Discovery of JND3229 as a New EGFR^{C797S} Mutant Inhibitor with *In Vivo* Mono-drug Efficacy

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Supporting Information

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EXPERIMENTAL SECTION

General Methods for Chemistry.

All reagents and solvents used were purchased from commercial sources and were of analytical grade. Flash chromatography was performed using 300 mesh silica gel. All reactions were monitored by thin-layer chromatography (TLC), using silica gel plates with fluorescence F254 and ultraviolet (UV) light visualization. ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz or a Bruker AV-500 spectrometer at 500MHz. ^{13}C NMR spectra were recorded on a Bruker AV-500 spectrometer at 125 MHz. Coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to an internal standard (TMS). Low resolution ESI-MS were recorded on an Agilent 1200 HPLC-MSD mass spectrometer and high resolution ESI-MS on an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer. Purity of all of the final compounds was determined by reverse-phase high performance liquid chromatography (HPLC) analysis to be >95%. HPLC instrument: Dionex Summit HPLC (Column: Diamonsil C18, 5.0μm, 4.6×250 mm (Dikma Technologies); detector: PDA-100 photodiode array; injector: ASI-100 autoinjector; pump: p-680A). A flow rate of 1.0 mL/min was used with mobile phase of MeOH in H_2O with 0.1% modifier (ammonia, v/v).

Ethyl4-(((1*r*,4*r*)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-2-(methylthio)pyrimidine-5-carboxylate (9). Compound **8** (7.33 g, 34.2 mmol) and potassium carbonate (7.88 g, 57.0 mmol) were added to a solution of **7** (6.63 g, 28.5 mmol) in DMF (30 mL). The resulting solution was stirred at 80 °C overnight. After cooling to room temperature, ice−water (300 mL) was added to the reaction mixture. The precipitate was filtered, and the filter cake was rinsed with additional cool water and then dried in a vacuum oven to give **9** (white solid, 9.96 g, yield 85%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.12 (d, *J* = 7.2 Hz , 1H), 4.70 (s, 1H), 4.29 (q, *J* = 7.2 Hz, 2H), 4.04−3.99 (m, 1H), 3.47 (s, 1H), 2.50 (s, 3H), 2.13−2.06 (m, 4H), 1.44 (s, 9H), 1.39−1.38 (m, 2H), 1.35 (t, *J* = 7.2 Hz, 3H), 1.33−1.30 (m, 2H). LRMS (ESI) for $C_{19}H_{30}N_4O_4S$ $[M + H]^+$, calcd: 410.5. Found: 411.2.

***tert*-Butyl((1*r*,4*r*)-4-((5-(hydroxymethyl)-2-(methylthio)pyrimidin-4-yl)amino)cyclohexyl) carbamate (10).** A suspension of $LiAlH_4$ (1.84 g, 48.4 mmol) in anhydrous THF (50 mL) was added dropwise to a solution of **9** (9.96 g, 24.2 mmol) in anhydrous THF (150 mL) under stirring for 1 h at −40 °C. The reaction mixture was stirred until the temperature was warmed to 0 °C, and then it was treated with 2 mL of water to decompose unreacted $LiAlH_4$. Then, a further 6 mL of water was added to the reaction mixture followed by 15% aqueous NaOH (2 mL). The resulting mixture was stirred for 15 min at room temperature and filtered through a pad of Celite. The Celite was washed with THF, and the washes were combined and then concentrated in vacuo. The concentrated mixture was poured into water and extracted with CH_2Cl_2. The organic layer was separated, washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/ MeOH stepwise elution, 40:1 to 20:1) to give **8** (white solid, 4.47 g, yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 5.83 (d, *J* = 7.6 Hz, 1H), 4.46 (s, 2H), 4.45 (s, 1H), 3.96−3.93 (m, 1H), 3.50 (s,

1H), 3.40 (s, 1H), 2.46 (s, 3H), 2.12−2.10 (m, 2H), 2.03−2.02 (m, 2H), 1.42 (s, 9H), 1.28−1.22 (m, 4H). LRMS (ESI) for $C_{17}H_{28}N_4O_3S$ $[M + H]^+$, calcd: 368.5. Found: 369.2.

