Supplementary Material

Inhibitor Screening using Immobilized Enzyme-Reactor Chromatography/Mass Spectrometry

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LC/MS Settings

Specific MS/MS parameters for each ion pair are shown in Table S1. The total scan time was 2 seconds per point.

Table S1

Species	Q1 (m/z)	Q3 (m/z)	Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
Adenosine	268	136	495	66	6.8	29.4	3.3
Inosine	269	137	495	49	4.0	22.0	4.0
EHNA*	278	119	495	90	10.0	52.0	4.0
EHNA	278	136	495	90	10.0	52.0	4.0

*Note: The weaker signal from this EHNA ion-pair was not used for data analysis but it's presence in the MRM method leads to a total scan time of 2 seconds, including 20 msec pause.

Compounds Tested:

#	Name
1	(-)- Sulpiride
2	(-)- Butaclamol.HCl
3	Haloperidol
4	4-Nitrophenethyl bromide
5	Spiperone
6	L-glutamicacid-γ-(p-nitroanilide).HCl
7	Dipyridamol
8	ATP
9	Glybenclamide
10	Glipizide
11	Benzamidine.HCl
12	Dextromethorphan.HBr
13	Pyrimethamine
14	N-Acetyl-L-Tryptophanamide
15	Trimethoprim
16	XTT.Na
17	Deoxycholic acid
18	2-Fluoro-2'-deoxyadenosine
19	Carbamylcholine.Cl
20	Chloropromazine.HCl
21	Acetopromazine.maleate

22	Nα-Benzoyl-DL-Arginine-p-nitroanalide
23	p-nitrophenyl butyrate
24	7-chloro-4-nitrobenz-2-oxa-1,3-diazole
25	N-benzoyl-L-Tyrosine ethyl ester
26	Acetaminophen
27	Caffeine
28	7-Azaindole
29	Cytidine 2':3'-cyclic monophosphate.Na
30	Nε-Acetyl-L-lysine
31	Acetylcholine.Cl
32	(+)- Tubocurarine.Cl
33	Uridine 5'-diphospho-n-acetyl glucosamine
34	Phospho(enol)pyruvate.K
35	(-)- Nicotine.H-tartarate
36	Adenine
37	Gly-gly
38	1,10-phenanthroline
39	EHNA
40	Sarcosine
41	Indole
42	Betaine
43	Trifluoperazine dihydrochloride
44	Activicin
45	p-nitrophenyl acetate
46	Guanylyl (2'-5') cytidine.NH_4
47	Acetylthiocholine.Cl
48	DL-Azatryptophan
49	+/- Epibatidine.2HCl

Relationship between IC_{50} and K_I:

The K_I value was determined by extrapolation of IC_{50} values to the point of zero substrate concentration, as shown in Figure 6b. The basis of this method is as follows, and is based on the derivation described by Cheng and Prusoff (ref 19).

The Michaelis-Menten equation is given by:

$$V_o = \frac{V_{\max}[S]}{K_m + [S]}$$

where V_0 is the initial rate of the enzyme catalyzed reaction. In the presence of a competitive inhibitor with an inhibition constant K_I and a concentration $[I]$, the rate of reaction will be reduced to V_I:

$$V_I = \frac{V_{max}[S]}{K_m(1+\frac{[I]}{K_i})+[S]}$$

By definition, if $[I]$ is equal to IC_{50} then $V_I = ½V_o$ and hence:

$$\frac{V_{max}[S]}{K_m+[S]} = \frac{2V_{max}[S]}{K_m(1+\frac{IC_{50}}{K_i})+[S]}$$

Rearranging and solving for IC_{50}:

$$\frac{1}{K_m+[S]} = \frac{2}{K_m(1+\frac{IC_{50}}{K_i})+[S]}$$

$$\therefore K_m(1+\frac{IC_{50}}{K_i})+[S] = 2K_m+2[S]$$

$$\therefore K_m(1+\frac{IC_{50}}{K_i}) = 2K_m+[S]$$

$$\therefore 1+\frac{IC_{50}}{K_i} = 2+\frac{[S]}{K_m}$$

$$\therefore \frac{IC_{50}}{K_i} = 1+\frac{[S]}{K_m}$$

$$\therefore IC_{50} = K_i(1+\frac{[S]}{K_m})$$

Rearranged in the form of a linear equation:

$$IC_{50} = K_i + \frac{K_i[S]}{K_m}$$

$$\therefore IC_{50} = \frac{K_i}{K_m}[S]+K_i$$

Hence in a plot of IC_{50} vs [S], the slope is K_i/K_m, the y-intercept is K_i, and the negative x-intercept is $-K_m$. Note that for the enzyme reactor column, product concentration, rather than reaction rate, is monitored. Thus, the equation will be valid only under conditions where the initial rate is proportional

to product concentration. As noted in the text, for our system conversions of 30% or less lead to errors of 7% or less in estimation of rate data from product concentrations.

K_m value in solution

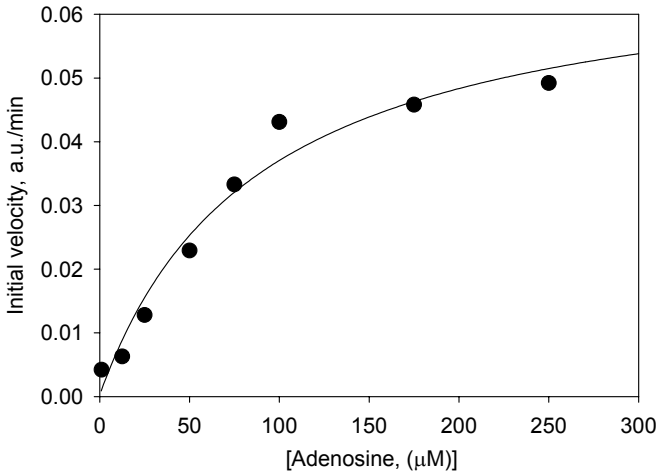

Figure S1. Assessment of K_m values for ADA in solution using an absorbance-based assay as described in reference 16. K_m was determined to be 89 μM.