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Supplemental information

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Supplemental Text and Figures

Resistance of *Mycobacterium tuberculosis* to indole 4-carboxamides occurs through alterations in drug metabolism and alterations in tryptophan biosynthesis

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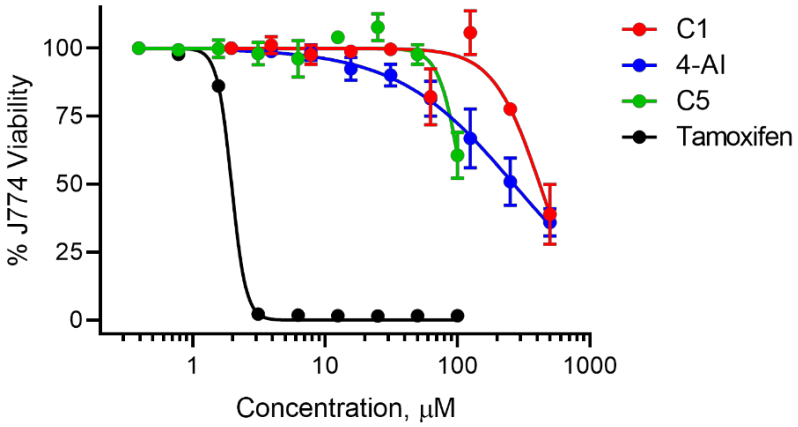


Figure S1. Cytotoxicity of indole-4-carboxamides and 4-aminoindole, related to Figure 1. Cytotoxicity of representative compounds in the study was measured using the CellTitre-Glo® luminescent cell viability assay. 10,000 J774 cells were seeded into wells of a 96-well plate and treated with the indicated compounds for 24 hrs. Data represent Mean ± SEM (n = 4).

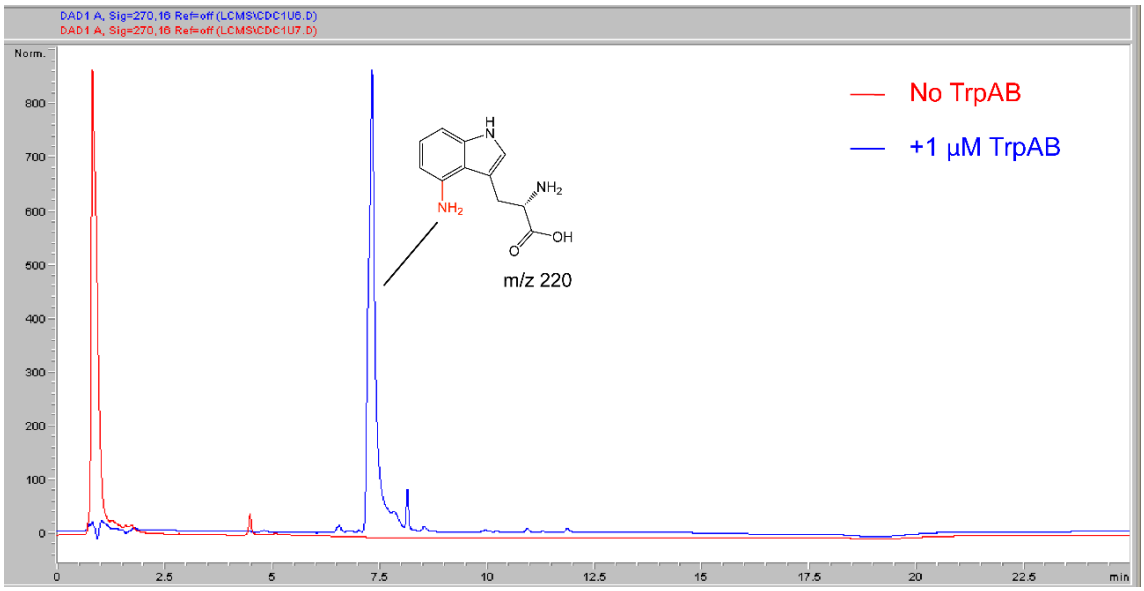


Figure S2. 4-Aminoindole is a substrate for TrpAB, related to Figure 6. 4-AI was incubated with 1µM TrpAB overnight before quenching the reaction with an equal volume of MeOH. A 2 µL aliquot of the reaction mixture was injected into the LC-MS to detect formation of 4-amino-L-Trp. Chromatograms are representative of three individual trials.

