Supporting Information

Discovery of Small Molecule RIP1 Kinase Inhibitors for the Treatment of Pathologies Associated with Necrotic Cell Death

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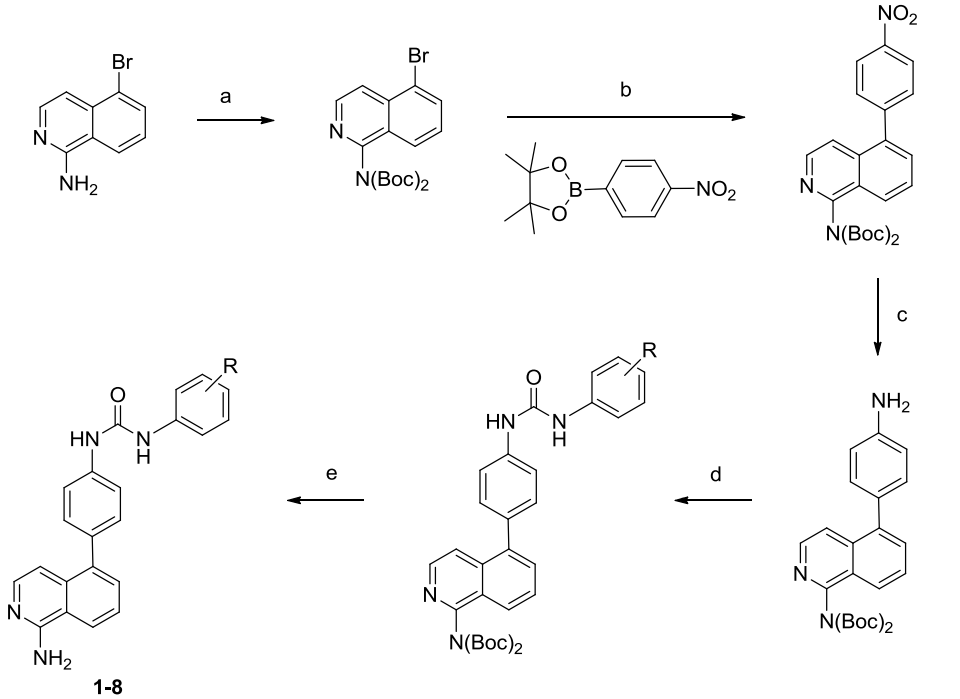
Synthesis of compounds

Aminoisoquinolines **1-8** were made according to the Schemes 1 and the representative procedure detailed below. The preparation of compound **2** is reported in Washio, Y. Preparation of aryl substituted isoquinoline derivatives as inhibitors of VEGFR2, TIE-2, and EphB4. PCT Int. Appl. **2005**, WO2005049576.

Scheme 1



Reagents and conditions: (a) $(Boc)_2O$, DMAP, TEA, MeCN, rt, 42% (b) $Pd(PPh_3)_4$, K_3PO_4, DMF/water, microwave 85°C, 70% (c) H_2, Pd/C, EtOH, rt, 100% (d) ArNCO, CH_2Cl_2, rt, 52-70% (e) TFA, CH_2Cl_2, rt, 42-90%

Preparation of bis(1,1-dimethylethyl) (5-bromo-1-isoquinolinyl)imidodicarbonate

To a solution of 5-bromoisoquinolin-1-amine (500 mg, 2.24 mmol), Boc_2O (1.56 ml, 6.72 mmol) and DMAP (55 mg, 0.45 mmol) and TEA (0.95ml, 6.82 mmol) in acetonitrile (5 mL) was stirred stirred at RT

for 5h. The reaction mixture was concentrated and the residue was purified by ISCO eluting with ethyl acetate/hexane (0-20%) to afford the title compound as (400 mg, 0.945 mmol, 42 % yield).

Preparation of bis(1,1-dimethylethyl) [5-(4-nitrophenyl)-1-isoquinolinyl]imidodicarbonate

A mixture of bis(1,1-dimethylethyl) (5-bromo-1-isoquinolinyl)imidodicarbonate (0.483 g, 1.14 mmol), 4,4,5,5-tetramethyl-2-(4-nitrophenyl)-1,3,2-dioxaborolane (0.426 g, 1.71 mmol) and potassium phosphate (0.727 g, 3.42 mmol) in N,N-dimethylformamide (12 mL) and water (4 mL) was degassed by bubbling nitrogen through for 5 minutes. Next, tetrakis(triphenylphosphine)palladium (0.04 g, 0.034 mmol) was added and the reaction microwaved at 85 ^{o}C for 30 minutes. Add a second equivalent of 4,4,5,5-tetramethyl-2-(4-nitrophenyl)-1,3,2-dioxaborolane (0.426 g, 1.712mmol) was then added and the reaction microwaves at 85 ^{o}C for additional 30 minutes. The reaction mixture was dilute with ethyl acetate and wash with water (2X), half-saturated brine (2X) and dried over MgSO4 and concentrated. The crude sample was purified via Isco normal phase chromatography (24 g column, 0% -25%, ethyl acetate/hexane). The pure product containing-fractions were combined and concentrated leading to the product: (0.373 g, 0.80 mmol, 70 % yield) as a yellow solid.

Preparation of bis(1,1-dimethylethyl) [5-(4-aminophenyl)-1-isoquinolinyl]imidodicarbonate

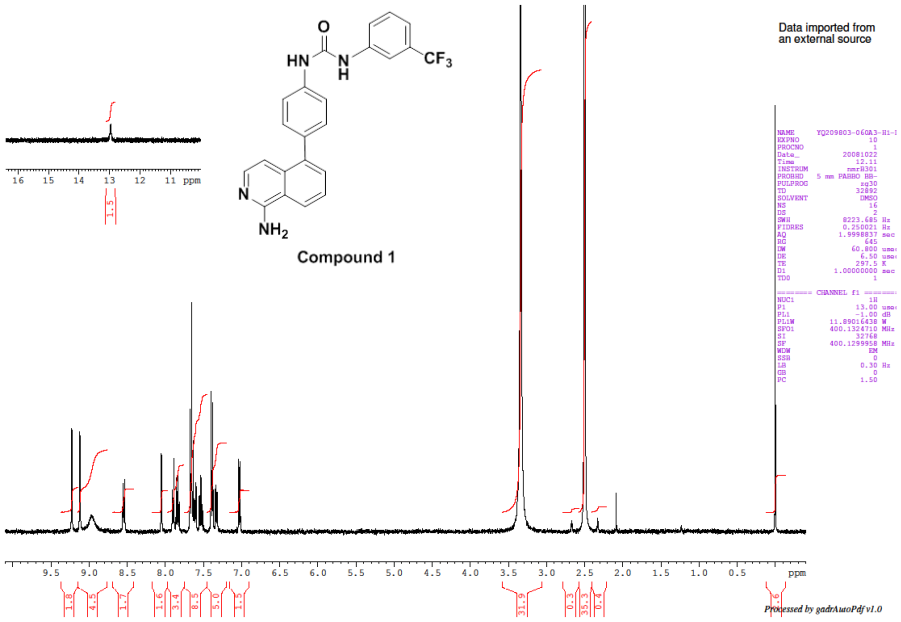
A mixture of bis(1,1-dimethylethyl) [5-(4-nitrophenyl)-1-isoquinolinyl]imidodicarbonate (0.373 g, 0.80 mmol) and 10% Pd/C (0.086 g, 0.81 mmol) in ethanol (10 mL) was stirred at RT under a balloon of hydrogen overnight. The reaction mixture was filtered through a pad of celite and concentrated to yield a yellow oil (0.37 g, 100 % yield) which was used as such without further purification.

