

Supplementary Table 1. Percent inhibition of a panel of protein kinases performed using ProfilerPro Kinase Selectivity Kits with 10 μ M Y-27632 or 200 nM OXA-06. Consistent data were generated for 183 kinases within this analysis (kinases that failed in one or more assays, or which did not generate data with CV values below 25% are not included). This table shows the inhibition percentages for every kinase tested.

Supplementary Figure 1. OXA-06 exhibits potent inhibition of PANC-1 cell migration and MYPT1 phosphorylation. A, Cell migration assay. PANC-1 cells were allowed to migrate towards growth medium supplemented with 10% FCS containing the indicated final concentrations of each inhibitor for 16 h. Assays were performed in duplicate wells, one for each inhibitor concentration, and are representative of three independent experiments. Data shown are the percent migrated cells relative to vehicle control (0.1% DMSO). B, Inhibition of MYPT1 phosphorylation in PANC-1 cells. The indicated concentrations of OXA-06 or Y-27632 were added to PANC-1 cells, and an ELISA capture assay was used to quantify the amount of phosphorylated (pT853) MYPT1. Data shown represent the percent decrease in phosphorylated MYPT1 relative to vehicle-treated cells.

Supplementary Figure 2. OXA-06 treatment does not significantly alter apoptosis or the activity regulators of G₁ progression in NSCLC lines. A, OXA-06 treatment did not induce apoptosis in suspended NSCLC cells. Apoptosis was measured by western blot analyses for cleaved PARP and caspase-3. Indicated cell lines were plated on Corning Ultra-Low Attachment Plates and treated with vehicle (DMSO), 10 μ M OXA-06, or 10 μ M staurosporine as a positive control.

Vehicle and OXA-06 treated cells in suspension were harvested as lysates after 48 h, and staurosporine treated cells after 6 h. The positions of cleaved PARP and caspase-3 are indicated by an arrow. B, Rb hyperphosphorylation and inactivation is not increased by OXA-06 treatment. Western blot analyses was done to monitor changes in phosphorylated Rb (pS608), with total Rb and β -actin determined to verify equivalent levels of total protein. A549 and H1299 cells were plated on Corning Ultra-Low Attachment Plates and treated with vehicle (DMSO) or OXA-06, and were harvested as lysates at 48 h.

Supplementary Figure 3. Full scans of western blots for Figure 4. Full scans corresponding to the indicated cell lines from Figure 4. Dotted lines indicate where the membranes were cut horizontally in order to blot with different antibodies. Exposure times of the scanned images are indicated on the right hand side. In some instances, different exposure times are shown for the different membrane slices.

Supplementary Figure 4. Full scans of western blots for Figure 5. Dotted lines indicate where the membranes were cut horizontally in order to blot with different antibodies. Exposure times of the scanned images are indicated on the right hand side. In some instances, different exposure times are shown for the different membrane slices.

Supplementary Table 1. Selectivity Comparison of OXA-06 and Y27632

Kinase	OXA-06 (0.2 mM)		Y27632 (10 mM)	
	% Inhibition	SD	% Inhibition	SD
ABL	7	6	17	11
ABL_E255K	1	3	-3	6
ABL_G250E	2	1	3	1
ABL_H396P	-1	1	1	2
ABL_Q252H	-5	2	-1	3
ABL_T315I	-4	0	5	8
ABL_Y253F	-2	2	-7	4
ALK	-2	6	0	5
AMPK A2/B1/G1	4	6	12	8
AMPKa1	10	6	21	3
ARG	-10	10	-10	15
ARK5	56	4	1	6
AURORA_A	-8	23	-10	24
AURORA_B	45	1	21	14
AXL	-31	5	-27	10
BLK	-12	9	-11	11
BMX	-8	10	-4	2
BRSK1	9	1	20	4
BRSK2	7	9	27	4
BTK	-18	19	-19	22
CAMK1	-2	7	0	2
CAMK1delta	-3	2	8	2
CAMKIIalpha	0	3	12	6
CAMKIIalpha	-27	8	-28	7
CAMKIIbeta	-3	10	-2	2
CAMKIIgamma	12	3	12	1
CAMKIV	8	0	7	2
CDK1_cyclinB	-8	1	3	2
CDK2_cyclinA	-3	9	25	7
CDK3_cyclinE	3	15	11	2
CDK5_p25	-3	3	15	1
CHK1	-5	10	-6	23
CHK2	0	10	20	7
CK1alpha1	-10	2	-8	0
CK1epsilon	2	8	4	1
CK1gamma1	-1	1	6	14
CK1gamma2	2	3	9	0
CK1gamma3	-2	1	1	4
CLK2	2	4	18	5
cRAF	0	5	1	6

