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Supplemental Data

An Inhibitor of Human Asparagine Synthetase

Suppresses Proliferation of an

L-Asparaginase-Resistant Leukemia Cell Line

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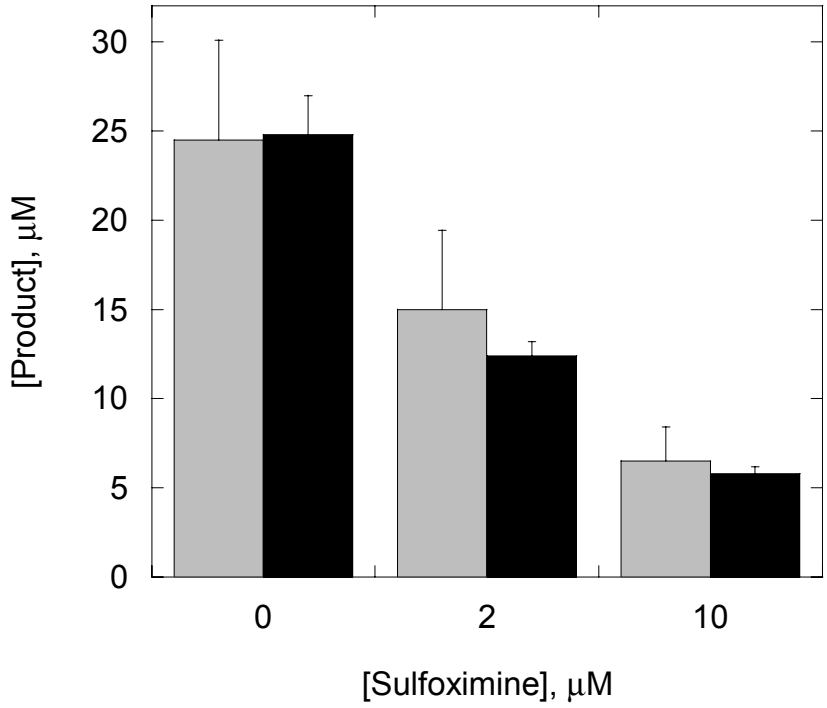
Table S1. Specific Activity of hASNS Samples Incubated in the Absence of Substrates with Various Concentrations of the Adenylated Sulfoximine **1**

	Specific Activity (U/mg)[a]	% Activity[a]
WT hASNS	0.46 (0.38)	100 (83)
WT hASNS + 5 μM **1**	0.38 (0.31)	83 (67)
WT hASNS + 10 μM **1**	0.26 (0.24)	56 (52)

[a] Values shown in parentheses are for enzyme/inhibitor samples that were not filtered through the G-50 column prior to the addition of substrates.

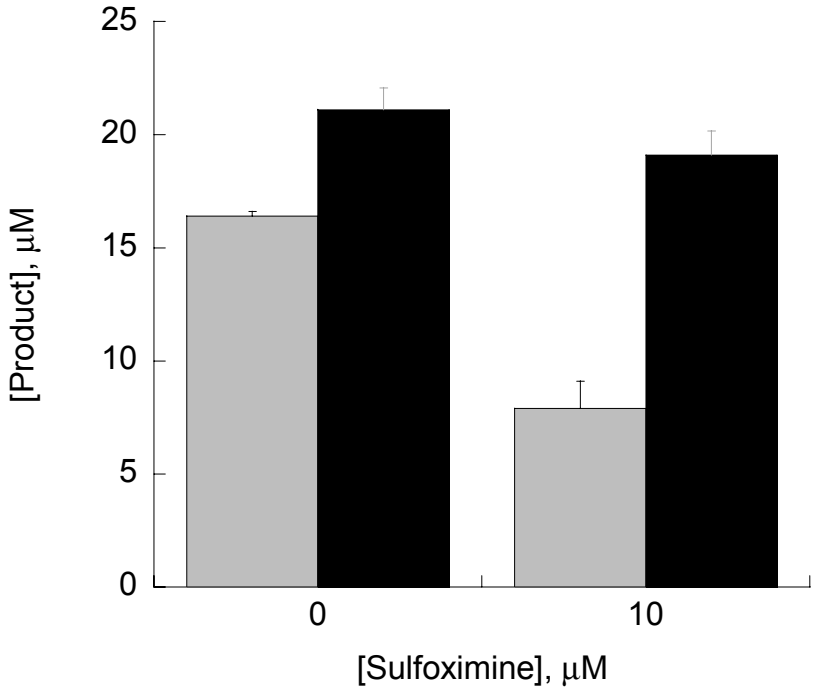
Procedures for these experiments are detailed in the main text of the manuscript. The higher activity values for enzyme samples that were passed through the G-50 column may be a consequence of the elimination of components present in the enzyme stock solution. Control experiments were also undertaken to ensure that the inhibitor **1** does not co-elute with the enzyme on passage through the G-50 column.

Figure S1. Effect of the Adenylated Sulfoximine **1** on Asn:PP_i Stoichiometry



ASNS activity was routinely measured by following the production of inorganic pyrophosphate (PP_i) because this is a simple continuous assay. In order to ensure that the inhibitor **1** did not affect the 1:1 ratio of Asn:PP_i, we performed control experiments in which asparagine production in the presence of **1** was measured independently using an end-point HPLC assay (grey bars) (see Experimental Procedures) and compared with PP_i detected using the continuous assay (black bars). The results, shown above, confirm that **1** does not affect the product stoichiometry. Errors represent the standard error of triplicate measurements.