***tert*-Butyl((1*r*,4*r*)-4-((5-formyl-2-(methylthio)pyrimidin-4-yl)amino)cyclohexyl)carbamate (11)**. MnO_2 (3.15 g, 36.3 mmol) was added to a solution of **8** (4.47 g, 12.1 mmol) in CH_2Cl_2 (120 mL). The resulting solution was stirred at room temperature overnight, and then the reaction mixture was filtered through a pad of Celite. Filtration and removal of the solvent gave an oily crude product that was subjected to flashcolumn chromatography (SiO_2, petroleum ether/ EtOAc stepwise elution, 3:1to 1:1) to give **11** (white solid, 4.09 g, yield 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.47 (s, 1H), 8.27 (s, 1H), 4.48 (s, 1H), 4.09-4.01 (m, 1H), 3.46 (s, 1H), 2.51 (s, 3H), 2.12−2.03 (m, 4H), 1.43 (s, 9H), 1.28−1.22 (m, 4H). LRMS (ESI) for $C_{17}H_{26}N_4O_3S$ $[M + H]^+$, calcd: 366.5. Found: 367.2

***tert*-Butyl((1*r*,4*r*)-4-((5-(((2-chlorophenyl)amino)methyl)-2-(methylthio)pyrimidin-4-yl)amino) cyclohexyl)carbamate (13)**. Compound **12** (1.42 g, 11.2 mmol) and AcOH (0.1 mL) were added to a solution of **11** (2.05 g, 5.58 mmol) in toluene (20 mL). The resulting solution was stirred at 110 °C overnight. After the amine was converted completely to the imine as determined by TLC, $NaBH_4$ (1.06 g, 27.9 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The resulting mixture was concentrated in vacuo, partitioned between CH_2Cl_2 and saturated $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was separated, washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude material was subjected to flash column chromatography (SiO_2, petroleum ether/EtOAc, 3:1) to give **13** (white solid, 2.13 g, yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.19 (t, J = 8.0 Hz, 1H), 6.86−6.76 (m, 2H), 5.61 (d, J = 4.0 Hz, 1H), 4.38 (s, 1H), 4.18−4.09 (m, 3H), 3.97−3.94 (m, 1H), 3.42 (s, 1H), 2.50 (s, 3H), 2.08-2.01 (m, 4H), 1.44 (s, 9H), 1.25-1.22 (m, 4H). LRMS (ESI) for $C_{23}H_{32}ClN_5O_2S$ $[M + H]^+$, calcd: 478.0. Found: 478.1.

***tert*-Butyl((1*r*,4*r*)-4-(3-(2-chlorophenyl)-7-(methylthio)-2-oxo-3,4-dihydropyrimido[4,5-*d*] pyrimidin-1(2*H*)-yl)cyclohexyl)carbamate (14)**. DIEA (2.37 mL, 13.4 mmol) and triphosgene (0.53 g, 1.78 mmol) were added to a solution of **13** (2.13 g, 4.46 mmol) in CH_2Cl_2 at 0 °C. The mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried withNa_2SO_4, filtered, and concentrated in vacuo to give **14** (white solid, 2.11 g, yield 94%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.34-7.29 (m, 3H), 4.68-4.60 (m, 1H), 4.54-4.48 (m, 2H), 4.32 (s, 1H), 3.46 (s, 1H), 2.67 (s, 3H), 2.12 (d, J = 8.0 Hz, 2H), 1.81 (d, J = 8.0 Hz, 2H), 1.45 (s, 9H), 1.27-1.22 (m, 4H). LRMS (ESI) for $C_{24}H_{30}ClN_5O_3S$ $[M + H]^+$, calcd: 504.0. Found: 504.2.