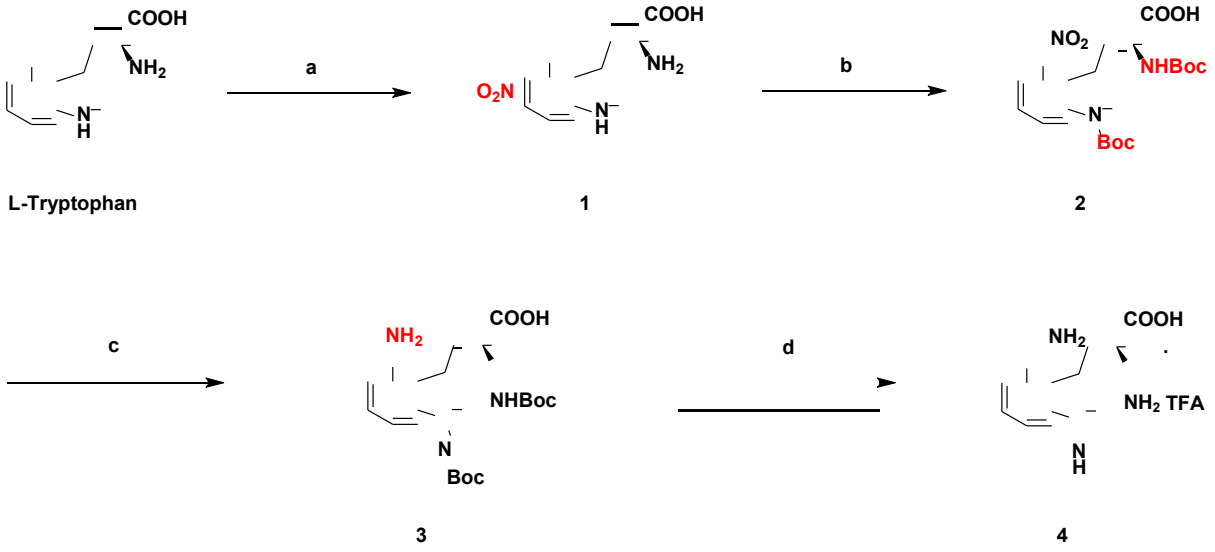


Figure S3. Chemical synthesis of 4-amino-L-Tryptophan, related to Figure 6. Reagents and conditions: (a) 70% HNO_3 (3 eq), AcOH, H_2O, rt, 4 h; (b) Boc anhydride (3 eq), DMAP (3 eq), NEt_3 (4 eq), DCM, rt, overnight; (c) Pd/C, H_2, rt, 3 h; (d) TFA, MC, rt, 2 h.

