Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Fragment growing to retain or alter the kinase selectivity of anchored hinge-binding fragments

Charlotte E. Allen, Amanda J. Welford, Thomas P. Matthews, John J. Caldwell and Ian Collins

Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, U.K.

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1. Details of kinases tested in substrate phosphorylation assay.

Kinase Name [a]	Kinome family [b]	[Enzyme] [c] (nM)	[ATP] (μM) [k]	Protein construct	Expression System	Average substrate conversion [m] (%)
MAPKAPK2	CAMK	0.099	4.6	Full length	Baculovirus	18
AurA	Other	2.401	9.7	Full length	Baculovirus	20
PKCζ	AGC	2.079	3.8	Full length	Baculovirus	23
RSK1	AGC	6.185	23.3	Full length	Baculovirus	21
PRAK	CAMK	3.174	5.0	Full length	Baculovirus	20
ERK1	CMGC	0.973	33.4	Full length	E. Coli	18
PKD2	CAMK	0.493	32.1	Full length	Baculovirus	21
CK1δ	CK1	2.041	16.3	Catalytic domain	E. Coli	18
CHK1	CAMK	2.208	33.0	Full length	Baculovirus	16
ABL	TK	0.401	14.0	Full length	Baculovirus	18
FYN	TK	0.220	36.0	Full length	Baculovirus	18
LYN	TK	0.976	17.0	Full length	Baculovirus	17
CHK2	TK	6.443	57.8	Full length	Baculovirus	20
MET	TK	2.712	79.5	Cytoplasmic domain	Baculovirus	19
LCK	TK	0.182	28.5	Full length	Baculovirus	17
SRC	TK	0.221	38.0	Full length	Baculovirus	20
GSK3β	CMGC	2.973	7.3	Full length	Baculovirus	19
ERK2	CMGC	1.236	62.1	Full length	E. Coli	19
PKA	AGC	0.461	1.7	Full length	Baculovirus	17
AKT2	AGC	0.259	186.1	Catalytic domain	Baculovirus	20
INSR	TK	21.951	871.8	Catalytic domain	Baculovirus	17
p38α	CMGC	1.600	396.5	Truncated protein	E. Coli	18
MSK1	AGC	1.856	21.2	Full length	Baculovirus	20
PKCβ2	AGC	1.115 [d]	84.8	Full length	Baculovirus	15
ROCK2	AGC	6.800	3.3	Catalytic domain	Baculovirus	23
CDK2	CMGC	1.877	57.6	Full length	Baculovirus	19
MST2	STE	22.974	36.6	Full length	Baculovirus	21

PKG1α	AGC	0.213 [e]	16.0	Full length	Baculovirus	18
PAK2	STE	0.114	103	Truncated	E. Coli	21
IGF1R	TK	56.721	320	Catalytic domain	Baculovirus	16
FGFR1	TK	4.714	495	Catalytic domain	Baculovirus	15
MARK1	CAMK	0.079	10.5	Full length	Baculovirus	15
CAMK2δ	CAMK	0.208 [f]	22.4	Full length	Baculovirus	20
BTK	TK	8.986	123	Full length	Baculovirus	19
c-TAK1	CAMK	1.165	66	Full length	E. Coli	16
* DYRK1a	CMGC	0.943	18.1	Full length	E. Coli	17
CaMK4	CAMK	0.362 [f]	3.9	Full length	Baculovirus	17
FLT3	TK	0.267	350	Catalytic domain	Baculovirus	18
HGK	STE	0.049	80	Catalytic domain	Baculovirus	16
KDR	TK	8.647 [g]	164.8	Cytoplasmic domain	Baculovirus	19
Raf-1	TKL	86.579	6.2	Truncated protein	Baculovirus	10
P70S6K	AGC	1.704	463	Catalytic domain	Baculovirus	17
IRAK4	TKL	5.916	196.5	Catalytic domain	Baculovirus	20
SGK	AGC	0.207	121.8	Truncated protein	Baculovirus	19
SYK	TK	3.425	33.5	Catalytic domain	Baculovirus	18

