

Intrabacterial Metabolism Obscures the Successful Prediction of an InhA Inhibitor of *Mycobacterium tuberculosis*

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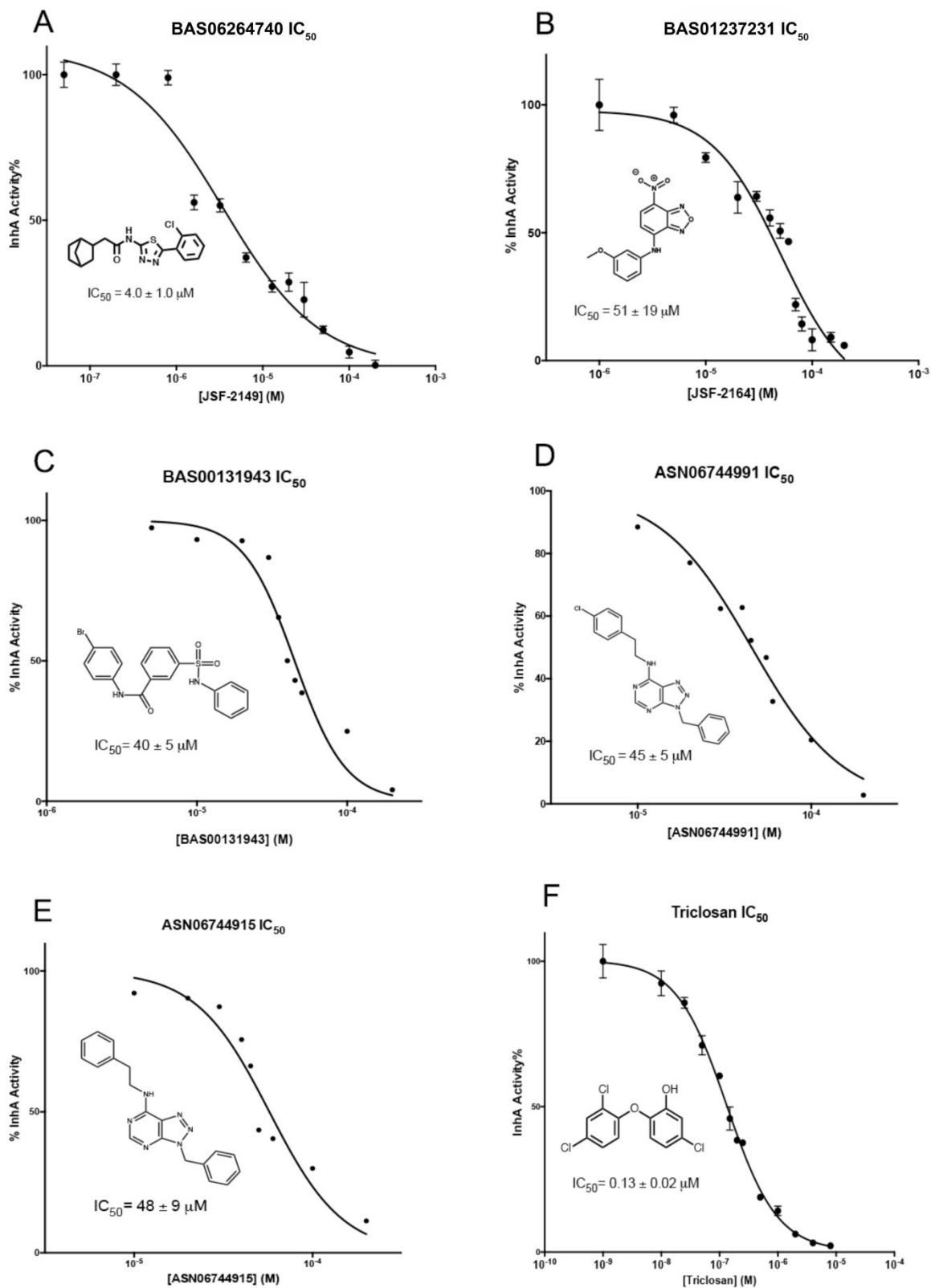


Figure S2. Synthetic scheme for JSF-2149.

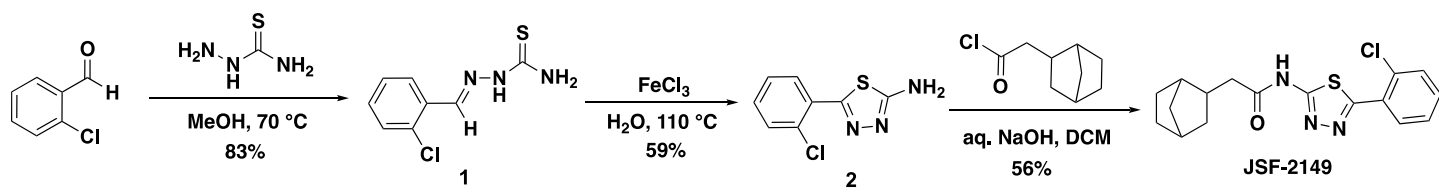


Figure S3. Synthetic scheme for JSF-2164 and its candidate metabolites.

