Supporting Information

Structure-guided Inhibitor Design Expands the Scope of Analog-Sensitive Kinase Technology

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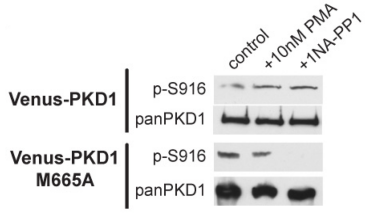
Supplementary Figure and Tables



Supplementary Figure S1. HEK 293T cells were transfected with Venus-Pkd1-*WT* or Venus-Pkd1-*AS2* and the cells were then treated with DMSO, 10nM PMA or 1μM 1NA-PP1. The cells were lysed and probed for total Pkd1 and p-S916-Pkd1.

S. Cerevisiae Kinases			
26			
Kinase	**Ref**	**GK**	**Inhib.**
Pho85	[1,2]	F82G	5uM 1NA-PP1
Elm1	[3]	T200G	25uM1NM-PP1
Kin28	[4]	L83G	6μM 1NA-PP1
Srb10	[4]	Y236G	24μM 1NA-PP1
Cla4	[5]	M640A & T710A	1NM-PP1
Cdc15	[6]	L99G	10μM 1NM-PP1
Cdc28	[7]	F88G	5μM 1NM-PP1
Mps1	[8]	M516G	10μM1NM-PP1
Ipl1	[9]	M181G & T244A	1NA-PP1
Ime2	[10]	M146G	20μM 1NA-PP1
Mek1	[11,12]	Q247G	1μM 1NA-PP1
Ire1	[13]	L745G	20μM 1NM-PP1
Prk1	[14]	M108G & C175A	40μM 1NA-PP1
Apg1	[15]	M102A	20μM 1NA-PP1
Cbk1	[16,17]	M429A	20μ 1NA-PP1
Hog1	[18,19]	T100G	12μM 1NM-PP1
Fus3	[7]	Q93G	5μM 1NM-PP1
Tpk1	[20-25]	M164G	1μM 1NM-PP1
Tpk2	[23-25]	M147G	1μM 1NM-PP1
Tpk3	[23-25]	M165G	1μM 1NM-PP1
Sch9	[24,25]	T492G	0.1 1NM-PP1
Tos3	[26]	L135G	2.5μM 2,3-dMB-PP1
Cdc7	[27]	L120A & V181A	15μM PP1
Cdc5	[28,29]	L158G, C96V	CMK
Snf1	[30,31]	I132G	10 μM 2NM-PP1
Bur1	[32]	L149G	6μM 3MB-PP1

S. Pombe Kinases			
15			
Kinase	**Ref**	**GK**	**Inhib.**
Hhp1	[33]	M84G	1NM-PP1
Cdc2	[33]	F84G	0.5μM 1NM-PP1
Cdk9	[33]	T120G	10μM1NM-PP1
Cka1	[33]	F117G	5μM 9Ch-ANPUR
Crk1	[33]	L87G	30μM 3BrB-PP1
Ksg1	[33]	L177G	5μM 1NM-PP1
Nak1	[33]	M77G	40μM 3BrB-PP1
Orb6	[33]	M170G	5μM 3BrB-PP1
Pat1	[33]	L95A	10μM 1NM-PP1
Plo1	[33]	L117G	10μM 3BrB-PP1
Ppk37	[33]	M537G	30μM 1NM-PP1
Prp4	[33]	F238A	30μM 3BrB-PP1
Shk1	[33]	M460A	20μM 3BrB-PP1
Sid2	[33]	M285G	40μM 3BrB-PP1
Ark1	[33,34]	L166A S229A	5μM 1NM-PP1

Mammalian Tyr Kinases				
18				
Kinase	**Ref**	**GK**	**Conc. Used µM**	**Inhib.**
v-Src	[35]	I338G	0.25	1NA-PP1
CSK	[36]	T266G	10	3IB-PP1
Fyn	[7]	T339G	0.005 (in vitro)	1NA-PP1
Lck	[37]	T316G	10	1NA-PP1
TrkA	[38]	F592A	0.1	1NM-PP1
TrkB	[38]	F616A	0.1	1NM-PP1
TrkC	[38]	F617A	0.1	1NM-PP1
Bcr-Abl	[39]	T316A	2	1NA-PP1
v-ErbB	[40]	T210A	2	1NA-PP1
Jak1	[41]	M956G	40	1NM-PP1
Jak3	[41]	M902G	40	1NM-PP1
Zap70	[42]	M414A C405V	10	3MB-PP1
Syk	[43]	M442A M429L R428Q	6	2,3DMB-PP1
EphB1	[44]	T697G	0.25	1NA-PP1
EphB2	[44]	T699A	0.25	1NA-PP1
EphB3	[44]	T706A	0.25	1NA-PP1
Ret	[45]	V805A	0.1	1NA-PP1
Yes	[46]	T346G	10	1NM-PP1

Mammalian Ser/Thr Kinases				
21				
Kinase	**Ref**	**GK**	**Conc. used in cells (μM)**	**Inhib.**
Cdk1	[47]	F80G	0.6	1NM-PP1
Cdk2	[48]	F80G	10	3MB-PP1
Cdk7	[49]	F91G	5	3MB-PP1
PKAα	[50]	M120A	10	1NM-PP1
PKAβ	[50]	M120G & K46I	10	1NM-PP1
PKCε	[51]	M486A	1	1NA-PP1
GRK2	[28,52]	L271G & C221V	2.5	1NA-PP1
JNK1	[53]	M108G & L168A	1	1NM-PP1
JNK2	[54]	M108G	10	1NM-PP1
CaMIIKα	[55,56]	F89G	5	1NM-PP1
Ire1α	[57]	I642G	5	1NM-PP1
Plk1	[58,59]	C67V & L130G	10	3MB-PP1
Mps1	[60,61]	M602A	5	3MB-PP1
Lkb	[62]	M129G	10	1NM-PP1
Akt1	[63,64]	M227G	2.5	3IB-PP1, PrIDZ
Akt2	[63,64]	M225G	2.5	PrIDZ
Akt3	[63,64]	M229G	2.5	PrIDZ
Pdk1	[65]	L159G	2	Cpac-BX
Mekk1	[28]	I1304G, C1238V	N.D.	N.D.
Pkd1	This Publication	M665A	1	1NA-PP1
Aurora B	[66]	L154G, H250Y	2	1NA-PP1
NDR	[67]	M166A, M152L, S229A	1	1NA-PP1

