Supplementary Information
Structural plasticity of malaria dihydroorotate dehydrogenases allows selective binding of diverse chemical scaffolds

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Supplementary Tables and Figures

Table S1. Steady-state kinetic analysis of wild-type *Pf*DHODH vs *Pf*DHODH$_{\Delta 384-413}$

	k_{cat} (s^{-1})	K_m (DHO) (μM)	K_m CoQ (μM)	IC_{50} DSM1
*Pf*DHODH	18±0.50	16±2.2	71±22	0.047 ± 0.02[a]
*Pf*DHODH$_{\Delta 384-413}$	18±0.43	21±2.1	105±39	0.016±0.0016

Table S2. *Pf*DHODH inhibitor complexes: X-ray diffraction data and refinement statistics

Data collection	DSM1	DSM2	DSM74
Resolution (Å)	50.0-2.02	50-2.4	50-2.7
Space group	P64	P64	P64
Cell dimensions	a=b=85.9, c= 138.4	a=b=85.9, c=138.7	a=b=85.4, c=138.4
	α=β=90º, γ=120º	α=β=90º, γ=120º	α=β=90º, γ=120º
Unique reflections	42,198	22,633	15,754
Completeness (%)	91.4 (67.9)	100 (100)	100 (99)
Redundancy	12 (3.6)	7.3 (7.5)	7.6 (7.7)
I/σ	45.7 (1.5)	41.9 (4)	45.2 (4)
R_{sym} (%)	5.8 (51.5)	5.3 (51.7)	6.5 (49.8)
Refinement			
Solvent (%)	62	63	63.6
R_{factor} (%)	23.1	22.5	23.6
R_{free} (%)	24.8	26.7	27.6
No. of atoms	3138	3193	3083
No. of water atoms	124	63	39
rms deviations			
Bond length (Å)	0.01	0.01	0.007
Bond angles (deg.)	1.3	1.24	1.04
Ramachandran plot			
Most favored (%)	90.9	92.3	89.9
Additionally allowed	8.8	7.4	9.8
Generously allowed	0.3	0.3	0.3

Table S3. Data collection details for the structures of DSM1, DSM15, DSM16 and DSM74.

Compound	Stepsize ^{o}/frame	Exposure (seconds)	Full & partial reflections
DSM1	0.9	30	29,175
DSM15	1.5	30	34,855
DSM16	2.0	30	29,924
DSM74	1.0	80	35,811

Table S4: Small molecule crystallographic data for the DSM1, DSM15, DSM16 and DSM74.

Property	DSM1	DSM15	DSM16	DSM74
Empirical Formula	C16 H13 Cl N5	C16 H12 N4 S	C16 H12 N4 O	C13 H10 F3 N5, 0.147(H_2O)
Formula Weight	310.76	292.36	276.30	295.91
Temperature K	130(2)	130(2)	130(2)	130(2)
Wavelength Å	0.71073	0.71073	0.71073	0.71073
Habit/color	prism / colorless	prism / colorless	plate / colorless	needle / colorless
Size (mm^3)	0.59 x 0.14 x 0.07	0.50 x 0.20 x 0.12	0.30 x 0.15 x 0.06	0.59 x 0.07 x 0.07
Crystal System, space group	Tetragonal, P 4_3 (No. 78)	Triclinic, P$\bar{1}$ (No. 2)	Monoclinic, $P2_1/c$ (No. 14)	Tetragonal, I $4_1/a$ (No. 88)
Unit Cell Dimensions				
a, Å	7.5871(3)	15.2471(3)	12.137(2)	35.812(3)
b, Å	7.5871(3)	18.8854(4)	10.8958(18)	35.812(3)
c, Å	24.9960(10)	36.1826(9)	9.8599(15)	3.9556(3)
α, ^{o}	90	100.728(7)	90.00	90
β, ^{o}	90	93.349(8) 99.481(12)	92.213(8) 90.00	90
γ, ^{o}	90			90
Volume (Å ^3)	1438.87(10)	676.7(3)	1303.0(4)	5073.1(7)
Density Mg/m^3	1.4435	1.435	1.408	1.550
Reflections Collected/Unique	2985 / 2985	3939 / 2378	4132/2329	3525/2066
Rint / Rσ	0.0980 / 0.1335	0.1140 / 0.1869	0.1494 / 0.2190	0.0956 / 0.1224
ϑm / completeness	25^{o} / 100.0%	25^{o} / 99.2%	25^{o} / 98.0%	25^{o} / 88.8%

Goodness of Fit	0.953	0.933	0.856	0.937
Final R indices [I>2σ(I)]				
R1	0.0604	0.0733	0.0586	0.0530
wR2	0.1350	0.1498	0.1012	0.1084
R indices (all data)				
R1	0.1207	0.1682	0.1772	0.1496
wR2	0. 1560	0. 1899	0.1286	0.1453

Table S5: Selected Bond distances for the structures DSM1, DSM15, DSM16 and DSM74.