***tert*-Butyl((1*r*,4*r*)-4-(3-(2-chlorophenyl)-7-(methylsulfonyl)-2-oxo-3,4-dihydropyrimido[4,5-*d*] pyrimidin-1(2*H*)-yl)cyclohexyl)carbamate (15).** m-CPBA (1.69 g, 8.38 mmol) was added to a solution of **14** (2.11 g, 4.19 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C under argon. The reaction mixture was allowed to warm to room temperature and then stirred for 4 h. The solution was diluted with CH_2Cl_2 and then treated with 50% $Na_2S_2O_3$/$NaHCO_3$ solution. The organic layer was separated, washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude material was subjected to flash column chromatography (SiO_2, CH_2Cl_2/MeOH stepwise elution, 40:1 to 20:1) to give **15** (white solid, 1.98

g, yield 88%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.54-7.51 (m, 1H), 7.38-7.34 (m, 3H), 4.84-4.63 (m, 3H), 3.94 (s, 1H), 3.51 (s, 1H), 3.33 (s, 3H), 2.69-2.66 (m, 2H), 1.94-1.91 (m, 1H), 1.84-1.81 (m, 2H), 1.67-1.63 (m, 1H), 1.45 (s, 9H), 1.29-1.24 (m, 2H). LRMS (ESI) for $C_{24}H_{30}ClN_5O_5S$ $[M + H]^+$, calcd:536.0. Found:536.2.

1-((1*r*,4*r*)-4-aminocyclohexyl)-3-(2-chlorophenyl)-7-((3-methyl-4-(4-methylpiperazin-1-yl)phenyl)amino)-3,4-dihydropyrimido[4,5-*d*]pyrimidin-2(1*H*)-one (18). Compound **16** (444 mg, 2.10 mmol) was added to a solution of **15** (938 mg, 1.75 mmol) in 2-butyl alcohol (5 mL), followed by trifluoroacetic acid (157 μL, 2.10 mmol). The reaction mixture was stirred for 18 h at 110 °C in a sealed tube. The reaction mixture was cooled to room temperature and concentrated in vacuo to give crude product **17** which was used in the next step without purification. Then trifluoroacetic acid (2 mL) was added to a solution of **17** in CH_2Cl_2 (5 mL), and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with CH_2Cl_2, basified with saturated aqueous $NaHCO_3$ to pH = 9, and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$, brine, dried with Na_2SO_4 and then concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH/NH_4OH, 40:1:0.4) to give **18** (white solid, 491 mg, yield 50% for twosteps).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.50-7.47 (m, 1H), 7.40-7.28 (m, 5H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.96 (s, 1H), 4.75-4.72 (m, 1H), 4.58-4.57 (m, 1H), 4.46−4.43 (m, 1H), 2.95-2.92 (m, 4H), 2.69-2.68 (m, 1H), 2.60-2.58 (m, 6H), 2.36 (s, 3H), 2.33 (s, 3H), 1.95 (d, *J* = 9.2 Hz, 2H), 1.74 (d, *J* = 8.8 Hz, 2H), 1.27-1.25 (m, 2H). LRMS (ESI) for $C_{30}H_{37}ClN_8O$ $[M + H]^+$, calcd: 561.1. Found: 561.3

***N*-((1*r*,4*r*)-4-(3-(2-chlorophenyl)-7-((3-methyl-4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxo-3,4-dihydropyrimido[4,5-*d*]pyrimidin-1(2*H*)-yl)cyclohexyl)prop ionamide (6).** DIEA (72 μL, 0.41 mmol) and propanoic acid (24 μL, 0.32 mmol) were added to a solution of HATU (122 mg, 0.32 mmol) in anhydrous CH_2Cl_2 (10 mL) at rt. After stirring for 10 min, **18** (115 mg, 0.21 mmol) was added to the resulting mixture , and then the mixture was stirred at room temperature for 0.5 h. The reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$, brine and dried with Na_2SO_4. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH/NH_4OH, 40:1:0.4) to give **6** (White solid, 116 mg, yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.57−7.54 (m, 1H), 7.50−7.48 (m, 1H), 7.37−7.27 (m, 3H), 7.23 (s, 1H), 7.15 (s, 0.5H), 7.03 (s, 0.5H), 6.95 (s, 1H), 5.27 (d, *J* = 8.0 Hz, 1H), 4.70−4.64 (m, 1H), 4.60−4.57 (m, 1H), 4.47−4.44 (m, 1H), 3.88-3.80 (m, 1H), 3.01−2.99 (m, 4H), 2.74 (br s, 6H), 2.48 (s, 3H), 2.33 (s, 3H), 2.19 (q, *J* = 7.6 Hz, 2H), 2.11-2.08 (m, 2H), 1.86−1.76 (m, 4H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 159.5, 158.0, 153.3, 153.1, 147.0, 139.6, 134.9, 133.7, 132.9, 130.7, 129.5, 129.3, 128.2, 123.0, 120.0, 118.8, 103.4, 55.9, 54.6, 51.8, 47.8, 47.1, 46.0, 33.2, 30.0, 27.7, 27.6, 18.0, 10.1. HRMS (ESI) for $C_{33}H_{41}ClN_8O_2$ $[M + H]^+$, calcd: 617.3114. Found: 617.3093. HPLC analysis: MeOH-H_2O (85:15), 13.44 min, 97.01% purity.