Plant Kinases			
3			
Kinase	**Ref**	**GK**	**Inhib.**
MPK4	[68]	Y124G	100μM 1NA-PP1
Pto	[28,69]	Y114A, L681I	10μM 1NM-PP1 10 μM 3MB-PP1
LeMPK3	[70]	T123A	1μM Bn-PP1(in vitro)

C. Elegans			
1			
Kinase	**Ref**	**GK**	**Inhib.**
Sad1	[71]	L123A	33μM 1NA-PP1

Supplementary Table S1. Analog-sensitive kinases reported in peer-reviewed publications. Information listed includes the kinase, associated references (Ref), gatekeeper mutation (GK), suppressor mutations in red, inhibitor sensitizing mutations in green, and the concentration and identity of inhibitor used in publication (Inhib).

Legend

< 40% Inhibition
40% - 80% Inhibition
≥ 80% Inhibition

		Compound	1NAPP1	1NMPP1	3MBPP1	3IB-PP1 (17)	3MSB-PP1 (18)
		Conc (nM)	1000	1000	1000	1000	1000
ABL1	Activity	Km app	26	14	16	-6	-10
ABL1 E255K	Activity	Km app	24	14	17	8	8
ABL1 G250E	Activity	Km app	18	9	13	24	19
ABL1 T315I	Activity	Km app	8	5	8	-2	-2
ABL1 Y253F	Activity	Km app	29	14	18	19	13
ABL2 (Arg)	Activity	Km app	19	16	13	5	3
ACVR1 (ALK2)	Binding					20	13
ACVR1B (ALK4)	Activity	Km app	65	-3	5	25	9

ACVR2B	Binding						
ADRBK1 (GRK2)	Activity	Km app	3	3	-1	12	9
ADRBK2 (GRK3)	Activity	Km app	6	-3	-6	3	3
AKT1 (PKB alpha)	Activity	Km app	0	2	-1	-6	-6
AKT2 (PKB beta)	Activity	Km app	-1	-2	0	6	6
AKT3 (PKB gamma)	Activity	Km app	9	1	1	2	1
ALK	Activity	Km app	1	3	-1	5	8
AMPK A1/B1/G1	Activity	Km app	2	1	-2	-18	-1
AMPK A2/B1/G1	Activity	Km app	9	4	8	10	12
AURKA (Aurora A)	Activity	Km app	6	0	4	0	-2
AURKB (Aurora B)	Activity	Km app	5	7	3	5	8
AURKC (Aurora C)	Activity	Km app	6	2	6	-10	-12
AXL	Activity	Km app	4	2	3	2	-1
BLK	Activity	Km app	38	16	15	12	11
BMPR1A (ALK3)	Binding						
BMX	Activity	Km app	26	52	25	41	47
BRAF	Binding					14	12
BRAF	Activity	100	29	19	26	8	11
BRAF V599E	Binding					9	2
BRAF V599E	Activity	100	17	9	32	12	11
BRSK1 (SAD1)	Activity	Km app	9	8	7	2	1
BTK	Activity	Km app	49	61	39	19	20
CAMK1 (CaMK1)	Activity	10	-16	-20	12	-17	-21
CAMK1 (CaMK1)	Activity	100					
CAMK1D (CaMKI delta)	Activity	Km app	-2	-5	-5	-1	3

CAMK2A (CaMKII alpha)	Activity	Km app	2	1	-3	12	14
CAMK2B (CaMKII beta)	Activity	Km app	14	15	9	19	21
CAMK2D (CaMKII delta)	Activity	Km app	4	0	-2	15	15
CAMK4 (CaMKIV)	Activity	Km app	-6	10	-5	-2	-1
CAMKK1 (CAMKKA)	Binding					9	6
CAMKK2 (CaMKK beta)	Binding					1	1
CDC42 BPA (MRCKA)	Activity	Km app	-2	-2	5	6	4
CDC42 BPB (MRCKB)	Activity	Km app	9	2	3	2	2
CDK1/cyclin B	Activity	Km app	2	2	3	8	10
CDK2/cyclin A	Activity	Km app	8	9	11	2	1
CDK5/p25	Activity	Km app	2	2	3	6	7
CDK5/p35	Activity	Km app	0	-2	2	10	16
CDK7/cyclin H/MNAT1	Activity	Km app	-5	14	-15	12	8
CDK8/cyclin C	Binding					12	16
CDK9/cyclin K	Binding					4	3
CDK9/cyclin T1	Activity	Km app	3	-2	-4	4	8
CHEK1 (CHK1)	Activity	Km app	3	6	4	-6	-6
CHEK2 (CHK2)	Activity	Km app	12	7	9	-3	-3
CHUK (IKK alpha)	Activity	10	4	-20	-12		
CHUK (IKK alpha)	Activity	Km app				3	-1
CLK1	Activity	Km app	7	7	7	13	12
CLK2	Activity	Km app	10	10	14	32	38
CLK3	Activity	Km app	4	7	6	1	5
CLK4	Binding						
CSF1R (FMS)	Activity	Km	40	28	20	30	19

