WHITE PAPER

THE SELECTIVITY PROFILE OF 35 SMALL MOLECULE PROTEIN KINASE INHIBITORS

INTRODUCTION

Protein kinases regulate most aspects of normal cellular life, such as differentiation and proliferation, whereas inappropriate phosphorylation is a cause of many diseases. As the regulation and roles of protein kinases have become better understood, the interest in developing orally active protein kinase inhibitors has increased, and has recently been validated by the success of several small molecule and antibody therapies (Gleevec®, Iressa®, Tarceva®, Herceptin®, Erbitux®, etc). With around 500 distinct protein kinases identified through the human genome project and around 30 kinase inhibitors in clinical trials or approved for human use, this class of protein continues to be a rich area for therapeutic drug development.

KINASE INHIBITOR PROFILING

While kinase inhibitors hold great promise for the treatment of many diseases, target selectivity remains a common problem in the development process. Inhibition of off-target kinases could produce undesired side-effects or toxicity, and as the development of such compounds continues, questions are arising around how to design multi-targetted kinase inhibitors.

To facilitate this by allowing a better understanding of compound inhibition patterns and structure-activity relationships (SAR), Upstate has used its unparalleled experience in the cloning, manufacturing and assaying of protein kinases to assemble the **Kinase**Profiler™ panel. This panel currently contains more than 240 kinases and is increasing in breadth continually. It already represents the largest and most disease-relevant panel in the industry, and we have augmented this with the introduction of clinically-relevant mutant versions of standard kinases. All assays are conducted in the gold-standard radiometric filter-binding format, so there is no deconvolution, interpretation or compound interference to worry about.

Customers can use the service to screen large numbers of compounds against a single kinase or screen that vital compound against the whole panel. They can observe and examine inhibition patterns to enable them to fully understand the SAR of their compound library, learn more about the specificities of their lead compounds, see if their leads hit any crucial, 'survival' kinases or determine rank orders of potency using our IC_{50} estimation service. The overall aim is to give them the flexibility to more quickly design potent and targeted compounds to enable the production of more effective drugs.

In order to demonstrate the type of information which can be obtained using **Kinase**Profiler™, we challenged our entire panel with 35 commercially available compounds which are reported to be relatively selective inhibitors of various protein kinases, and this data is presented on pages 2 and 3.

