

A novel allosteric inhibitor of the uridine diphosphate *N*-acetylglucosamine pyrophosphorylase from *Trypanosoma brucei*

Michael D. Urbaniak¹, Iain T. Collie¹, Wenxia Fang², Tonia Aristotelous¹, Susanne Eskilsson¹, Olawale G. Raimi², Justin Harrison¹, Iva Hopkins Navratilova¹, Julie A. Frearson¹, Daan M. F. van Aalten^{2,3} and Michael A. J. Ferguson^{1*}

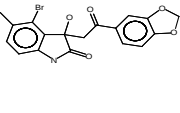
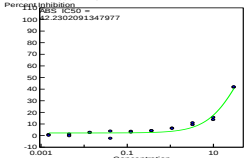
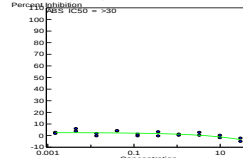
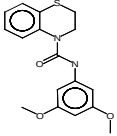
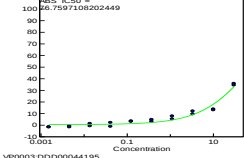
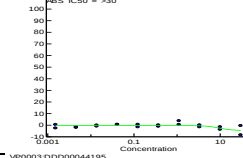
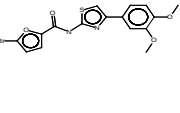
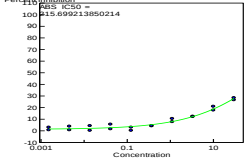
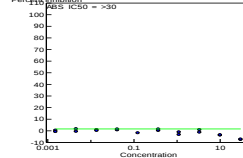
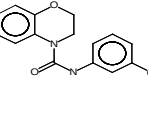
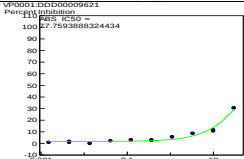
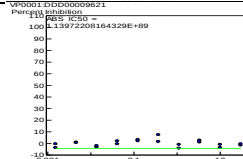
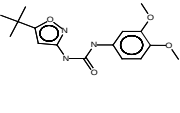
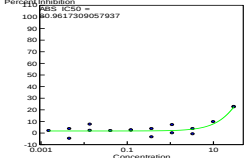
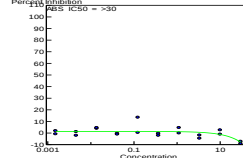
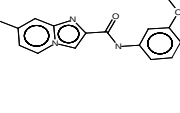
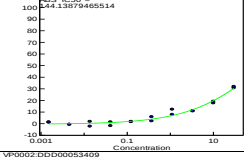
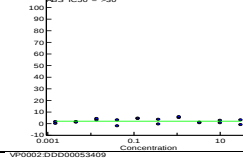
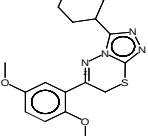
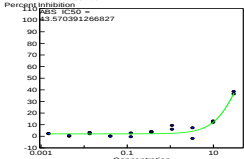
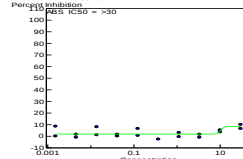
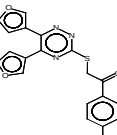
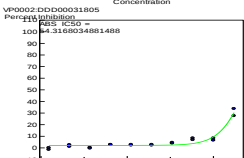
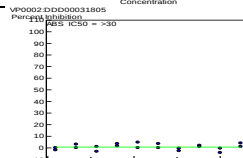
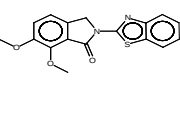
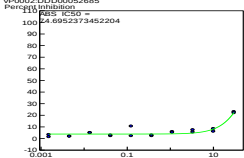
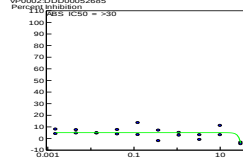
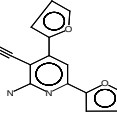
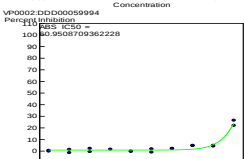
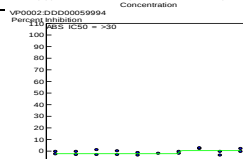
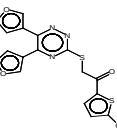
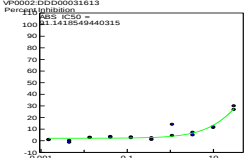
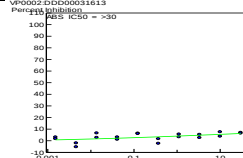
¹Division of Biological Chemistry and Drug Discovery, ²Division of Molecular Microbiology and ³MRC Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