		app						
CSK	Activity	Km app	28	29	17	28	27	
CSNK1A1 (CK1 alpha 1)	Activity	Km app	12	1	13	18	17	
CSNK1D (CK1 delta)	Activity	Km app	38	14	70	90	89	
CSNK1E (CK1 epsilon)	Activity	Km app	91	96	85	100	100	
CSNK1G1 (CK1 gamma 1)	Activity	Km app	0	2	-2	6	3	
CSNK1G2 (CK1 gamma 2)	Activity	Km app	-2	-1	-2	14	11	
CSNK1G3 (CK1 gamma 3)	Activity	Km app	0	2	2	6	4	
CSNK2A1 (CK2 alpha 1)	Activity	Km app	6	7	3	5	5	
CSNK2A2 (CK2 alpha 2)	Activity	Km app	4	3	1	4	3	
DAPK1	Activity	Km app	-3	12	3	2	-1	
DAPK3 (ZIPK)	Activity	Km app	0	2	-1	2	-3	
DCAMKL2 (DCK2)	Activity	Km app	2	1	-1	7	9	
DDR1	Binding							
DDR2	Binding					6	4	
DMPK	Binding					6	7	
DNA-PK	Activity	Km app						
DYRK1A	Activity	Km app	4	2	3	-3	-4	
DYRK1B	Activity	Km app	12	11	10	-10	-9	
DYRK3	Activity	Km app	0	2	0	3	3	
DYRK4	Activity	Km app	2	2	1	1	2	
EEF2K	Activity	Km app	2	3	1	20	18	
EGFR (ErbB1)	Activity	Km app	28	24	36	56	65	
EGFR (ErbB1) L858R	Activity	Km app	40	29	34	48	27	

EGFR (ErbB1) L861Q	Activity	Km app	34	17	30	34	20
EGFR (ErbB1) T790M	Activity	Km app	48	38	44	66	81
EGFR (ErbB1) T790M L858R	Activity	Km app	65	53	56	66	55
EPHA1	Activity	Km app	93	83	71	48	47
EPHA2	Activity	Km app	75	43	44	22	13
EPHA3	Binding						
EPHA3	Activity	Km app	45	18	21	12	4
EPHA4	Activity	Km app	83	43	46	36	25
EPHA5	Activity	Km app	86	56	61	40	21
EPHA7	Binding					6	8
EPHA8	Activity	Km app	67	51	40	39	24
EPHB1	Activity	Km app	63	44	43	34	27
EPHB2	Activity	Km app	78	58	58	38	25
EPHB3	Activity	Km app	48	35	52	31	14
EPHB4	Activity	Km app	73	62	63	45	25
ERBB2 (HER2)	Activity	Km app	9	19	25	21	32
ERBB4 (HER4)	Activity	Km app	10	38	35	63	85
FER	Activity	Km app	29	24	15	12	15
FES (FPS)	Activity	Km app	20	13	4	15	15
FGFR1	Activity	Km app	13	8	17	7	7
FGFR2	Activity	Km app	16	10	12	11	9
FGFR3	Activity	Km app	1	4	-3	14	15
FGFR3 K650E	Activity	Km app	5	2	4	25	22

FGFR4	**Activity**	**Km app**	14	-2	5	20	15
FGR	**Activity**	**Km app**	71	42	55	52	33
FLT1 (VEGFR1)	**Activity**	**Km app**	5	5	4	10	9
FLT3	**Activity**	**Km app**	13	12	20	28	35
FLT3 D835Y	**Activity**	**Km app**	16	14	-2	1	13
FLT4 (VEGFR3)	**Activity**	**Km app**	9	8	20	21	17
FRAP1 (mTOR)	**Activity**	**Km app**	0	0	-3	6	-5
FRK (PTK5)	**Activity**	**Km app**	89	47	74	60	33
FYN	**Activity**	**Km app**	45	37	32	37	39
GRK4	**Activity**	**Km app**	-10	-9	-12	-3	-5
GRK5	**Activity**	**Km app**	-4	1	-5	4	3
GRK6	**Activity**	**Km app**	-5	-4	-6	1	-1
GRK7	**Activity**	**Km app**	-4	-6	-6	-2	-3
GSG2 (Haspin)	**Activity**	**Km app**				28	49
GSK3A (GSK3 alpha)	**Activity**	**Km app**	5	6	2	9	11
GSK3B (GSK3 beta)	**Activity**	**Km app**	4	5	7	-3	-3
HCK	**Activity**	**Km app**	59	31	31	17	11
HIPK1 (Myak)	**Activity**	**Km app**	1	0	-1	5	5
HIPK2	**Activity**	**Km app**				4	5
HIPK3 (YAK1)	**Activity**	**Km app**					
HIPK4	**Activity**	**Km app**	9	9	8	12	10
IGF1R	**Activity**	**Km app**	4	7	2	11	10
IKBKB (IKK beta)	**Activity**	**Km**	4	5	4	5	6

		app					
IKBKE (IKK epsilon)	Activity	Km app	1	-2	-4	-1	-6
INSR	Activity	Km app	7	6	4	4	4
INSRR (IRR)	Activity	Km app	-6	-5	-6	24	21
IRAK1	Activity	Km app				7	11
IRAK4	Activity	Km app	5	2	4	-3	-2
ITK	Activity	Km app	6	4	3	6	4
JAK1	Activity	Km app	0	-6	-3	5	3
JAK2	Activity	Km app	-2	-1	4	-8	-10
JAK2 JH1 JH2	Activity	Km app	3	4	4	-3	-2
JAK2 JH1 JH2 V617F	Activity	Km app	-6	-5	-7	7	6
JAK3	Activity	Km app	-6	-6	-5	3	8
KDR (VEGFR2)	Activity	Km app	24	21	41	25	21
KIT	Activity	Km app	38	24	21	-4	-5
KIT T670I	Activity	Km app	7	5	10	-2	-1
KIT V654A	Binding					3	6
LCK	Activity	Km app	52	35	36	24	20
LIMK1	Binding					0	-5
LIMK2	Binding					-3	1
LRRK2	Activity	Km app	13	-7	5	-13	3
LRRK2 G2019S	Activity	Km app	19	10	1	4	8
LTK (TYK1)	Activity	Km app	-2	1	-2	0	1
LYN A	Activity	Km app	82	55	59	49	39
LYN B	Activity	Km app	81	55	55	43	33