Supplementary Table 1. Selectivity Comparison of OXA-06 and Y27632

Kinase	OXA-06 (0.2 mM)		Y27632 (10 mM)	
	% Inhibition	SD	% Inhibition	SD
CSF_1R	1	4	-2	0
CSNK1delta	1	9	1	17
DAPK1	5	6	1	12
DCAMKL1	-4	2	-2	4
DCAMKL2	-3	9	0	4
DDR2	38	18	24	10
DYRK1A	17	19	19	17
DYRK1B	-5	4	5	5
DYRK3	-3	3	3	1
DYRK4	-10	4	-7	0
EGF_R	-5	3	4	6
EGFR_T790M	-1	4	3	4
EGFR_T790M_L858R	-8	5	-8	3
EPHA1	-3	4	0	1
EPHA2	-1	2	2	1
EPHA3	-8	3	-5	2
EPHA4	-17	12	-14	12
EPHA5	-5	5	-4	6
EPHA8	-4	2	-7	13
EPHB1	-10	1	-5	1
EPHB2	1	5	-2	4
EPHB3	-3	0	0	1
EPHB4	-3	2	2	4
ERBB4	10	4	7	5
ERK1	0	13	2	10
FER	-9	10	-5	3
FES	-5	7	3	0
FGFR1	4	1	13	2
FGFR1_V561M	8	7	13	4
FGFR2	-7	1	-4	1
FGFR2_N549H	-9	0	-1	1
FGFR3	1	2	6	2
FGFR3_K650E	-17	5	-16	9
FGFR4	-5	3	2	11
FGR	-2	5	0	10
FLT1	-8	0	-7	4
FLT3	14	2	11	2
FLT3_D835Y	15	10	16	6
FLT4	-11	3	-4	3
FRK	2	1	5	5

Supplementary Table 1. Selectivity Comparison of OXA-06 and Y27632

Kinase	OXA-06 (0.2 mM)		Y27632 (10 mM)	
	% Inhibition	SD	% Inhibition	SD
FYN	-6	3	2	1
GSK3alpha	3	1	18	1
GSK3beta	-7	1	6	3
HCK	0	0	-3	2
HGK	5	3	8	8
HIPK1	-6	1	-7	5
HIPK2	1	2	-5	2
IGF_1R	-8	8	5	4
IKKbeta	56	10	82	9
IRAK4	2	0	7	6
ITK	-4	6	6	4
JAK2	39	4	3	1
KDR	-3	6	-6	3
LCK	-10	11	8	6
LTK	2	1	5	2
LYN	-10	8	-2	5
LYNB	-12	6	-8	0
MAP4K2	-12	1	-2	2
MAPK1	-10	3	-3	1
MAPK11	-4	1	-4	3
MAPK12	1	8	6	7
MAPK13	4	3	3	3
MAPK14_T106M	1	5	6	7
MAPKAPK2	-10	2	3	2
MAPKAPK3	-1	1	3	1
MAPKAPK5	-1	2	2	3
MARK1	6	5	23	6
MARK2	5	9	8	8
MARK3	12	16	10	14
MARK4	-4	2	1	1
MELK	7	1	2	3
MER	-1	3	-3	2
MET_M1250T	-5	3	-9	6
MINK1	5	1	1	6
MNK1	16	1	78	1
MSK1	43	11	43	15
MSK2	-29	13	-48	1
MST1	19	20	59	9
MST3	7	3	10	4
NEK1	1	14	-3	19

