Supporting Information

Thermodynamic Investigation of Inhibitor Binding to 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase

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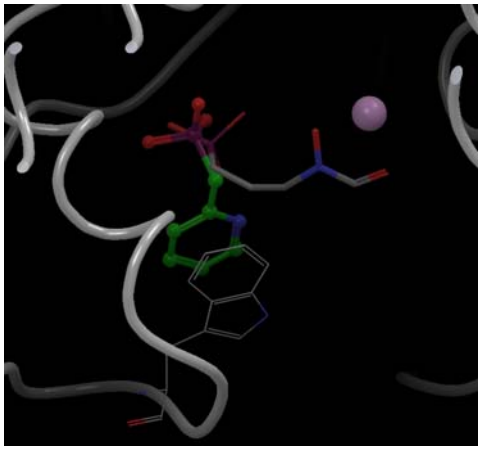
A. 

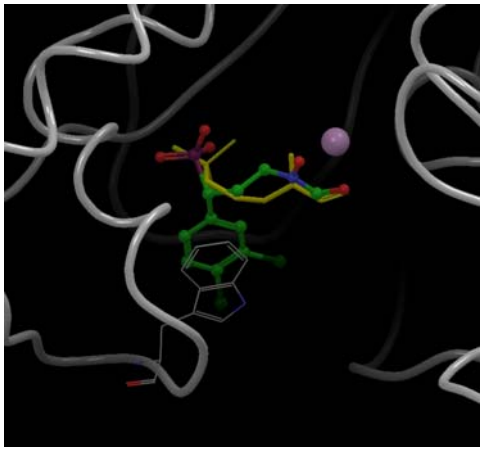
B. 

Figure S1. (**A**) Superimposed crystal structures of the *Ec*DXR:**1** and *Ec*DXR:**4** complexes,[1] showing the two phosphonate groups are located in the same site, but **4** does not occupy the hydroxamate/Mg^{2+} binding pocket. Mg^{2+} is shown as a pink sphere; (**B**) Superimposition of the *Ec*DXR:**1** crystal structure with a modeled structure of the *Ec*DXR:**3** complex,[1] showing the hydrophobic 3,4-dichlorophenyl group of **3** is located in the pyridine binding site of **4** (having a favorable π-π stacking interaction with the indole ring of Trp211).

Experimental Section

Inhibitor. Compounds **1** – **6** were from the same batches for our previous work.[1-3] Compounds were characterized by ^{1}H NMR on a Varian (Palo Alto, CA) 400-MR spectrometer and the purities monitored by a Shimadzu Prominence HPLC with a Zorbax C18 or C8 column (4.6 x 250 mm) or using ^{1}H (at 400 MHz) absolute spin-count quantitative NMR analysis with imidazole as an internal standard.

Expression and purification of DXR. Recombinant *E. coli* and *M. tuberculosis* DXR (*Ec*DXR and *Mt*DXR) enzymes were produced using our previous methods in ~90% purity (SDS-PAGE).[3] *Plasmodium falciparum* DXR (*Pf*DXR, catalytic domain 75-488) was cloned from *P. falciparum* genomic DNA (Malaria Research and Reference Reagent Resource Center, Manassas, VA) using 5'-GCGGATCCAAGAAACCAATTAATGTAGC-3' and 5'-GCAAGCTTCTATGAAGAATTATGTTTGTTGT-3' as forward and reverse primers, respectively, and inserted into pQE30 expression vector (Qiagen). The correctness of insert was verified by sequencing. The plasmid was transformed into *E. coli* (M15 strain) and cultured in LB medium containing kanamycin (25 μg/mL) and ampicillin (50 μg/mL). Upon reaching an optical density of ~0.6 at 600 nm, *Pf*DXR expression was induced by adding 0.2 mM isopropylthiogalactoside (IPTG) for 5 hours at 37°C. Cells were harvested and disrupted and His6-tagged recombinant *Pf*DXR was purified using HisTrap HP column (Ni-affinity, GE Healthcare) followed by Superdex 75 (GE Healthcare) column chromatography. *Pf*DXR was obtained with >90% purity (SDS-PAGE).

Isothermal titration calorimetry. ITC experiments were performed using a Nano ITC LV (190 μL) instrument from TA Instruments (New Castle, DE) at 279 K, at which DXR enzymes are stable during the experiments (lasting for ~2h). A 190 μL solution containing a DXR enzyme (40 μM), 2 mM NADPH and 150 mM NaCl with or without a divalent metal ion (1 mM Mg^{2+} or Mn^{2+}) in 50 mM HEPES buffer (pH = 7.6) was added into the sample cell. An inhibitor (0.4 - 0.6 mM) solution in the same buffer (50 μL) was transferred into the syringe of the instrument. Upon reaching temperature equilibrium, 25 injections (2 μL each) of the inhibitor were used for the titration. ITC data were then

imported into NanoAnalyze software (TA Instruments, New Castle, DE). The titration baseline was corrected and the ΔH and K_a values for the binding of the inhibitor were obtained by fitting into the independent model in the software. All experiments were repeated for at least one time to ensure the reported data are reliable.

Enzyme inhibition assays. For *Pf*DXR, we first determined the K_m value for the substrate DXP. The kinetic assay was performed in a 96-well microplate using the purified *Pf*DXR (50 nM), 1 mM $MnCl_2$, 100 μM NADPH and an increasing concentration of DXP (10 - 1000 μM) in 50 mM HEPES buffer (pH = 7.6) containing 50 μg/mL BSA (bovine serum albumin). The process was monitored at 340 nm with a Beckman DTX-880 microplate reader. The data were imported into Prism (version 5.0, GraphPad Software, Inc., La Jolla, CA) and the K_m value was determined to be 106 μM by fitting to the Michaelis-Menten model in the software. The inhibition assays in triplicate were performed using *Pf*DXR (50 nM), an increasing concentration of an inhibitor, 1 mM $MnCl_2$, 100 μM DXP and 100 μM NADPH in 50 mM HEPES buffer (pH = 7.6) containing 50 μg/mL BSA (bovine serum albumin). Inhibitors were pre-incubated with *Pf*DXR for 5 min at 30 °C, before adding DXP to initiate the reaction. The IC_{50} values were then obtained by using a standard dose response curve fitting in Prism. If $IC_{50} >>$ [*Pf*DXR] (50 nM), the K_i values were calculated using the formula $K_i = IC_{50}/(1+[S]/K_m)$, where [S] is the concentration of DXP (100 μM) and K_m is the literature value of 106 μM. If $IC_{50} < 10 \times$ [*Pf*DXR], the K_i values were calculated by fitting the initial velocities to the Morrison tight inhibition model in Prism, using the K_m value for DXP (106 μM) as well as the concentration of *Pf*DXR (50 nM). The inhibition assays for *Ec*DXR and *Mt*DXR were performed similarly, as described in detail in our previous work.

References for Supporting Information

(1) Deng, L.; Endo, K.; Kato, M.; Cheng, G.; Yajima, S.; Song, Y. *ACS Med. Chem. Lett.* **2011**, *2*, 165.

(2) Deng, L.; Sundriyal, S.; Rubio, V.; Shi, Z.; Song, Y. *J. Med. Chem.,* **2009**, *52*, 6539.

(3) Deng, L.; Diao, J.; Chen, P.; Pujari, V.; Yao, Y.; Cheng, G.; Crick, D. C.; Prasad, B. V. V.; Song, Y. *J. Med. Chem.*, **2011**, *54*, 4721.