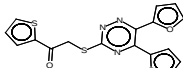
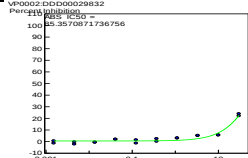
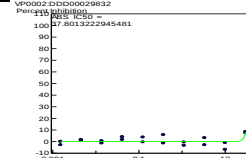
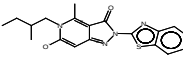
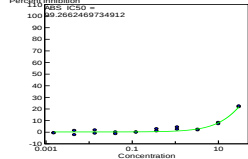
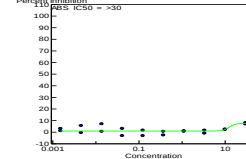
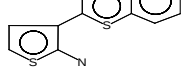
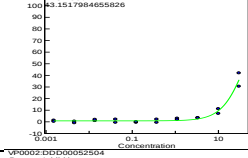
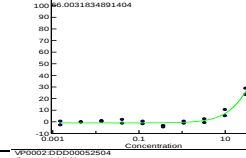
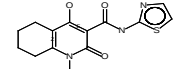
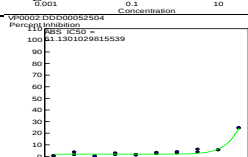
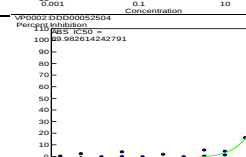
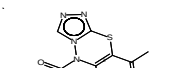
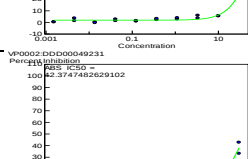
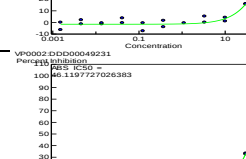
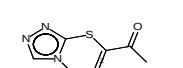
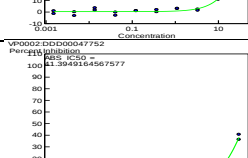
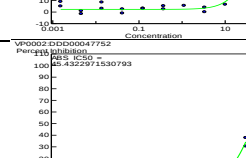
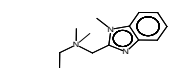
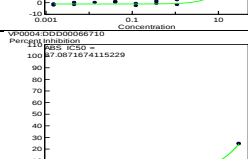
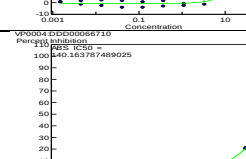
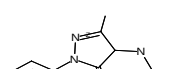
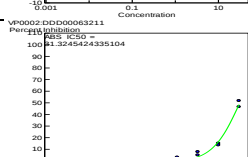
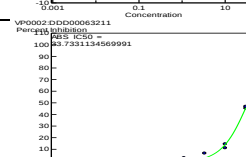
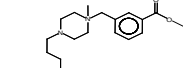
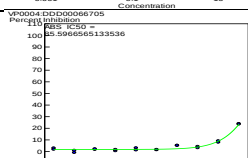
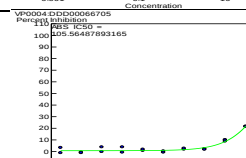
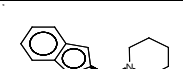
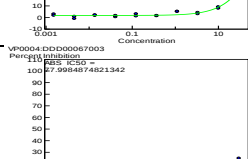
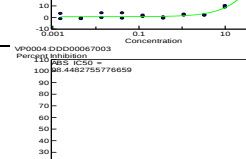
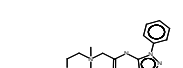
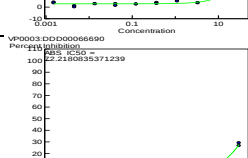
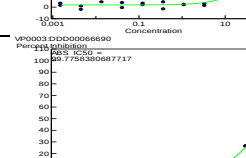
*To whom correspondence should be addressed, m.a.j.ferguson@dundee.ac.uk