KINASE INHIBITION DATA GENERATED BY KINASEPROFILER™

	PP2 [1µM]	PP3 [1µM]	LY294002 [50µM]	SB203580 [10µM]	HA1077 [10µM]	Roscovitine [10µM]	Rottlerin [10µM]	EGCG [10µM]	K252c [10µM]	Staurosporine [1µM]	SU6656 [1µM]	AG1296 [10µM]	Olomoucine [50µM]	Ro31-8220 [1µM]	AG183 [10µM]	AG18 [10µM]	AG1024 [10µM]	AG538 [10µM]	AG1478 [10µM]	H89 [10µM]	Y27632 [10µM]	Alsterpaullone [1µM]	Indirubin monoxime [10µM]	CDK2/5 inhibitor [10µM]	JNK inhibitor II [10µM]	KT5720 [1µM]	ST638 [10µM]	ZM336372 [10µM]	GW583340 [10µM]	GW441756 [10µM]	API-2 [10µM]	PD153035 [1µM]	JAK inhibitor I * [1µM]	UO126 [10µM]
Abl(h)	96	0	0	24	0	0	49	0	1	93	81	0	0	0	4	0	99	73	95	0	0	67	92	7	64	0	22	43	0	90	0	78	0	0
AMPK(r)	1	7	8	2	27	3	0	38	90	98	86	7	10	80	51	13	65	46	31	90	22	56	98	25	84	23	12	0	0	75	0	0	75	0
Aurora-A(h)	7	0	3	3	2	0	93	10	94	95	99	3	26	3	40	31	56	62	69	51	0	6	100	9	94	98	90	2	17	100	0	37	77	0
Axl(h)	0	1	21	0	3	11	68	47	78	93	89	11	8	57	22	6	40	34	56	25	0	22	93	0	78	64	36	0	14	92	0	34	46	0
Bmx(h)	89	11	22	39	0	0	0	1	27	93	0	0	0	44	16	0	0	0	51	3	0	0	73	0	53	47	0	0	0	22	0	13	49	0
CDK1/cyclinB(h)	0	0	16	0	0	77	0	2	65	98	55	0	53	96	11	0	7	17	2	7	3	83	92	47	80	3	0	0	0	44	0	0	9	0
CDK2/cyclinA(h)	2	0	18	0	3	93	0	0	81	98	32	0	84	97	15	0	3	8	0	20	24	93	94	78	81	21	0	0	0	32	0	0	16	2
CDK2/cyclinE(h)	12	0	28	0	10	96	0	23	77	98	26	0	92	98	36	2	0	17	0	50	44	96	99	88	86	14	8	0	0	47	0	0	20	0
CDK5/p35(h)	4	0	33	8	20	97	7	24	93	99	60	0	91	96	17	1	2	21	0	48	32	95	98	80	89	19	0	0	0	50	11	0	23	0
CHK1(h)	0	0	0	2	6	0	0	0	88	93	45	0	0	82	15	5	0	28	16	79	0	77	89	25	86	82	0	0	0	63	0	6	83	0
CHK2(h)	11	0	3	5	51	29	82	19	94	93	80	0	73	57	39	4	11	51	70	74	4	90	95	7	97	57	50	48	30	90	0	42	94	0
CK1_(h)	92	45	79	97	13	85	38	26	47	21	33	65	50	8	14	9	80	35	64	48	4	11	96	9	96	0	38	26	2	96	17	39	23	27
CK2(h)	0	8	93	0	0	0	0	0	49	0	0	0	10	0	71	85	0	71	1	0	0	0	20	0	72	0	0	0	0	37	3	0	17	0
cKit(h)	63	9	58	0	0	16	0	95	67	95	79	84	12	24	82	0	6	39	25	0	0	5	97	5	81	0	33	96	0	89	0	4	0	9
CSK(h)	38	15	0	11	3	13	10	101	38	74	13	0	6	11	79	0	0	51	15	7	5	1	25	4	21	0	3	6	0	2	8	9	0	0
cSRC(h)	97	40	26	74	2	9	1	21	47	96	97	12	0	12	24	3	89	53	96	9	1	82	97	29	62	13	75	18	11	97	0	81	21	3
DAPK1(h)	0	0	45	6	22	0	0	1	40	98	0	0	15	46	9	1	59	18	10	46	0	0	81	0	71	0	4	0	0	11	0	0	95	0