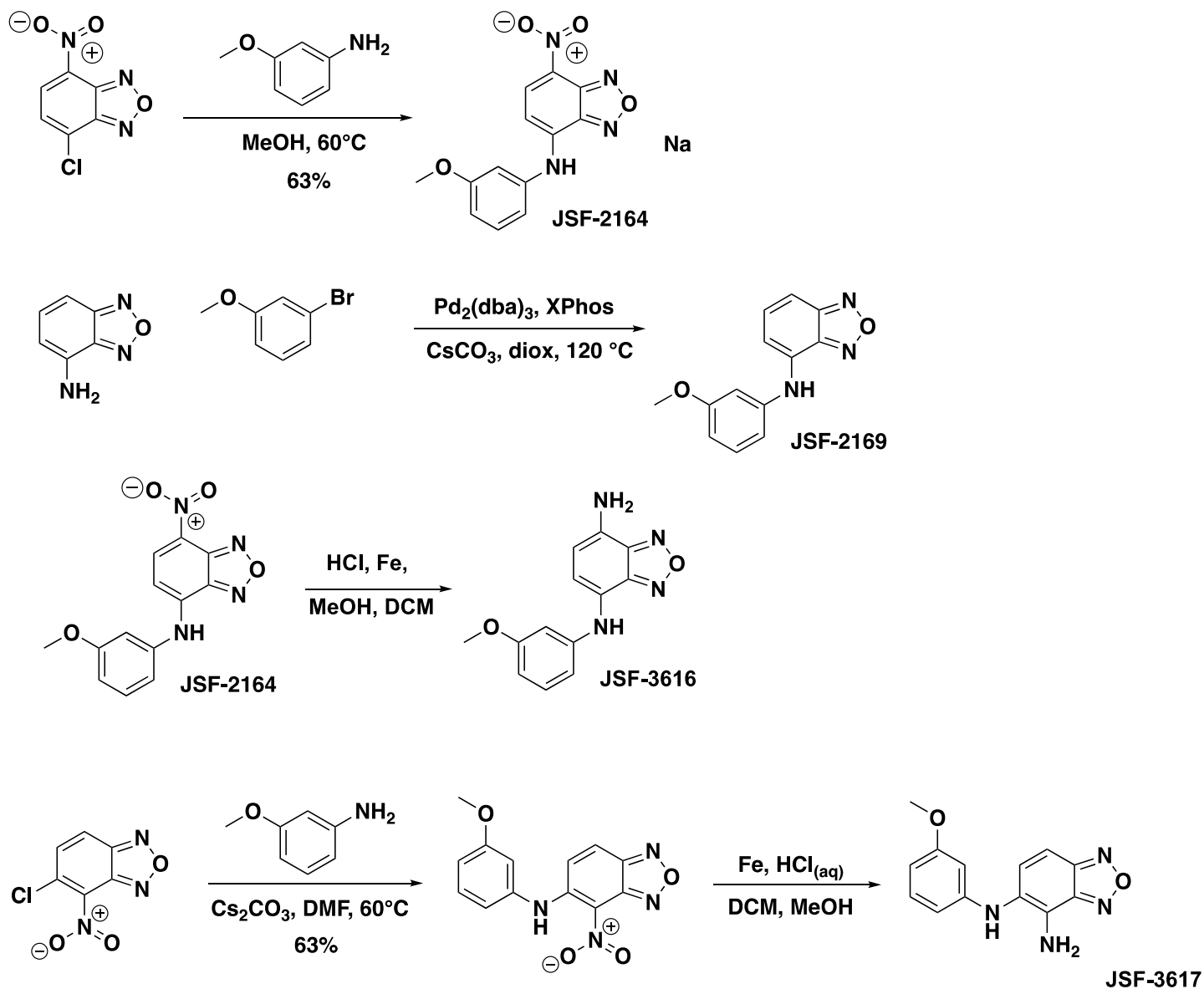


Figure S4. InhA activity in the presence of Tween-20. InhA activity ($\mu\text{M/s}$) was measured at a range of concentrations of Tween-20 (0.001X – 1X CMC), where CMC = 60 mg/L. InhA activity without Tween-20 was 0.10 $\mu\text{M/s}$. InhA catalytic activity reached a plateau from 0.001 CMC until approximately the detergent's CMC. Therefore, a range of Tween-20 concentrations from 0.01 CMC to 0.5 CMC may be used for testing compound aggregation. The error bars quantify the standard errors for each measurement made in biological triplicates.