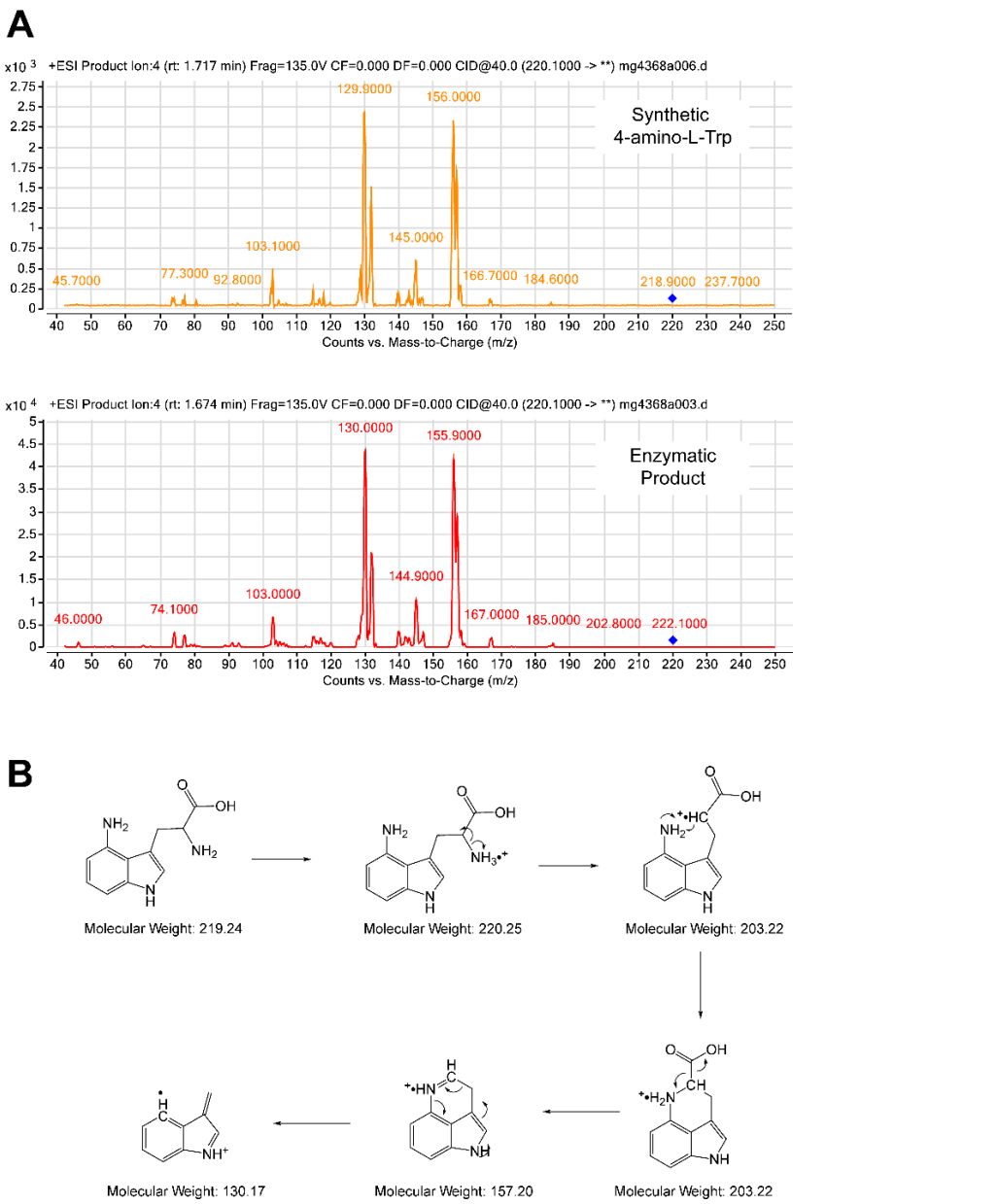


Figure S4. Fragmentation patterns of synthetic 4-amino-L-trp match the enzyme reaction product observed from 4-AI, related to Figure 6 (A) Triple quadrupole tandem MS fragmentation of synthetic 4-amino-L-Trp (top) and the enzymatic product obtained by incubating TrpAB with 4-AI (bottom). (B) Possible mechanism of fragmentation of 4-amino-L-Trp to account for the observed m/z in panel A and in Figure 6B and 6C.

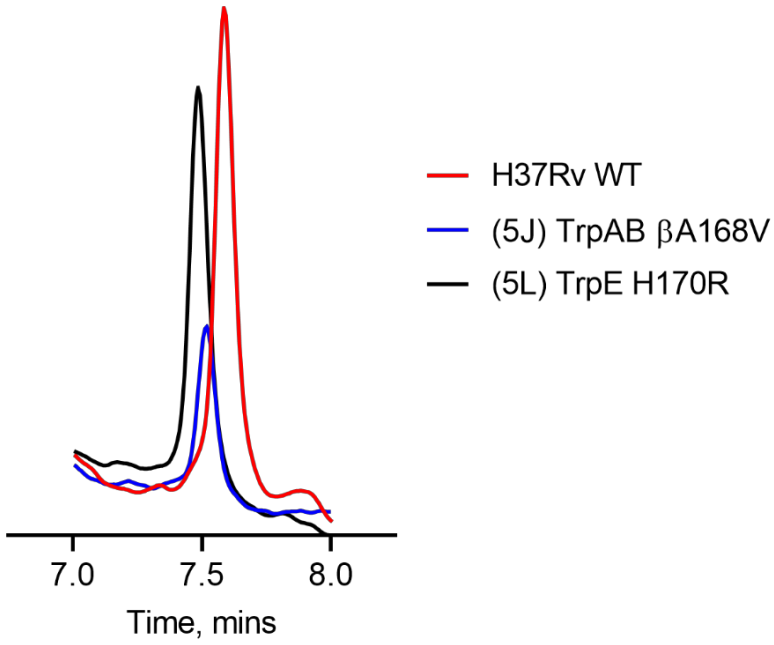


Figure S5. TrpAB mutant is less active than wild type in converting 4-AI to 4-a-Trp, Related to Figure 6. LC-MS chromatograms (270 nm) obtained when 4-AI was incubated with H37Rv WT or representative resistant mutants. The peak represent an m/z of 220.1 accounting for intracellular formation of 4-amino-L-Trp.