Protein Kinase Assay

$EGFR^{L858R/T790M}$ kinase protein was purchased from Eurofins Scientific. $EGFR^{L858R/T790M/C797S}$ kinase proteins were purchased from BPS Bioscience. Tyrosine kinase activity was evaluated by an enzyme-linked-immunosorbent assay (ELISA) in 96-well plates pre-coated with 2.5 μg/well poly(Glu, Tyr)

$_{4:1}$ (Sigma) as a substrate. Fifty microliters of 10 μmol/L ATP solution diluted in reaction buffer [50 mmol/L HEPES, pH 7.4, 20 mmol/L $MgCl_2$, 0.1 mmol/L $MnCl_2$, 0.2 mmol/L Na_3VO_4, and 1 mmol/L DTT] was added to each well. Various concentrations of compounds diluted in DMSO were added to each reaction well. A total volume of 1 μL DMSO was used as the vehicle control. The kinase reaction was initiated by adding purified tyrosine kinase proteins diluted in 49 μL of kinase reaction buffer. After incubation for 1 h at 37 °C, the plate was washed three times with phosphate buffered saline containing 0.1% Tween 20 (PBST). Next, 100 μL of antiphosphotyrosine (PY99; 1:500 dilution) antibody was added. After 0.5 h incubation at 37 °C, the plate was washed three times and goat anti-mouse IgG horseradish peroxidase (100 μL of a 1:2000 dilution) diluted in PBST containing 5 mg/mL BSA was added. The plate was reincubated at 37 °C for 0.5 h and washed as before. Finally, 100 μL of color development solution (0.03% H_2O_2 and 2 mg/mL o-phenylenediamine in 0.1 mol/L citrate buffer, pH 5.4) was added and the plate was incubated at room temperature until color emerged. The reaction was terminated by the addition of 50 μL of 2 mol/L H_2SO_4, and the A490 was measured using a multi-well spectrophotometer (SpectraMax Plus384, Molecular Devices).

Cell Proliferation Inhibition Assay

Cell proliferation was evaluated using the Resazurin (7-hydroxy-3H- phenoxazin-3-one 10-oxide) Assay. BaF3-EGFR$^{19D/T790M/C797S}$and BaF3-EGFR$^{L858R/T790M/C797S}$ cell lines were built by Jian Ding's laboratory, and cultured in RPMI-1640 (GE Healthcare) supplemented with 10% fetal bovine serum (Gibico) and 1μg/ml puromycin (Sigma). Cells were seeded in 96-well plates (10,000 cells per well) and grown overnight. The cells were treated with various concentrations of the compounds for 72 h. Then 10 μL of resazurin solution (500 μM resazurin solution in DPBS, Sigma) were added to each well， and the cells were incubated for 2h at 37° C. The Fluorescence signals were measured at an excitation wavelength at 540 nm and an emission wavelength at 590 nm using a microplate reader (Synergy2, BioTek). The percent inhibition rate for cell proliferation was calculated as [1 - ($A_{540/590}$treated/ $A_{540/590}$control)] × 100%. The IC_{50} value was obtained using the Logit method.