Preparation of bis(1,1-dimethylethyl) (5-{4-[({[3-(trifluoromethyl)phenyl]amino}carbonyl)amino]-phenyl}-1-isoquinolinyl)imidodicarbonate

A mixture of bis(1,1-dimethylethyl) [5-(4-aminophenyl)-1-isoquinolinyl]imidodicarbonate (0.07 g, 0.16 mmol) in dichloromethane (40 mL) was added 3-(trifluoromethyl)phenyl isocyanate (0.045 g, 0.24 mmol) and the solution stirred at RT for 15 hours. The reaction mixture was concentrated and the crude sample was purified via Isco normal phase chromatography (24 g column, 1:4 ethyl acetate/hexane). The pure product containing-fractions were combined and concentrated leading to the product: (0.07 g, 70 % yield) as a colorless oil.

Preparation of 1-(4-(1-aminoisoquinolin-5-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea; Compound 1

To a mixture of bis(1,1-dimethylethyl) (5-{4-[({[3-(trifluoromethyl)phenyl]amino}carbonyl)amino]-phenyl}-1-isoquinolinyl)imidodicarbonate (0.07 g, 0.11 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (4 mL) and the solution stirred at RT for 14 hours. The reaction mixture was concentrated and sodium bicarbonate solution (5 mL) was added. The resulting white solid suspension was filtered off, washed with water and dried to give the product (0.048 g, 78 % yield) as a white solid.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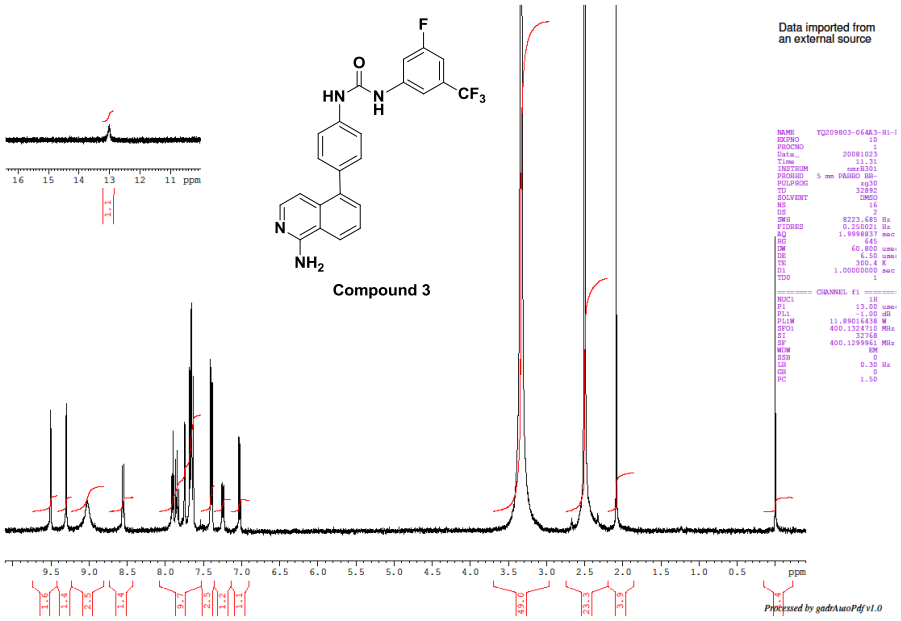
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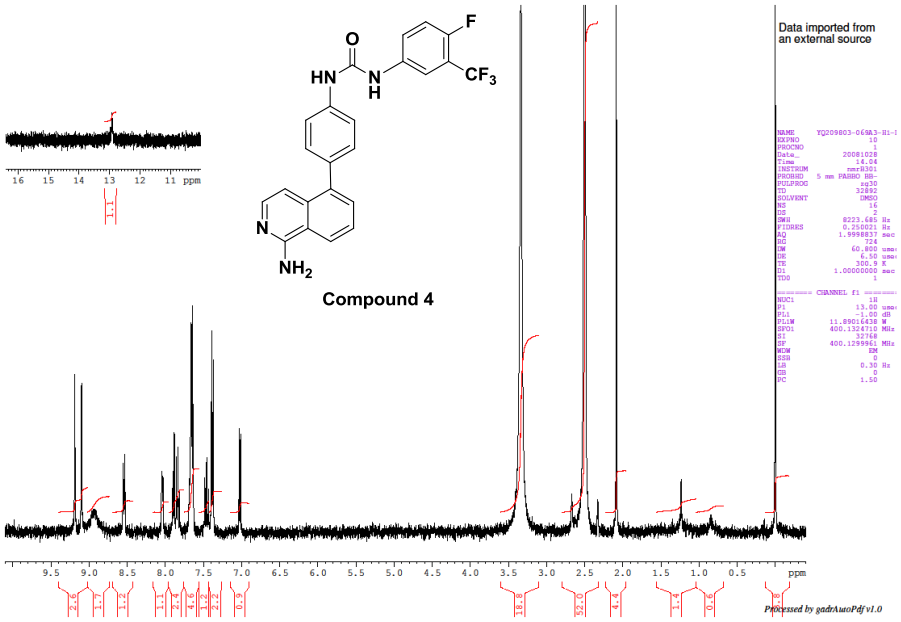
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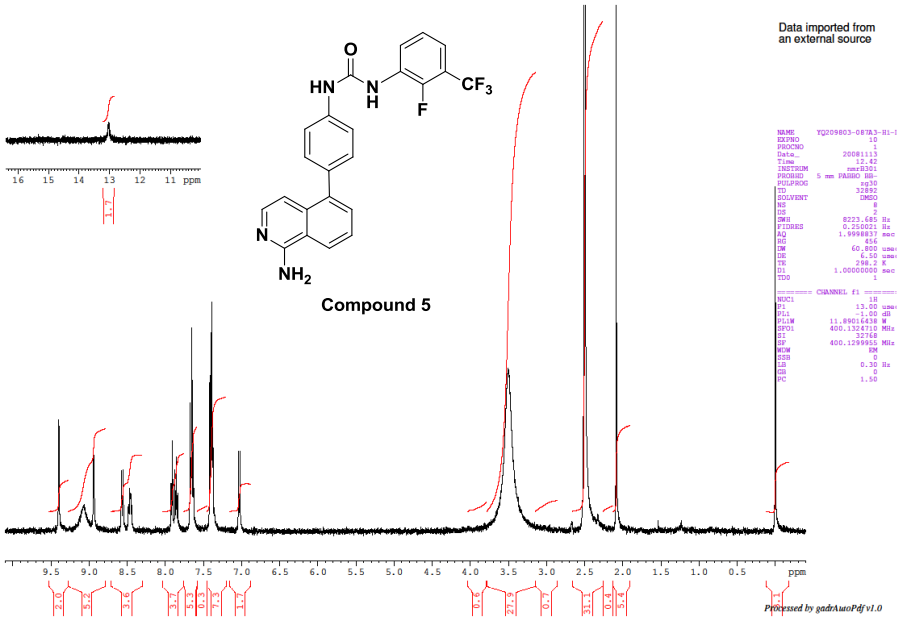
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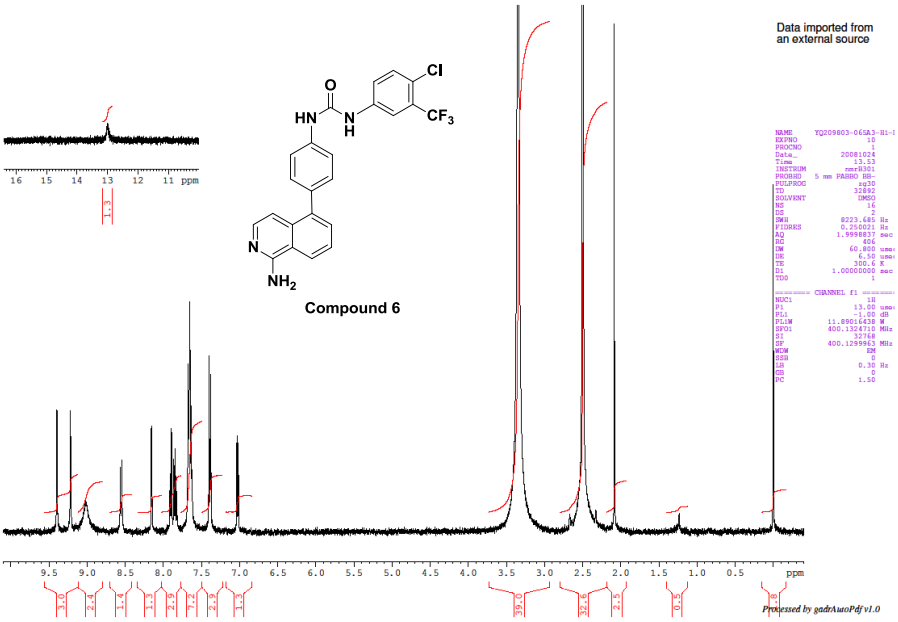
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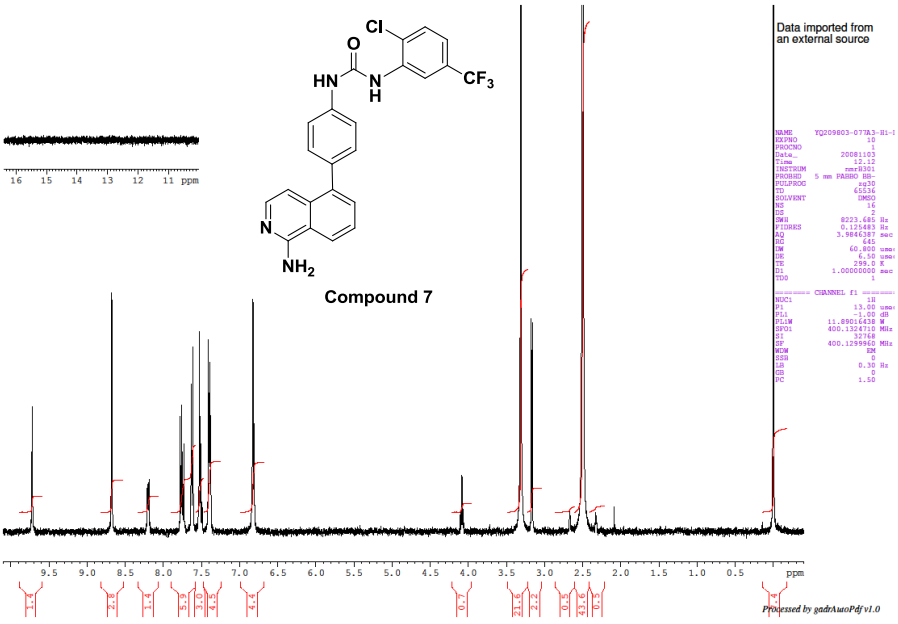
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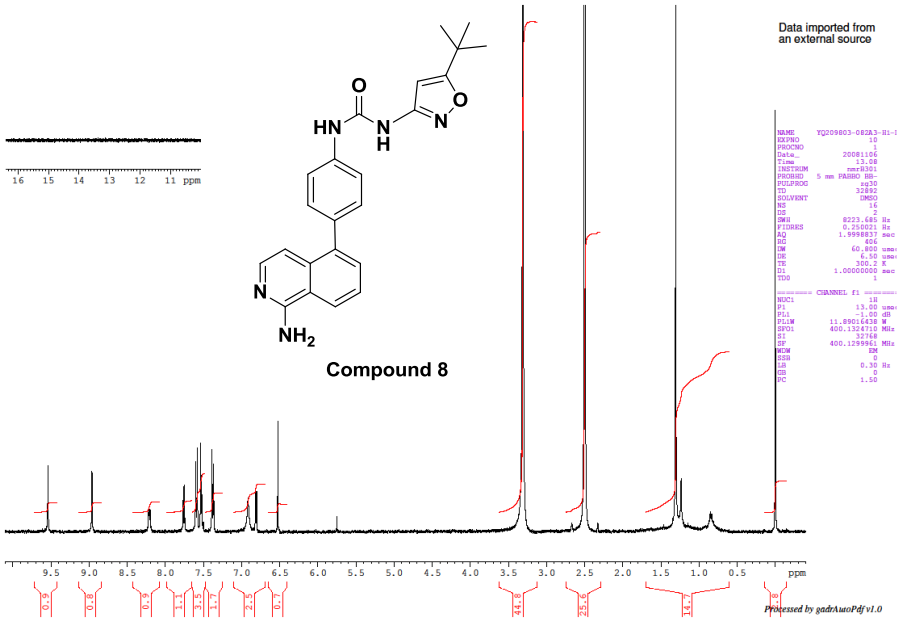
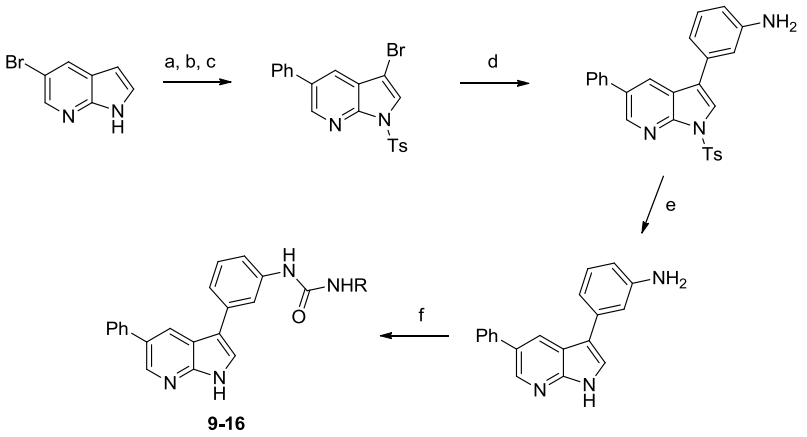


Compounds **9-16** were made according to the Schemes 2 and the representative procedure detailed below.