DYRK2(h)	2	9	26	1	0	6	0	69	91	50	0	0	64	6	15	49	12	74	53	7	0	41	78	14	96	7	0	0	0	40	33	27	25	8
EGFR(h)	51	5	17	54	13	0	0	97	24	37	0	0	15	6	80	0	0	19	98	15	3	0	19	0	0	0	68	3	97	0	0	98	0	0
EphB4(h)	95	9	16	56	0	1	0	98	3	36	9	0	0	5	73	0	0	0	89	2	0	0	85	0	23	0	11	53	0	43	0	75	0	0
Flt1(h)	74	0	13	75	30	0	27	41	94	91	62	53	15	85	20	4	25	67	87	84	0	50	91	18	94	64	30	7	0	94	0	68	59	10
Flt3(h)	30	5	44	26	59	21	40	34	83	94	75	71	33	84	26	6	51	66	71	68	6	69	93	46	84	85	32	0	3	97	8	53	76	10
Flt4(h)	88	0	69	62	46	28	62	37	98	98	82	79	70	96	35	13	77	76	96	88	0	71	100	11	98	86	62	78	0	99	0	84	76	4
GSK3_(h)	0	0	65	37	0	0	23	0	32	91	0	0	26	95	68	3	41	22	0	0	0	95	92	0	50	19	17	0	0	42	0	0	2	0
GSK3ß(h)	0	0	56	64	0	0	20	0	21	89	0	0	12	89	39	2	38	10	0	0	0	99	86	0	29	0	0	0	0	27	0	0	0	0
Hck(h)	90	21	32	51	0	0	76	0	28	87	80	0	0	0	11	0	85	46	81	0	0	36	91	16	58	24	40	89	0	84	4	70	0	2
IGF-1R(h)	0	0	10	0	0	0	0	53	32	63	0	0	0	0	15	0	0	0	0	0	0	0	85	0	30	0	16	0	0	55	1	0	7	3
IR(h)	0	0	5	0	0	7	0	0	29	41	0	0	0	0	0	0	0	0	0	0	0	0	89	0	40	0	6	0	0	74	0	0	14	0
KDR(h)	59	8	36	54	14	9	0	36	93	93	61	67	26	68	25	18	42	42	78	63	9	42	91	9	90	50	38	29	3	92	16	60	34	0
Lck(h)	96	0	1	35	0	0	0	77	0	98	90	0	0	1	0	0	76	77	85	0	0	25	94	0	7	0	29	91	0	90	0	52	32	0
Lyn(h)	99	55	62	89	12	11	44	95	95	99	99	28	0	32	14	3	93	64	99	39	16	87	99	31	86	39	73	94	0	98	0	97	31	0
MAPK1(h)	0	4	14	4	0	19	32	62	56	35	0	0	41	13	1	0	0	3	18	26	0	8	11	0	20	19	0	0	0	6	0	0	56	8
MAPKAP-K2(h)	0	0	0	0	0	0	56	2	49	85	0	0	0	15	0	0	0	0	0	26	0	45	32	0	31	44	0	0	0	0	0	0	44	0
MAPKAP-K3(h)	0	0	0	0	0	0	2	0	14	21	0	0	0	0	0	0	0	0	0	0	0	8	0	0	2	0	0	0	0	0	0	0	6	0
Met(h)	0	0	52	42	0	26	0	0	83	92	81	2	0	41	26	0	28	19	42	0	0	11	86	0	68	38	0	0	0	53	0	14	0	0
MSK1(h)	7	0	5	15	70	0	23	14	46	91	0	19	0	91	0	0	9	8	0	89	61	3	74	0	45	40	0	0	0	16	0	0	38	0
MSK2(h)	26	0	17	17	81	0	48	25	84	88	6	3	0	90	1	6	39	22	0	90	59	3	93	0	68	79	3	0	0	69	0	0	79	0
NEK2(h)	0	5	13	0	0	8	2	0	41	68	54	10	1	6	21	0	4	51	18	0	0	0	31	3	42	0	34	0	0	74	0	4	4	0
p70S6K(h)	14	3	10	24	88	0	14	17	91	101	10	0	0	99	4	31	75	23	14	100	37	0	94	0	84	40	1	0	0	78	0	0	45	2
PAK2(h)	8	6	14	11	3	5	1	15	37	98	4	0	14	21	6	0	2	17	2	13	0	79	67	0	32	29	2	0	2	9	0	0	19	0
PDGFR_(h)	0	0	2	0	9	55	0	0	80	98	92	0	48	31	0	0	0	0	18	87	0	33	97	0	91	5	43	0	0	72	0	3	0	0