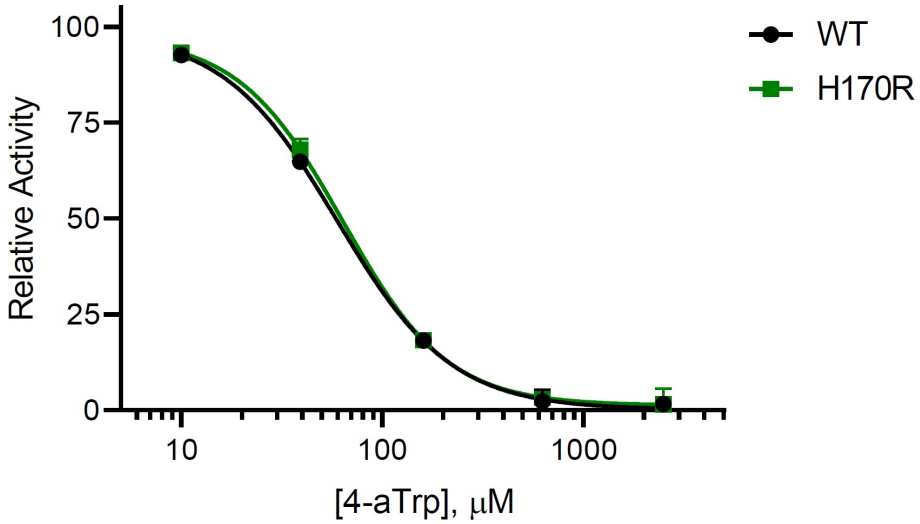


Figure S6. 4-Amino-Trp does not cause feedback inhibition of TrpE, Related to Figure 6. Inhibition of TrpE by 4-AI occurs with a high IC50 value of 60 μM. In addition, the mutant H170R which alleviates allosteric regulation by tryptophan, supporting that 4-aTrp does not inhibit TrpE.