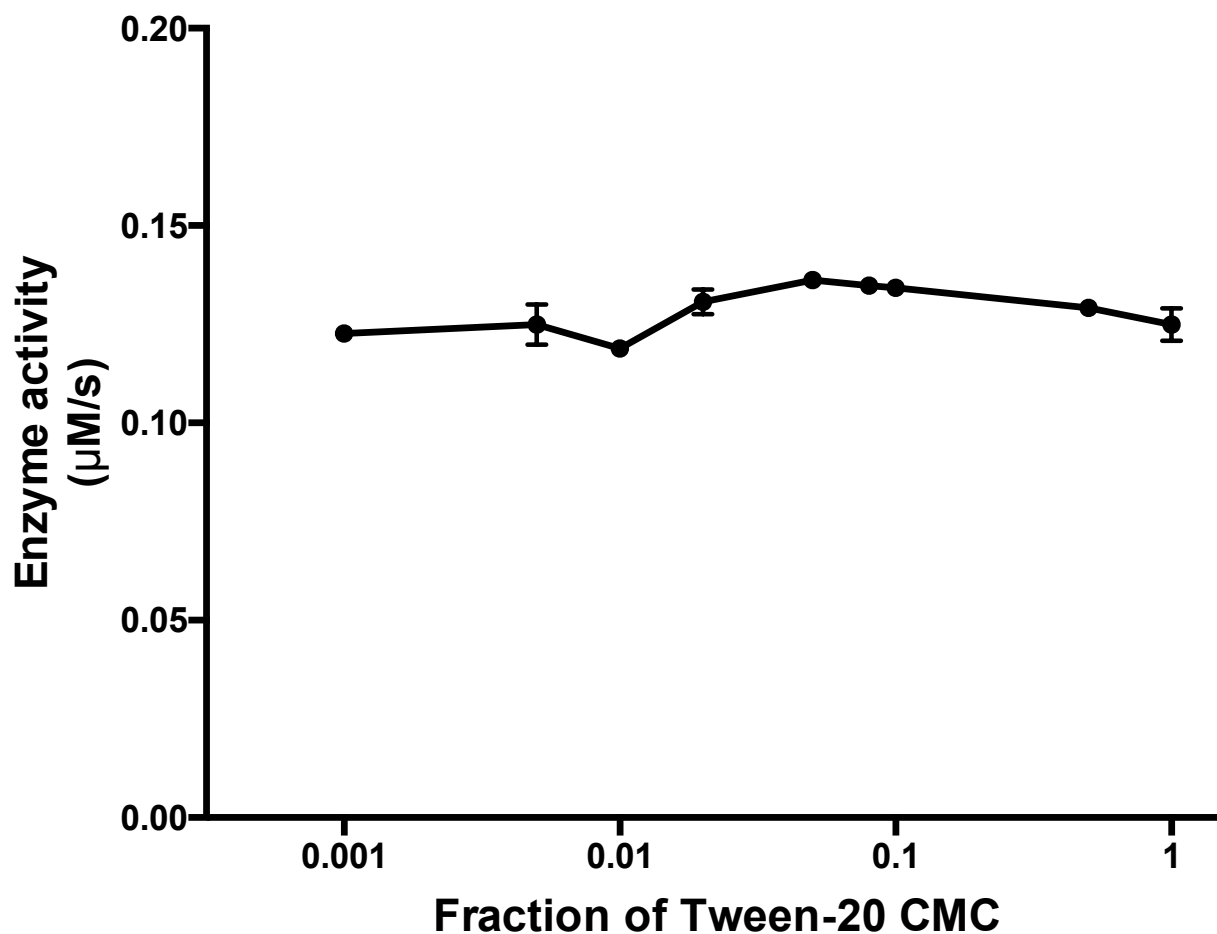


Figure S5. JSF-2149 exhibited detergent-dependent inhibition of purified InhA. InhA dose-response curves with or without 0.01 CMC Tween-20 were generated and the IC_{50} was calculated as mean \pm standard error for (A) JSF-2149, (B) Triclosan as a negative control, and (C) benzyl benzoate as a positive control. The error bars showed standard errors of each measurement in biological triplicates.