AURB	Other	2.374	4.7	Full length	Baculovirus	25
FGFR2	TK	8.073 [h]	84	Catalytic domain	Baculovirus	17
FGFR3	TK	6.915 [h]	300	Catalytic domain	Baculovirus	17
ABL Q252H	TK	0.156	57.5	Truncated protein	Baculovirus	17
AURC	Other	5.397	10	Full length	Baculovirus	23
FGFR4	TK	6.205 [h]	46.1	Catalytic domain	Baculovirus	16
EGFR	TK	3.404 [h]	10.4	Catalytic domain	Baculovirus	18
ABL T315I	TK	0.211	11.7	Truncated protein	Baculovirus	18
IKKβ	Other	12.780	22	Full length	Baculovirus	16
MAPKAPK3	CAMK	0.210	38.6	Full length	E. Coli	18
P38β2	CMGC	1.027	106	Full length	E. Coli	14
TSSK1	CAMK	4.445	29.3	Full length	Baculovirus	16

PKG1β	AGC	0.218 [e]	20	Full length	Baculovirus	14
CAMK2β	CAMK	0.303 [i]	107.3	Catalytic domain	Baculovirus	10
P38δ	CMGC	16.806	4.1	Full length	E. Coli	21
TSSK2	CAMK	1.255	16.1	Full length	Baculovirus	19
ABL H396P	TK	0.141	28	Truncated protein	Baculovirus	17
PDGFRα	TK	11.686 [h]	732.3	Catalytic domain	Baculovirus	16
FGFR2 N549H	TK	4.532 [h]	5.5	Catalytic domain	Baculovirus	18
HCK	TK	0.335	59.6	Catalytic domain	Baculovirus	18
FLT3 D835Y	TK	0.443	48.5	Catalytic domain	Baculovirus	17
FER	TK	14.384 [h]	5.3	Catalytic domain	Baculovirus	12
CAMK2γ	CAMK	0.320 [i]	25.3	Catalytic domain	Baculovirus	20
MSK2	AGC	10.529	52.4	Full length	Baculovirus	18
P38γ	CMGC	7.289	2.7	Full length	E. Coli	17
PKD1	CAMK	0.230	47.6	Full length	Baculovirus	17
MARK2	CAMK	0.128	11.0	Full length	Baculovirus	14
BMX	TK	2.673 [h]	7.2	Full length	Baculovirus	14
CSNK1A1	CK1	1.749	6.0	Full length	Baculovirus	18
PKD3	CAMK	0.942	34.1	Full length	Baculovirus	19
BRSK1	CAMK	1.254	236	Full length	Baculovirus	17
NEK2	Other	8.808	155	Full length	Baculovirus	19
PIM1	CAMK	0.627	329.3	Full length	Baculovirus	17
SGK2	AGC	11.315	69.0	Full length	Baculovirus	24
SGK3	AGC	1.589	8.0	Full length	Baculovirus	23
ARG	TK	0.162	57.4	Truncated protein	Baculovirus	16
DCAMKL2	CAMK	22.377	115.5	Full length	Baculovirus	17
RSK2	AGC	2.918	17.9	Full length	Baculovirus	19
RSK3	AGC	2.025	19.1	Full length	Baculovirus	21
BRSK2	CAMK	1.700	127.7	Truncated protein	Baculovirus	15
PCKα	AGC	0.309 [j]	18.0	Truncated protein	Baculovirus	21
PKCβ1	AGC	0.373 [j]	27.5	Truncated protein	Baculovirus	21
PKCγ	AGC	0.812 [j]	22.5	Truncated protein	Baculovirus	29
PKCδ	AGC	1.047 [j]	30.6	Truncated protein	Baculovirus	22

PKCε	AGC	0.231 [j]	25.9	Truncated protein	Baculovirus	22
PKCη	AGC	0.305 [j]	35.0	Truncated protein	Baculovirus	15
PKCθ	AGC	0.109 [j]	56.3	Truncated protein	Baculovirus	21