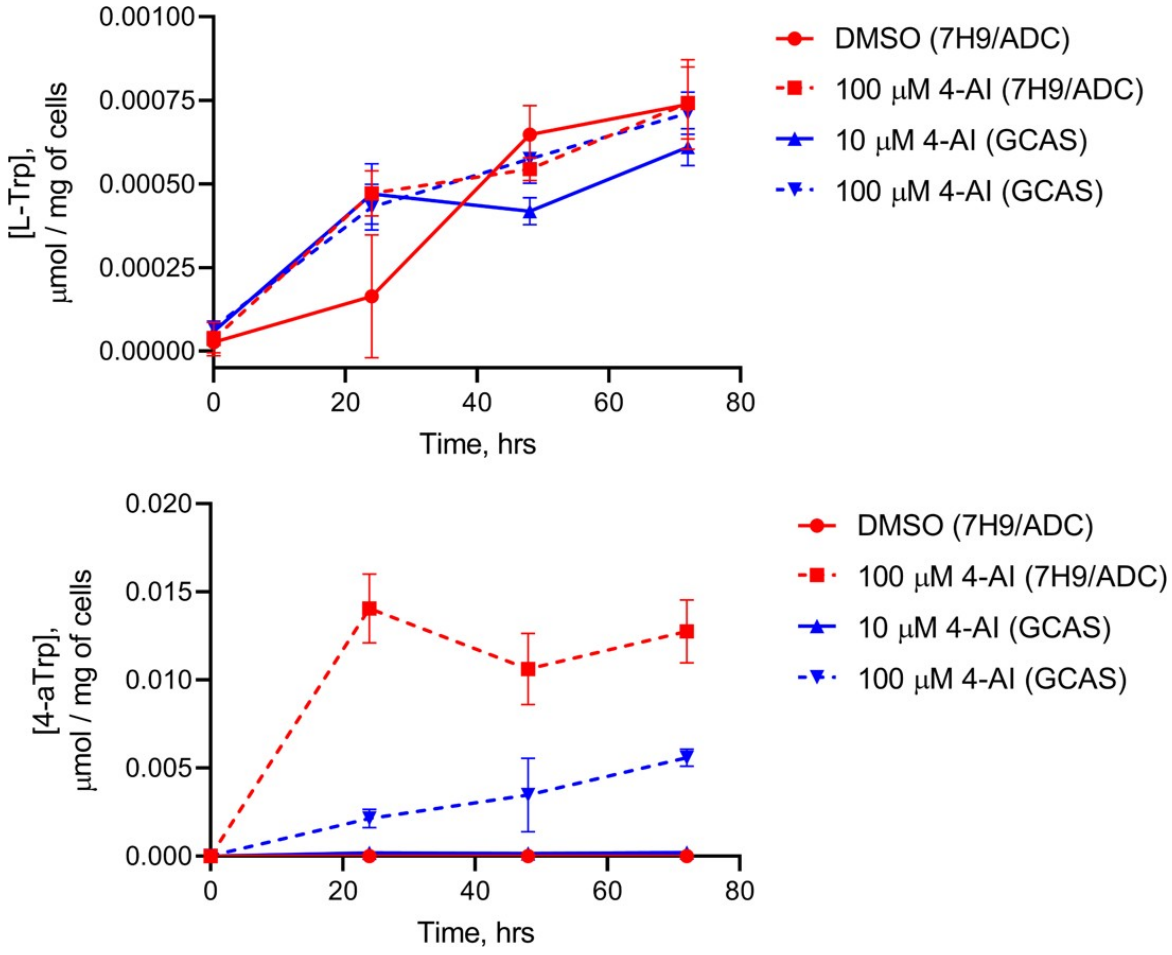


Figure S7. 4-AI incorporation into 4-a-Trp does not depress cellular levels of L-Trp, Related to Figure 6. Incorporation of 4-AI into whole cells of Mtb and the impact on intracellular L-tryptophan (top) and 4-aTrp (bottom) levels.

Table S1. Minimum Inhibitory Concentration (MIC) of Indole-4-carboxamides in various growth media against Mtb and other mycobacteria, related to Table 1.

Compound	MIC vs ***M. tuberculosis*** **H37Rv**, μM			MIC vs ***M. bovis BCG***, μM		MIC vs ***M. smegmatis*** **mc^{2}155**, μM
	7H9/ADC	7H9/GCas	GAST-Fe	7H9/ADC	7H9/GCas	7H9/GCas
C1	12.5	1.56	6.25	100	6.25	>100
C2	25	3.12	25	>100	12.5	>100
C3	>100	6.25	>100	>100	50	>100
C4	25	3.12	50	>100	12.5	>100

Table S2. Minimum Inhibitory Concentration (MIC) of 4-aminoindole and the corresponding free carboxylic acid building blocks of the NMMV03 compounds against Mtb H37Rv, related to Figure 2.