Scheme 2



Reagents and conditions: (a) $PhB(OH)_2$, $Pd(dppf)Cl_2$ K_2CO_3, dioxane/water, 99%; (b) Br_2, $CHCl_3$; (c) TsCl, Bu_4NSO_4, CH_2Cl_2, 6N NaOH, 68% for 2 steps; (d) 3-aminophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, 120 ^oC, 92%; (e) KOH, MeOH, 92%; (f) RNCO, Et_3N, CH_2Cl_2, 60–90%

Preparation of 5-Phenyl-1 H-pyrrolo[2,3-blpyridine.

[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(ll) (1.04 g, 1.27 mmol) was added in one portion to a suspension of 5-bromo-1 H-pyrrolo[2,3-b]pyridine (5 g, 25.4 mmol), phenylboronic acid (3.7 g, 30.5 mmol) and potassium carbonate (1 0.5g, 76.1 mmol) in 2.5:1 dioxane/water (253 mL). The reaction mixture was placed under N_2 atmosphere and heated in an oil bath set to 80 °C. After heating for 22.5 hours, the reaction mixture was cooled to room temperature, acidified with 6N HCl, and partitioned between ethyl acetate (100 mL) and water (100 mL). The mixture was filtered through a pad of Celite, and the layers of the filtrate were separated. The aqueous layer was extracted with ethyl acetate (2 X 50 mL portions). The combined organics were washed with saturated aqueous sodium chloride (100 mL), dried over sodium sulfate and concentrated. The residue was dissolved in methanol (200 mL), 15g of DOWEX 50WX2-400 ion exchange resin was added, and the mixture was stirred gently

for 3 hours. The resin was collected by filtration and washed with methanol (2 X 100 mL portions), dichloromethane (100 mL), and methanol (100 mL). The product was released from the resin by washing with 4N ammonia in methanol (3 X 100 mL portions). The 4N ammonia methanol washings were concentrated in vacuo to provide 5-phenyl-1H-pyrrolo[2,3-b]pyridine as a light brown solid (4.86 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) 10.33 (br s, 1H), 8.62 (s,.1 H), 8.22 (s, 1H), 7.66 (d, J =8 Hz, 2H), 7.52 (t, J =7.6 Hz, 2H), 7.43 (m, 2H), 6.62 (s, 1H). LC-MS (ES) m/e 195 $(M + H)^+$.

Preparation of 3-bromo-5-phenyl-1H-pyrrolo[2,3-b]pyridine

Bromine (1.27 mL, 3.95 g, 24.7 mmol) was added over a period of 35 minutes to a solution of 5-phenyl-1 H-pyrrolo[2,3-b]pyridine (4.8 g, 24.7 mmol) in chloroform (247 mL). The reaction mixture was stirred at room temperature for 15 min. and then concentrated in vacuo. The pale orange foam containing 3-bromo-5-phenyl-1 *H*pyrrolo[2,3-b]pyridine was carried directly to the next reaction without further purification.

Preparation of 3-bromo-5-phenyl-1-tosyl-1H-pyrrolo[2,3-b]pyridine

Tetrabutylammonium hydrogen sulfate (100 mg, catalytic) was added to a mixture of 3-bromo-5-phenyl-1 H-pyrrolo[2,3-b]pyridine (24.7 mmol, 1 equiv) and p-toluenesulfonylchloride (5.65 g, 29.6 mmol) in a bilayer of dichloromethane (308 mL) and 6N NaOH (50 mL). The reaction mixture was stirred for 1 hour and then diluted with water (100 mL). The reaction mixture was filtered through a plug of Celite, and the filtrate layers were separated. The aqueous layer was extracted with dichloromethane (100 mL). The combined organics were dried over sodium sulfate and were concentrated. Purification of the residue by ISCO chromatography (eluting with dichloromethane, with a gradient of 0-10% ethyl acetate) afforded 3-bromo-1-[(4methylphenyl)sulfonyl]-5-phenyl-1 H-pyrrolo[2,3-b]pyridine (7.19 g, 68% over two steps) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) 88.71 (s, 1H), 8.12 (d, J =8, 2H), 7.97 (s, 1H), 7.84 (s, 1H), 7.60 (d, J = 7.2 Hz, 2H), 7.51 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 6.8 Hz, 1H), 7.32 (d, J =8.4 Hz, 2H), 2.41 (s, 3H). LC-MS (ES) m/e 427 $(M + H)^+$.

Preparation of 3-(5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)aniline.

To a solution of 3-bromo-1-[(4methylphenyl)sulfonyl]-5-phenyl-1 H-pyrrolo[2,3-b]pyridine (1 g, 2.34 mmol) in DMF (18 mL) was added 3-aminophenylboronic acid (0.47 g, 3.04 mmol), tetrakis(triphenylphosphine)palladium(0) (0.277 g, 0.24 mmol) and saturated aqueous sodium carbonate (3 mL) and microwaved at 120 ^{o}C with stirring for 10 minutes. The combined organics were dried over sodium sulfate and were concentrated. The product was isolated by SCX cartridge via capture and release reaction: a SCX cartridge was rinsed with MeOH, and the crude mixture was dissolved in MeOH and loaded on the cartridge. The cartridge was rinsed with methanol and dichloromethane successively. The product was released by eluting with a 2M ammonia solution in methanol , followed by concentration in vacuo. Purification of the residue by ISCO chromatography (eluting with dichloromethane) afforded 3-(5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)aniline (0.95 g, 92% yield).

Preparation of 1-(2-fluoro-5-(trifluoromethyl)phenyl)-3-(3-(5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)phenyl)urea (Compound 12)

3-(5-Phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)aniline (0.02 g, 0.07 mmol) in THF (5 mL) was treated with 3-(trifluoromethyl)phenyl isocyanate (0.016 g, 0.077 mmol) and the solution stirred at RT for 16 hours.

The product was isolated by SCX cartridge via capture and release reaction as described previously: (0.024 g, 70 % yield).