Bond	**DSM1**	**DSM15**	**DSM16**	**DSM74**
C7 – N1/S1/O1/N1	1.438(6)[a]	1.769(5)	1.424(3)	1.42(2)/1.44(2)
C1 – N1/S1/O1/N1	1.313(6)	1.740(4)	1.338(3)	1.344(4)
C1 – N2	1.381(5)	1.362(6)	1.358(4)	1.372(4)
N2– N3	1.364(5)	1.363(5)	1.372(3)	1.382(4)
N3 – C5	1.318(6)	1.312(6)	1.343(4)	1.327(5)
C5 – N4	1.364(6)	1.362(6)	1.352(4)	1.348(5)
N4 – C4	1.320(6)	1.334(6)	1.340(4)	1.333(4)
C4 – N2	1.368(6)	1.373(6)	1.379(4)	1.377(4)
C4 – N5	1.342(6)	1.364(6)	1.333(4)	1.341(4)
N5 – C3	1.368(6)	1.323(6)	1.327(4)	1.337(5)
C2 – C3	1.379(7)	1.403(6)	1.411(4)	1.393(5)
C1 – C2	1.404(6)	1.376(5)	1.360(4)	1.372(5)

[a] Standard mean. Error of the last digit is in parenthesis.

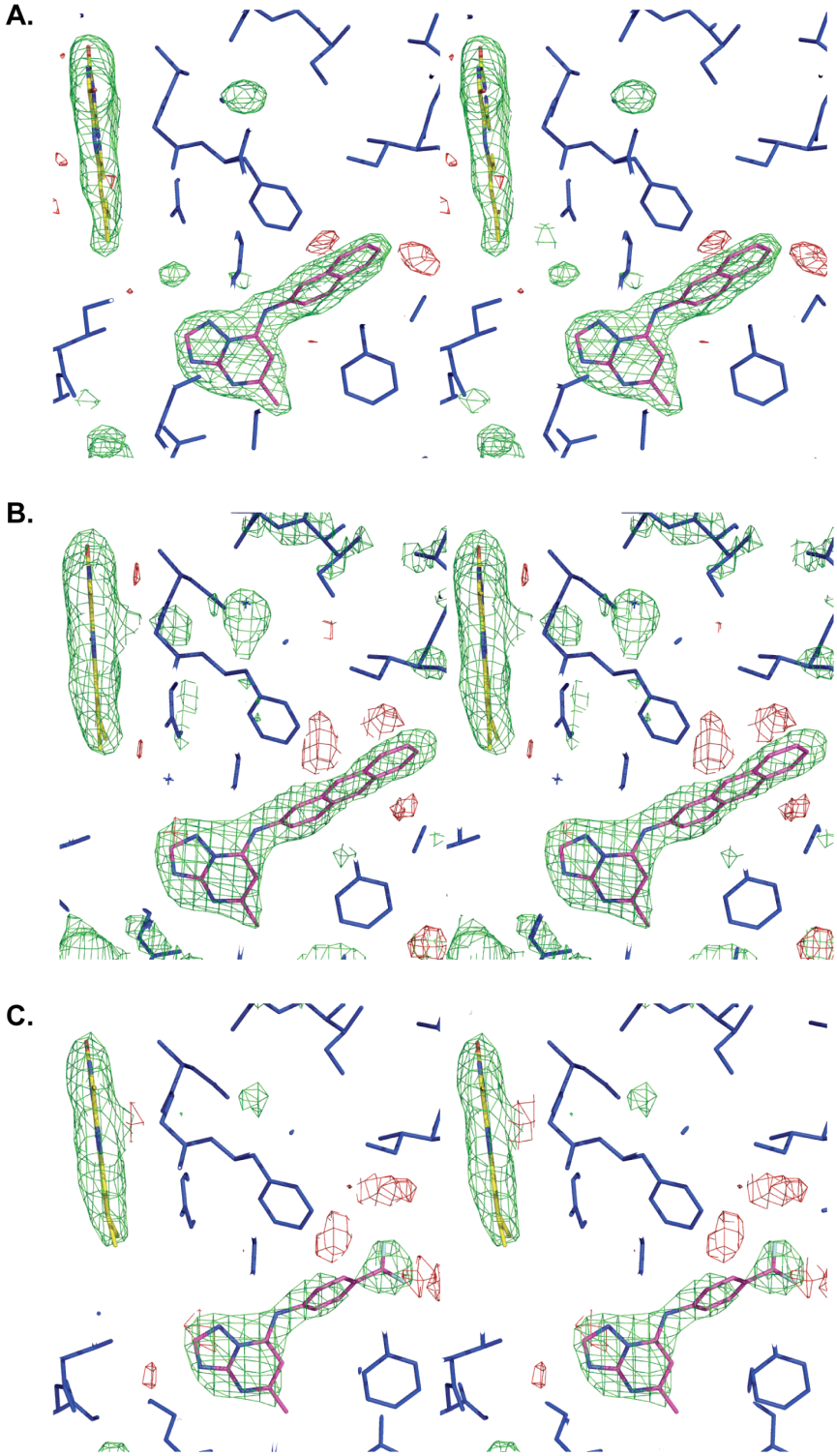


Figure S1. Stereo electron density difference maps (F_o-F_c) for *Pf*DHODH bound to A) DSM1, B) DSM2 and C) DSM74. Maps were calculated based on the original molecular replacement solution (without cofactors and inhibitors included). The final structure is overlayed on the map. Inhibitor is displayed in pink, FMN is displayed in yellow, and the protein is displayed in dark blue. Difference maps of A) and C) are contoured at 3σ while B) is contoured at 2σ. Positive density is displayed in green and native density is displayed in red.

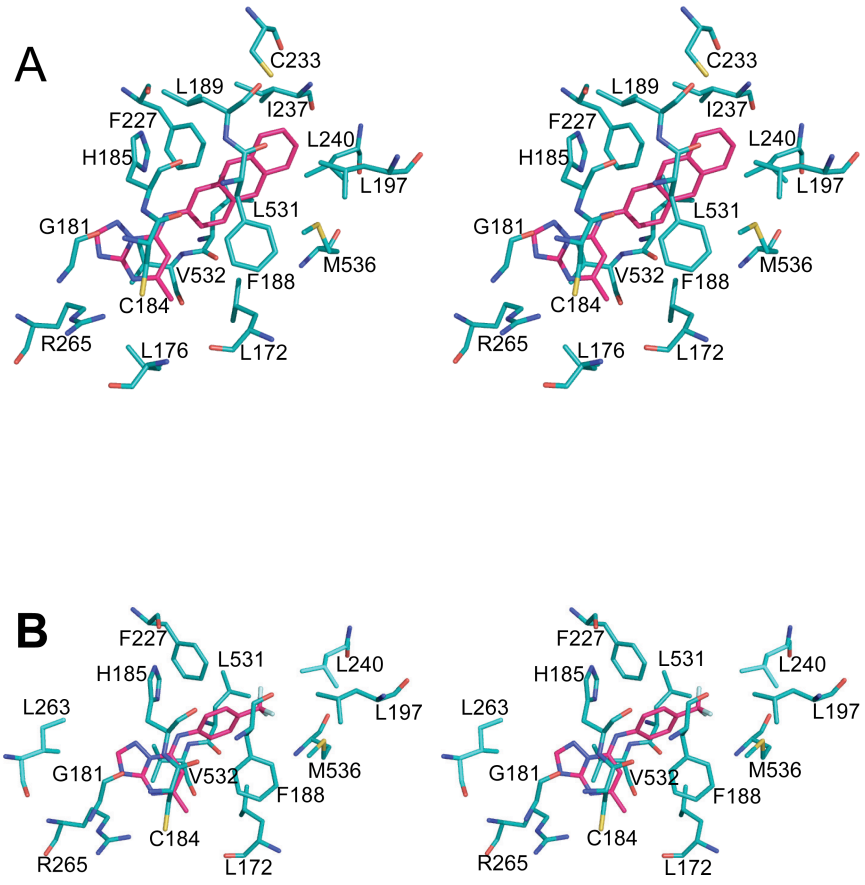
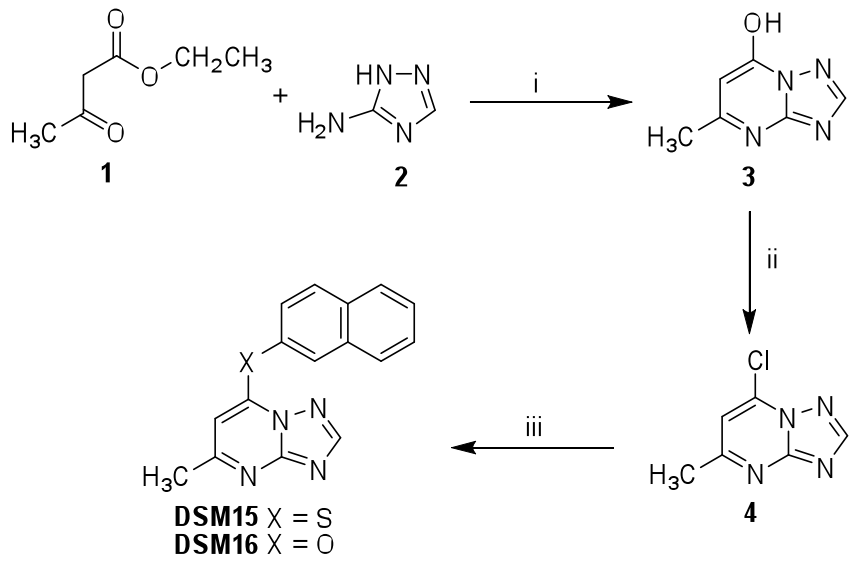