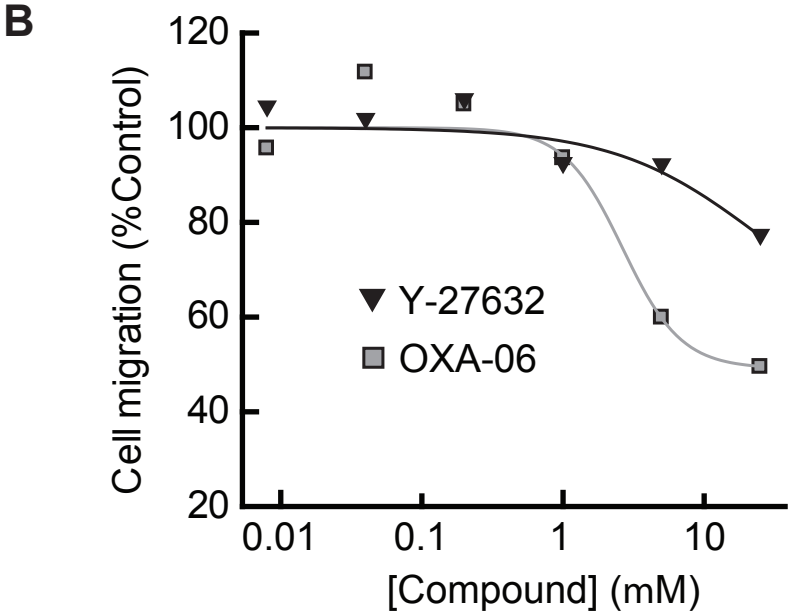
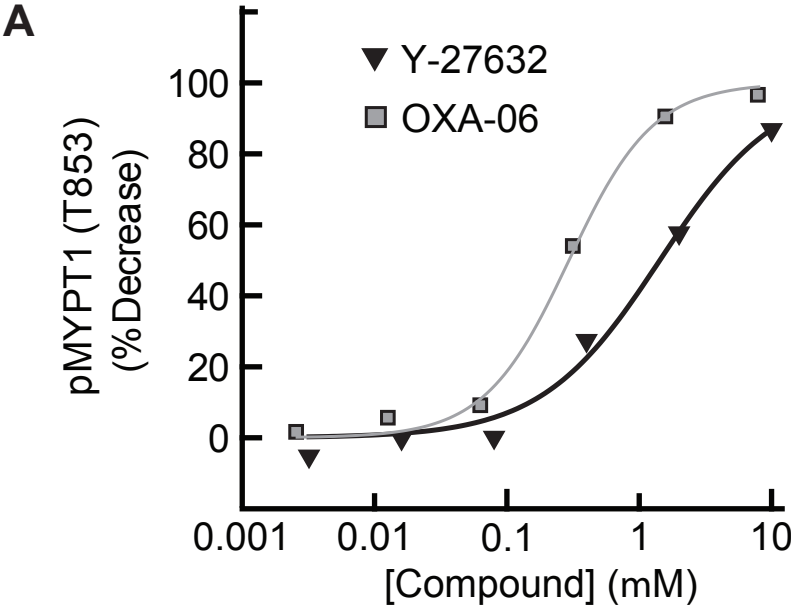
Supplementary Table 1. Selectivity Comparison of OXA-06 and Y27632

