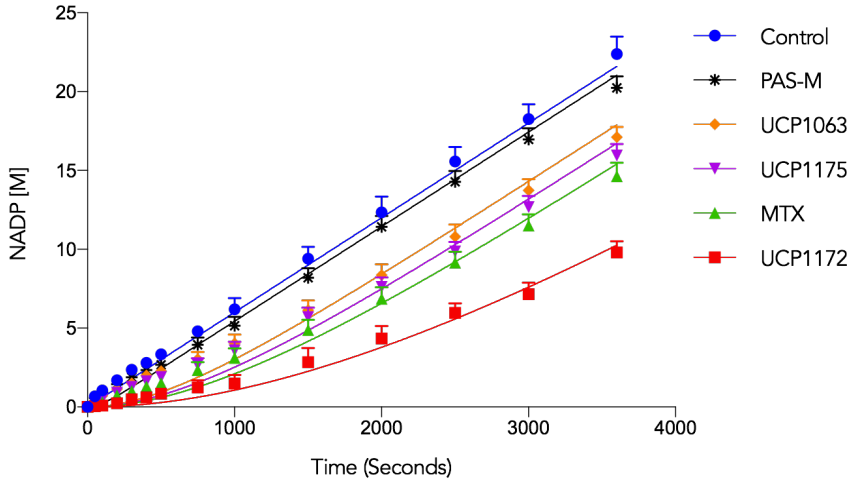
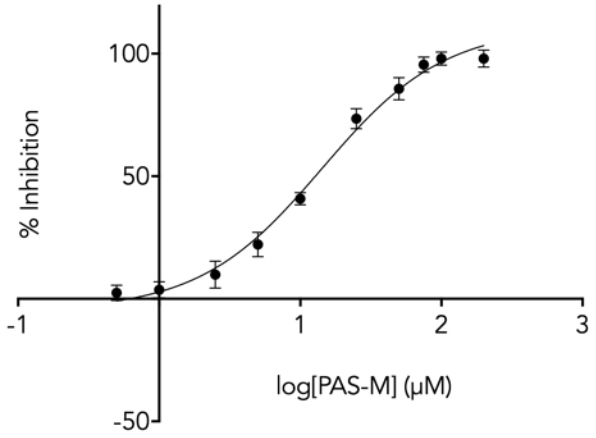
Figure S1. Jump-dilution progress curves for Mtb DHFR inhibitors (PLAs, MTX, and PAS-M), Related to Table 1.



Legends indicate the inhibitors tested against MtbDHFR. Reactions were conducted under conditions where compound rebinding after dissociation is minimized and the observed time constant for reactivation is indicative of residence time. Progress curves were fit into integrated rate Equation 1 (STAR methods) using GraphPad Prism 7.0 to yield initial velocity (V_i), steady-state velocity (V_s), and the observed recovery rate constant (k_{obs}). k_{obs} was then used to calculate residence time using Equation 2 (STAR methods and Table 1). Constraint for k_{obs} value was set to be greater than zero. Error bars, indicating standard deviation, are from triplicates.

Figure S2. Dose-response curve of PAS-M FDTS inhibition, Related to Figure 4.



Curve fitting was performed using nonlinear regression and (log)inhibitor vs. response equation in GraphPad Prism 7.0. with a standard slope (Hill slope= 1.0). The model was as Y=Bottom + (Top-Bottom)/(1+10^((X-LogIC50))) where X values are logarithms of concentration, Y=response (%inhibition), Bottom and Top= plateaus in same units as Y and LogIC50= same log unit as X. No constraint was set for the model. Error bars, indicating standard deviation, are from triplicates.

Figure S3. Crystal structure of Mtb FDTS tetramer bound with FAD (yellow) and dUMP (magenta), Related to Figure 4.