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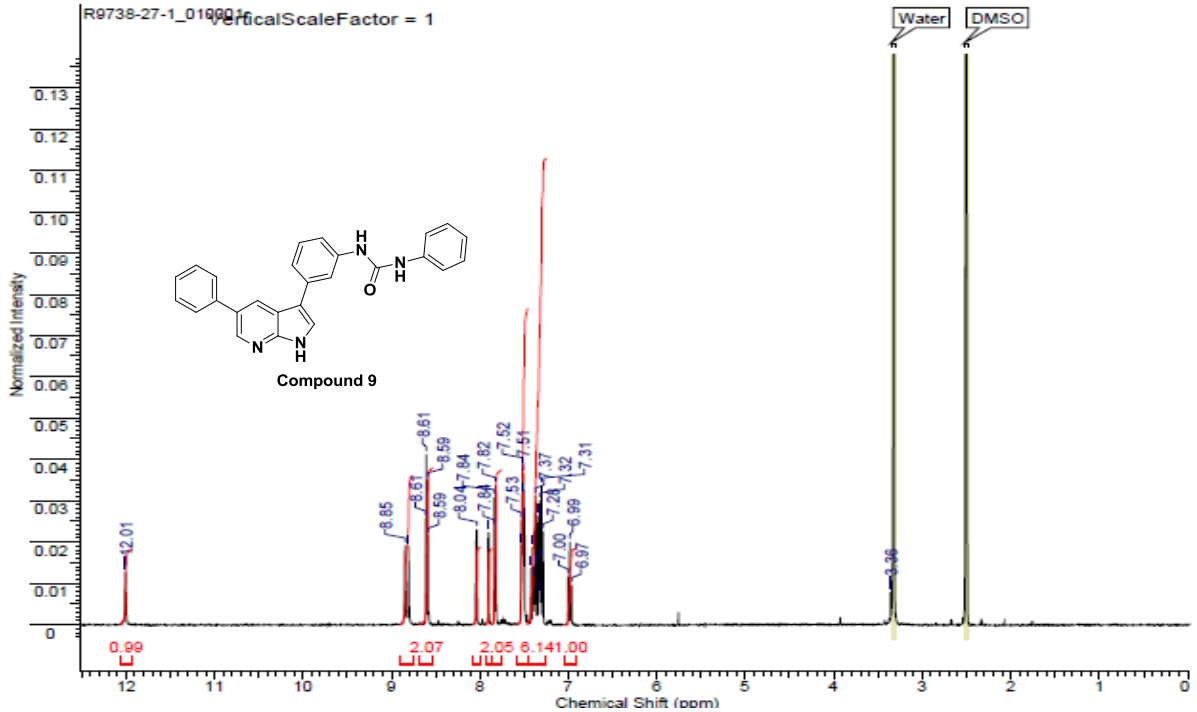

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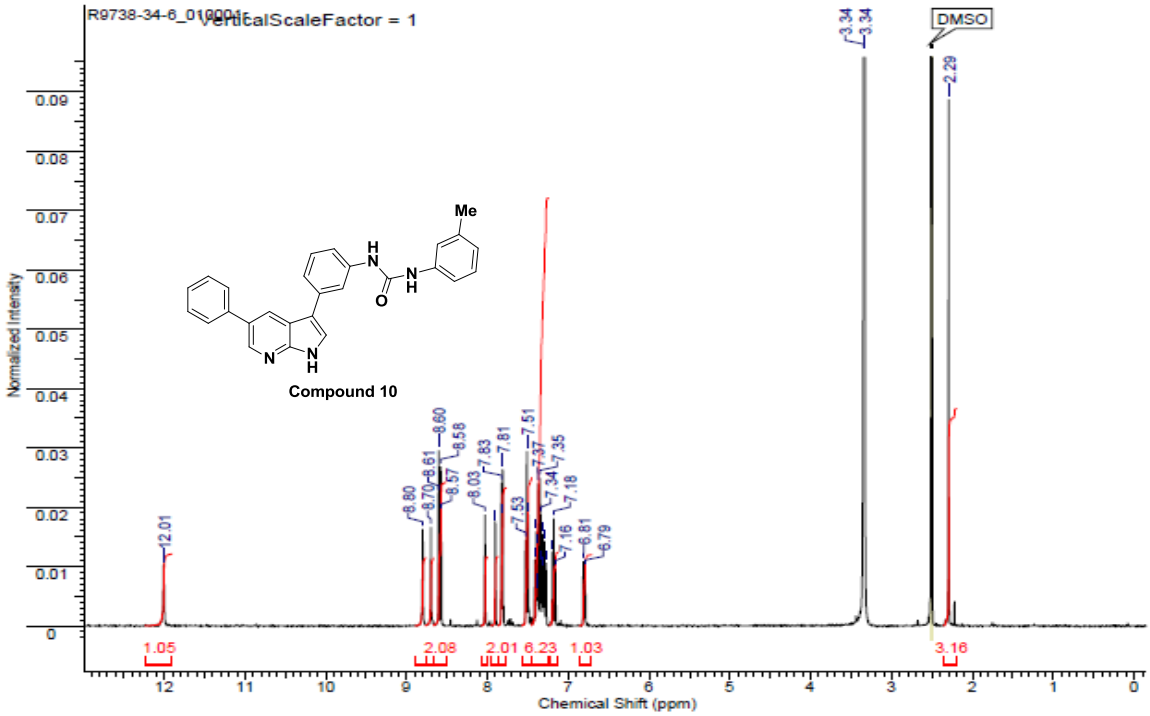

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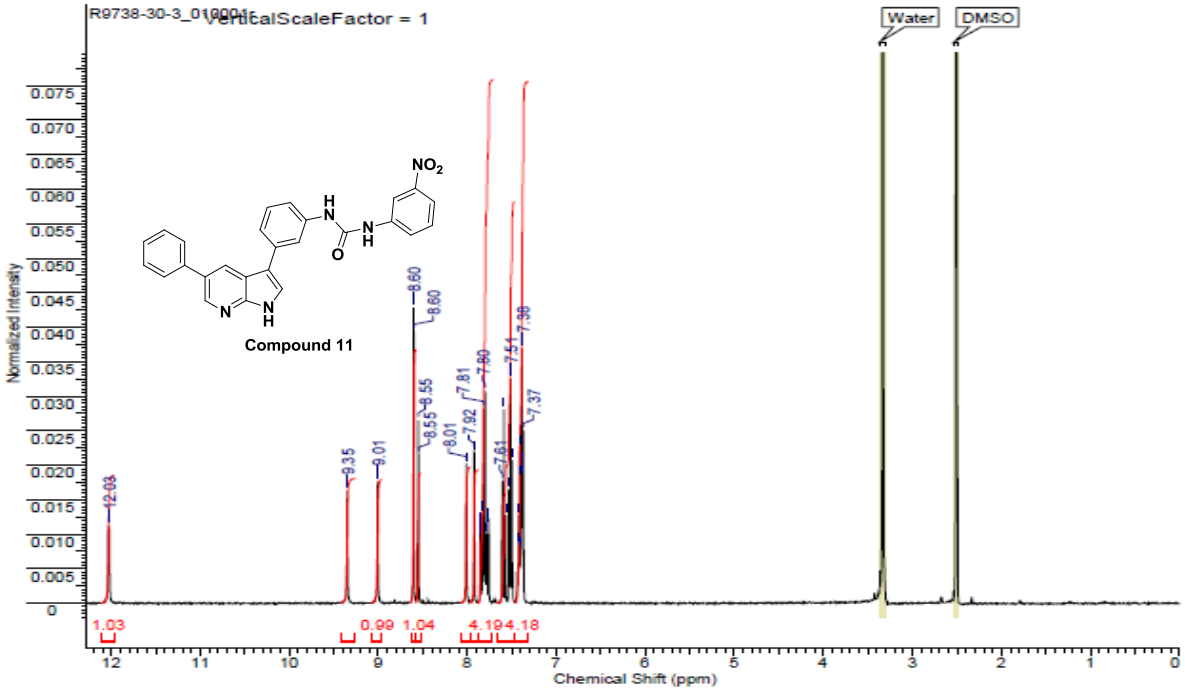