Table S1: [a] 91 Kinases used in the microfluidic peptide phosphorylation assay. Human protein was used in each case except * (Rat Dyrk1a); [b] TK = Tyrosine kinase; TKL = Tyrosine kinase-like, STE = Homolog's of yeast Sterile 7, Sterile 11, Sterile 20 kinase, AGC = Containing PKA, PKG, PKC families, CAMK = Calcium/calmodulin-dependant kinase, CMGC = Containing CDK, MAPK, GSK3b, CLK families; [c] Concentration of kinase enzyme used. Reactions were conducted in a common buffer, 100 mM HEPES, pH 7.5, 4% DMSO, 0.003% Brij-35, 0.004% Tween, with fixed peptide sequence concentration of 1.5 µM and 10 mM $MgCl_2$ co-factor except: [d] 10 mM $MgCl_2$, 0.02 ug/mL PS/PMA; [e] 10 mM $MgCl_2$, 10 uM cGMP; [f] 10 mM $MgCl_2$, 1 mM $CaCl_2$, 6.7 ug/mL Calmodulin; [g] 10 mM $MgCl_2$, 0.05% CHAPSO; [h] 10 mM $MgCl_2$, 10 mM $MnCl_2$; [i] 10 mM $MgCl_2$, 1 mM $CaCl_2$, 6.7 ug/mL Calmodulin; [j] 10 mM $MgCl_2$, 10 ug/mL PS/PMA; [k] Concentration of ATP used in enzyme reaction, equivalent to the $K_{m,ATP}$ determined for each individual enzyme. [m] Conversion of substrate peptides to phospho-peptides in standard incubation of 90 min.

2. Experimental protocol for kinase activity (substrate phosphorylation) assays.

Assays were carried out using ProfilerPro assay kits 1-4 (Caliper Life Sciences). Reagents were prepared and handled as described in the manufacturer's instructions. Assay plates were stored at -20 ^{o}C until required. Test compounds were prepared in DMSO solution, at the appropriate concentration, and were diluted with 57 μL of reconstitution buffer (Caliper Life Sciences). The reagent plates containing ATP and fluorescence-labelled substrate peptides were thawed at 28^{o}C for 60 min, and the assay plates containing the kinase enzymes were thawed at 28^{o}C for 15 min. All assay plates were centrifuged for 1 min. Aliquots of the solutions of test compounds in DMSO/reconstitution buffer (16 μL) were added to the assay plate containing the enzymes, followed by aliquots (10 μL) the appropriate ATP/substrate peptide solution. The assay plates were centrifuged for 1 min then incubated at 28^{o}C for 90 min. Aliquots of termination buffer (45 μL, Caliper Life Sciences) were added to each well and the assay plates were centrifuged for 1 min. Electrophoretic separation and quantification of the fluorescence of phosphorylated peptide (product) and unphosphorylated peptide (substrate) in each well was carried out using a LabChip EZReaderII (Caliper LifeSciences) equipped with a 4-channel sipper chip.

