Electronic Supplementary Material (ESI)

Identification of P218 as a potent inhibitor of *Mycobacterium ulcerans* DHFR

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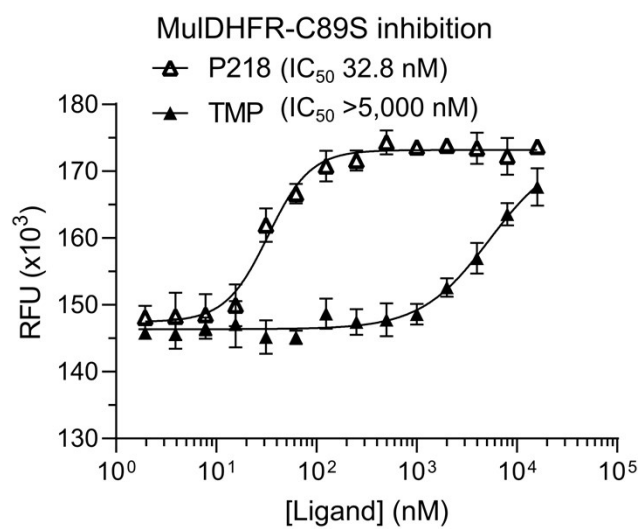
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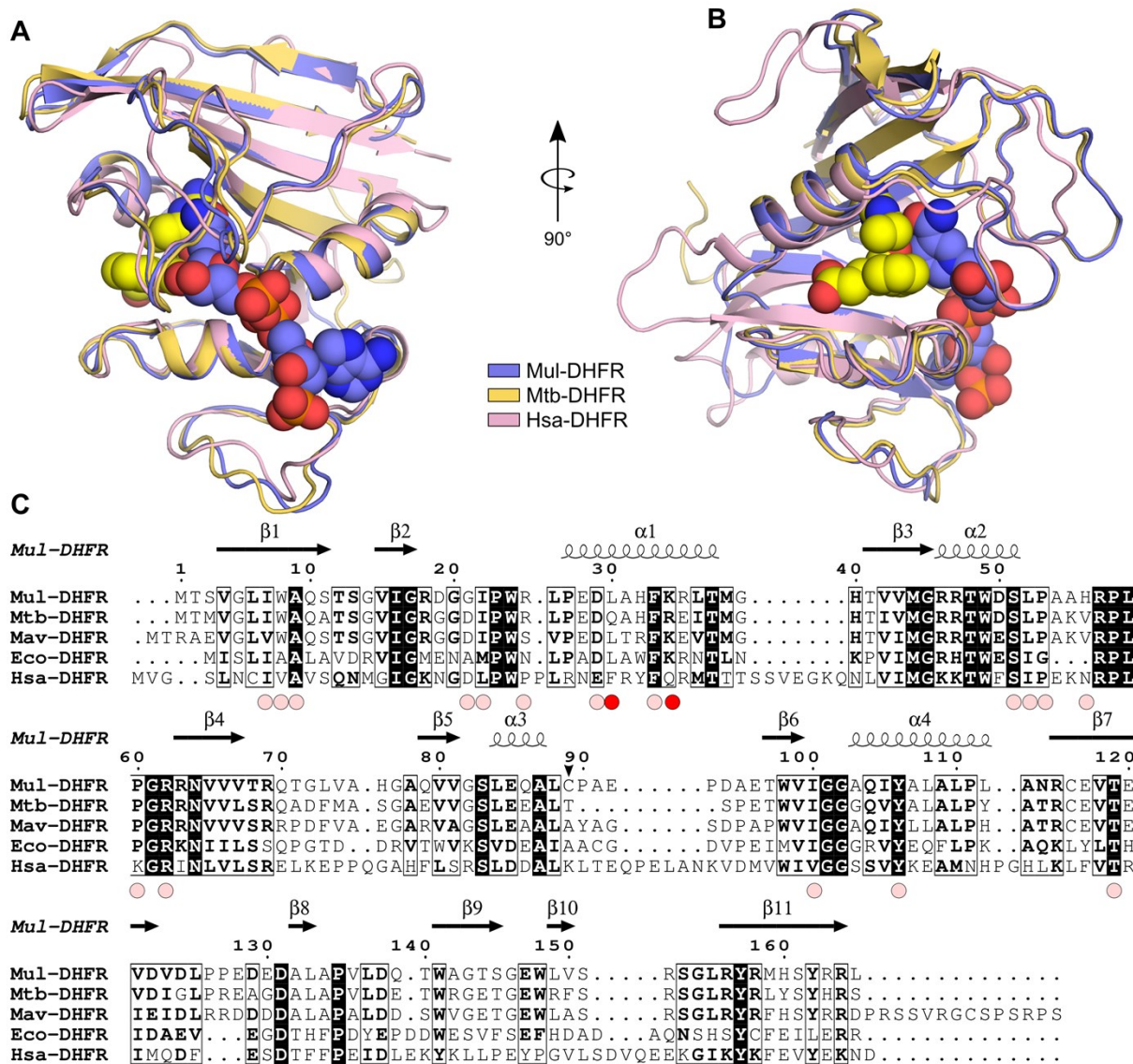* rafael.counago@unicamp.br; bart.staker@seattlechildrens.org

Supplementary Table S1. Crystallographic data and refinement statistics for MulDHFR-C89S crystals.