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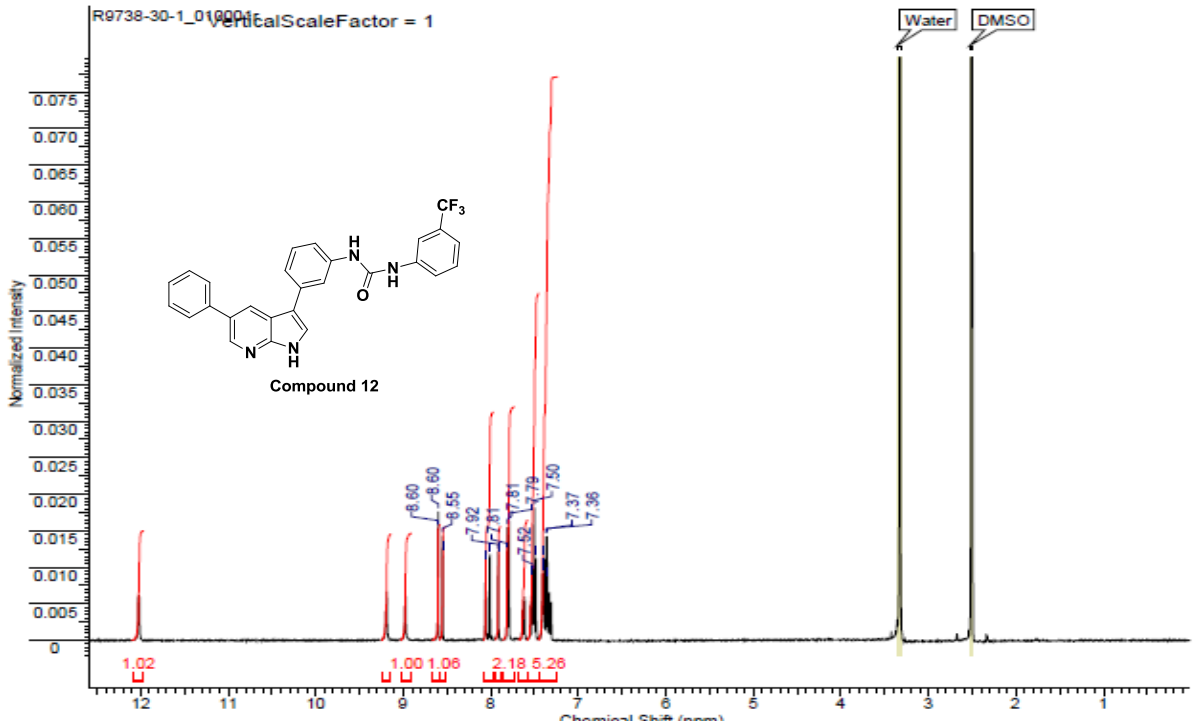

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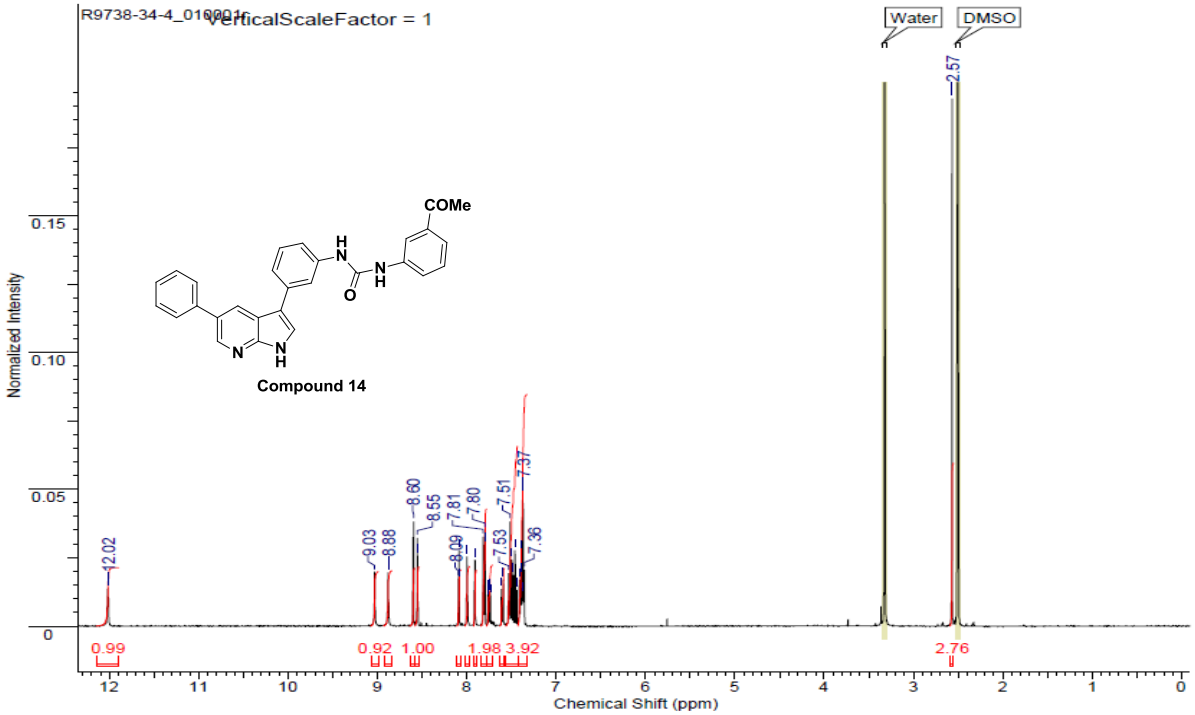

