Supporting Information (Revised version)

Design of Inhibitors of Orotidine Monophosphate Decarboxylase using Bioisosteric Replacement and Determination of Inhibition Kinetics

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Figure S-1. The assays were done in 100 mM Tris buffer containing 1 mM TCEP at pH 7.5 and 55 °C.

(A) The thermogram (top panel) and the corresponding isotherm (bottom panel) generated upon titrating 6-aza-UMP (**3**) into a solution of ODCase. The enzyme concentration was 14 μM. The ligand concentration in the syringe was 10 mM. The volume of the first injection was 1 μL and those of all subsequent injections were 5 μL each, resulting in a 35 μM- increment in concentration for each injection.

(B) The thermogram (top panel) and the corresponding isotherm (bottom panel) generated upon titrating 6-cyano-UMP (**5)** into a solution of ODCase. The enzyme concentration was 50 μM. The ligand concentration in the syringe was 35 mM. The volume of the first injection was 1 μL and those of the subsequent injections were 5 μL resulting in an increment of 122 μM concentration per each injection.

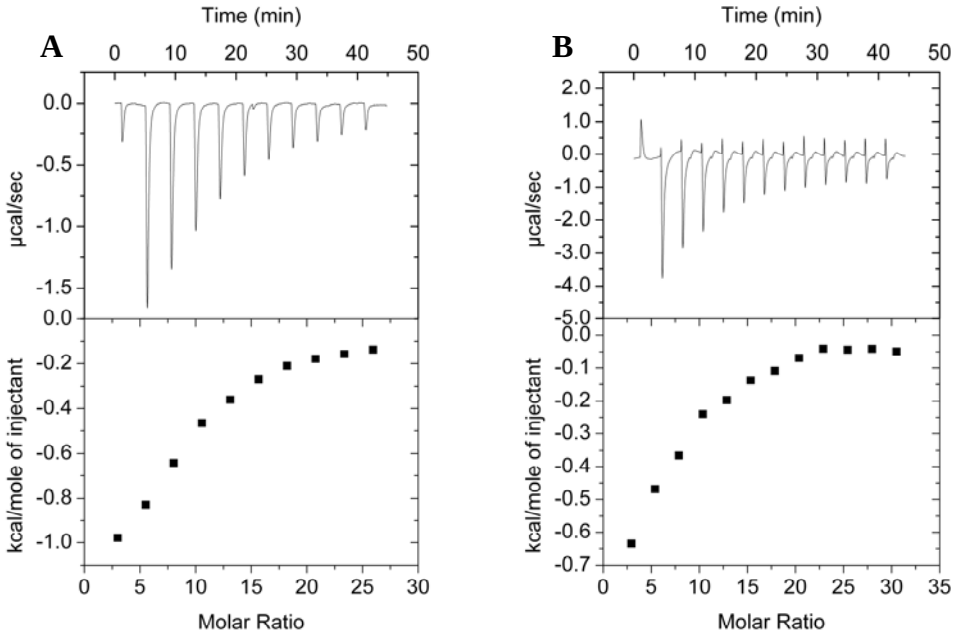
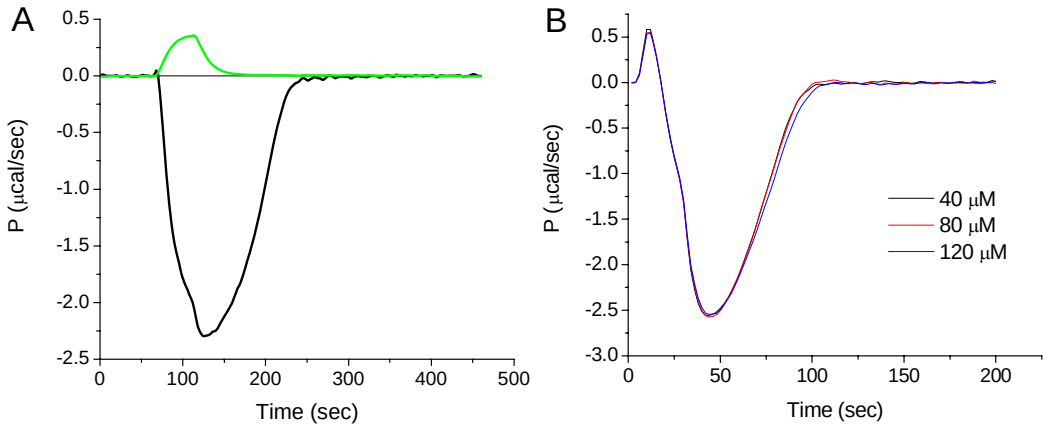


Figure S-2. (A) The dilution heat due to UMP (40 μM) injected into an ODCase solution (20 nM) (green) is compared to the rate of OMP decarboxylation (black) after the single injection of OMP (40 μM). Addition of UMP to an ODCase solution does not generate any observable heat, but only produced the heat of dilution (small peak in green after the injection) and indicated that the binding of UMP to ODcase is not favored. This is also evident from the kinetics experiments from other groups, where the K_i for UMP is 0.2 mM and K_M for OMP is 0.7 μM (for the yeast enzyme). (B) ITC experiments were performed to investigate product inhibition in the catalytic reaction by ODCase. Consecutive injections of OMP (three injections, each injection was 20 μL, OMP concentration 40 μM) into the same ODCase enzyme solution(20 nM) were performed, and the heat was recorded with respect to time. The rate of the enzyme catalysis as seen in the isotherms after each injection was identical (black, red and blue lines, indicating the three different injections of 40 μM OMP, and the cumulative concentrations of UMP after the 1st, 2nd and 3rd injection) indicating that the product (UMP) present in the solution as a result of the first reaction did not influence the rate of catalysis by ODCase after the second or third injections of OMP. In other words, UMP is a very weak inhibitor of ODCase catalysis, at best, and does not show any observable influence on the isotherms in the ITC experiments.



Purity for Compounds 5 and 6.

Purities of the compounds **5** and **6** were tested on a Waters Delta 660 HPLC system attached to a PDA detector. Profiles of the purity are described below. Column was a Waters Spherisorb (ODS2) reverse phase with an I.D. of 5 μm (4.6x100 mm length). All solvents were purchased from commercial sources, filtered through Waters membranes 47 mm GHP 0.45μm, Pall Corporation and degassed with helium. Injection samples were filtered using Waters Acrodisc ® Syringe Filters 13 mm PTFE 0.2 μm.

Compound #	Solvent	Flow rate (mL/min)	Retention time (min)	Purity (from integration)
5	AcOH:AcCN:H_2O (2.4:20:77.8)	0.5	1.5	99.2 (λ_{max} 281 nm)
5	AcOH:H_2O (3%)	0.5	3.5	100 (λ_{max} 281 nm)
6	AcOH:H_2O (3%)	0.5	3.05	100 (λ_{max} 271 nm)
6	AcOH:AcCN:H_2O (2.4:20:77.8)	0.5	2.6	100 (λ_{max} 271 nm)