PDGFRB(h)	75	13	6	16	5	5	0	103	34	105	30	40	0	26	77	0	0	23	3	13	0	2	80	0	17	6	0	84	8	55	0	0	0	0
PDK1(h)	2	4	1	2	0	0	14	0	97	99	0	0	0	55	0	0	0	0	0	4	0	16	83	0	62	94	0	0	0	7	0	0	91	0
Pim-1(h)	6	8	102	8	13	3	50	84	98	99	2	6	13	101	79	60	84	89	82	36	7	22	94	0	93	62	38	9	10	84	3	65	81	10
PKA(h)	27	11	0	13	75	6	0	6	27	99	0	0	5	72	0	13	0	0	5	96	11	10	23	4	9	18	0	0	0	0	0	0	0	0
PKB_(h)	0	0	37	14	38	0	5	2	21	95	0	0	0	81	12	0	0	14	1	95	24	0	42	0	21	12	0	0	0	3	0	0	8	0
PKBß(h)	0	0	19	24	0	0	53	22	25	96	0	0	0	59	2	0	0	9	0	72	0	0	15	0	0	4	0	0	0	10	0	0	16	0
PKB_(h)	19	1	25	22	65	0	20	4	38	98	0	0	0	100	0	0	6	0	0	97	9	0	55	0	29	13	0	0	0	4	0	0	53	0
PKCßII(h)	3	0	13	6	27	0	0	11	88	96	0	0	1	100	4	1	0	0	0	64	14	0	11	0	12	0	0	0	0	0	0	0	33	0
PRAK(h)	0	3	36	0	0	0	61	9	71	79	3	0	0	0	0	0	32	2	0	18	0	11	69	0	82	0	0	0	0	60	0	0	41	0
ROCK-I(h)	1	0	9	26	65	21	0	0	53	97	28	0	0	10	0	0	51	0	27	83	80	17	76	0	54	0	3	37	0	75	0	16	15	0
ROCK-II(h)	0	0	22	0	91	0	0	0	95	96	59	0	0	97	0	0	6	0	43	94	94	32	61	12	41	88	0	0	0	37	0	3	45	0
Rsk2(h)	0	0	4	0	71	20	12	0	93	98	62	14	21	99	5	0	21	7	37	91	66	45	94	6	61	72	12	0	0	70	0	6	63	0
SAPK2a(h)	0	0	3	98	71	24	1	0	96	6	73	30	28	98	11	1	32	20	55	89	76	60	96	0	79	82	5	0	0	86	0	25	18	42
SAPK2b(h)	48	0	0	97	0	0	0	0	6	0	0	0	0	0	0	0	0	0	54	0	0	0	0	23	19	0	0	87	0	0	0	20	50	0
SAPK3(h)	0	5	13	19	0	0	0	3	16	69	0	0	6	0	0	0	0	4	0	3	0	0	5	0	37	0	0	0	0	0	0	0	15	0
SAPK4(h)	0	0	4	10	0	0	0	4	50	67	0	0	0	0	14	0	6	9	0	0	0	0	5	0	33	0	0	0	0	0	0	0	23	0
SGK(h)	0	0	2	0	19	0	13	0	85	94	0	0	14	94	4	4	29	7	10	82	0	0	88	50	83	59	0	0	0	59	0	0	45	0
Syk(h)	0	5	10	15	5	0	49	100	93	100	12	0	0	23	1	0	0	70	18	16	0	56	94	0	68	84	0	0	0	58	0	0	11	0
Tie2(h)	7	0	15	63	0	0	33	53	76	95	64	0	0	6	0	0	20	31	51	15	0	0	79	0	45	0	27	0	6	18	0	17	0	0
TrkA(h)	26	1	80	0	0	14	0	85	97	99	94	47	9	95	59	6	64	18	73	61	28	33	94	24	95	93	65	28	21	96	33	47	97	44
TrkB(h)	13	0	59	0	0	0	21	2	99	100	99	20	1	97	0	0	0	22	29	18	0	12	100	0	99	94	24	6	0	100	0	11	95	0
Yes(h)	102	62	31	75	13	16	70	99	80	98	99	22	13	40	43	0	86	57	99	29	0	85	94	9	70	9	63	0	0	99	0	95	42	0
ZAP-70(h)	0	0	0	0	0	0	0	96	11	98	0	0	0	0	78	0	0	66	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

>80% inhibition 50-79% inhibition

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