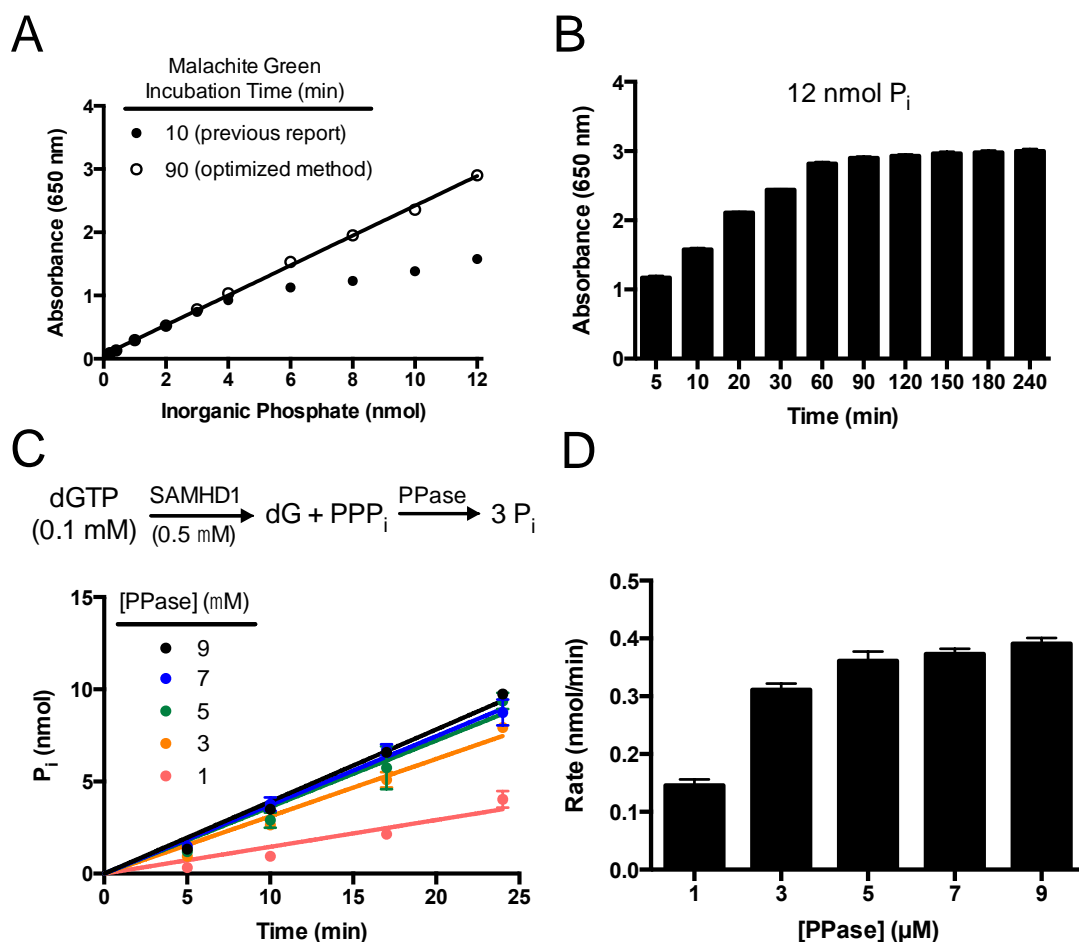


***Supporting Information***

Development of a High-Throughput Enzyme-Coupled Screen of SAMHD1

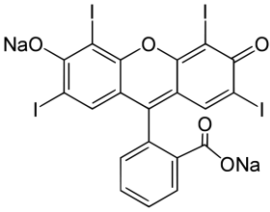
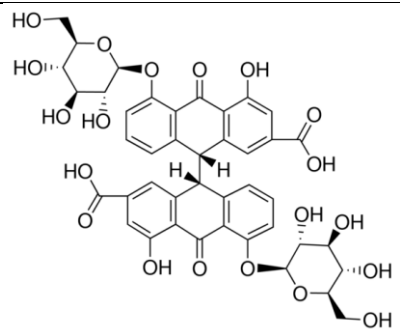
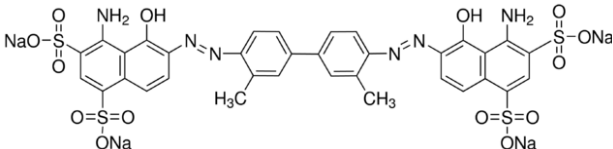
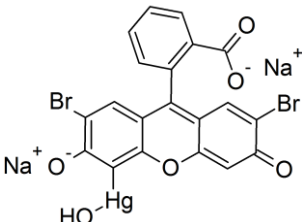
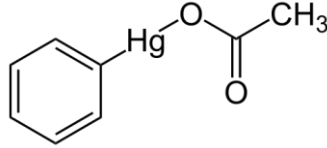
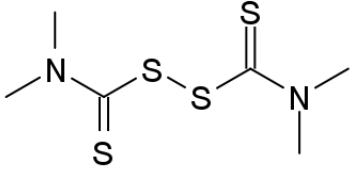
dNTPase Activity