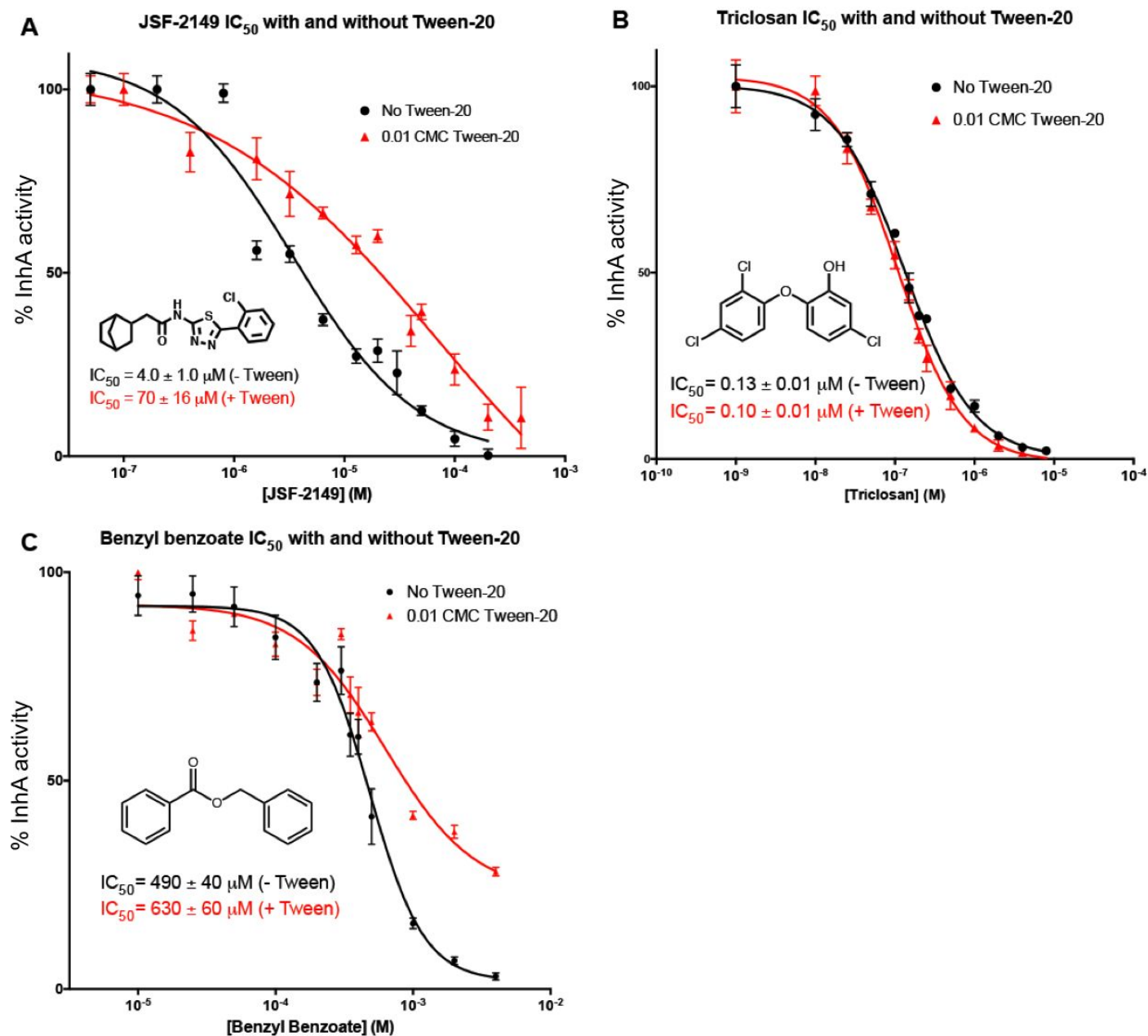


Figure S6. RIF *in vitro* activity versus the ss18b strain. Strain ss18b was starved without streptomycin supplementation in 7H9+ADS media for 2 weeks at $OD_{595} = 0.3$. RIF activity was then measured by REMA. The error bars showed standard errors of each measurement in biological triplicates.