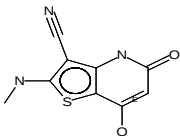
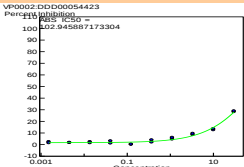
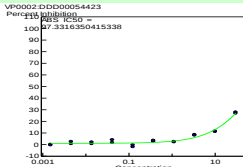
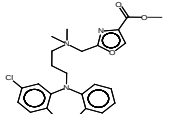
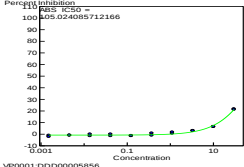
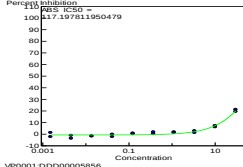
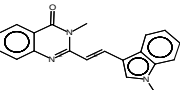
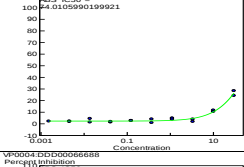
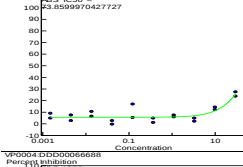
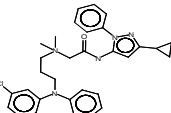
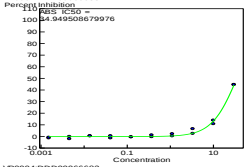
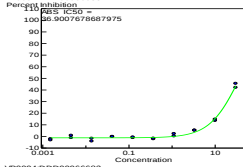
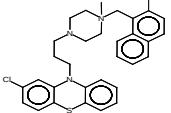
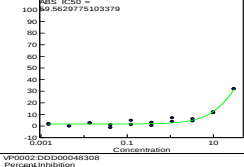
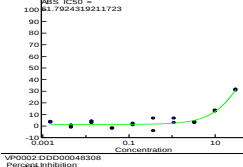
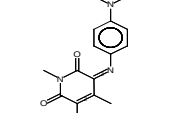
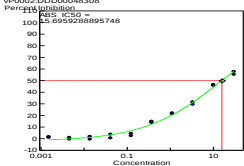
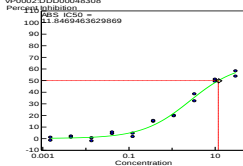
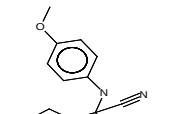
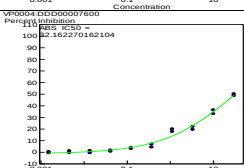
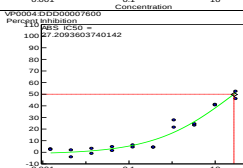
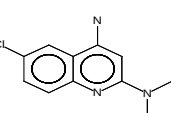
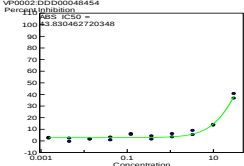
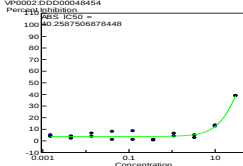
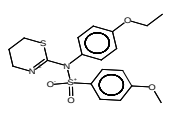
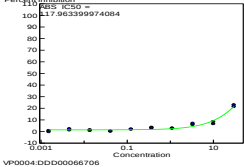
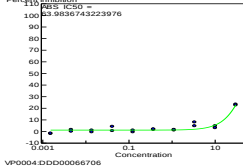
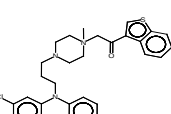
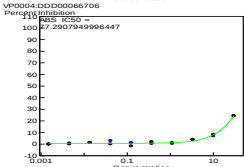
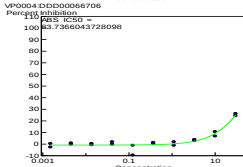
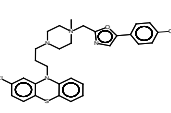
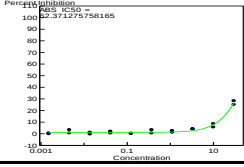
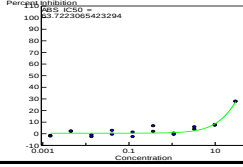
Supporting information

Table S1. *TbUAP* initial hit compounds. Potency of the 73 initial hits against *TbUAP* in the coupled assay and against the coupling enzyme *E. coli* pyrophosphorylase alone.

