Supplemental Data

Figure 1. Effect of ATP concentration on inhibition of PI3K-α by PKI-402; IC50 values at 25, 100, 250, and 500 mM ATP.

PKI-402: IC50 nM

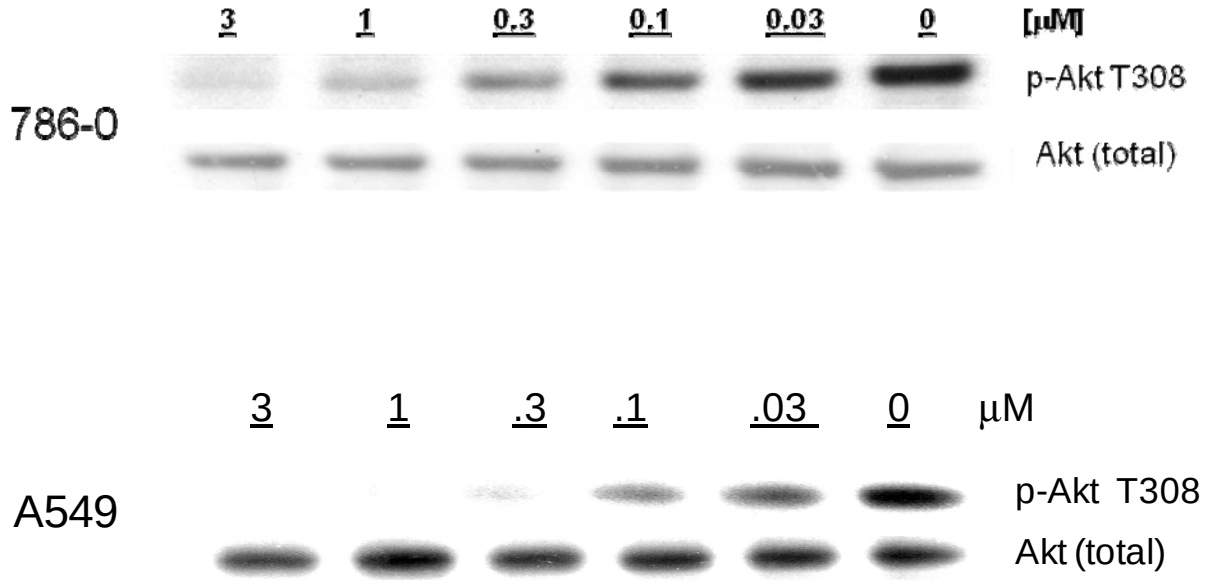
ATP [μM]	25	100	250	500	
	0.6	0.8	3.8	4.5	
ATP [μM]	**25**	**100***	**250***	**500***	*Observed ratio IC50/IC50 at 25 μM
		1.4	6.1	7.3	
Theoretical^		**2**	**4**	**7.3**	

^Cheng & Prusoff (1973) Biochemical Pharm. 22:3099

Figure 2. Kinase Selectivity Data for PKI-402. Invitrogen 236 Human Kinase Panel run with 10 µM PKI-402 and with ATP concentration at respective Km for each enzyme.