MAP2K1 (MEK1)	Binding						22	38
MAP2K1 (MEK1)	Activity	100	5	15	9	1	4
MAP2K1 (MEK1) S218D S222D	Binding						18	28
MAP2K2 (MEK2)	Binding						12	21
MAP2K2 (MEK2)	Activity	100	14	8	8	-4	0
MAP2K3 (MEK3)	Binding						16	14
MAP2K6 (MKK6)	Binding						7	10
MAP2K6 (MKK6)	Activity	100	4	4	3	-11	-16
MAP2K6 (MKK6) S207E T211E	Binding						17	9
MAP3K10 (MLK2)	Binding						11	13
MAP3K11 (MLK3)	Binding						0	1
MAP3K14 (NIK)	Binding						-1	-2
MAP3K2 (MEKK2)	Binding						31	15
MAP3K3 (MEKK3)	Binding						62	35
MAP3K5 (ASK1)	Binding						6	5
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	Binding						2	4
MAP3K8 (COT)	Activity	100	14	10	12	26	22
MAP3K9 (MLK1)	Activity	Km app	1	3	0	-6	0
MAP4K2 (GCK)	Activity	Km app	7	2	4	4	6
MAP4K4 (HGK)	Activity	Km app	92	56	28	8	1
MAP4K5 (KHS1)	Activity	Km app	69	31	38	26	30
MAPK1 (ERK2)	Activity	Km app	4	6	3	2	0
MAPK10 (JNK3)	Binding						21	18
MAPK10 (JNK3)	Activity	100	4	5	6	-6	-7
MAPK11 (p38 beta)	Activity	Km app	12	15	16	14	15
MAPK12 (p38 gamma)	Activity	Km app	1	2	2	6	8
MAPK13 (p38 delta)	Activity	Km app	2	3	4	11	11
MAPK14 (p38 alpha)	Activity	100	15	26	24	7	12

MAPK14 (p38 alpha) Direct	Activity	Km app					-1	-1
MAPK3 (ERK1)	Activity	Km app	6	4	5	9	7	
MAPK8 (JNK1)	Binding					1	3	
MAPK8 (JNK1)	Activity	100	-7	-11	-5	-7	3	
MAPK9 (JNK2)	Binding					15	18	
MAPK9 (JNK2)	Activity	100	0	6	4	9	3	
MAPKAPK2	Activity	Km app	-2	0	-1	-6	-6	
MAPKAPK3	Activity	Km app	2	-1	3	-1	-1	
MAPKAPK5 (PRAK)	Activity	Km app	-2	0	0	1	1	
MARK1 (MARK)	Activity	Km app	6	4	3	10	10	
MARK2	Activity	Km app	11	8	7	14	13	
MARK3	Activity	Km app				1	-12	
MARK4	Activity	Km app				-2	-1	
MATK (HYL)	Activity	Km app	-2	0	-3	13	10	
MELK	Activity	Km app	21	17	16	16	16	
MERTK (cMER)	Activity	Km app	5	5	7	21	19	
MET (cMet)	Activity	Km app	-2	-1	0	3	3	
MET M1250T	Activity	Km app	-2	-5	-4	3	3	
MINK1	Activity	Km app	89	52	37	40	28	
MKNK1 (MNK1)	Activity	Km app						
MKNK2 (MNK2)	Binding							
MLCK (MLCK2)	Binding					6	4	
MST1R (RON)	Activity	Km app	2	2	3	-2	-2	
MST4	Activity	Km app	25	13	13	1	3	
MUSK	Activity	Km	-3	2	-2	28	29	

		app					
MYLK (MLCK)	Binding					2	1
MYLK2 (skMLCK)	Activity	Km app	0	5	-2	-3	3
NEK1	Activity	Km app	9	8	10	-8	1
NEK2	Activity	Km app	8	6	9	5	3
NEK4	Activity	Km app	3	3	3	8	5
NEK6	Activity	Km app	8	3	0	3	3
NEK7	Activity	Km app	3	4	-1	24	25
NEK9	Activity	Km app	-6	-5	-8	2	2
NLK	Binding					34	20
NTRK1 (TRKA)	Activity	Km app	4	10	16	40	33
NTRK2 (TRKB)	Activity	Km app	6	10	46	63	62
NTRK3 (TRKC)	Activity	Km app	20	12	34	66	66
NUAK1 (ARK5)	Activity	Km app				3	10
PAK1	Activity	Km app				20	26
PAK2 (PAK65)	Activity	Km app	-3	11	9	6	5
PAK3	Activity	Km app	7	7	3	11	3
PAK4	Activity	Km app	16	1	17	2	9
PAK6	Activity	Km app	14	10	11	14	14
PAK7 (KIAA1264)	Activity	Km app	8	8	4	10	13
PASK	Activity	Km app	2	3	3	-2	2
PDGFRA (PDGFR alpha)	Activity	Km app	7	3	10	11	9
PDGFRA D842V	Activity	Km app	0	-4	3	3	3
PDGFRA T674I	Activity	Km	-1	5	-2	20	21