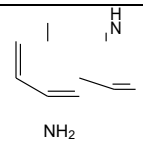
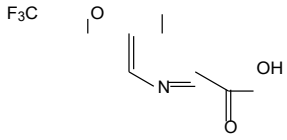
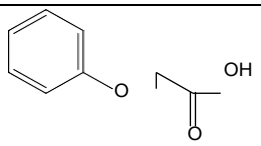
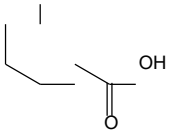
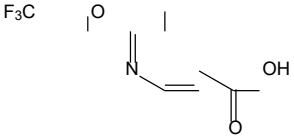
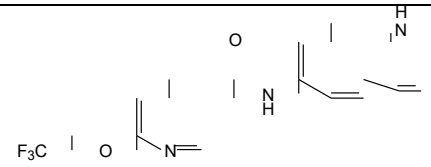
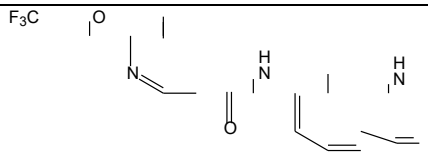
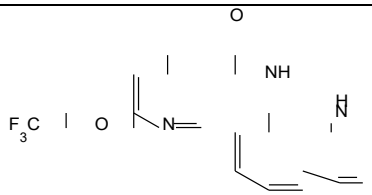
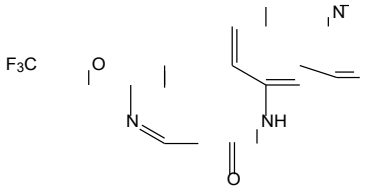
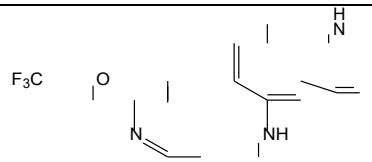
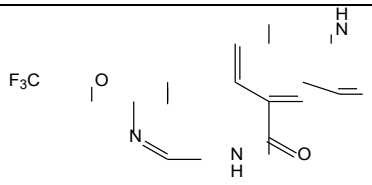
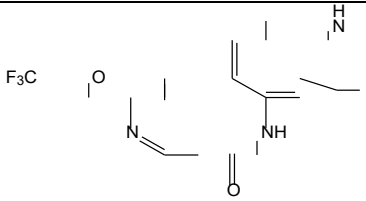
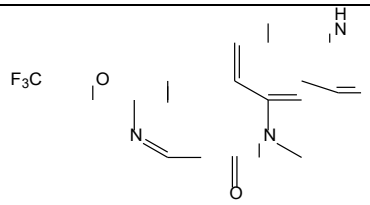
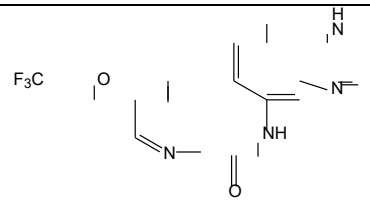
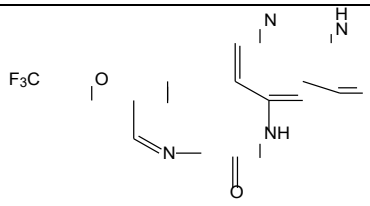
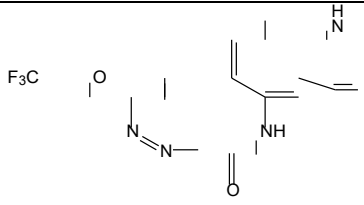
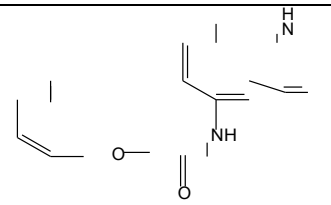
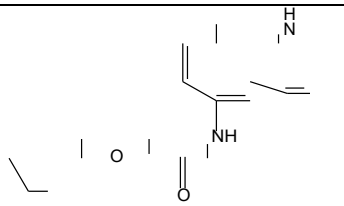
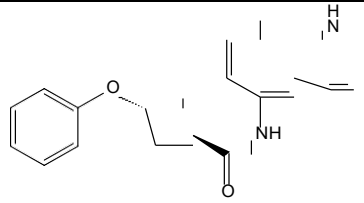
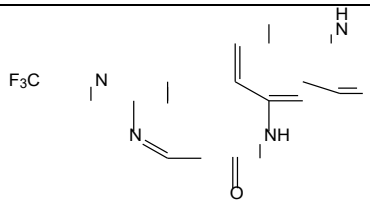
Compound	Structure	MIC, µM (7H9/GCas)
4-aminoindole		4.68
C1-COOH		50
C2-COOH		>100
C3-COOH		>100
C4-COOH		>100

Table S3. MIC of similar compounds lacking the 4-aminoindoleamide, related to Figure 2. Minimum Inhibitory Concentration (MIC) of representative members of the compound cluster against Mtb H37Rv.

Compound	Structure	MIC, μM (7H9/GCas)
C6		50
C7		50
C8		9.4
C9		50
C10		50
C11		50
C12		50

C13		25
C14		50
C15		50
C16		2.3
C17		1.56
C18		3.13
C19		3.13
C20		4.7

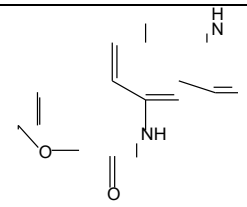
C21		6.25

Table S4. C5-resistant mutants remain sensitive to indole-4-carboxamides, related to Figure 5. Mtb H37Rv $C5^R$ mutant MIC against indole-4-carboxamides and the corresponding mutations found via whole genome sequencing.