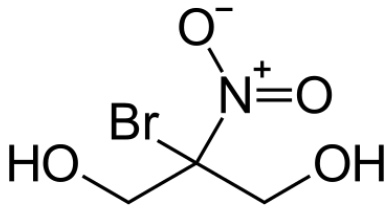
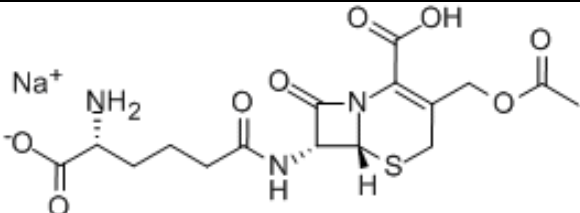
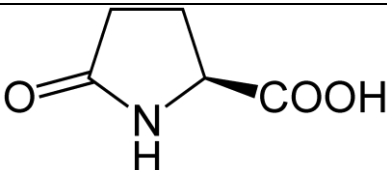
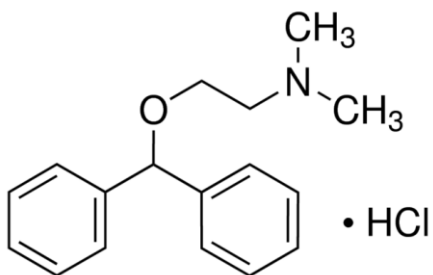
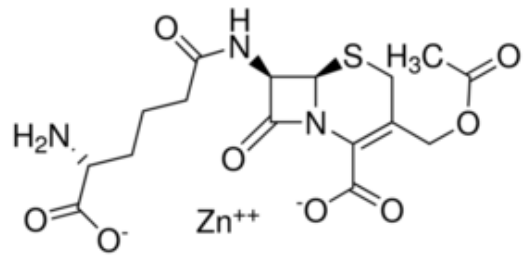
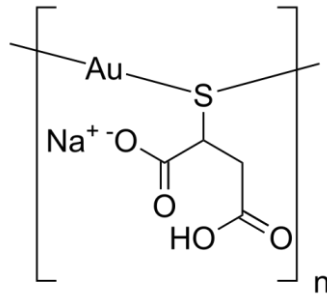
Kyle J. Seamon and James T. Stivers

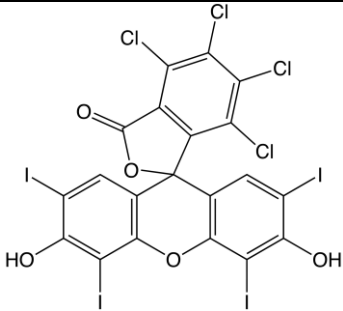
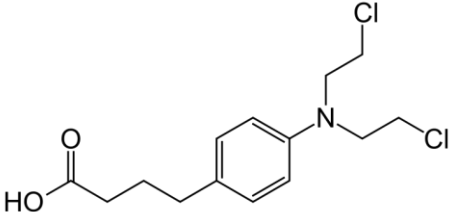
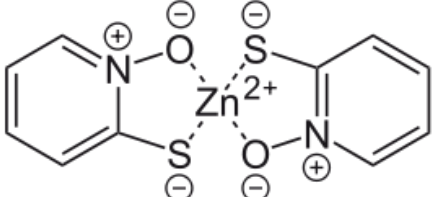