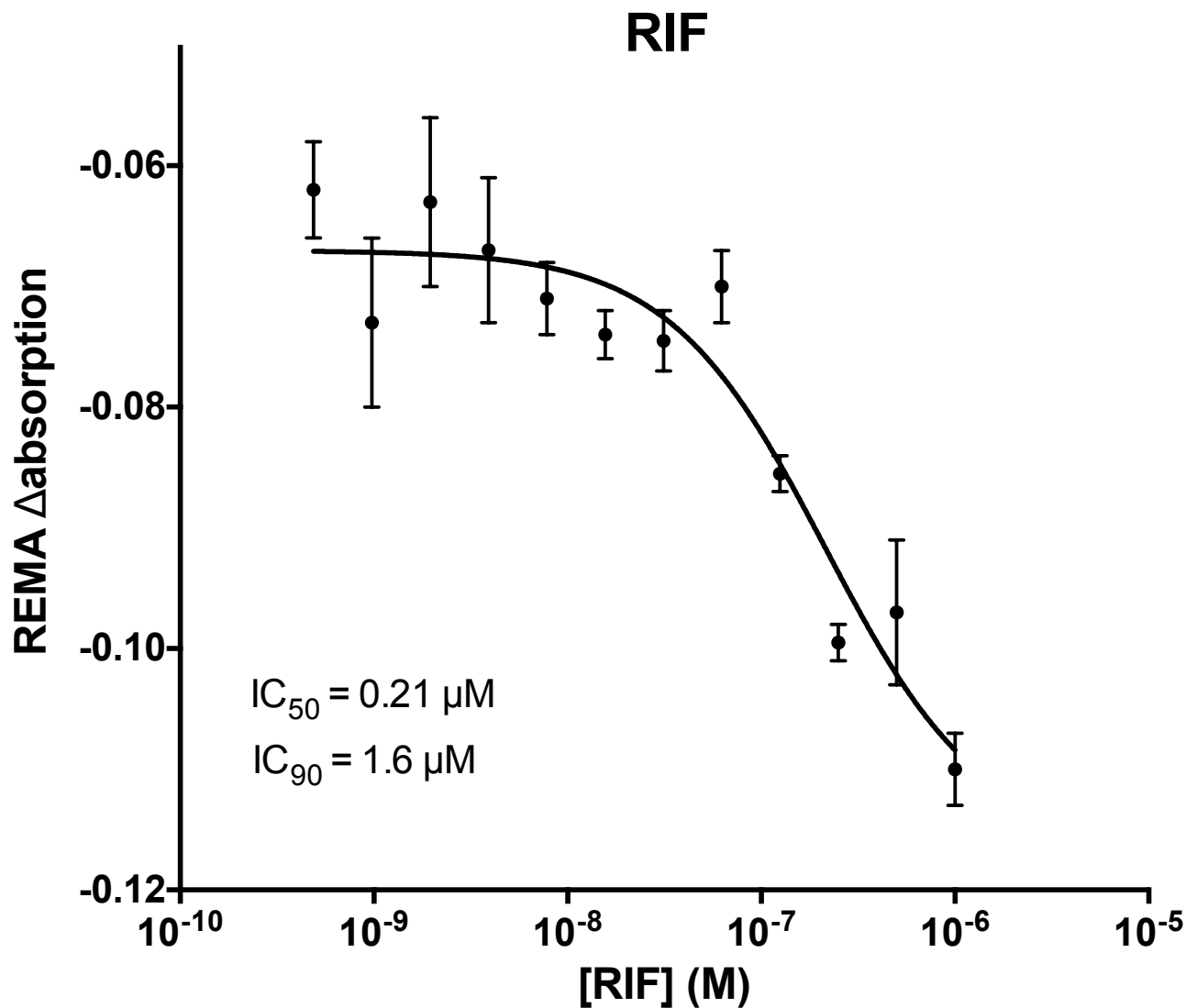


Figure S7. *inhA* promoter mutation in 16x6 resulted in *inhA* over-expression. (A) Mutation *c(-15)t* in strains 16x6 and mc²4914 as determined by whole-genome and Sanger sequencing. (B) Expression levels of *inhA* and *mabA* in mutant strains as compared to expression in H37Rv, as assayed via qPCR and quantified by expression of 16S rRNA. The error bars showed standard errors of each measurement in biological triplicates. *inhA* and *mabA* expression in the mutant strains were compared to those in the wild type strain followed by statistical analysis with an unpaired Student's t-test. *** $p < 0.001$

