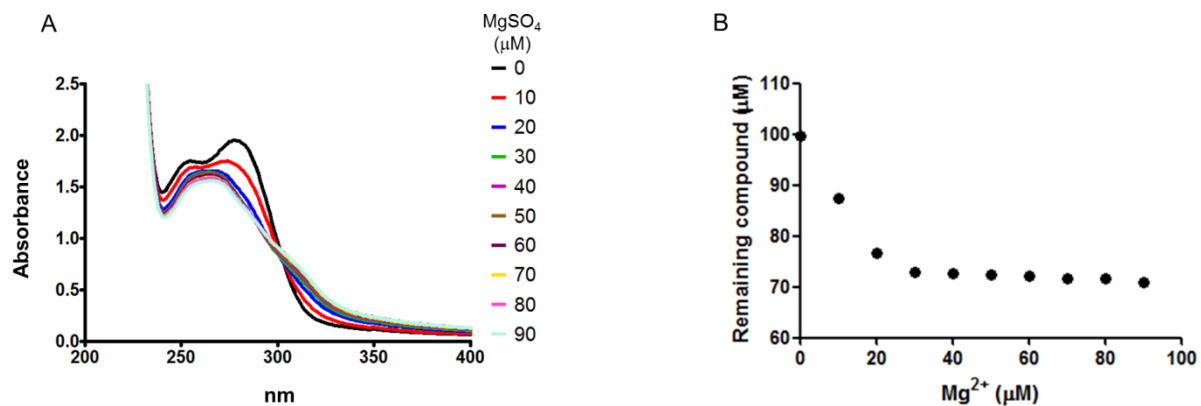


**Figure S1.** The *Mtb* MIC of the pyrimidinetrione amides tracks with HepG2 mitochondrial toxicity with a few notable exceptions. The mitochondrial toxicity against HepG2 (in  $\mu\text{M}$ ) observed during growth on galactose as carbon source was compared to the MIC against *Mtb* H37Rv. Data from Table 1.

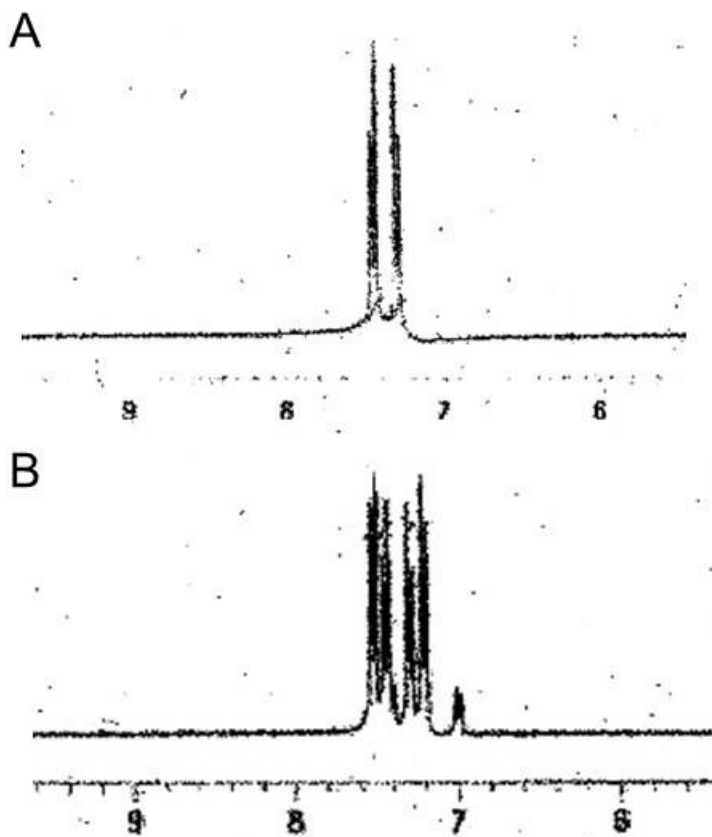


**Figure S2.** MtCorA homology model showing that pyrimidinetrione amide resistance conferring mutations map to regions involved in regulating opening of the Mg<sup>2+</sup> tunnel. The homology model of the MtCorA is based on the *Thermotoga maritana* CorA (TmCorA)