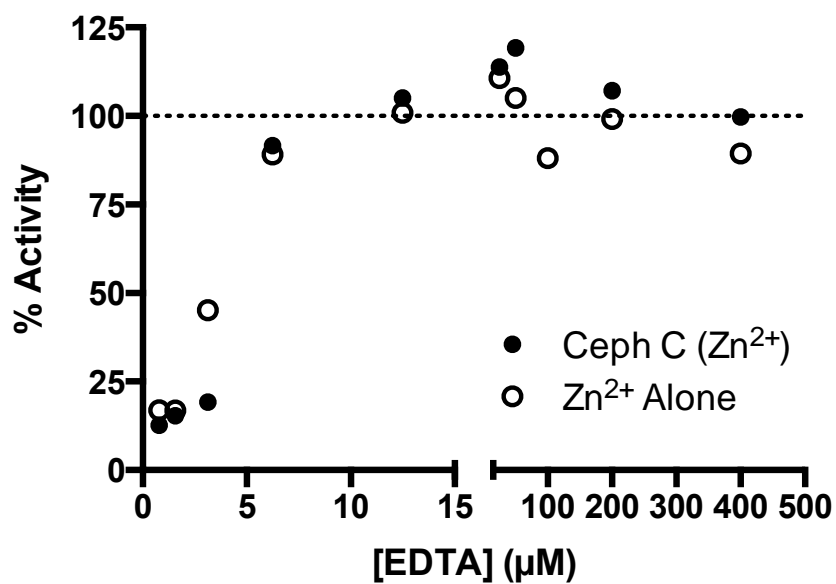
**Figure S1.** Development of the enhanced enzyme-coupled MG assay for  $P_i$ . **(A)** Standard curves for the detection of phosphate by the enhanced MG assay. An increased linear range and signal yield is obtained by increasing the incubation time for color development to 90 min (open circles). Previous versions of the MG assay have only used a 10 min incubation time, which limits the linear range and color yield (closed circles). **(B)** Time course for the development of MG color. The color yield from 12 nmol of a  $P_i$  standard (the maximal phosphate present at 100% SAMHD1 reaction) is complete after 90 minutes and remains stable for an additional 2.5 hours. **(C)** Background-corrected plots of phosphate-production against time were collected in the presence of fixed  $[SAMHD1]$  (0.5  $\mu\text{M}$ ) and  $[dGTP]$  (0.1 mM) with varying  $[PPase]$  (1 to 9  $\mu\text{M}$ ). **(D)** The rates of phosphate production from the linear regression slopes in panel (C) show that the overall rate is zero-order in PPase when its concentration exceeds 5  $\mu\text{M}$ . Thus, the rate-limiting step is the production of PPPi by SAMHD1.

**Table S1.** Structures of 6σ hits with percent activity remaining in primary and secondary assays

Compound #	Inhibitor	Structure	% Activity (1° Screen)	% Activity (2° TLC)
1	Erythrotyrosine (Na <sup>+</sup> Salt)		33	62
2	Sennoside A		39	80
3	Evans Blue (Na <sup>+</sup> Salt)		10	3
4	Merbromin (Na <sup>+</sup> Salt)		14	3
5	Phenylmercuric Acetate		30	<0.01
6	Thiram		45	24

7	Bronopol		23	22
8	Cephalosporin C (Na <sup>+</sup> Salt)		13	2
9	Pidolic Acid		19	106
10	Diphenhydramine (HCl Salt)		45	157
11	Cephalosporin C (Zn <sup>2+</sup> Salt)		12	<0.01
12	Aurothiomalate (Na <sup>+</sup> Salt)		33	14

13	Rose Bengal		43	10
14	Chlorambucil		31	135
15	Pyrithione Zinc		42	44



**Figure S2.** Rescue of SAMHD1 inhibition by Ceph C (Zn<sup>2+</sup>) and Zn<sup>2+</sup> alone with the addition of the Zn<sup>2+</sup> chelator EDTA. Reactions of SAMHD1 were prepared with a fixed inhibitor concentration (5 μM) and addition of increasing concentrations of EDTA. SAMHD1 activity is completely rescued with a near-stoichiometric concentration of EDTA.