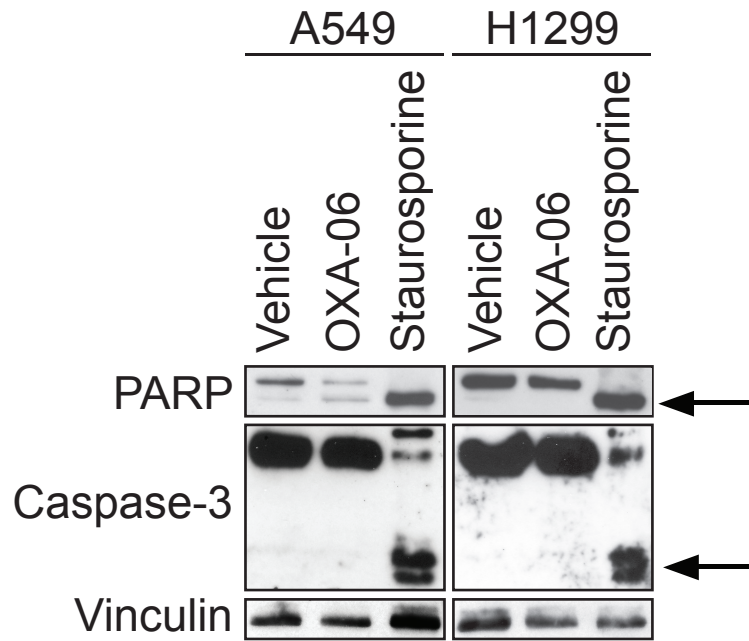
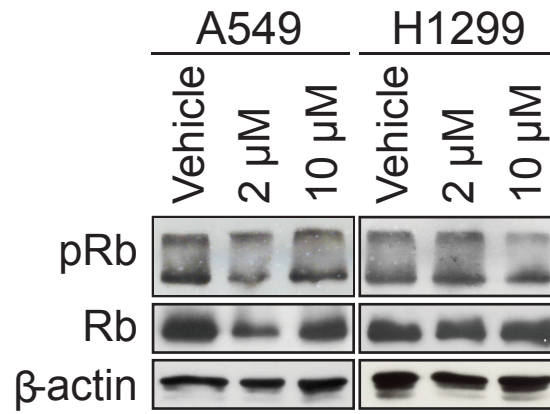
Kinase	OXA-06 (0.2 mM)		Y27632 (10 mM)	
	% Inhibition	SD	% Inhibition	SD
P38alpha	-5	4	-8	3
PAK2	-2	0	3	2
PAK3	-9	1	-10	5
PAK4	4	3	1	2
PAK5	9	6	13	14
PASK	-1	8	1	2
PDGFRa_D842V	5	5	19	15
PDGFRa_V561D	-7	1	-14	5
PDGFRalpha	-26	8	-34	12
PDGFRbeta	-2	17	-11	6
PHKG2	-4	2	-11	4
PIM1	-7	3	2	8
PIM2	-18	14	2	9
PIM3	-8	13	-3	14
PKA	98	0	29	1
PKBalpha	3	10	0	5
PKBbeta	6	3	2	5
PKBgamma	12	14	3	10
PKC_mu	-4	2	9	0
PKCalpha	5	3	15	12
PKCbeta1	-3	1	52	4
PKCbetall	-5	5	-7	6
PKCdelta	36	9	55	0
PKCepsilon	26	4	84	2
PKCeta	27	4	81	4
PKCgamma	29	2	-4	12
PKCiota	8	2	18	1
PKCnu	5	3	18	9
PKCtheta	12	12	82	3
PKCzeta	-13	15	8	1
PKG	72	6	64	9
PKG1b	73	4	68	11
PRKD2	0	19	9	11
PRKX	56	5	78	2
PYK2	-9	6	-8	4
RET	-1	11	-3	1
RET_V804L	18	5	8	3
RET_Y791F	6	3	-2	5
ROCK1	94	1	94	0
ROCK2	95	2	95	2

Supplementary Table 1. Selectivity Comparison of OXA-06 and Y27632

Kinase	OXA-06 (0.2 mM)		Y27632 (10 mM)	
	% Inhibition	SD	% Inhibition	SD
RON	-8	1	-17	8
ROS	16	14	26	0
RSK1	6	1	51	8
RSK2	17	2	70	3
RSK3	10	5	82	4
RSK4	9	4	40	0
S6K	40	0	1	24
SGK	7	9	6	10
SGK2	-25	3	-17	2
SGK3	7	4	7	9
SRC	-4	1	0	3
SRMS	4	1	13	7
STK10	58	10	87	5
STK3	3	1	17	5
SYK	4	14	5	12
TEC	3	0	2	4
TRKB	21	1	8	12
TRKC	-1	2	-5	4
TSSK1	9	1	6	2
TSSK2	-9	6	-8	3
TXK	0	1	-4	1
TYRO3	-10	2	-7	7
ZIPK	19	1	20	3

Supplementary Figure 1



A**B**

Supplementary Figure 3A

Vigil et al.