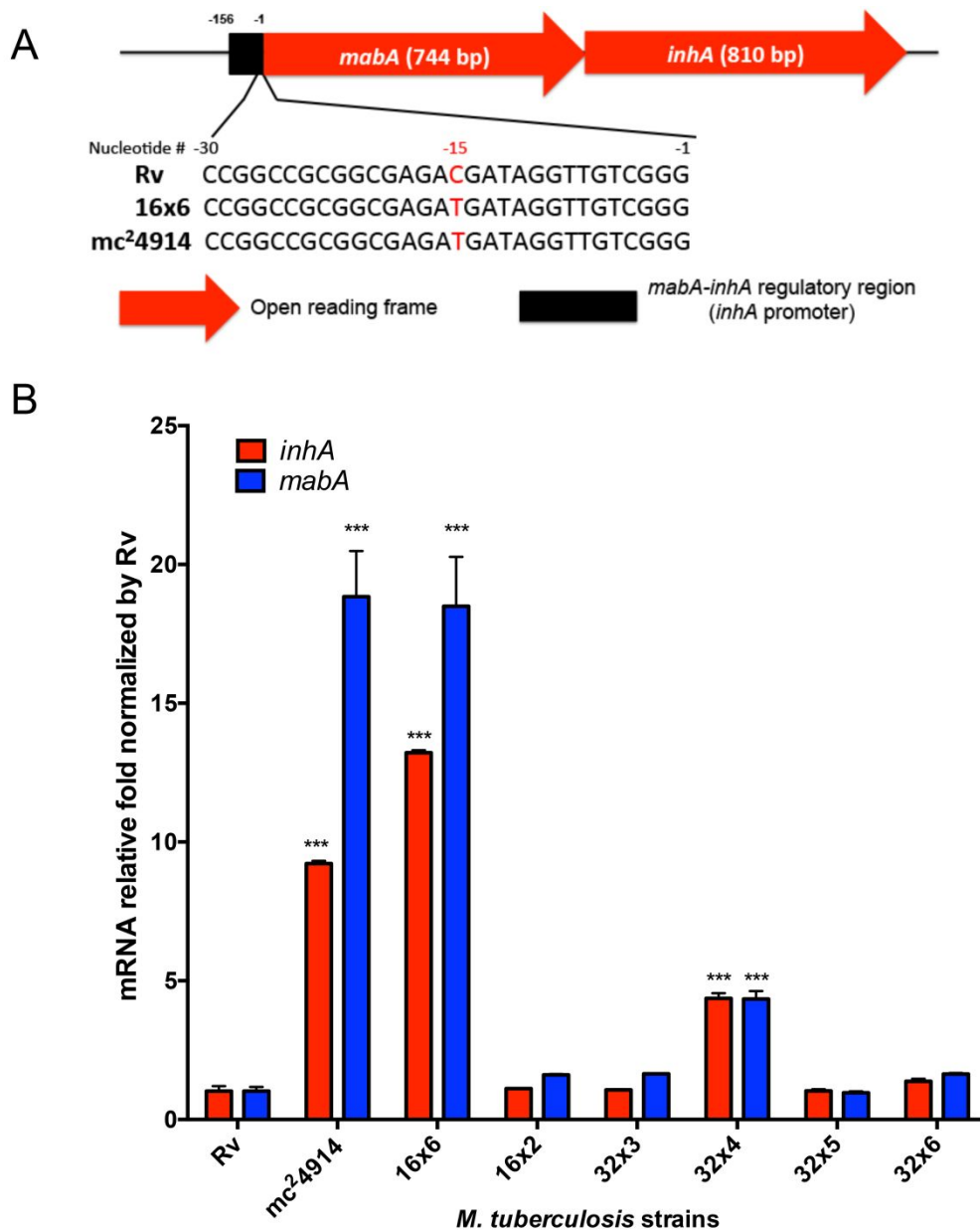


Figure S8. LC-MS data supporting identification of JSF-3617 as intrabacterial metabolite M1. Metabolite with m/z of 257.1030 was purified from bacterial lysate followed by analysis via (A and B) high-resolution mass spectrometry and (C) LC-MS co-elution of isolated M1 with synthetic JSF-3617.