Table S2. Commercial analogues of hit compounds. Potency of the 30 analogues of **1** and **2** against *TbUAP* in the coupled assay and against the coupling enzyme *E. coli* pyrophosphorylase alone.

	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2			UAP + pyrophosphatase			Pyrophosphatase alone			SEL. DIFF.	
3	1		42.2		41.7	>30		-3.8	45.4	Yes
4	2		76.8		35.1	>30		-4.4	39.6	Yes
5	DDD00044195		215.7		27.5	>30		-7.4	34.9	Yes
6	DDD00009621		77.8		30.4	>30		-1.0	31.4	Yes
7	DDD00041394		81.0		22.6	>30		-8.2	30.7	Yes
8	DDD00029810		144.1		31.3	>30		1.1	30.3	Yes
9	DDD00053409		43.6		37.4	>30		8.3	29.0	Yes
10	DDD00031805		54.3		30.7	>30		2.6	28.1	Yes
11	DDD00052685		74.7		22.5	>30		-4.0	26.5	Yes
12	DDD00059994		61.0		24.3	>30		0.8	23.4	Yes
13	DDD00031613		91.1		28.3	>30		6.7	21.6	Yes

	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2	UAP + pyrophosphatase					Pyrophosphatase alone			SEL. DIFF.	
14	DDD00029832		85.4		23.0		37.8	8.0	15.0	Yes
15	DDD00049295		99.3		22.1		>30	7.5	14.6	
16	DDD00061182		43.2		36.2		66.0	26.1	10.1	
17	DDD00052504		61.1		24.5		90.0	15.9	8.5	
18	DDD00049231		42.4		38.0		46.1	33.0	5.0	
19	DDD00047752		41.4		38.4		45.4	34.2	4.2	
20	DDD00066710		87.1		24.5		140.2	20.6	4.0	
21	DDD00063211		31.3		49.2		33.7	46.1	3.1	
22	DDD00066705		85.6		23.7		105.6	21.6	2.1	
23	DDD00067003		78.0		23.5		98.4	21.6	1.9	
24	DDD00066690		72.2		27.9		99.8	26.4	1.5	

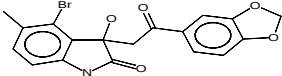
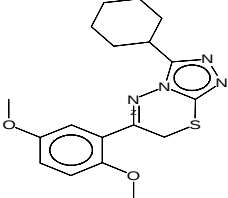
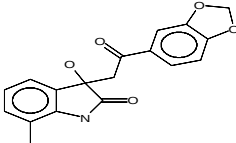
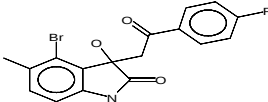
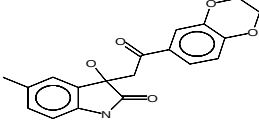
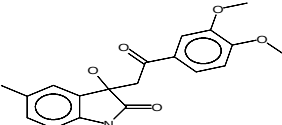
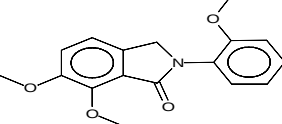
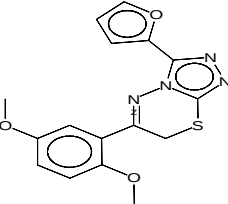
	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2			UAP + pyrophosphatase			Pyrophosphatase alone			SEL. DIFF.	
25	DDD00054423		102.9		28.6	97.3		27.4	1.1	
26	DDD00066678		105.0		21.5	117.2		20.4	1.1	
27	DDD00005856		74.0		26.3	73.9		25.5	0.9	
28	DDD00066688		34.9		44.6	36.9		43.8	0.7	
29	DDD00066683		59.6		31.9	61.8		31.2	0.7	
30	DDD00048308		15.7		56.5	11.8		55.9	0.5	
31	DDD00007600		32.2		49.4	27.2		49.2	0.1	
32	DDD00048454		43.8		38.6	40.3		38.9	-0.3	
33	DDD00030814		118.0		22.1	64.0		23.1	-1.0	
34	DDD00066706		77.3		24.3	83.7		25.3	-1.0	
35	DDD00066682		62.4		26.7	63.7		27.8	-1.0	