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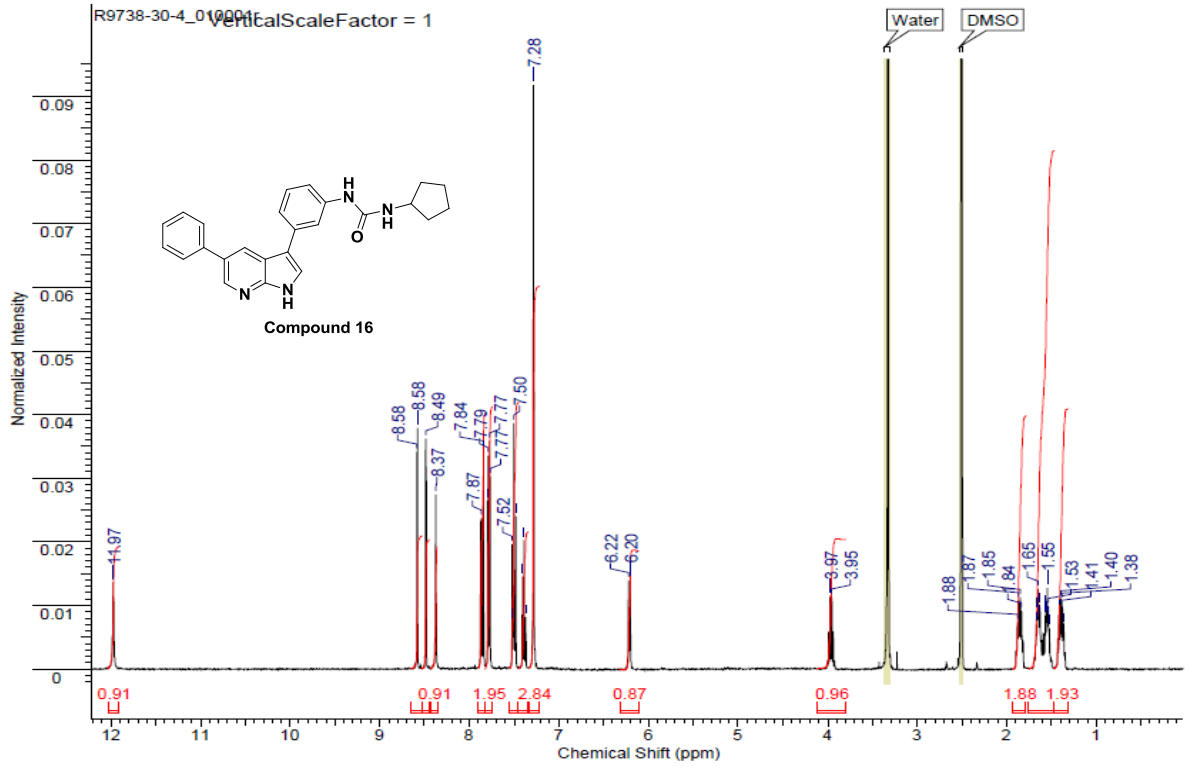
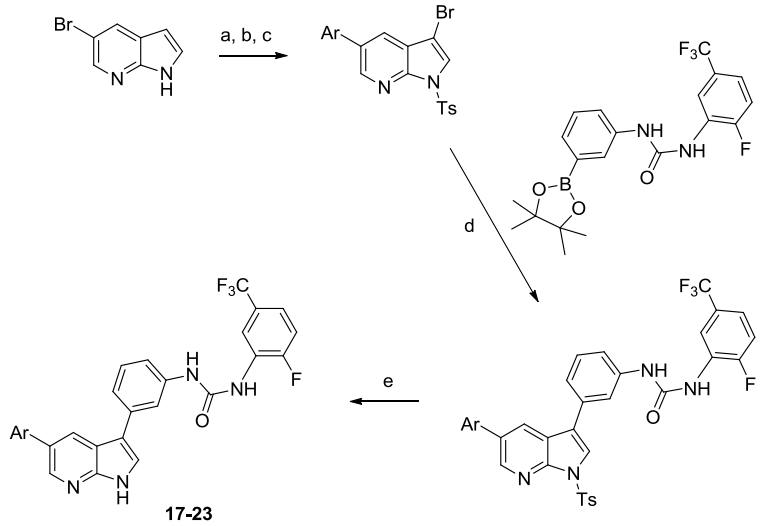


Compounds **17-23** were made according to the Schemes 3 and the representative procedure detailed below. The 3-bromo-5-aryl-1H-pyrrolo[2,3-b]pyridines were obtained following the steps described above for 3-bromo-5-phenyl-1-tosyl-1H-pyrrolo[2,3-b]pyridine.



Reagents and conditions: (a) $ArB(OH)_2$, $Pd(dppf)Cl_2$ K_2CO_3, dioxane/water; (b) Br_2, $CHCl_3$; (c) TsCl, Bu_4NSO_4, CH_2Cl_2, 6N NaOH; (d) $Pd(PPh_3)_4$, Na_2CO_3, DME, 120 ^oC; (e) NaOH, MeOH;

Preparation of 1-(2-fluoro-5-(trifluoromethyl)phenyl)-3-(3-(5-aryl-1-tosyl-1H-pyrrolo[2,3-b]pyridin-3-yl)phenyl)urea.

3-Bromo-5-aryl-1H-pyrrolo[2,3-b]pyridines (0.1 mmol) and N-[2-fluoro-5-(trifluoromethyl)phenyl]-N-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]urea (55 mg, 0.13 mmol) were dissolved in DME (1 mL) and aqueous Na_2C0_3 (2M, 0.25 mL). To the resulting solution was added $Pd(PPh_3)_4$ (10 mol%) and the reaction mixture was microwaved at 120 ^oC for 15 min. The mixture was then filtered through Celite and washed with MeOH. The crude compound in methanol was carried on to the next step as such.

Preparation of 1-(2-fluoro-5-(trifluoromethyl)phenyl)-3-(3-(5-aryl-1H-pyrrolo[2,3-b]pyridin-3-yl)phenyl)urea

To the crude solution of 1-(2-fluoro-5-(trifluoromethyl)phenyl)-3-(3-(5-aryl-1-tosyl-1H-pyrrolo[2,3-b]pyridin-3-yl)phenyl)urea in methanol was added 6N $NaOH_{(aq)}$ (0.1 mL). The resulting solution was stirred at 50 ^oC for 1 hour. The reaction mixture was dilute with water, extracted with dichloromethane (2X) and the organic phase dried over $MgSO_4$ and concentrated. The crude sample was purified via a mass directed auto purification HPLC/MS system to give the title compound.

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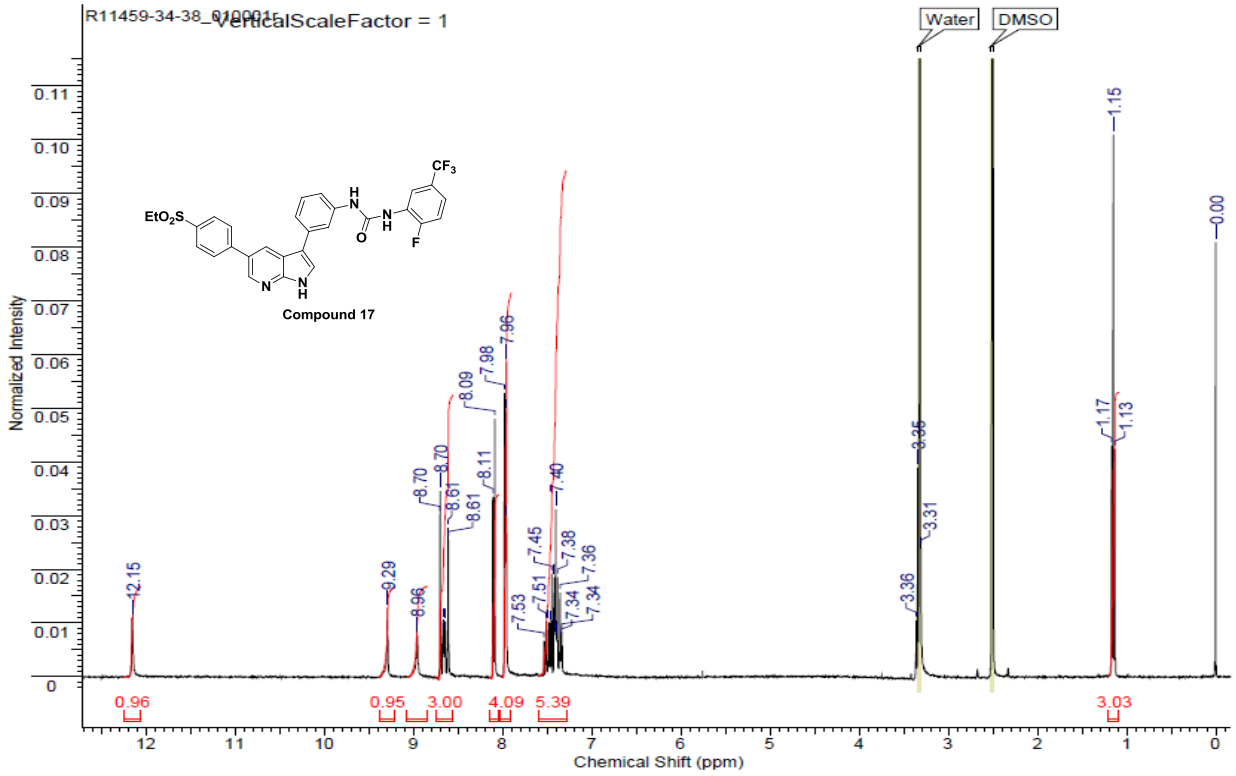