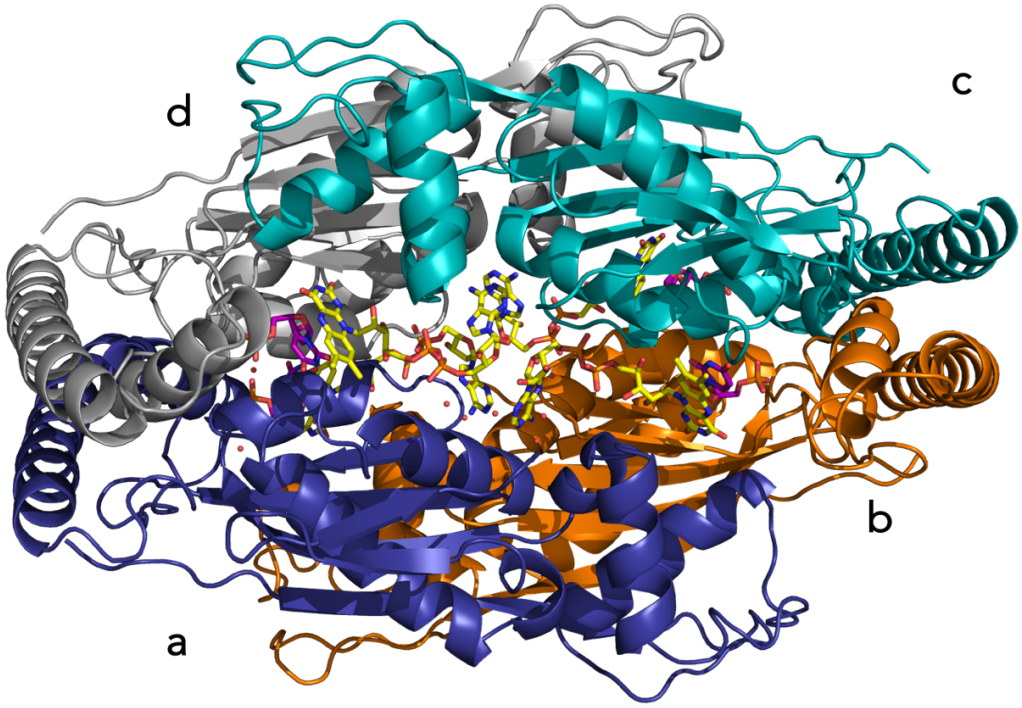


Table S1. Kinetic parameters of Mtb DHFR, Hu DHFR, and Mtb Rv2671, Related to Table 1.

Enzyme	*K*cat (1/sec)	Km (μM)		Vmax (μmol/mL/min)	
		DHF	**NADPH**	**DHF**	**NADPH**
MtbDHFR	1.67	9.8	11.6	0.023	0.019
MtbRv2671	0.69	40	9.83	0.015	0.021
HuDHFR	0.47	8	12.56	0.012	0.013

Table S2. *In vitro* selection of Mtb strains resistant to INCAs, Related to Table 2.

Compounds	Mutation rate[a]	Wild-type MIC (μg/mL)	Mutant MIC (μg/mL)	Fold potency loss[b]	PAS MIC (μg/mL)
UCP1172	$2.4 \times 10^{-8} \pm 0.5$	0.03	1	32	16
UCP1175	$8.5 \times 10^{-8} \pm 0.39$	0.125	4	32	16

[a] per bacterium per generation

[b] Fold potency loss= mutant MIC/wild-type MIC

Table S3. Crystallography data collection and refinement statistics, Related to Figure 3 and STAR methods.

Complex	MtbDHFR:NADPH:1172	MtbDHFR:NADPH:1175	MtbD \HFR:NADPH:PASOX	HuDHFR:NADPH:1172	MtbRV2671:NADPH:1063
PDB ID	6DDP	6DDS	6DDW	6DE4	6DE5
Data collection statistics					
Beamline	SSRL 14-1	SSRL 14-1	SSRL 14-1	SSRL 14-1	NSLS AMX 17 D-1
Space group	P1	P1	P41	C2221	C2221
Cell dimensions					
a, b, c (Å)	57.77, 60.55, 60.63	59.20, 60.80, 60.72	60.43, 60.43, 58.95	104.61, 104.66, 144.33	70.44, 94.63, 75.49
α, β, γ (°)	90.04, 90.02, 90.03	90.15, 89.93, 90.01	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	34.42-1.461	27.00-1.72	42.22-1.40	42.37-2.41	56.52-2.30
Highest resolution shell (Å)	1.53-1.46	1.75-1.72	1.44-1.40	2.47-2.41	2.40-2.30
Completeness%	99.4 (98.2)	96.8 (93.9)	86.7 (43.5)	99.7 (97.2)	98 (98.2)
R_{sym}	0.020 (0.239)	0.028 (0.128)	0.013 (0.151)	0.082 (0.779)	0.091 (0.236)
R_{pim}	0.020 (0.239)	0.028 (0.128)	0.013 (0.151)	0.031 (0.297)	0.091 (0.236)
R_{meas}	0.029 (0.338)	0.040 (0.182)	0.018 (0.214)	0.088 (0.835)	0.091 (0.236)
Redundancy	1.5 (1.4)	1.6 (1.5)	2 (1.9)	7.7 (7.7)	1.9 (1.8)
Mean $I/\sigma(I)$	13.8 (3.0)	9.0 (4.3)	24 (3.8)	16.4 (4.2)	5.5 (3.3)
Refinement statistics					
Resolution (Å)	34.42-1.461	27.00-1.72	42.22-1.40	42.37-2.41	56.52-2.30
No. of reflections	118402	70016	36214	30735	11313
R_{factor}/R_{free}	0.2029/0.2253	0.1817/0.2056		0.1812/0.2205	0.1870/0.2477
No. of atoms (protein, ligand, solvent)	5652	5957	1523	3420	1969
rmsd bond length (Å)	0.007	0.008	0.007	0.008	0.010
rmsd bond angle (°)	1.29	1.27	1.36	1.05	1.23
Ramachandran plot analysis (%)					
Most favored	98.1	97.3	97.5	97.9	95.5
Outliers	0.2	0.3	0	0.3	0.4

Scheme S1. Synthesis of PAS-M, Related STAR methods.