	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2			UAP + pyrophosphatase			Pyrophosphatase alone			SEL. DIFF.	
36	DDD00003950		58.2		25.9	51.9		28.1	-2.2	
37	DDD00006722		55.0		33.0	45.3		35.6	-2.5	
38	DDD000021254		44.9		34.7	42.0		37.3	-2.6	
39	DDD000066721		85.6		22.8	130.5		25.9	-3.1	
40	DDD000046926		90.7		36.2	1026.8		39.5	-3.3	
41	DDD000066679		92.7		25.8	94.2		29.3	-3.4	
42	DDD000066681		61.3		28.9	56.5		32.5	-3.6	
43	DDD000066709		50.2		30.6	54.6		34.7	-4.0	
44	DDD000066720		69.2		28.1	59.2		32.3	-4.2	
45	DDD000050065		110.1		21.9	72.5		26.3	-4.4	
46	DDD000066703		43.1		35.6	39.7		40.4	-4.7	

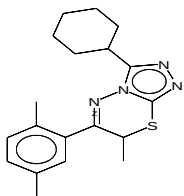
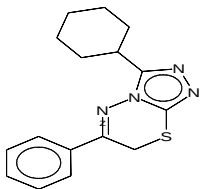
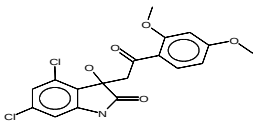
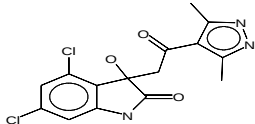
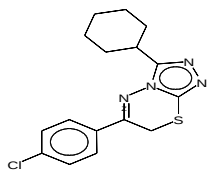
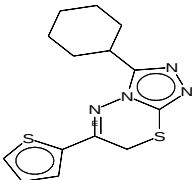
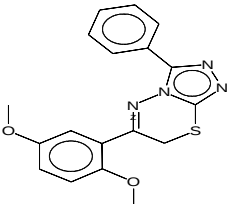
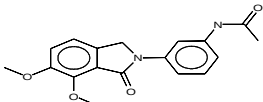
	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2			UAP + pyrophosphatase			Pyrophosphatase alone			SEL. DIFF.	
47	DDD00066702		55.2		29.1	46.2		33.9	-4.8	
48	DDD00057504		59.0		26.6	56.9		31.5	-4.9	
49	DDD00066701		51.9		33.2	45.5		38.1	-4.9	
50	DDD00066684		64.9		24.5	54.0		29.5	-4.9	
51	DDD00066707		59.9		26.5	54.1		31.5	-5.0	
52	DDD00066712		64.3		27.7	52.5		32.8	-5.1	
53	DDD00066693		95.7		24.5	72.5		29.8	-5.3	
54	DDD00022561		24.5		53.8	17.5		60.1	-6.3	
55	DDD00049489		117.2		18.9	77.8		25.5	-6.6	
56	DDD00066689		39.6		38.6	34.3		45.3	-6.7	
57	DDD00066680		35.6		45.0	28.8		51.7	-6.7	