Western Blot Analysis

Cells were collected and suspended in lysis buffer (100 mmol/L Tris-HCl, pH6.8, 200 mmol/L DTT, 4% SDS, 0.2% bromophenol blue, 20% glycerol). Equivalent amounts of proteins were loaded and separated by 10% SDS-PAGE and transfered to nitrocellulose membranes. Western blot analysis was subsequently performed using standard procedures. Antibodies used for immune detection of proteins p-EGFR (Y1068; #3777S), EGFR (#4267S), and Tubulin (#2128L) were purchased from Cell Signaling technology.

In Vivo Efficacy for Mouse Tumor Xenografts.

4-6 weeks-old BALB/c mice were purchased from the Shanghai Laboratory Animal Research Center (Shanghai, China). All studies conformed the Animal Care and Use Committee guidelines of China. Approximately 1.5 mm^3 BaF3-EGFR$^{19D/T790M/C797S}$ tumor tissue was implanted subcutaneously into the right flank per mice. The mice were randomized into vehicle and treated groups. Forty eight hours later (day 0), for efficacy studies, mice were dosed either vehicle or inhibitors using the indicated doses for maximum 10 days. The average tumor volume (TV) was measured with vernier calipers every 2 or 3 days

then was calculated with the formula $V = (L \times W^2)/2$, and L stands for length and W is width, the tumor growth inhibition (TGI) = [1- RTV(treated)/ RTV(control)] × 100%. Meanwhile, the body weight of mice was measured.

Immunohistochemical (IHC) Assays

After the treatment, BaF3-EGFR$^{19D/T790M/C797S}$ xenograft mouse models were harvested and fixed in formalin. Tumor samples were embedded in paraffin and prepared in sections (4μm). The immunohistochemical analysis was conducted by Shanghai ZuoChengBio Ltd., and the assay was operated according to the manufacturer's instructions. Sections were stained with p-EGFR (Y1068; #3777S, Cell Signaling Technology) antibody using optimized protocols.

Protein expression, crystallization and structure determination

Construct spanning residues 696-1022 of EGFR$^{T790M/C797S}$ were expressed and purified using the baculovirus/insect cell system as described.[1] Crystals used in this study were prepared by hanging drop vapor diffusion. For co-crystallization, the compound JND3229 was added to 10.5 mg/mL EGFR$^{T790M/C797S}$ proteins to a final concentration of 2mM and incubated at 4°C for two hours before setting up the crystallization tray. The reservoir solution for growing crystals was 0.05 M HEPES pH 8.0, 0.2 M $ZnSO_4$, 25% PEG6000. The diffraction data were collected on beamline BL19U1 at Shanghai Synchrotron Radiation Facility (SSRF). The diffraction data were processed using HKL3000.[2] The structure was solved by molecular replacement with Phaser using the previously determined EGFR L858R+ANP structure (PDB ID 2itv)[1] as the search model. Repeated rounds of manual refitting and crystallographic refinement were then performed using COOT [3] and Phenix.[4] The inhibitor was modeled into the closely fitting positive Fo-Fc electron density and included in following refinement cycles. Topology and parameter files for the inhibitor were generated using Phenix.[4] The diffraction data collection and refinement statistics were summarized in Table 1.

Table S1. Data collection and refinement statistics of complex crystal structure of EGFR$^{T790M/C797S}$ with JND3229.

	T790M/C797S + JND3229
Data collection	
Space group	I23
Cell dimensions	
a, *b*, *c* (Å)	148.7, 148.7, 148.7
α,β,γ(°)	90.0, 90.0, 90.0
Resolution (Å)	50.0-2.65 (2.71-2.65)
R_{merge}	0.127 (0.353)
I/σ	6.0 (2.0)
Completeness (%)	100.0 (100.0)
Redundancy	18.0 (17.7)
Refinement	
Resolution (Å)	47.0-2.65
No. reflections	16080
R_{work} / R_{free}	0.232/0.272
No. atoms	

Protein	2278
Ligand/ion	45
Water	38
B-factors	
Protein	66.5
Ligand/ion	64.4
Water	64.4
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.138
Ramachandran Plot	
Favored regions	272 (94.44%)
Allowed regions	16 (5.56%)
Outliers	0 (0.00%)

§ Values in parentheses are for highest-resolution shell.