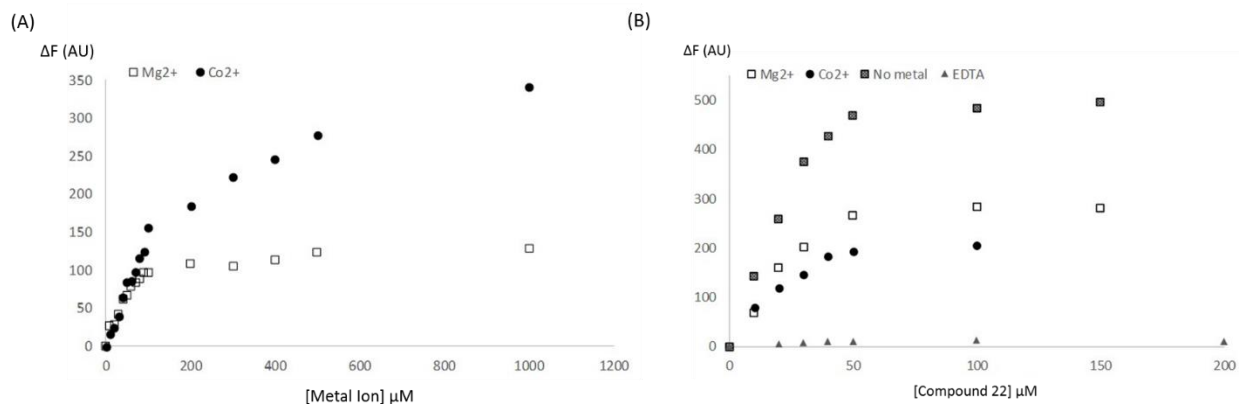
structure. A. Aligned MtCorA homology model (cyan) with TmCorA (dark blue). B. The full-length structure of MtCorA with an aligned monomer of TmCorA (dark blue). The locations of the MtCorA mutated residues were labeled in blue (A317S), magenta (M300L, G299S) and red (E212D). Mg<sup>2+</sup> ions in orange. Region with positively charged residues were in Basic Ring box and with negatively charged residues were in Acidic Ring box.



**Figure S3. The pyrimidinetrione amides directly bind to Mg<sup>2+</sup> ions.** A. UV spectrum of 100 μM compound **10** by serial addition of 10 μM MgSO<sub>4</sub>. B. Reduction of compound **10** by serial addition of 10 μM MgSO<sub>4</sub>.



**Figure S4.** The pyrimidinetrione amide **10** binds  $Mg^{2+}$ . The Aromatic region of  $^1H$ -NMR spectra of compound **10**. (A) No  $Mg^{2+}$  (B) With  $Mg^{2+}$ .



**Figure S5.** CorA binds to compound **10** and the divalent cations  $Mg^{2+}$  and  $Co^{2+}$ . (A) The binding affinity of the ligands,  $Mg^{2+}$  and  $Co^{2+}$ , to CorA was determined using tryptophan fluorescence quenching. Changes in intrinsic fluorescence intensity upon ligand binding was plotted against ligand concentration. (B) The binding affinity of compound **10** to CorA in the presence of 1mM  $Mg^{2+}$ ,  $Co^{2+}$  or without metal ions was determined using tryptophan fluorescence quenching.

## Supplementary Tables

**Table S1:** Compound **10** dependent growth inhibition of Gram-positive bacteria and *Mycobacterium smegmatis* can be rescued by Mg<sup>2+</sup> supplementation.

Organism	MIC ( $\mu$ M) in presence of:		
	-	+ 200 $\mu$ M MgCl <sub>2</sub>	+ 400 $\mu$ M MgCl <sub>2</sub>
<i>M. smegmatis</i>	3.1	ND	>50
<i>B. subtilis</i>	6.2	>50	>50
<i>S. aureus</i>	0.07	3.1	>50

Magnesium concentration in 7H9 and LB is 400 and 200  $\mu$ M, respectively.

**Table S2:** Mutations in *corA* that confer pyrimidinetrione amide resistance in Mtb.

compound	Mutation Frequency	Number of strains sequenced for <i>corA</i>	Fold increase in MIC	SNP in <i>corA</i>
<b>1</b>	8.90E-08	4	>250 fold	E212D, M300V
<b>10</b>	1.99E-08	6	>128 fold	E212D, G299S, M300V
<b>12</b>	2.18E-08	2	>64 fold	E212D
<b>13</b>	3.41E-08	2	8 fold	E212D
<b>15</b>	4.41E-10	2	8 fold	E212D, M300L

**Table S3:** Minimum magnesium concentrations required for growth of wild type and *corA* mutant strains of *Mtb*.

	H37Rv (parental)	<i>corA</i> :E212D	<i>corA</i> :A317S
Minimum Mg <sup>2+</sup> conc. to grow	0.92 μM	0.23 mM	0.23 mM
Fold of change	1	250	250

**Table S4:** Thermostability of CorA E212D in the presence of metal cations, EDTA and compound **10**.

CorA + additive	°C thermal shift
none	60
0.125mM MgCl <sub>2</sub>	75
1mM compound <b>10</b>	≥95
1mM compound <b>10</b> / 0.125mM MgCl <sub>2</sub>	≥95

**Table S5:** The apparent dissociation constant ( $K_d(\text{app})$ ) of CorA E212D with metal cations and compound **10**.

CorA additives in fluorescence shift assay	$K_d(\text{app})$ μM
Mg <sup>2+</sup>	38.9 ± 13.6
Compound <b>10</b>	10.0 ± 0.8
Compound <b>10</b> / 1mM Mg <sup>2+</sup>	52.7 ± 4.1