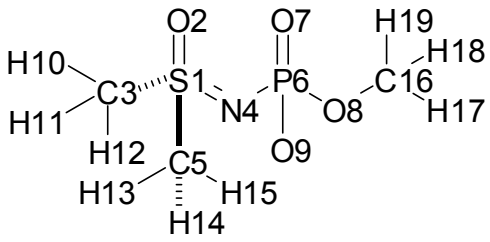
Figure S2. Effect of the Adenylated Sulfoximine **1** on Asn:Glu Stoichiometry



In order to evaluate the effect of the inhibitor **1** on the ratio of Asn:Glu, we performed control experiments in which asparagine (grey bars) and glutamate (black bars) production in the presence of **1** were measured independently using an end-point HPLC assay (see Experimental Procedures). Errors represent the standard error of triplicate measurements. In these experiments, hASNS (2.5 μg) was added to solutions of 5mM ATP, 10 mM L-aspartic acid, 10 mM $MgCl_2$ and 25 mM L-glutamine in 100 mM EPPS buffer, pH 8, in the absence or presence of the adenylated sulfoximine **1** (10 μM) at 37 ^{o}C. The reaction was quenched after 20 min, and the amino acids derivatized using DNFB. The amount of DNP-glutamate and DNP-asparagine were then measured by HPLC.

Table S2. Structure and Electronic Properties of the PM3-Optimized Phosphorylated Sulfoximine **2**

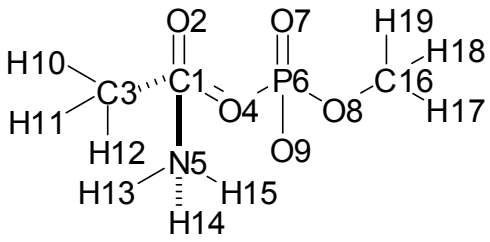
Numbers in the table refer to the atoms shown in the following structure (C5 is the equivalent of the ammonia nitrogen N5 in model transition state **3**).



Atom	X	Y	Z	Partial Charge (e)	Bond	Length (Å)
S1	-2.251	0.495	0.361	2.001	S1-O2	1.54
O2	-1.683	1.705	1.129	-0.977	S1-C3	1.77
C3	-1.222	0.182	-1.042	-0.473	S1-C5	1.77
N4	-2.503	-0.932	1.14	-1.258	S1-N4	1.65
C5	-3.81	0.963	-0.331	-0.476	C5-H13	1.10
P6	-2.108	-1.522	2.739	2.135	C5-H14	1.10
O7	-0.925	-2.518	2.678	-1.111	C5-H15	1.10
O8	-3.552	-2.515	2.883	-0.696		
O9	-2.17	-0.486	3.877	-1.104	Bond Angle	Angle (deg)
H10	-0.171	0.024	-0.757	0.137		
H11	-1.532	-0.712	-1.602	0.131	C5-S1-O2	108.2
H12	-1.23	1.02	-1.755	0.132	C5-S1-N4	106.2
H13	-3.721	1.825	-1.008	0.132	C5-S1-C3	104.4
H14	-4.273	0.153	-0.914	0.132	O2-S1-N4	120.1
H15	-4.539	1.247	0.443	0.137	O2-S1-C3	108.7
C16	-3.694	-3.221	4.071	0.1	O4-S1-C3	108.1
H17	-2.834	-3.105	4.741	0.028		
H18	-3.817	-4.282	3.824	0.015		
H19	-4.598	-2.867	4.58	0.015		

Table S3. Structure and Electronic Properties of the PM3-Optimized Transition State **3** for Ammonia Attack on an Acylphosphate

Numbers in the table refer to the atoms shown in the following structure.



Atom	X	Y	Z	Partial Charge (e)	Bond	Length (Å)
C1	-0.825	0.955	1.099	0.263	C1-O2	1.36
O2	0.536	0.955	1.099	-0.648	C1-C3	1.53
C3	-1.52	2.316	1.099	-0.126	C1-N5	1.64
O4	-1.02	0.07	0.069	-0.603	C1-O4	1.37
N5	-1.35	0.154	2.426	0.513	N5-H13	1.00
P6	0.68	-0.239	-0.397	2.005	N5-H14	1.00
O7	1.429	0.729	-1.349	-0.94	N5-H15	1.00
O8	-0.364	-1.103	-1.556	-0.668		
O9	1.419	-1.365	0.365	-0.976	Bond Angle	Angle (deg)
H10	-1.137	2.954	0.288	0.079		
H11	-2.601	2.226	0.928	0.021	N5-C1-O2	108.7
H12	-1.342	2.87	2.03	0.016	N5-C1-O4	104.3
H13	-1.32	0.744	3.232	-0.021	N5-C1-C3	106.9
H14	-2.298	-0.138	2.303	-0.006	O2-C1-O4	98.2
H15	-0.801	-0.659	2.616	0.038	O2-C1-C3	117.1
H16	0.212	-1.99	-2.45	0.178	O4-C1-C3	120.7
H17	1.308	-1.987	-2.424	-0.015		
H18	-0.119	-1.735	-3.465	-0.052		
H19	-0.142	-3.003	-2.22	-0.058		