The kinases Profiling Study

For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 %SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05 %Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

Table S2. Matrix of Compound Screen for JND3229 (Assigned the name as D3003 in the assay)

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
AAK1	20
ABL1(E255K)-phosphorylated	0.35
ABL1(F317I)-nonphosphorylated	1.1
ABL1(F317I)-phosphorylated	7.8
ABL1(F317L)-nonphosphorylated	0.1
ABL1(F317L)-phosphorylated	0.2
ABL1(H396P)-nonphosphorylated	0
ABL1(H396P)-phosphorylated	0
ABL1(M351T)-phosphorylated	0.3
ABL1(Q252H)-nonphosphorylated	0
ABL1(Q252H)-phosphorylated	0.05
ABL1(T315I)-nonphosphorylated	3.5
ABL1(T315I)-phosphorylated	2.2
ABL1(Y253F)-phosphorylated	0.05
ABL1-nonphosphorylated	0
ABL1-phosphorylated	0.05
ABL2	0.35
ACVR1	2.9
ACVR1B	2.8
ACVR2A	0
ACVR2B	0
ACVRL1	7.8
ADCK3	19
ADCK4	0
AKT1	100
AKT2	46
AKT3	31
ALK	16
ALK(C1156Y)	6.8
ALK(L1196M)	4.6
AMPK-alpha1	9.1
AMPK-alpha2	17
ANKK1	100
ARK5	16
ASK1	100
ASK2	0
AURKA	19
AURKB	36
AURKC	26
AXL	0
BIKE	1.5
BLK	0
BMPR1A	5.4
BMPR1B	0
BMPR2	9
BMX	0.4
BRAF	13
BRAF(V600E)	3.7

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
BRK	0.05
BRSK1	54
BRSK2	100
BTK	0
BUB1	0
CAMK1	1
CAMK1B	19
CAMK1D	0
CAMK1G	43
CAMK2A	78
CAMK2B	91
CAMK2D	91
CAMK2G	98
CAMK4	100
CAMKK1	97
CAMKK2	95
CASK	83
CDC2L1	80
CDC2L2	66
CDC2L5	85
CDK11	100
CDK2	55
CDK3	87
CDK4	1.8
CDK4-cyclinD1	0.3
CDK4-cyclinD3	3
CDK5	97
CDK7	7.2
CDK8	32
CDK9	98
CDKL1	99
CDKL2	4.2
CDKL3	0
CDKL5	100
CHEK1	32
CHEK2	33
CIT	6.9
CLK1	53
CLK2	39
CLK3	59
CLK4	100
CSF1R	0.05
CSF1R-autoinhibited	0.05
CSK	0.05
CSNK1A1	7.9
CSNK1A1L	22
CSNK1D	22
CSNK1E	0.8
CSNK1G1	83

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
CSNK1G2	87
CSNK1G3	83
CSNK2A1	92
CSNK2A2	100
CTK	18
DAPK1	50
DAPK2	81
DAPK3	53
DCAMKL1	1.9
DCAMKL2	3.8
DCAMKL3	0.95
DDR1	1.5
DDR2	0
DLK	8.1
DMPK	8.2
DMPK2	2.5
DRAK1	1.2
DRAK2	99
DYRK1A	99
DYRK1B	16
DYRK2	79
EGFR	2.8
EGFR(E746-A750del)	0
EGFR(G719C)	0.15
EGFR(G719S)	0.65
EGFR(L747-E749del, A750P)	10
EGFR(L747-S752del, P753S)	2.5
EGFR(L747-T751del,Sins)	8.1
EGFR(L858R)	2.4
EGFR(L858R,T790M)	1.4
EGFR(L861Q)	0.15
EGFR(S752-I759del)	2.4
EGFR(T790M)	0.3
EIF2AK1	31
EPHA1	0.15
EPHA2	0
EPHA3	0.35
EPHA4	0
EPHA5	0.5
EPHA6	0
EPHA7	53
EPHA8	0.25
EPHB1	0.2
EPHB2	0
EPHB3	0
EPHB4	0
EPHB6	0
ERBB2	0
ERBB3	0

