Supporting Information

for

Application of *Mycobacterium smegmatis* as a surrogate to evaluate drug leads against *Mycobacterium tuberculosis*

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General/ Chemicals and Reagents

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Difco Middlebrook 7H10 agar, Middlebrook 7H9 broth, Tryptic soy agar, Tryptic soy broth, MOPS, tris(hydroxymethyl)aminomethane, 2-mercaptoethanol, sucrose and triton-X 100 were purchased from Sigma-Aldrich. OADC enrichment was purchased from Fisher Scientific. Magnesium chloride and potassium chloride were obtained from Fisher. Resazurin (Alamar blue) was purchased from Sigma-Aldrich.

***M. smegmatis* (ATCC607) cells grown in 0.5% Tween 80 in Middlebrook 7H9 (0.4% glycerol)**

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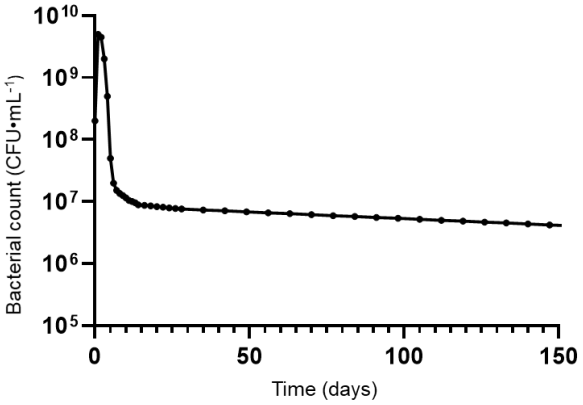


M. smegmatis (ATCC607) cells were grown and CFU were performed over time. Each point represents the average of three independent experiments.

Example of CFU of *M. smegmatis* (ATCC)

CFU: 552 (dilution: 1.6 x10^7)

Determination of Minimum Inhibitory Concentration (MIC).

The inhibitors were dissolved in DMSO or water, to make a final concentration of 1 mg per 100 μL. This concentration was used as the stock solution for all the studies. Stock solution aliquots were stored at 4 °C for the duration of experiments. As described above,

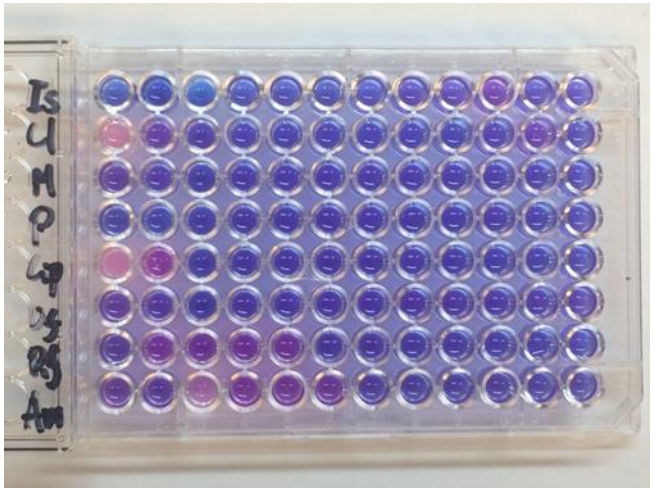
bacterial cultures at 0.5 optical density, was treated with serial dilutions of inhibitors in aerobic conditions and incubated at 37 °C for 4 days. 20 µL of Alamar blue was added and incubated in a static incubator at 37 °C for 4-6 h. The lowest concentration at which the color of alamar blue was completely retained as blue was read as the MIC. (Pink = Growth, Blue = No growth) The absorbance measurements were done using a Biotek Synergy XT, 96 well plate reader at 570 nm and 600 nm.

An MIC experiment against *M. smegmatis* (ATCC607) in Middlebrook 7H9 in the presence of 0.5% tween 80 and 0.4% glycerol

	1	2	3	4	5	6	7	8	9	10	11	12	
	0.012	0.024	0.048	0.097	0.19	0.39	0.78	1.56	3.125	6.25	12.5	25	
A													
B													
C													
D													
E													
F													
G													
H													

A: Isoniazid MIC 0.012 (MIC 0.7 determined previously))
B: Clofazimine MIC 0.024
C: Meropenem MIC 0.024
D: Pyrazinamide MIC <0.012
E: Ciprofloxacin MIC 0.048
F: Ofloxiacin MIC 0.024
G: Rifampicin MIC 0.39 (MIC: 0.79 (determined previously)
H: Amikacin MIC 1.56 (MIC: 0.097 (determined previously)

All experiments were triplicated.



Risazurin 20 uL (4.5 mg/25 mL) at 37 ºC for 6h

MIC experiment against *M. smegmatis* (ATCC607) in Middlebrook 7H9 in ADC enrichment (10%)

Molecule	*M. smegmatis* MIC μg•mL^{-1} [a]	*M. tuberculosis* $H_{37}Rv$ MIC μg•mL^{-1}
Isoniazid (INH)	>25	0.02-0.2[b]
Rifampicin (RIF)	6.25-12.5	0.05-0.1[b]
Ethambutol (EMB)	>25	1.0-5.0[b]
Amikacin (AMK)	0.39	2.0-4.0[b]
Capreomycin (CAP)	3.25	2.0-4.0[b]
Ethionamide	25.0	0.5-2.0[b]
Moxifloxacin	3.2-12.5	0.25-2.0[b]
Ciprofloxacin	3.2-6.25	0.25-2.0[b]

[a] Microplate Alamar (Risazurin) blue assays were applied. All MIC data (24h) were generated in this studies. A similar MIC trend was observed in the MIC studies with Brain Heart Infusion Broth (24h). [b]The MIC data were cited from databases and/or literatures.

Genetic analyses of *M. smegmatis* resistant strains

The target genes, *embB*, *rpoB*, *embB*, *rrs*, *inhA*, *katG*, and *ahpC*, were amplified using the purified genomic DNA as template by PCR using high fidelity DNA polymerase (BioLabs) and gene specific primers listed in Table S1.

Table S1

Primer name	Primer sequence (5'-3')
rpoBfor-99	GTC GTT GCG TCC AGG GTT CTG
rpoBrev+81	GAA GTT GAC GTC TAG CAC G
rpoBfor735	GAA GTT GAC GTC TAG CAC G
rpoBfor1491	GAA CCC GTT CGG CTT CAT CGA G
rpoBfor2192	GCC TGG TCG AAG AGG ACG TG
embBfor-65	GCC ATC GAG GAA GGA TCG ACG
embBfor498	GAT TTC CGG TGA GGA CGC GG
embBfor1047	GCT GCT GTC CCG TGA GGT GCT G
embBfor1625	GAT GTT GCG GCG CAA GCA CAT CG
embBfor2208	GTT CTG GTG GAA CCG GAT TCG
embBRev+37	GAG TCG CGT ATG AAA CCG CAC G
rrsFor-87	GGT GGA TGT TTT TGA TGC CAG
rrsFor586	GAA AAC TCA CAG CTT AAC TGT G
rrsRev+93	GTT TGC TGT GCA CCA AGC CGG
inhAfor-60	CAT GGG CCA CTA GTC AAA AG
inhArev+39	GAA CGA CAG CAG CAG CAA G
katGfor-99	GAG CTG GTG TTG GTC ATG GAC G
katGfor618	GCT TGC CGA CGA ACG ACA CAG
katGfor1312	GAA CGA ATG ATC TGG CAG G
katGrev+79	GTT CGC TGT GAA AGT CAC ACG

A mid-log phase (Optical density – 0.5) was used. The optical density was monitored at 600 nm using a 96 well microplate reader.