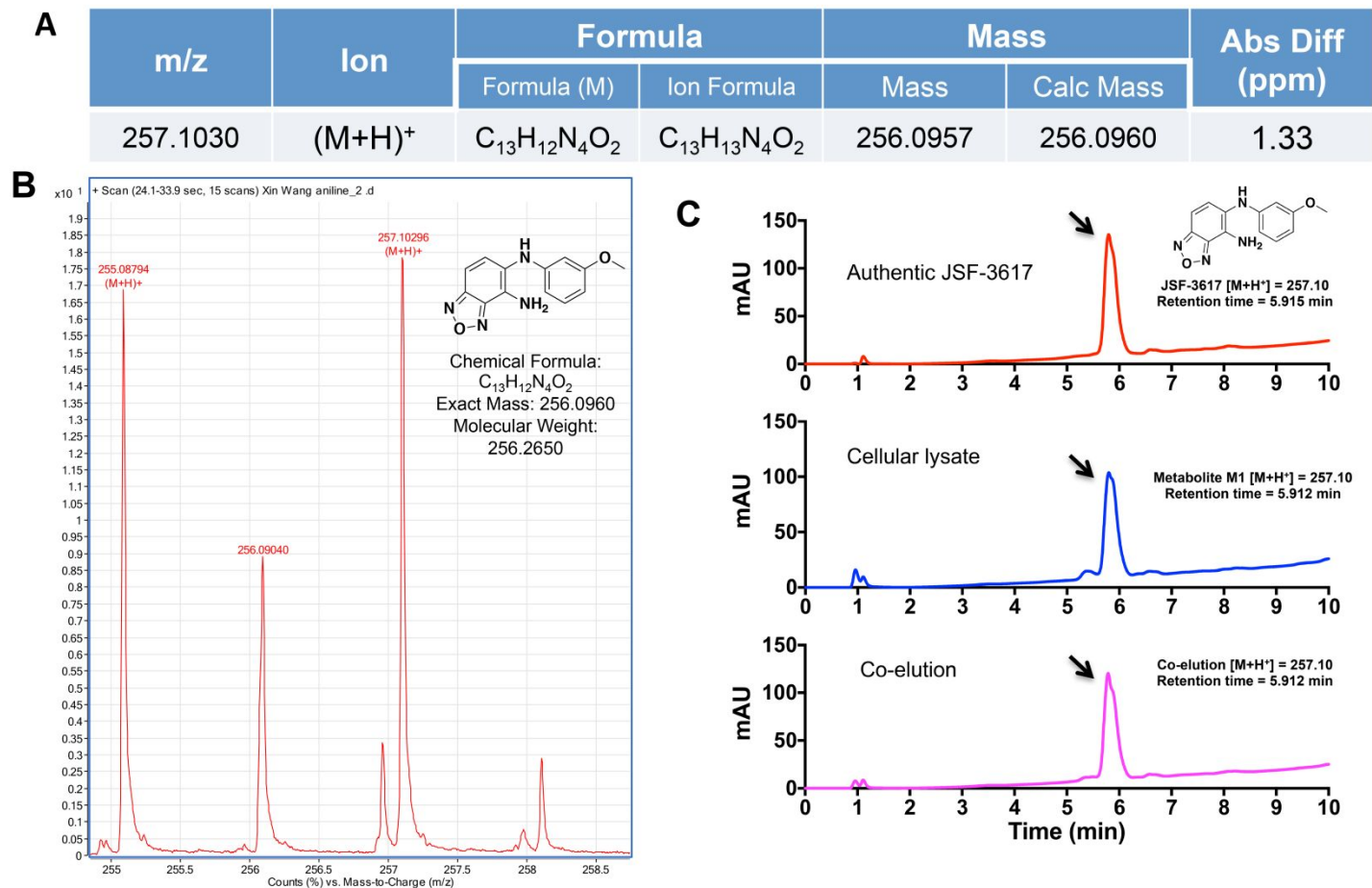


Figure S9. LC-MS data supporting identification of JSF-3616 as intrabacterial metabolite M2. Metabolite with m/z of 257.1030 was purified from bacterial lysate followed by analysis via (A and B) high-resolution mass spectrometry and (C) LC-MS co-elution of isolated M2 with synthetic JSF-3616.