$C5^R$ Strain	**MIC, μM** (7H9/GCas)						
	C1	**C2**	**C3**	**C4**	**4-AI**	**C5**	**INH**
WT	3.12	6.25	12.5	6.25	6.25	0.39	0.16
A1 (*trpB* F188S)	1.56	3.12	12.5	3.12	6.25	>100	0.16
A2 (*trpB* Y200C)	6.25	6.25	12.5	6.25	6.25	>100	0.16
A4 (*trpA* P65L)	3.12	6.25	12.5	6.25	6.25	>100	0.16
B2 (*trpA* D136N)	3.12	6.25	12.5	6.25	6.25	>100	0.16

Table S5. Oligonucleotides used in this study, related to STAR methods.

Primers for Psmyc promoter swap	GTGTTCTAGAGGATCGTCGGCACC	P_{smyc}-F
	GCGCCCATGGAGATACCTCCTTAATTAAGCATGCGGATC	P_{smyc}-R
Primers for AmiC cloning	GCTACCATGGGCATGTCGCGCGTACACG	AmiC_pMV306-F
	GCTAATCGATCTACTCGGCGATATTTGGGGCGTGG	AmiC_pMV306-R
Primers for TrpB recombineering	GAGACCGGTGCCGGCCAGCACGGGGTCGCCACGGTCACCGCATGCGCATTGCTCGGCCTGGACTGTGTC	TrpB-A134V_Rec
	GCGCTAAACGTGGCCCGGATGCGATTGCTGGGTGTCGAAGTCGTCGCGGTTCAGACGGGCTCGAAAACG	TrpB-A168V_Rec
Primer for TrpE recombineering	CTGTTGCTGGCCACCGATGTGGCGGCGGTCGATCGCCACGAGGGCACCATCACGTTGATCGCCAACGCC	TrpE-H170R_Rec
Primer for RpsL recombineering	GCGGGCAACCTTCCGAAGCGCCGAGTTCGGCTTCCTCGGAGTGGTGGTGTACACGCGGGTGCATACACC	RpsL-K43R_Rec
Primers for confirming TrpB	CGATCTGGTTACCGCGGG	TrpB_RecCheck-F
	CCCGGTCGGCAAGTAACC	TrpB_RecCheck-R
Primers for confirming TrpE	GCCCGACTCCTTGCGC	TrpE_RecCheck-F
	CCGATGACCACCCAGGG	TrpE_RecCheck-R
Primers for confirming RpsL	AGTTTGAGGCAAGCTATG	RpsL_RecCheck-F
	CCCTTCAACAGAACCTTG	RpsL_RecCheck-R
Primers for TrpE overexpression	GCTACATATGCACGCCGACCTC	TrpE_pET28a-Exp-F
	GCTAAAGCTTTTAGCAGCCACTGCG	TrpE_pET28a-Exp-R
Primers for TrpE H170R mutagenesis	GCGGTCGATC**g**CCACGAGGGC	TrpE-H170R-F
	CGCCACATCGGTGGCCAG	TrpE-H170R-R
Primers for TrpB overexpression	GCTAAAGCTTATGAGTGCTGCCATC	TrpB_pRSFDuet-F
	GCTAAAGCTTTCAGTCGTTGCCCAG	TrpB_pRSFDuet-R
Primers for TrpA overexpression	GCTACATATGGTGGCGGTGGAAC	TrpA_pRSFDuet-F
	GCTACTCGAGTCATGCGGACATCC	TrpA_pRSFDuet-R
Primers for AmpR for TrpA cloning	CATGCCATGGGAATGGTGGCGGTGGAACAG	TrpA_AmpR-F
	CCCAAGCTTTCATGCGGACATCCCTAG	TrpA_AmpR-R
Primers for TrpB mutagenesis A168V	GTCGCCACGG**t**CACCGCATGC	TrpB-A168V_SDM-F
	CCCGTGCTGGCCGGCACC	TrpB-A168V_SDM-R
Primers for TrpB mutagenesis D261G	TTTCTCGATG**g**CCCAGGCGTA	TrpB-D261G_SDM-F
	CGCATGAAAAATACCAATGGC	TrpB- D261G_SDM-R
Primers for TrpCF expression	GCTACATATGATGATGCAAACCGTTTTAGC	Ec_TrpCF_Exp-F
	GCTAAAGCTTTTAATATGCGCGCAGCG	Ec_TrpCF_Exp-R