Kinase Tested	% Inh.	Kinase Tested	% Inh.	Kinase Tested	% Inh.	Kinase Tested	% Inh.	Kinase Tested	% Inh.	Kinase Tested	% Inh.
ABL1	2	CSF1R (FMS)	15	FGR	7	MAP4K2 (GCK)	2	PASK	26	ROS1	7
ABL1 E255K	5	CSK	3	FLT1 (VEGFR1)	-3	MAP4K4 (HGK)	-7	PDGFRA (PDGFR alpha)	-12	RPS6KA1 (RSK1)	2
ABL1 G250E	1	CSNK1A1 (CK1 alpha 1)	14	FLT3	14	MAP4K5 (KHS1)	17	PDGFRA D842V	4	RPS6KA2 (RSK3)	0
ABL1 T315I	2	CSNK1D (CK1 delta)	15	FLT3 D835Y	7	MAPK1 (ERK2)	6	PDGFRA T674I	2	RPS6KA3 (RSK2)	-11
ABL1 Y253F	-3	CSNK1E (CK1 epsilon)	36	FLT4 (VEGFR3)	8	MAPK10 (JNK3)	10	PDGFRA V561D	-3	RPS6KA4 (MSK2)	24
ABL2 (Arg)	-1	CSNK1G1 (CK1 gamma 1)	-4	FRAP1 (mTOR)	98	MAPK11 (p38 beta)	22	PDGFRB (PDGFR beta)	5	RPS6KA5 (MSK1)	7
ACVR1B (ALK4)	2	CSNK1G2 (CK1 gamma 2)	1	FRK (PTK5)	14	MAPK12 (p38 gamma)	6	PDK1	12	RPS6KA6 (RSK4)	9
ADRBK1 (GRK2)	9	CSNK1G3 (CK1 gamma 3)	2	FYN	4	MAPK13 (p38 delta)	9	PHKG1	7	RPS6KB1 (p70S6K)	0
ADRBK2 (GRK3)	2	CSNK2A1 (CK2 alpha 1)	10	GRK4	0	MAPK14 (p38 alpha)	16	PHKG2	12	SGK (SGK1)	-7
AKT1 (PKB alpha)	1	CSNK2A2 (CK2 alpha 2)	16	GRK5	4	MAPK3 (ERK1)	2	PIM1	12	SGK2	-3
AKT2 (PKB beta)	6	DAPK3 (ZIPK)	9	GRK6	0	MAPK8 (JNK1)	10	PIM2	7	SGKL (SGK3)	-3
AKT3 (PKB gamma)	3	DCAMKL2 (DCK2)	20	GRK7	2	MAPK9 (JNK2)	9	PKN1 (PRK1)	9	SRC	17
ALK	20	DYRK1A	11	GSK3A (GSK3 alpha)	17	MAPKAPK2	0	PLK1	14	SRC N1	10
AMPK A1/B1/G1	-4	DYRK1B	6	GSK3B (GSK3 beta)	8	MAPKAPK3	9	PLK2	28	SRMS (Srm)	20
AMPK A2/B1/G1	3	DYRK3	12	HCK	-5	MAPKAPK5 (PRAK)	8	PLK3	7	SRPK1	9
AURKA (Aurora A)	14	DYRK4	3	HIPK1 (Myak)	9	MARK1 (MARK)	16	PRKACA (PKA)	-7	SRPK2	3
AURKB (Aurora B)	2	EEF2K	19	HIPK4	9	MARK2	14	PRKCA (PKC alpha)	5	STK22B (TSSK2)	10
AURKC (Aurora C)	11	EGFR (ErbB1)	9	IGF1R	13	MATK (HYL)	11	PRKCB1 (PKC beta I)	-3	STK22D (TSSK1)	4
AXL	8	EGFR (ErbB1) L858R	-10	IKBKB (IKK beta)	0	MELK	39	PRKCB2 (PKC beta II)	4	STK23 (MSSK1)	14
BLK	5	EGFR (ErbB1) L861Q	4	IKBKE (IKK epsilon)	-3	MERTK (cMER)	10	PRKCD (PKC delta)	6	STK24 (MST3)	6
BMX	7	EGFR (ErbB1) T790M	-5	INSR	10	MET (cMet)	19	PRKCE (PKC epsilon)	12	STK25 (YSK1)	5
BRAF	80	EGFR (ErbB1) T790M L858R	0	INSRR (IRR)	29	MET M1250T	6	PRKCG (PKC gamma)	3	STK3 (MST2)	0
BRAF V599E	89	EPHA1	13	IRAK4	-7	MINK1	0	PRKCH (PKC eta)	11	STK4 (MST1)	6
BRSK1 (SAD1)	8	EPHA2	5	ITK	7	MST1R (RON)	7	PRKCI (PKC iota)	-14	SYK	3
BTK	11	EPHA3	7	JAK1	-7	MST4	5	PRKCN (PKD3)	5	TAOK2 (TAO1)	-2
CAMK1D (CaMKI delta)	6	EPHA4	4	JAK2	-1	MUSK	-1	PRKCQ (PKC theta)	4	TBK1	7
CAMK2A (CaMKII alpha)	12	EPHA5	9	JAK2 JH1 JH2	-8	MYLK2 (skMLCK)	8	PRKCZ (PKC zeta)	4	TEK (Tie2)	-2
CAMK2B (CaMKII beta)	9	EPHA8	5	JAK2 JH1 JH2 V617F	2	NEK1	5	PRKD1 (PKC mu)	8	TYK2	5
CAMK2D (CaMKII delta)	7	EPHB1	5	JAK3	-6	NEK2	1	PRKD2 (PKD2)	4	TYRO3 (RSE)	11
CAMK4 (CaMKIV)	-2	EPHB2	10	KDR (VEGFR2)	14	NEK4	16	PRKG1	0	YES1	7
CDC42 BPA (MRCKA)	1	EPHB3	5	KIT	30	NEK6	20	PRKG2 (PKG2)	14	ZAP70	-4
CDC42 BPB (MRCKB)	2	EPHB4	7	KIT T670I	8	NEK7	31	PRKX	4		
CDK1/cyclin B	0	ERBB2 (HER2)	8	LCK	19	NEK9	12	PTK2 (FAK)	2		
CDK2/cyclin A	3	ERBB4 (HER4)	8	LTK (TYK1)	-1	NTRK1 (TRKA)	13	PTK2B (FAK2)	3		
CDK5/p25	0	FER	5	LYN A	6	NTRK2 (TRKB)	16	PTK6 (Brk)	8		
CDK5/p35	3	FES (FPS)	8	LYN B	12	NTRK3 (TRKC)	24	RAF1 (cRAF) Y340D Y341D	57		
CHEK1 (CHK1)	2	FGFR1	-2	MAP2K1 (MEK1)	18	PAK2 (PAK65)	11	RET	1		
CHEK2 (CHK2)	0	FGFR2	0	MAP2K2 (MEK2)	16	PAK3	6	RET V804L	4		
CLK1	17	FGFR3	17	MAP2K6 (MKK6)	1	PAK4	0	RET Y791F	5		
CLK2	4	FGFR3 K650E	4	MAP3K8 (COT)	28	PAK6	-9	ROCK1	-7		
CLK3	9	FGFR4	5	MAP3K9 (MLK1)	9	PAK7 (KIAA1264)	3	ROCK2	-5		

