2-aminoimidazoles collapse mycobacterial proton motive force and block the electron transport chain

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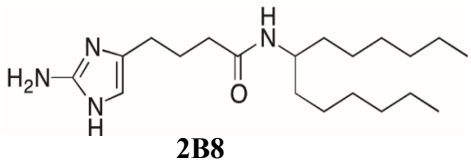
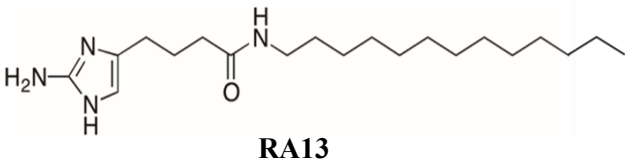
Figure S1.



2B8

Active



RA13

Inactive

Figure S1. Structures of 2-AI compounds.

Figure S2.

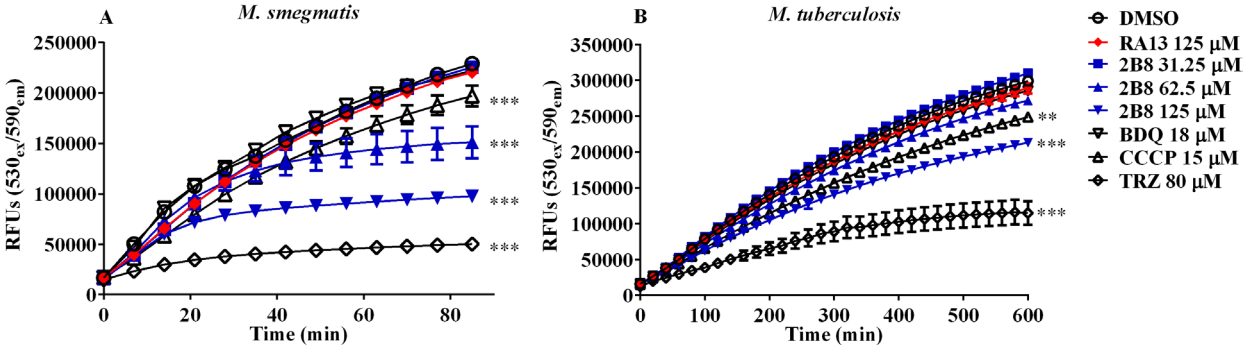


Figure S2. 2B8 inhibits alamarBlue® reduction by mycobacteria. *M. smegmatis* and *M. tuberculosis* were treated with 2-AI compounds or drugs targeting mycobacterial bioenergetics, while being monitored for alamarBlue® reduction immediately following treatment. A) After 80 min, 62.5 and 125 µM 2B8, 15 µM CCCP, and 80 µM TRZ inhibited alamarblue® reduction by *M. smegmatis*. B) AlamarBlue® reduction by *M. tuberculosis* was inhibited with 15 µM CCCP, 80 µM TRZ and 125 µM 2B8 (but not 62.5 µM). For both *M. smegmatis* and *M. tuberculosis*, incubation with 125 µM RA13 and 18 µM BDQ did not have an effect. Experiments were repeated three separate times and representative data are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA.

Table S1. MICs of 2-AI compounds against *M. smegmatis* and *M. tuberculosis* in the presence or absence of albumin.

	M. tuberculosis H37Rv mc^2 6206		*M. smegmatis*	
	+0.5% BSA	No BSA	+0.5% BSA	No BSA
2B8	250-500	250	125-250	62.5
RA13	> 1000	> 1000	> 1000	> 1000

All MIC values are μM.

Table S2: Summary of bioenergetics effects induced by tested compounds.

Read-out	2B8	RA13	CCCP	BDQ	TRZ	KCN
Mechanism of action	Uncoupler & ETC block at unknown site	Negligible	Uncoupler & Proton ionophore	ATP synthase Inhibitor & Uncoupler	NDH-2 inhibitor & Uncoupler	ETC block at cytochrome c oxidase
alamarBlue® reduction	Strong ↓	No effect	Weak ↓	No effect	Strong ↓	N/T
Δψ collapse	Strong	No effect	Strong	No effect	Intermediate	N/T
ΔpH collapse	Strong	Weak	Intermediate	Intermediate	Strong	N/T
O2 consumption rate	↑ then ↓	Weak ↑	↑ then ↓	↑	↑	N/T
[ATP]i	Strong ↓	No effect	Intermediate ↓	Strong ↓	Strong ↓	N/T
ETC activity	Strong ↓	Weak ↓	Weak ↑	No effect	Strong ↓	No effect
NADH/NAD+ ratio	Strong ↑	No effect	No effect	Intermediate ↑	Strong ↑	N/T
NADH oxidation	Strong ↓	No effect	Weak ↑	Intermediate ↓	Strong ↓	Strong ↓
Rescue by CFZ	Partial	N/T	No effect	N/T	No	Yes
ß-lactam potentiation	Strong ↑	Marginal ↑	Intermediate ↑	Weak ↑	Intermediate ↑	N/T

↓= Inhibition
↑= Induction
N/T= Not tested

Table S3. Comparison of the effects induced by a low or high concentration of 2B8.

	Low (≤ 62.5 μM)	High (> 62.5 μM)
Proposed action	Uncoupler	ETC blocker
Acute (≤ 2 h)		
Δψ collapse	Strong	Strong
ΔpH collapse	Low-Strong	Strong
O2 consumption rate	↑	↓ to basal
ETC activity	No effect	↓
NADH oxidation	No effect	↓
[ATP]i	No effect	No effect
NADH/NAD+ ratio	No effect-↑	↑
Delayed (> 2 h)		
alamarBlue® reduction	No effect	↓
[ATP]i	No effect-↓	↓
NADH/NAD+ ratio	No effect-↑	↑

↓= Inhibition
↑= Induction