3. Inhibition of kinases by compounds 1 - 13.

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13
Test concentration (μM)	1000	70	95	0.2	8	2	0.06	0.02	420	90	9	0.35	0.1
Kinase													
ABL	42	47.5	78	0	57.5	13.5	13	6.5	69	21.5	12	0	0
Abl(Q252H)	29	39	67.5	0	87.5	17	0	0	64	15.5	6	0	4
Abl(T315I)	58	22	53	0	53	1	0	0	73	31	10	0	0
Abl(H396P)	44.5	42.5	69.5	4.5	20.5	2.5	0.5	0	71	19	16	1	1.5
Arg	40	33	50.5	4.5	3.5	7.5	5	3	54.5	23	6.5	0	1.5
BTK	19	0	17	0	4.5	0.5	0	1	55	0	11	0	0
BMX	22	0	0	4	42.5	2	0	0	62.5	5.5	11	0	3
SRC	28.5	37.5	62	24.5	1.5	0	0	0	62.5	22	19.5	7.5	3
FYN	24	31.5	64.5	16	1	5	3	12.5	64.5	21	29.5	2	0
LCK	29	38	68.5	35	48.5	4.5	0	1	67.5	15.5	39	5.5	0
LYN	31.5	35	65.5	18	51	0.5	0	1	68	32	42	8	0
Hck	30	18.5	48.5	35	19.5	3	7	0	69.5	22.5	29	11	4
EGFR	13.5	16.5	19.5	2.5	33	3.5	0	2.5	29	32	12	0	0.5
IGF1R	51.5	0	20.5	5	30	0	0	0	15	0	10	3.5	9.5
INSR	29	0	23.5	11	16.5	7.5	6	10.5	8	0	2.5	7	0
MET	55	0	5.5	9.5	9	0	0	0	23	0	0	2.5	0
FGFR1	64.5	43	72	13	36.5	1.5	0	0	97	61.5	55.5	0	2.5
FGFR3	63.5	27.5	27	0	0.5	0	0	3.5	95	30.5	43.5	0	5.5
FGFR2	62	27	36	4	29.5	1.5	0	0	93	7	30	0	0
FGFR2(N549H)	42	35.5	40	7	0	1	5	0	89.5	42.5	47.5	6.5	7.5
FGFR4	28.5	11.5	10	1.5	27.5	0	2	1	75.5	26	0	1.5	0
KDR	89.5	64	72.5	9	50	0	0	0	95.5	82.5	63.5	0	14
PDGFR_alpha	31.5	16.5	37	0.5	0	5	0	0	32.5	11	23	9.5	8.5
FLT3	75.5	36	49.5	7.5	0	0	0	0	85.5	62.5	89.5	21	7
Flt3(D835Y)	76	69	86	16	4.5	0	0	0.5	93	82	95	63	27
Fer	18	30	28	2.5	1.5	13.5	4	7.5	70.5	28	11	1	0
IRAK4	65	15	34	13	25.5	1.5	0	4.5	74.5	54.5	56.5	5.5	11.5
c-Raf	20	39.5	39.5	3.5	2.5	7	3.5	3.5	52	0	7.5	7	4
PAK2	13.5	26	57.5	11	24.5	3.5	1.5	6	67.5	55.5	3	1.5	0
MST2	63	88	86.5	2	73	17.5	3	0	99	87.5	81	0.5	11.5
HGK	90	42	39.5	3	11.5	0	1	3	78.5	98.5	54.5	3	0
CSNK1A1	73.5	45.5	53.5	4	32.5	9.5	1	5	60.5	0	13	2	2.5
CK1d	94.5	46	77.5	8.5	25.5	0	0.5	0	81.5	26	39.5	10.5	9.5
ROCK2	99.5	88.5	93.5	97	89.5	44	0	35.5	101	103	53	2	0
AKT1	65.5	64.5	81	94	96	83	34	44.5	61.5	91.5	52	0	0
SGK1	94	48	52.5	33	59	27	56	21.5	83.5	77	51	4.5	0
SGK3	80.5	58	41.5	41	83	34.5	42.5	0	63	72	44	5	0
SGK2	64	74	50.5	23.5	69	46.5	9	13.5	76.5	84.5	48.5	0	5.5
PKC-alpha	43	56	69.5	77.5	56.5	18	30	0	64.5	41	14.5	0	0
PKC-beta1	46	73.5	77	53	50.5	16.5	3.5	0	70	68.5	22.5	2	3
PKCb2	21	0	0	38.5	88.5	62.5	0	2	38	0	23.5	0	0
PKC-gamma	57	64	71	80	86	57.5	3.5	7.5	66	66.5	34.5	0	6
PKC-eta	52	78.5	86.5	36.5	82	56.5	51	34.5	82	66.5	54.5	1	0
PKC-epsilon	38	44	49	46.5	34	2.5	1.5	4.5	73.5	0	14.5	1	0
PKC-delta	45.5	60	72	37.5	67	33	2	3.5	78.5	66	37.5	0	0