		app					
PDGFRA V561D	Activity	Km app	20	15	29	22	15
PDGFRB (PDGFR beta)	Activity	Km app	12	-4	9	-2	-2
PDK1	Activity	100	3	0	2	18	18
PDK1 Direct	Activity	Km app				-1	1
PHKG1	Activity	Km app	6	7	5	5	5
PHKG2	Activity	Km app	-1	0	2	-9	-5
PI4KA (PI4K alpha)	Activity	10				1	-7
PI4KB (PI4K beta)	Activity	Km app				17	14
PIK3C2A (PI3K-C2 alpha)	Activity	Km app				-1	4
PIK3C2B (PI3K-C2 beta)	Activity	10				11	-1
PIK3C2B (PI3K-C2 beta)	Activity	100					
PIK3C3 (hVPS34)	Activity	Km app				-13	-9
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	Activity	Km app	0	-35	-29	-14	-4
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	Activity	Km app				13	8
PIK3CG (p110 gamma)	Activity	Km app	12	4	10	10	-9
PIM1	Activity	Km app	-4	-2	0	1	6
PIM2	Activity	Km app	1	0	2	-8	-7
PKN1 (PRK1)	Activity	Km app	8	12	5	-7	5
PLK1	Activity	Km app	5	4	6	1	-6
PLK2	Activity	Km app	7	13	9	-3	4
PLK3	Activity	Km app	16	5	8	-6	-7
PRKACA (PKA)	Activity	Km app	12	41	38	56	53
PRKCA (PKC alpha)	Activity	Km app	13	6	8	-3	0

PRKCB1 (PKC beta I)	Activity	Km app	5	6	0	7	7
PRKCB2 (PKC beta II)	Activity	Km app	-4	1	-5	9	7
PRKCD (PKC delta)	Activity	Km app	4	6	9	-1	3
PRKCE (PKC epsilon)	Activity	Km app	-2	2	1	26	30
PRKCG (PKC gamma)	Activity	Km app	4	-2	2	3	6
PRKCH (PKC eta)	Activity	Km app	2	3	4	8	15
PRKCI (PKC iota)	Activity	Km app	11	7	7	13	15
PRKCN (PKD3)	Activity	Km app	69	65	72	77	100
PRKCQ (PKC theta)	Activity	Km app	22	15	17	5	12
PRKCZ (PKC zeta)	Activity	Km app	1	5	-4	1	3
PRKD1 (PKC mu)	Activity	Km app	59	56	65	79	102
PRKD2 (PKD2)	Activity	Km app	75	83	83	85	101
PRKG1	Activity	Km app	-1	0	0	7	6
PRKG2 (PKG2)	Activity	Km app	4	6	4	-4	0
PRKX	Activity	Km app	4	4	5	1	2
PTK2 (FAK)	Activity	Km app	20	11	10	12	10
PTK2B (FAK2)	Activity	Km app	5	1	-2	4	1
PTK6 (Brk)	Activity	Km app	98	55	87	9	11
RAF1 (cRAF) Y340D Y341D	Binding					28	22
RAF1 (cRAF) Y340D Y341D	Activity	100	14	1	10	38	22
RET	Activity	Km app	79	65	67	47	46
RET V804L	Activity	Km app	11	15	10	3	2
RET Y791F	Activity	Km app	84	70	71	55	48

RIPK2	Binding					75	57
ROCK1	Activity	Km app	-1	6	0	5	3
ROCK2	Activity	Km app	3	3	-2	-13	-9
ROS1	Activity	Km app	6	5	1	11	13
RPS6KA1 (RSK1)	Activity	Km app	1	0	4	4	1
RPS6KA2 (RSK3)	Activity	Km app	4	3	3	-5	-6
RPS6KA3 (RSK2)	Activity	Km app	3	4	2	4	2
RPS6KA4 (MSK2)	Activity	Km app	25	12	11	3	8
RPS6KA5 (MSK1)	Activity	Km app	5	2	6	8	10
RPS6KA6 (RSK4)	Activity	Km app	1	5	6	2	7
RPS6KB1 (p70S6K)	Activity	Km app	4	4	4	0	-1
SGK (SGK1)	Activity	Km app	0	2	1	-9	-8
SGK2	Activity	Km app	6	5	0	-2	-7
SGKL (SGK3)	Activity	Km app	-1	-1	-1	-5	-3
SLK	Binding					5	5
SNF1LK2	Activity	Km app				9	7
SPHK1	Activity	Km app				-4	-2
SPHK2	Activity	10				-36	2
SPHK2	Activity	100					
SRC	Activity	Km app	65	27	36	16	7
SRC N1	Activity	Km app	61	21	28	23	13
SRMS (Srm)	Activity	Km app	22	7	11	22	21
SRPK1	Activity	Km app	5	5	4	6	4
SRPK2	Activity	Km app	0	0	3	-3	1

STK16 (PKL12)	Binding					11	3
STK17A (DRAK1)	Binding					10	6
STK22B (TSSK2)	Activity	Km app	2	4	2	6	4
STK22D (TSSK1)	Activity	Km app	5	4	5	0	1
STK23 (MSSK1)	Activity	Km app	6	5	6	4	3
STK24 (MST3)	Activity	Km app	22	9	9	0	4
STK25 (YSK1)	Activity	Km app	11	1	5	-2	-3
STK3 (MST2)	Activity	Km app	13	4	3	5	8
STK33	Binding					3	1
STK4 (MST1)	Activity	Km app	14	8	5	12	18
SYK	Activity	Km app	7	10	4	-10	-3
TAOK2 (TAO1)	Activity	Km app	3	-1	1	-4	2
TAOK3 (JIK)	Binding					9	6
TBK1	Activity	Km app	7	8	5	9	9
TEC	Binding					3	3
TEK (Tie2)	Activity	Km app	-2	0	-4	-5	3
TGFBR1 (ALK5)	Binding						
TNK2 (ACK)	Binding					29	31
TTK	Binding					0	0
TXK	Activity	Km app					
TYK2	Activity	Km app	0	0	1	10	10
TYRO3 (RSE)	Activity	Km app	9	3	2	24	21
WEE1	Binding					-3	-1
WNK2	Binding					-3	-4
YES1	Activity	Km app	54	42	52	42	39
ZAK	Binding					1	1

ZAP70	**Activity**	**Km app**	-7	-5	-6	14	10

Supplementary Table S2. Kinase inhibitor profiling data was provided by SelectScreen® Kinase Profiling Services from Life Technologies. 1μM of each inhibitor (1NA-PP1, 1NM-PP1, 3MB-PP1, 17 and 18) was screened against a panel of several hundred protein kinases in duplicate. The average % activity inhibited is listed for each compound against each kinase.