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
ERBB4	1.8
ERK1	46
ERK2	86
ERK3	58
ERK4	77
ERK5	2.9
ERK8	27
ERN1	25
FAK	5.3
FER	0.05
FES	0.25
FGFR1	0.4
FGFR2	0.15
FGFR3	0.8
FGFR3(G697C)	0.95
FGFR4	0.55
FGR	0
FLT1	0.45
FLT3	0.05
FLT3(D835H)	2.1
FLT3(D835V)	0
FLT3(D835Y)	12
FLT3(ITD)	1.2
FLT3(ITD,D835V)	0
FLT3(ITD,F691L)	0
FLT3(K663Q)	11
FLT3(N841I)	12
FLT3(R834Q)	1.9
FLT3-autoinhibited	19
FLT4	0.05
FRK	0.05
FYN	0.2
GAK	2.7
GCN2(Kin.Dom.2,S808G)	7.2
GRK1	66
GRK2	83
GRK3	76
GRK4	2.3
GRK7	86
GSK3A	100
GSK3B	41
HASPIN	87
HCK	0.15
HIPK1	84
HIPK2	82
HIPK3	1.8
HIPK4	24
HPK1	91
HUNK	8.8

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
ICK	100
IGF1R	60
IKK-alpha	78
IKK-beta	13
IKK-epsilon	0
INSR	49
INSRR	73
IRAK1	18
IRAK3	75
IRAK4	100
ITK	71
JAK1(JH1domain-catalytic)	2.9
JAK1(JH2domain-pseudokinase)	10
JAK2(JH1domain-catalytic)	0
JAK3(JH1domain-catalytic)	0
JNK1	0.4
JNK2	0.25
JNK3	0.85
KIT	0.05
KIT(A829P)	1.5
KIT(D816H)	0
KIT(D816V)	0
KIT(L576P)	0
KIT(V559D)	0.05
KIT(V559D,T670I)	3.5
KIT(V559D,V654A)	0
KIT-autoinhibited	0.2
LATS1	2.1
LATS2	0
LCK	0.15
LIMK1	3.9
LIMK2	0
LKB1	22
LOK	0
LRRK2	20
LRRK2(G2019S)	9.3
LTK	5.1
LYN	0
LZK	21
MAK	80
MAP3K1	0
MAP3K15	88
MAP3K2	0
MAP3K3	0.1
MAP3K4	2.1
MAP4K2	4.5
MAP4K3	0.3
MAP4K4	3.9
MAP4K5	0

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
MAPKAPK2	100
MAPKAPK5	100
MARK1	27
MARK2	1.8
MARK3	1
MARK4	66
MAST1	46
MEK1	0
MEK2	0
MEK3	1.8
MEK4	0.15
MEK5	0.1
MEK6	30
MELK	7.2
MERTK	2
MET	4.5
MET(M1250T)	4.9
MET(Y1235D)	6.8
MINK	2.5
MKK7	96
MKNK1	81
MKNK2	41
MLCK	100
MLK1	1.8
MLK2	7.2
MLK3	1
MRCKA	59
MRCKB	19
MST1	1.2
MST1R	46
MST2	66
MST3	2.4
MST4	3.6
MTOR	86
MUSK	37
MYLK	80
MYLK2	66
MYLK4	100
MYO3A	44
MYO3B	11
NDR1	24
NDR2	25
NEK1	50
NEK10	34
NEK11	0.6
NEK2	1.2
NEK3	99
NEK4	100
NEK5	16