PKC-theta	29	67.5	72	31.5	67.5	13	4.5	3.5	67.5	32	52.5	4	5
PKCz	31.5	0	0	1	67.5	20.5	0	5.5	46	0	22	0	19
MSK1	87	72.5	76	90	68	0.5	5.5	5.5	78.5	82.5	60	8	0.5
MSK2	81	68	54	82.5	50	15	0	3.5	76	69.5	52	0	13.5
p70S6K	92	29.5	49	87.5	68	3.5	0	0	84.5	6.5	14.5	0	0
RSK2	68.5	75	87.5	55	32	2	0.5	2	95	99	95.5	42	59.5
RSK1	60.5	48	83.5	38	11.5	7.5	19.5	11.5	87	98.5	91	37	48.5
RSK3	71	81	89	40	28	0	0	2.5	98	100	95.5	44	60.5
PKA	96.5	96	97.5	100	29.5	21	17	13	92.5	95.5	33.5	0	0
PKGa	90.5	97.5	92	88.5	14	10	0	9.5	98	93	15.5	4.5	0
PKG1-beta	87	95.5	93	88.5	76.5	8.5	0	14.5	95.5	96	12.5	0	0
PKD1	54	54.5	44	8	75.5	26	32.5	0	70	82	76	3	5
CAMK4	52	77.5	85	74	43	12	6	7.5	62	41.5	31.5	0.5	3.5
CAMK2	37.5	17.5	45.5	16	25	8	0	7.5	63.5	4	42.5	0	0
CaMKII_beta	30	6.5	2	11.5	81	25.5	0	0.5	20.5	1.5	4	0	0
CaMKII_gamma	24.5	16	30	15	20.5	0	0	0	42	15.5	24	0	0
DCAMKL2	15	20.5	35	10	21	1.5	0	1	41.5	39	3	4.5	6
MAPKAPK2	67	77	56.5	10.5	81	33	0	0	74	54	19.5	0	0
MAPKAPK3	0.5	1.5	24	4	81.5	45.5	7	0	96.5	98	74	7	0
PRAK	77	20.5	35	8.5	51.5	7	0	0	40	51.5	0	4	3.5
BRSK2	28	43	40	13	56.5	2.5	0	5	60.5	39.5	36	0	28.5
BRSK1	22	29.5	44	11	56.5	14.5	0	0	51.5	63	29.5	0	16.5
MARK1	93.5	52	97	51	74	34.5	4.5	0	4.5	40.5	2	5	0
MARK2	75	61	67	41.5	37	0	0	0	88	90	73	0	2
c-TAK1	77	6.5	18	27.5	3	3	0	0	86.5	0	30	0.5	6
CHK1	52	72	75.5	46	0	1.5	0	0	88.5	37.5	91	83	92
TSSK2	37	10	23.5	7	23.5	6.5	3	4.5	24.5	44	0	0	0
TSSK1	52.5	62	89	67	25.5	0	0	0	71	62	41.5	0	0
PIM1	20	26	42.5	0	9	2	0	2.5	31.5	14	36.5	7	9.5
PKD3	62.5	72.5	61.5	12.5	0	0	0	0	85	81	80	0	0
PKD2	49.5	77.5	77.5	13	28.5	6.5	1.5	6	70	93	68	0	0
CHK2	74	57.5	74	59	0	0	0	0.5	85	52.5	49	0	0
CDK2	45.5	34	39.5	7.5	7	0	1	6	75	53	26.5	6	1.5
Erk2	23.5	29	55.5	7	7.5	2.5	4.5	0	68	43.5	1.5	0	0
Erk1	36	28.5	59	6	8.5	1	0	4	78.5	48	8.5	0	1
p38a	0	0	6.5	5	7	2	0	7	0	0	2.5	2	1
p38-beta2	0	1.5	11	2.5	12.5	5.5	9	4.5	1.5	0	0	1	0
p38-delta	51	5	13	11	5.5	8	0	1.5	34	10.5	2	0	2.5
p38-gamma	64	15	13	5	5	1.5	0	0	41.5	7.5	4.5	1.5	5.5
GSK3b	32	37.5	55	3.5	0	5	0	0	90	68.5	94	3.5	0
DYRK1a	64.5	61	79	11	54	0.5	0	0	77	79	7.5	1.5	3.5
AurA	88	68	85	49	87.5	64.5	7.5	0	96	98	23.5	6.5	0
AurB	95	85	97	87.5	76	34	0	15	96	98.5	31	4	0
AurC	96	73	87.5	73.5	34	13.5	0	7	95.5	99	11.5	7.5	1
IKK-beta	95.5	90.5	91	81	13.5	1	2	9.5	87	74.5	83	2	3.5
NEK2	2.5	15.5	0	4.5	0	0	0	0	27	0	6	0.5	2

Table S2: Percentage inhibition of kinases by compounds **1-13** (mean of n = 2 determinations) tested at the specified concentrations. Measured inhibition of less than zero is recorded as 0%.