Data Collection			
Structure	**Src-1NA**	**Src-1NM**	**Src-3MB**
Space Group	P212121	P1	P1
Unit Cell Dimensions	a= 50.96, b=72.72 , c=171.98 , α=90 , β=90 , γ =90	a=42.3 , b=62.7 , c=74.3 , α=100.4 , β=91.0 , γ=90.1	a=42.0 , b=55.8 , c=63.0 , α=90.0 , β=90.1 , γ=88.9
Number protein molceules/assymetric unit	1	2	2
X-ray Source	CHESS A1	ALS 5.0.1	ALS 5.0.1
Resolution (Å)	2.3	2.84	2.4
Total Reflections	101969	16738	21085
Unique Reflections	28882	16738	18807
Completeness (%)	98.7 (99.6 in 2.38-2.3 shell)	94.7	95.1
Model Refinement			
Resolution (Å)	25-2.3	32.93-2.84	33.87-2.41
Rwork/Rfree	0.213/0.25	0.230/0.287	0.232/0.281
Rmsd from ideality in bond length (Å)	0.018	0.008	0.014
Rmsd from ideality in Angles (°)	1.38	1.12	1.4
Number of Protein Atoms In Model	3607	3874	7685
Number of Drug atoms In Model	24	50	43

Number of waters	278	37	46
Favored/Allowed/Outliers in the Ramachandran Plot (%)	97.3/2.7/0	95.6/4.4/0	96.6/3.4/0

Supplemental Table S3. Data collection and refinement statistics for X-ray crystal structures of Src(T338G) in complex with PP inhibitors.

Supplementary Experimental

Compound Characterization

1-*tert*-Butyl-3-(2,3-dimethylphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (1). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.81 (s, 9H), 2.17 (s, 3H), 2.33 (s, 3H), 5.25 (br s, 2H), 7.20 (m, 3H), 8.31 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.5, 20.6, 29.3, 60.3, 100.9, 125.9, 127.9, 130.7, 132.6, 136.2, 138.2, 142.0, 153.7, 154.6, 157.8; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.17968.

3-Benzyl-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (2). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 4.29 (s, 2H), 4.89 (br s, 2H), 7.20 (d, J = 7 Hz, 2H), 7.25 (t, J = 7 Hz, 1H), 7.32 (t, J = 7 Hz, 2H), 8.23 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.2, 35.2, 59.9, 100.7, 127.3, 128.4, 129.4, 138.2, 140.9, 154.5, 154.7, 157.6; HRMS (EI) molecular ion calculated for $C_{16}H_{19}N_5$ 281.16405, found 281.16410.

1-*tert*-Butyl-3-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (3). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.74 (s, 9H), 3.11 (t, 2H), 3.22 (t, 2H), 6.32 (s, 2H), 7.17 (d, 2H), 7.25 (m, 3H), 8.17 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.2, 31.3, 34.8, 61.1,

99.7, 126.7, 128.5, 128.8, 140.6, 143.2, 147.8, 152.3, 155.1; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.17888.

1-*tert*-Butyl-3-cyclopentylmethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (4). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (m, 2H), 1.53 (m, 2H), 1.65 (m, 2H), 1.71 (m, 2H), 1.73 (s, 9H), 2.28 (m, 1H), 2.86 (d, J = 8 Hz, 2H), 5.69 (br s, 2H), 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.0, 29.2, 32.4, 35.2, 39.7, 59.7, 100.5, 142.1, 153.0, 154.0, 158.0; HRMS (EI) molecular ion calculated for $C_{15}H_{23}N_5$ 273.19535, found 273.19565.

1-*tert*-Butyl-3-(1-phenethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (5). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.71 (d, J = 7 Hz, 3H), 1.79 (s, 9H), 4.38 (q, J = 7 Hz, 1H), 5.06 (br s, 2H), 7.19 (d, J = 7 Hz, 2H), 7.22 (m, 1H), 7.30 (t, J = 7 Hz, 2H), 8.16 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.0, 29.1, 40.1, 60.0, 100.0, 127.2, 127.4, 129.3, 144.5, 145.2, 153.6, 154.5, 157.3; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.18043.

1-*tert*-Butyl-3-(2-methylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (6). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77 (s, 9H), 2.30 (s, 3H), 4.25 (s, 2H), 5.09 (br s, 2H), 6.98 (d, J = 8 Hz, 1H), 7.14 (m, 3H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.8, 29.1, 33.1, 59.9, 100.9, 126.7, 127.3, 128.3, 130.8, 136.1, 136.8, 140.6, 154.3, 154.5, 157.8; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.17922.

1-*tert*-Butyl-3-(2-chlorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (7). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78 (s, 9H), 4.38 (s, 2H), 5.41 (br s, 2H), 7.00 (dd, J_1 = 7 Hz, J_2 = 2 Hz, 1H), 7.15 (m, 2H), 7.39 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 1H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.1, 31.9, 60.0, 100.7, 127.4, 128.4, 129.6, 130.1, 133.3, 135.6, 139.4, 154.4, 154.5, 157.7; HRMS (EI) molecular ion calculated for $C_{16}H_{18}N_5Cl$ 315.12507, found 315.12449.

