Supporting Information

Crystal structure of the anthrax drug target, *Bacillus anthracis* dihydrofolate reductase

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Contents

Supporting Table 1	page S2
Supporting Figure 1	page S3
Supporting Figure 2	pages S4-S5
Supporting Figure 3	page S6
Supporting References	page S7

Supporting Table 1: Reported IC₅₀ values (in μ M) for DHFR inhibitors

	MTX	TMP	MANIC	Iclaprim ^e	AR-709
baDHFR ^{a,b}	0.015	77.2	104.2	n.d.	n.d.
hsDHFR ^c	0.024	> 1000	n.d.	> 300	> 300
saDHFR ^d	n.d.	45	n.d.	0.007	0.9
spDHFR ^d	n.d.	42	n.d.	0.008	0.09

^a The IC₅₀ values for MTX and MANIC are reported from the present work.

^b The IC₅₀ value for TMP against baDHFR was reported in ¹.

^c The IC₅₀ values for MTX and TMP against hsDHFR were reported in ¹, and the IC₅₀ value for AR-709 against hsDHFR was reported in ².

^d The IC₅₀ values of AR-709 against hsDHFR, against DHFR from a TMP resistant *Staphylococcus aureus* mutant (saDHFR; F98Y) and against DHFR from a TMP resistant *Streptococcus pneumoniae* mutant (spDHFR; I100L) were reported in ².

^e The IC₅₀ values shown for Iclaprim (formerly AR-100) were reported in ³ and ⁴.

n.d.: not determined

Supporting Figures

Supporting Figure 1. MANIC shows inhibitory activity against baDHFR. DHFR activity was measured at 340 nm (to monitor the oxidation of NADPH to NADP⁺) over the course of 90 s in the absence and in the presence of different concentrations of MANIC. We determined that the IC₅₀ of MANIC for baDHFR is 104 μ M. The concentration of enzyme and NADPH were 50 nM and 12.5 μ M, respectively. The concentration of the DHF substrate was held constant at 125 μ M, which is assumed to be saturating. However, in a separate set of experiments, MANIC showed competitive inhibition of baDHFR at lower (2.5, 5, 10, 12.5 and 15 μ M) substrate concentrations.

a **b**

Supporting Figure 2. Structural differences between pathogenic prokaryotic DHFRs.

(a) Stereo view of the superposition of *Lactobacillus casei* (lcDHFR; dark blue) and *Mycobacterium tuberculosis* (mtDHFR; red) DHFRs onto baDHFR (green), all shown as C α traces. Regions of significant contiguous divergence between C α atoms are labeled. The MTX molecule shown (green) is from the baDHFR structure. The superposition was performed using Coot⁵. (b) An up close stereo view of the active sites of the superposed DHFR molecules, with residue differences between the three structures shown (coloring as in panel A). Contacts to the pteridine ring of MTX are conserved but interactions formed

with the *p*-ABA-Glu tail differ between the structures. Note the Gly (baDHFR) → Pro (lcDHFR and mtDHFR) substitution at position 52 of the baDHFR sequence. This corresponds to position 61 in hsDHFR and is also a Pro.

Supporting Figure 3. The diaminopyrimidine iclaprim may bind preferentially to baDHFR compared to hsDHFR. Stereo view of iclaprim (orange) modeled into the baDHFR active site. Iclaprim was docked into the baDHFR (carbon: green, oxygen: red, nitrogen: blue, sulfur: orange) active site and then the complex was energy minimized. The resultant conformation of iclaprim reveals that it does not clash with baDHFR but forms very close contacts with Phe 34, Phe 31 and Pro 61 of hsDHFR (carbon: cyan, oxygen: red, nitrogen: blue, sulfur: orange).

Supporting References

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