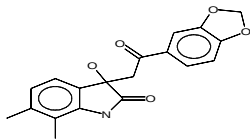
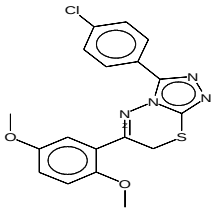
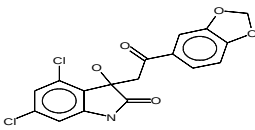
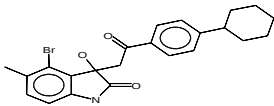
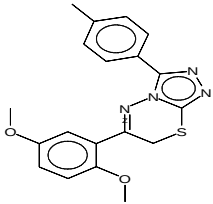
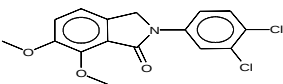
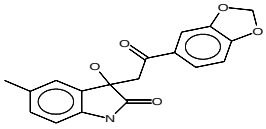
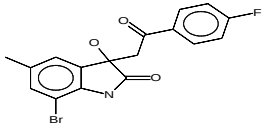
	A	B	C	D	E	F	G	H	I	J
	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
1										
2			UAP + pyrophosphatase			Pyrophosphatase alone			SEL. DIFF.	
58	DDD00066714		95.5		21.5	85.3		28.3	-6.8	
59	DDD00066699		45.1		35.2	35.8		42.3	-7.1	
60	DDD00066713		31.9		48.0	25.5		56.0	-8.0	
61	DDD00004306		39.3		41.6	30.1		49.6	-8.0	
62	DDD00066685		53.5		29.3	42.0		37.4	-8.1	
63	DDD00066723		35.6		41.6	30.1		50.0	-8.4	
64	DDD00023951		109.0		28.5	64.1		37.0	-8.5	
65	DDD00066716		50.6		33.2	38.9		42.0	-8.8	
66	DDD00066698		36.9		40.3	30.0		49.7	-9.5	
67	DDD00066677		36.7		41.7	29.2		51.5	-9.8	
68	DDD00066704		47.2		31.1	36.9		41.4	-10.3	

	A	B	C	D	E	F	G	H	I	J
1	Compound ID	Structure	AVERAGE ABS IC50 (UAP + PPase)	AVERAGE ABS IC50 - Graphical Result (UAP + PPase)	PI AVE @ 30uM (UAP + PPase)	AVERAGE ABS IC50 (PPase alone)	AVERAGE ABS IC50 - Graphical Result (PPase alone)	PI AVE @ 30uM (PPase alone)	PI UAP - PI PPase @ 30uM	HPLC activity @ 30uM (Yes/No)
2	UAP + pyrophosphatase					Pyrophosphatase alone			SEL. DIFF.	
69	DDD00066711		40.6		36.1	32.6		46.5	-10.5	
70	DDD00066700		43.3		37.6	29.7		50.5	-12.9	
71	DDD00066694		36.6		39.9	28.0		53.5	-13.6	
72	DDD00071188		73.7		23.6	47.4		37.3	-13.7	
73	DDD00066695		43.3		38.4	28.8		52.2	-13.9	
74	DDD00066718		33.1		46.0	24.6		59.9	-13.9	
75	DDD00066717		55.3		30.7	31.0		49.7	-19.1	
76										

Compound ID	Structure	TbUAP coupled assay		Pyrophosphorylase assay	
		AVERAGE IC50 uM	AVERAGE HILLSLOPE	AVERAGE IC50 uM	AVERAGE HILLSLOPE
1		36.61	0.92	>300	-0.85
DDD00053409		48.93	0.65	549.81	0.87
7		475.97	1.56	>100	-19.85
DDD00085843		>100	-0.43	>100	-0.27
3		>100	-4.16	>100	-0.93
DDD00085847		>100	-29.96	>100	-0.80
DDD00085853		>100	-16.38	>100	-1.46
DDD00085854		>100	0.97	>100	11.32

DDD00085855		317.56	0.75	>100	2.31
DDD00085856		>100	-1.46	>100	-0.60
DDD00085857		>100	-9.09	>100	-0.28
DDD00085858		566.30	0.94	>100	0.06
DDD00085859		98.76	0.59	575.43	0.73
DDD00085861		>100	-0.50	>100	-0.56
DDD00085863		>100	-1.56	>100	-1.64
DDD00085864		317.23	0.64	449.47	0.84

DDD00085865		256.18	0.67	225.94	0.86
DDD00085866		>100	3.22	>100	-0.14
DDD00085867		>100	-1.01	>100	-0.52
DDD00085868		>100	-15.76	>100	-0.99
DDD00085869		>100	1.86	>100	0.37
DDD00085870		197.47	1.21	>100	0.12
DDD00085871		448.93	0.64	>100	8.95
DDD00085872		>100	-0.77	>100	-19.83

6		365.49	0.76	>100	-0.95
DDD00085876		>100	0.22	>100	-0.07
5		348.25	0.91	>100	-0.72
DDD00085880		>100	-1.81	>100	-1.36
DDD00085882		252.07	0.89	292.51	1.01
DDD00085883		>100	-1.10	>100	-0.99
4		224.48	1.40	>100	-2.06
DDD00085885		>100	-1.74	>100	-1.01