1-*tert*-Butyl-3-(2-methoxybenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (8). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77 (s, 9H), 3.89 (s, 3H), 4.25 (s, 2H), 5.77 (br s, 2H), 6.89 (m, 2H), 7.18 (m, 2H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.4, 29.2,

55.5, 59.8, 100.4, 110.8, 121.5, 126.5, 128.0, 130.6, 141.1, 154.3, 154.3, 155.7, 158.0; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5O$ 311.17461, found 311.17545.

1-*tert*-Butyl-3-(3-methylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (3MB). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 2.29 (s, 3H), 4.24 (s, 2H), 4.95 (br s, 2H), 6.99 (d, J = 7 Hz, 1H), 7.00 (s, 1H), 7.06 (d, J = 8 Hz, 1H), 7.20 (t, J = 8 Hz, 1H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.4, 29.2, 35.1, 59.9, 100.7, 125.4, 128.1, 129.1, 129.1, 138.1, 139.0, 141.1, 154.4, 154.7, 157.7; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.17981.

1-(*tert*-butyl)-3-(3-chlorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (10). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 3.79 (s, 3H), 3.25 (s, 2H), 7.06 (d, 1H), 7.19 (m, 3H), 8.26 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.2, 34.8, 60.1, 77.2, 100.6, 126.4, 127.5, 128.5, 130.4, 135.1, 139.8, 140.2, 154.6, 157.5; HRMS (EI) molecular ion calculated for $C_{16}H_{19}N_5{}^{35}Cl$ 316.1324, found 316.1314.

3-(3-Methoxybenzyl)-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (11). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 3.74 (s, 3H), 4.25 (s, 2H), 4.89 (s, 2H), 6.72 (s, 1H), 6.79 (m, 2H), 7.22 (s, 1H), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.4, 35.4, 55.4, 60.1, 100.9, 112.5, 114.7, 120.8, 130.5, 140.1, 141.0, 154.7, 154.9, 157.8, 160.5; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5O$ 311.1824, found 311.1835.

1-*tert*-Butyl-3-(4-methylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (12). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 2.30 (s, 3H), 4.24 (s, 2H), 4.94 (br s, 2H), 7.08 (d, J = 8 Hz, 2H), 7.11 (d, J = 8 Hz, 2H), 8.21 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.0, 29.2, 34.8, 59.9, 100.6, 128.3, 130.0, 135.1, 136.9, 141.3, 154.5, 154.7, 157.7; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5$ 295.17970, found 295.18068.

1-*tert*-Butyl-3-(4-chlorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (13). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77 (s, 9H), 4.25 (s, 2H), 5.16 (br s, 2H), 7.11 (d, J = 8 Hz, 2H), 7.27 (d, J = 8 Hz, 2H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.1, 34.5, 60.0, 100.6, 129.3, 129.6, 133.1, 136.6, 140.1, 154.5, 154.6, 157.6; HRMS (EI) molecular ion calculated for $C_{16}H_{18}N_5Cl$ 315.12507, found 315.12545.

1-*tert*-Butyl-3-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (14). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78 (s, 9H), 3.76 (s, 3H), 4.22 (s, 2H), 4.91 (br s, 2H), 6.84 (d, J = 9 Hz, 2H), 7.11 (d, J = 9 Hz, 2H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.2, 34.3, 55.2, 59.9, 100.6, 114.6, 129.4, 130.0, 141.4, 154.5, 154.7, 157.6, 158.7; HRMS (EI) molecular ion calculated for $C_{17}H_{21}N_5O$ 311.17461, found 311.17454.

3-(3-Trifluoromethylbenzyl)-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (15). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.789 (s, 9H), 4.349 (s, 2H), 4.917 (s, 2H), 7.360 (d, 1H), 7.430 (t, 1H), 7.506 (s, 1H), 7.516 (d, 1H), 8.262 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.144, 34.961, 60.172, 77.205, 100.601, 124.108, 125.042, 129.640, 131.265, 131.652, 139.124, 139.474, 154.646, 154.699, 157.387; ESI-HRMS $[MH]^+$ calculated for $C_{17}H_{18}F_3N_5$ 350.1587, found 350.1581.

3-(3-Bromobenzyl)-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (16). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 4.28 (s, 2H), 5.73 (s, 2H), 7.13 (d, 1H), 7.20 (t, 1H), 7.36 (s, 1H), 7.40 (d, 1H), 8.21 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.2, 34.7, 60.8, 100.1, 123.5, 126.9, 130.7, 130.8, 131.4, 140.0, 141.0, 151.5, 153.8, 156.0; HRMS (EI) molecular ion calculated for $C_{16}H_{18}BrN_5$ 359.07456, found 359.07423.

3-(3-Iodobenzyl)-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (17). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 4.24 (s, 2H), 4.90 (s, 2H), 7.04 (t, 1H), 7.13 (d, 1H), 7.58 (s, 1H), 7.60 (d, 1H), 8.26 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.4, 34.9, 60.3, 95.4, 100.8, 127.8, 131.0, 136.6, 137.5, 140.0, 140.8, 154.8, 154.9, 157.7; HRMS (EI) molecular ion calculated for $C_{16}H_{18}IN_5$ 407.0685, found 407.0705.

3-(3-Methylthiolbenzyl)-1-*tert*-butyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (18). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.791 (s, 9H), 2.414 (s, 3H), 4.250 (s, 2H), 4.886 (s, 2H), 6.937 (d, 1H), 7.097 (s, 1H), 7.123 (d, 1H), 7.225 (t, 1H), 8.243 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.567, 29.190, 35.105, 60.013, 77.205, 100.685, 124.924, 125.076, 126.283, 129.602, 138.942, 139.853, 140.468, 154.585, 154.767, 157.585; HRMS (EI) $[MH]^+$ calculated for $C_{17}H_{21}N_5S$ 328.1577, found 328.1583.