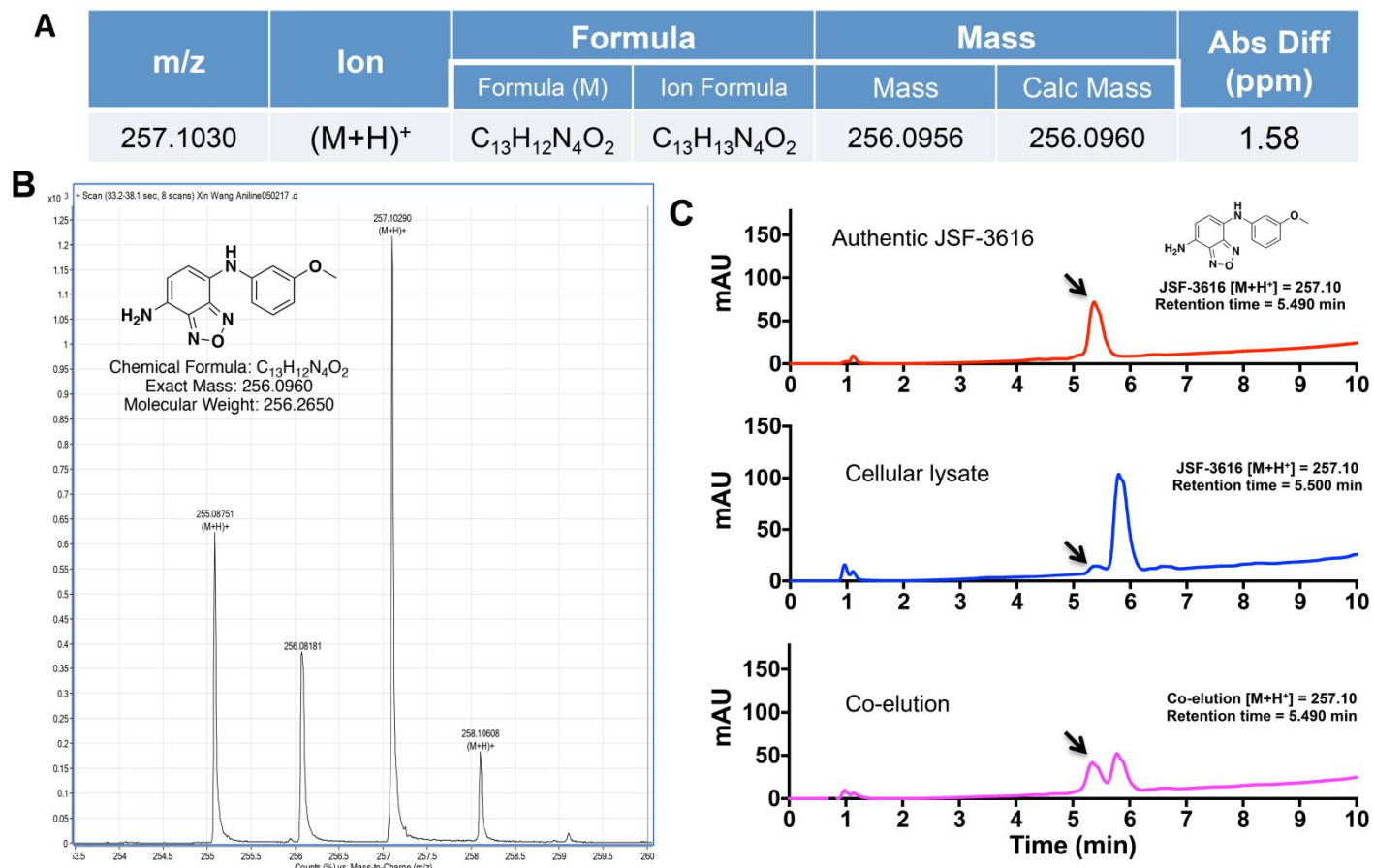


Table S1. 370 compounds that passed the initial docking filters and were scored with two recently validated Bayesian dual-event models.

Please view file : Table S1 DockingFiltered370_wCB2_nCombined.xlsx

Table S2. Antitubercular activity of 5 lowest-scoring compounds amongst the 370 candidates passing docking filters.

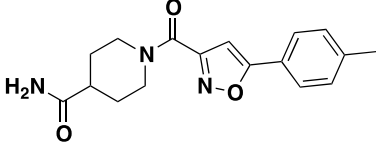
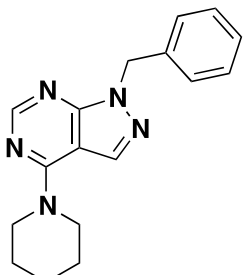
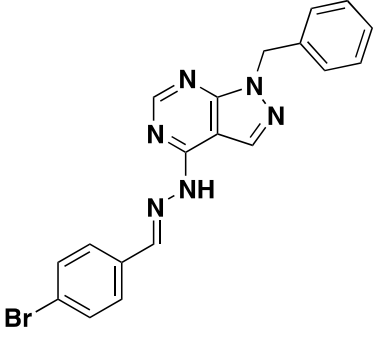
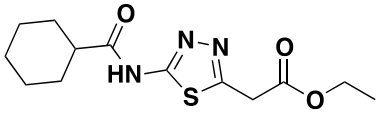
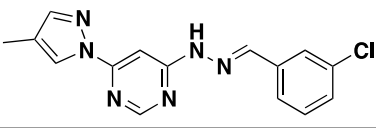
Asinex ID #	Structure	MIC vs. <i>M. tuberculosis</i> H37Rv (μM)	TAACF-CB2 dose-response and cytotoxicity model score
BAS09529894		250	-8.61
BAS02224699		125	-5.34
BAS00367862		250	-4.34
BAS04834575		250	-3.73
BAS00317511		125	-3.46

Table S3. Profiling of select spontaneous JSF-2164-resistant mutants.

Strain	Gene(s) mutated (Amino acid change)	MIC (μM)		
		JSF-2164	INH	Pretomanid
H37Rv	/	8.0	0.16	0.078
16x2	<i>fbiC</i> (F567S)	250	0.078	>40
16x6	<i>fgd1</i> (frameshift at L138) <i>inhA</i> promoter <i>c(-15)t</i>	250	1.25	>40
32x4	<i>tmk</i> promoter <i>g(-66)t</i>	31	1.25	0.16
32x5	<i>fbiC</i> (F566S)	250	0.64	>40

Table S4. MIC values for JSF-2164, INH, and pretomanid versus JSF-2164-resistant transposon mutants.

Please view file : Table S4 Transposon_MICs.xlsx