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
NEK6	71
NEK7	89
NEK9	86
NIK	85
NIM1	59
NLK	0.2
OSR1	5.5
p38-alpha	0.2
p38-beta	0
p38-delta	58
p38-gamma	60
PAK1	3.3
PAK2	49
PAK3	11
PAK4	8.9
PAK6	5.2
PAK7	0.3
PCTK1	2.1
PCTK2	9.4
PCTK3	86
PDGFRA	0
PDGFRB	0
PDPK1	98
PFCDPK1(P.falciparum)	0
PFPK5(P.falciparum)	65
PFTAIRE2	53
PFTK1	73
PHKG1	100
PHKG2	66
PIK3C2B	4.1
PIK3C2G	43
PIK3CA	100
PIK3CA(C420R)	98
PIK3CA(E542K)	100
PIK3CA(E545A)	100
PIK3CA(E545K)	100
PIK3CA(H1047L)	88
PIK3CA(H1047Y)	96
PIK3CA(I800L)	74
PIK3CA(M1043I)	86
PIK3CA(Q546K)	100
PIK3CB	98
PIK3CD	58
PIK3CG	61
PIK4CB	59
PIKFYVE	35
PIM1	94
PIM2	100
PIM3	88

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
PIP5K1A	77
PIP5K1C	71
PIP5K2B	3.5
PIP5K2C	0.1
PKAC-alpha	0.2
PKAC-beta	1.4
PKMYT1	48
PKN1	69
PKN2	20
PKNB(M.tuberculosis)	0.2
PLK1	100
PLK2	97
PLK3	69
PLK4	29
PRKCD	5.7
PRKCE	0
PRKCH	14
PRKCI	13
PRKCQ	26
PRKD1	1.9
PRKD2	2.7
PRKD3	0
PRKG1	66
PRKG2	76
PRKR	69
PRKX	96
PRP4	5.5
PYK2	5.3
QSK	1.4
RAF1	68
RET	0
RET(M918T)	0.1
RET(V804L)	0.05
RET(V804M)	0.25
RIOK1	2.6
RIOK2	33
RIOK3	7.5
RIPK1	52
RIPK2	0.6
RIPK4	93
RIPK5	2.4
ROCK1	54
ROCK2	24
ROS1	28
RPS6KA4(Kin.Dom.1-N-terminal)	92
RPS6KA4(Kin.Dom.2-C-terminal)	100
RPS6KA5(Kin.Dom.1-N-terminal)	100
RPS6KA5(Kin.Dom.2-C-terminal)	74
RSK1(Kin.Dom.1-N-terminal)	83

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
RSK1(Kin.Dom.2-C-terminal)	17
RSK2(Kin.Dom.1-N-terminal)	12
RSK2(Kin.Dom.2-C-terminal)	0.15
RSK3(Kin.Dom.1-N-terminal)	56
RSK3(Kin.Dom.2-C-terminal)	64
RSK4(Kin.Dom.1-N-terminal)	15
RSK4(Kin.Dom.2-C-terminal)	1.1
S6K1	26
SBK1	5
SGK	86
SgK110	0
SGK2	97
SGK3	89
SIK	0.9
SIK2	1.1
SLK	0.3
SNARK	0.55
SNRK	94
SRC	0.1
SRMS	4.9
SRPK1	5.1
SRPK2	17
SRPK3	20
STK16	0
STK33	7.2
STK35	3.2
STK36	0.2
STK39	55
SYK	1.2
TAK1	1.2
TAOK1	0.05
TAOK2	0
TAOK3	0.9
TBK1	4.4
TEC	1.2
TESK1	0
TGFBR1	2.5
TGFBR2	2.7
TIE1	15
TIE2	0.75
TLK1	33
TLK2	87
TNIK	0.25
TNK1	0.3
TNK2	0.2
TNNI3K	1.2
TRKA	0
TRKB	1.8
TRKC	0.9

Target	D3003
Gene Symbol	%Ctrl @ 1000nM
TRPM6	99
TSSK1B	2.7
TSSK3	25
TTK	0.9
TXK	0.15
TYK2(JH1domain-catalytic)	0
TYK2(JH2domain-pseudokinase)	100
TYRO3	15
ULK1	3
ULK2	0.3
ULK3	0.1
VEGFR2	0
VPS34	20
VRK2	73
WEE1	2.9
WEE2	4.5
WNK1	100
WNK2	4.1
WNK3	51
WNK4	64
YANK1	6.9
YANK2	29
YANK3	0.55
YES	0.15
YSK1	8.7
YSK4	0
ZAK	0.3
ZAP70	6.1

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