1-*tert*-Butyl-3-(2,3-dimethylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (19). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78 (s, 9H), 2.19 (s, 3H), 2.28 (s, 3H), 4.28 (s, 2H), 4.86 (s, 2H), 6.88 (d, 1H), 7.02 (t, 1H), 7.08 (d, 1H), 8.23 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.3, 20.6, 29.2, 34.0, 59.9, 100.9, 126.1, 126.3, 129.2, 135.7, 136.0, 137.8, 141.1, 154.4, 154.6, 157.7; HRMS (EI) molecular ion calculated for $C_{18}H_{23}N_5$ 309.19535, found 309.19515.

1-*tert*-Butyl-3-(3,4-dimethylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (20). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 2.20 (s, 3H), 2.21 (s, 3H), 4.21 (s, 2H), 5.45 (s, 2H), 6.91 (d, 1H), 6.96 (s, 1H), 7.07 (d, 1H), 8.18 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.4, 19.8, 29.2, 34.8, 60.4, 100.2, 125.7, 129.6, 130.6, 135.0, 136.0, 137.9, 142.4, 151.5, 153.9, 156.4; HRMS (EI) molecular ion calculated for $C_{18}H_{23}N_5$ 309.19535, found 309.19542.

1-*tert*-Butyl-3-(2,5-dimethylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (21). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 2.20 (s, 3H), 2.25 (s, 3H), 4.22 (s, 2H), 4.94 (s, 2H), 6.81 (s, 1H), 6.98 (d, 1H), 7.08 (d, 1H), 8.23 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.3, 21.0, 29.2, 33.1, 59.9, 100.9, 128.1, 129.1, 130.9, 133.6, 135.9, 136.3, 140.9, 154.3, 154.6, 157.7; HRMS (EI) molecular ion calculated for $C_{18}H_{23}N_5$ 309.19535, found 309.19386.

1-*tert*-Butyl-3-(3,5-dimethylbenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (22). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80 (s, 9H), 2.25 (s, 6H), 4.20 (s, 2H), 5.00 (s, 2H), 6.80 (s, 2H), 6.88 (s, 1H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.3, 29.2, 35.1, 59.9, 100.7, 126.2, 129.0, 138.0, 138.9, 141.3, 154.4, 154.7, 157.7; HRMS (EI) molecular ion calculated for $C_{18}H_{23}N_5$ 309.19535, found 309.19439.

1-*tert*-Butyl-3-(3,4-dichlorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (23). White powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (s, 9H), 4.24 (s, 2H), 5.07 (s, 2H), 7.02 (dd, 1H), 7.29 (d, 1H), 7.37 (d, 1H), 8.26 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.1, 34.3, 60.2, 100.6, 127.6, 130.2, 131.0, 131.4, 133.2, 138.4, 139.2, 154.6, 154.7, 157.5; HRMS (EI) molecular ion calculated for $C_{16}H_{17}N_5Cl_2$ 349.08610, found 349.08621.

Kinase inhibition assays *in vitro*

Fyn Kinase. Glutathione S-transferase (GST) fused Fyn proteins were expressed in *E. coli* and purified on glutathione beads as described previously[35]. In the Fyn kinase assay, various concentrations of inhibitor were incubated with 50 mM Tris (pH 8.0), 10 mM $MgCl_2$, 1.6 mM glutathione, 1 mg/mL BSA, 0.1 mg/mL peptide substrate (IYGEFKKK), 3.3% DMSO, and 11 nM (2 μCi) [γ-^{32}P]ATP (6000 Ci/mmol, NEN), and Fyn kinase in a total volume of 30 μL for 30 min. Reaction mixtures (27 μL) were spotted onto a phosphocellulose disk, and washed with 0.5% H_3PO_4. The transfer of ^{32}P was measured by standard scintillation counting. IC_{50} values were defined to be the concentration of inhibitor at which the radioactivity counts remaining on the phosphocellulose disk were inhibited by 50%.

Src Kinase. 6xHis-tagged Src (257-533) was expressed in BL-21 *ecoli* cells as previously described[72]. Kinase reaction was carried out as described for Fyn kinase above.

CK1δ and CK1ε. Casein Kinase 1δ (catalog # PV3665) and Casein Kinase 1ε (catalog # PV3500) were purchased from Life Technologies and assayed under the following conditions: 50mM TRIS (pH 8.0), 10mM $MgCl_2$, 0.4 mg/mL casein, 2.5 mM DTT, 2% DMSO, 5nM kinase, 100μM ATP, 0.1 mg/mL BSA, 1μCi ^{32}P-ATP and various concentrations of inhibitors.

PKD1 and PKD2. PKD1 (catalog # PV3791) and PKD2 (catalog # PV3758) were purchased from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2mM DTT, 2% DTT, 0.1 mg/mL BSA, 100 μM ATP, 3 μCi ^{32}P-ATP, 7 nM kinase and various concentrations of inhibitors.

RET and ACK. RET (catalog # PV3819) and ACK (catalog # PV4807) were purchase from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 0.1 mg/mL BSA, 2% DMSO, 200 μM Abltide, 10 nM kinase, 100 μM ATP, 1μCi ^{32}P-ATP and variable concentrations of inhibitors.

EPHA1 and PTK6. EPHA1 (catalog # 3841) and PTK6 (catalog # 3291) were purchased from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2.5 mM DTT, 0.1 mg/mL BSA, 2% DMSO, 0.2 mg/mL poly[Glu, Tyr] 4:1, 2 nM kinase, 100 μM ATP, 1μCi ^{32}P-ATP and variable concentrations of inhibitors.

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