Data collection	
Crystal	Native
PDB ID	6UWW
X-ray source	APS LS-CAT 21-ID-D
Wavelength (Å)	0.8666
Space group	$P\ 1\ 2_1\ 1$
Cell dimensions	
a, b, c (Å)	28.73, 66.20, 44.52
α, β, γ (°)	90.000, 91.614, 90.000
Resolution (Å)	50-0.92 (0.94-0.92)
No. of unique reflections*	112,854 (8,042)
R_{merge} (%)	3.6 (49.1)
Mean I/σI	14.66 (2.01)
Mean $CC_{1/2}$	99.9 (74.8)
Completeness (%)	98.1 (94.7)
Redundancy	3.5 (2.9)
Refinement Statistics	
Resolution (Å)	50-0.92 (0.94-0.92)
R_{work} / R_{free} (%)	13.15 (21.59) / 14.54 (22.67)
No. of atoms / Mean B-factor (Å)	
Protein atoms	1,331 / 12.2
Solvent atoms	271/ 29.8
NADPH/P218 atoms	84 / 13.9
RMSD bond lengths	0.009 Å
RMSD bong angles	1.29°
Ramachandran plot (%)	
Favored	98.8
Allowed	1.2
Outliers	0

Data for the outmost shell are given in parentheses.

Supplementary Figure S1 - Enzyme inhibition of mutant MulDHFR-C89S by TMP (filled symbols) and P218 (empty symbols). The half-maximal inhibitory concentration (IC_{50}) for each compound is shown in parenthesis. Data shown are mean $\pm$ SD of triplicates.

Supplementary Fig. S2 - The structure of MulDHFR-C89S bound to P218 and NADPH reveals a conserved DHFR architecture. (A-B) Superposition of MulDHFR-C89S (blue cartoon) onto the P218-NADPH-bound structures of *M. tuberculosis* (yellow cartoon, PDB ID5U26) and human (pink cartoon, PDB ID 4DDR)[1] enzymes. P218 and NADPH, as seen in the MULDHFR-C89S co-structure, are shown as van der Waal spheres. (C) Structure-based sequence alignment of *M. ulcerans* (Mul-), *M. tuberculosis* (Mtb-), *M. avium* (Mav-), *E. coli* (Eco-), and human (Hsa-) DHFR. Pink circles indicate structurally-equivalent residues within a 4 Å radius of ligand P218, as seen in our MulDHFR-C89S structure. Red circles indicate structurally-equivalent residues thought responsible for sterically preventing P218 binding to the human enzyme. The black arrowhead indicates the position of Cys89 in MulDHFR mutated to a serine to improve protein

crystallization. Absolutely conserved residues are indicated by a black background. Similar residues are shown in bold and framed in a box. The secondary structure (α-helices, shown as coils; and β-sheets, shown as arrows), and the numbering shown in the top line are for MulDHFR. Protein sequence / structures used in were: Mul-DHFR (UniProt ID A0PQG8, PDB ID 6UWW) (this work), Mtb-DHFR (UniProt ID P9WNX1, PDB ID 5U26), Mav-DHFR (UniProt ID O30463, PDB ID 2W3W), Eco-DHFR (UniProt ID P0ABQ4, PDB ID 1RF7)[2], and Hsa-DHFR (UniProt ID P00374, PDB ID 4DDR)[1]. Structural alignment by PROMALS3D [3].

Supplementary References:

1   Y. Yuthavong, B. Tarnchompoo, T. Vilaivan, P. Chitnumsub, S. Kamchonwongpaisan, S. A. Charman, D. N. McLennan, K. L. White, L. Vivas, E. Bongard, C. Thongphanchang, S. Taweechai, J. Vanichtanankul, R. Rattanajak, U. Arwon, P. Fantauzzi, J. Yuvaniyama, W. N. Charman and D. Matthews, *Proc. Natl. Acad. Sci.*, 2012, **109**, 16823–16828.

2   M. R. Sawaya and J. Kraut, *Biochemistry*, 1997, **36**, 586–603.

3   J. Pei and N. V. Grishin, *Bioinformatics*, 2007, **23**, 802–808.