Figure S2. Inhibitor binding sites of *Pf*DHODH bound to A. DSM2 and B. DSM74 displayed as stereo pairs for residues within 4Å of the bound inhibitor. Inhibitors are displayed in pink, and residues are displayed in turquoise.



[a] Reagents and conditions: (i) AcOH, 3.5 h, reflux, 55%; (ii) $POCl_3$, 45 min., reflux, 58%; (iii) 2-Thionaphthol/2-Naphthol, K_2CO_3, DMF, 20 h, rt, 70-72%.

Fig S3. Synthetic strategy for compounds DSM15 and DSM16[a].

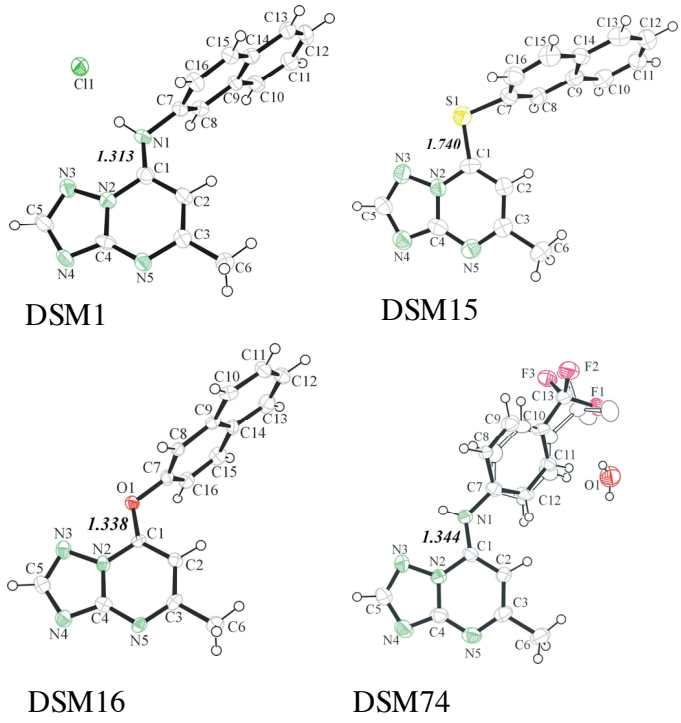


Fig. S4. ORTEP drawing of DSM compounds with thermal ellipsoids at the 50% probability level. The disordered units for DSM74 are differentiated by depicting one half in normal coloring, the other in contours only. The N1-C1 bond distances (Å) are displayed.