Figure 3. Renal carcinoma cell lines 786-0 and A498 exposed to PKI402 for 4 h, lysates then probed for suppression of p-Akt (T308), and for total Akt. Inhibition of protein phosphorylation was quantified from Western blots analyzed on the BioRad Fluor-S MultiImager (Hercules, CA), using Quantity One Analysis software.



IC50 μM	
Cell Line	**p-Akt T308**
786-0	0.223
A549	0.097

Figure 4. In Vitro Profile of PKI-402 (Figure2, Panel A). MDA-MB-361 lysates probed with Actin Ab.

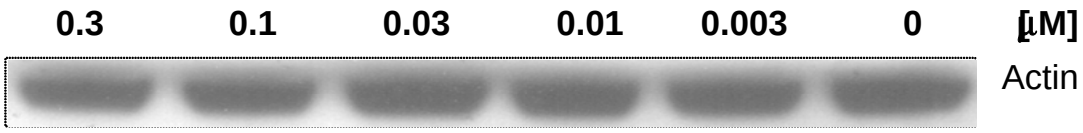


Figure 5. MDA-MB-361 cell survival after 24 h exposure to PKI-402 (0.001 to 3.0 mM), followed by removal of compound, wash, and subsequent 24 h incubation in normal growth medium. Surviving cells were quantified using Thermo Scientific Cellomics ArrayScan VTI HCS Reader.