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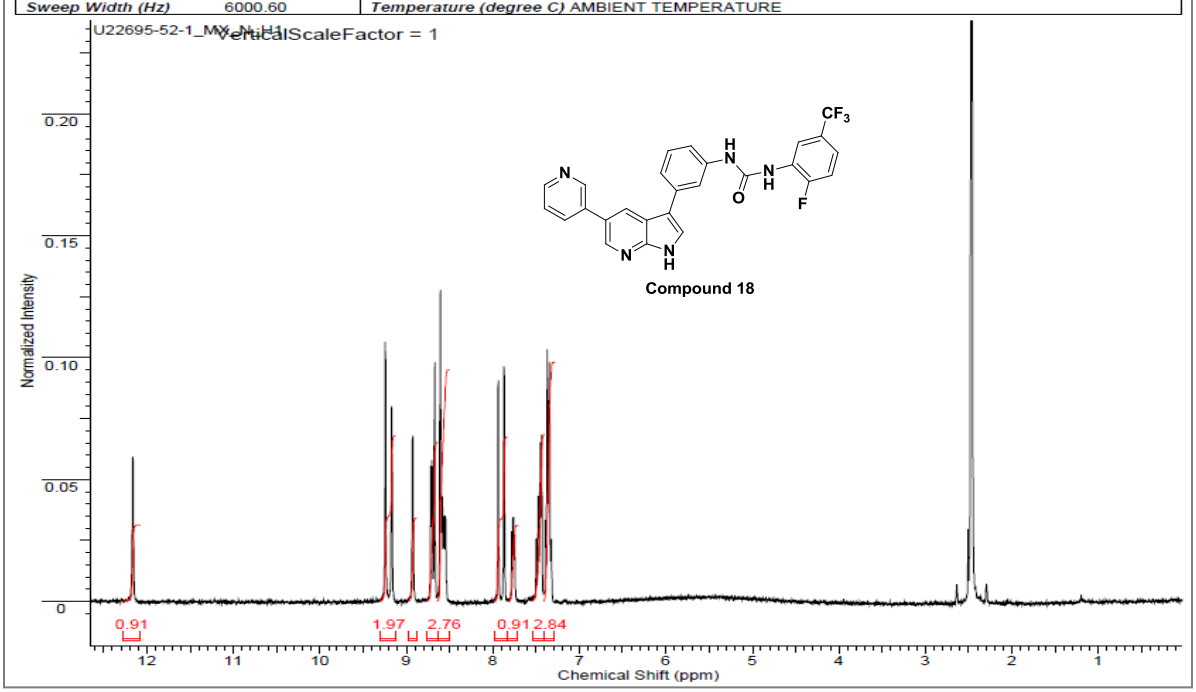
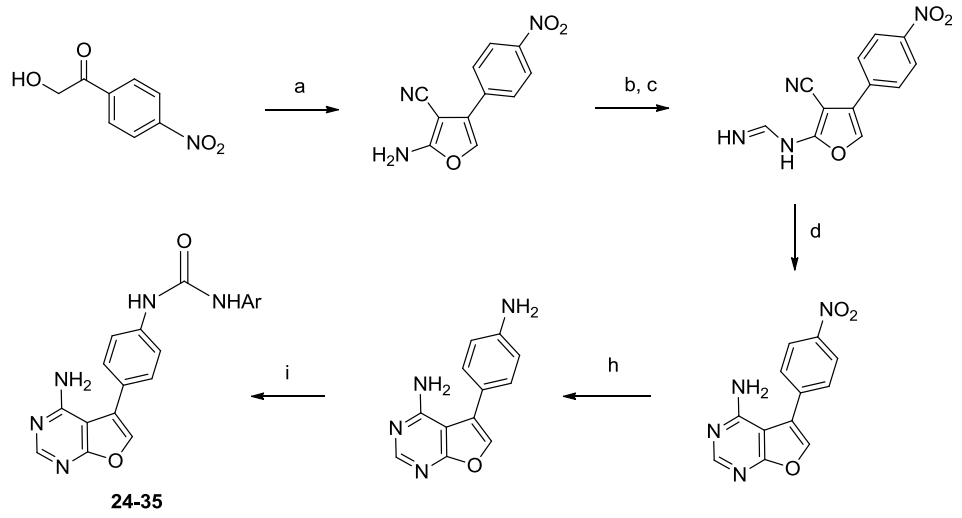


Compounds **24-35** were made according to the Schemes 4 below as described in Hirst G. C.; Arnold L. D.; Burchat A.; Wishart N.; Calderwood D.; Wada C. K.; Michaelides, M. R.; Ji Z.; Muckey M. *PCT Int. Appl.* **2003**, WO 2003080064.

Scheme 4



24-35

Reagents and conditions: (a) malononitrile, Et_2NH, MeOH, rt, 23%; (b) $HC(OEt)_3$, $(NH_4)_2SO_4$, reflux; (c) NH_3, EtOH, rt; 88% for 2 steps; (d) 1,2-dichlorobenzene, microwave 200°C, 41% (h) Fe, NH_4Cl, EtOH-H_2O, 80 °C, 70%; (i) ArNCO, CH_2Cl_2, rt, 42% for 3,5-bis(trifluoromethyl)phenyl isocyanate.

Preparation of 2-amino-4-(4-nitrophenyl)furan-3-carbonitrile

To a stirred mixture of 2-hydroxy-1-(4-nitrophenyl)ethanone (2 g, 11 mmol) and malononitrile (0.89 g, 12.1 mmol) in methanol (10 mL) was added diethylamine (0.34 g, 4.6 mmol) and the reaction stirred at room temperature for 1 hour. The reaction mixture was then poured into cold water (200 mL) and extracted with ethyl acetate (3 x 100 mL). The organic phases were dried over magnesium sulfate and evaporated under reduced pressure to give the crude product which was purified by chromatography on a silica gel column (eluting with petroleum ether/ ethyl acetate 1:1) to afford the product as a red solid (0.7 g, 23% yield).

Preparation of N-(3-cyano-4-(4-nitrophenyl)furan-2-yl)formimidamide

A mixture of 2-amino-4-(4-nitrophenyl)furan-3-carbonitrile (0.46 g, 2 mmol) and ammonium sulfate (0.29 g, 2.2 mmol) in triethylformate (9 mL) was heated to reflux for 4 h. The reaction mixture was cooled to -20°C and then treated with 2M ammonia in ethanol (20 mL) and the reaction was allowed to warm to room temperature and stirred overnight. The resulting precipitate was filtered off, washed with water and ethanol and dried to give the product as a light-yellow solid (0.45 g, 87% yield).

Preparation of 5-(4-nitrophenyl)furo[2,3-d]pyrimidin-4-amine

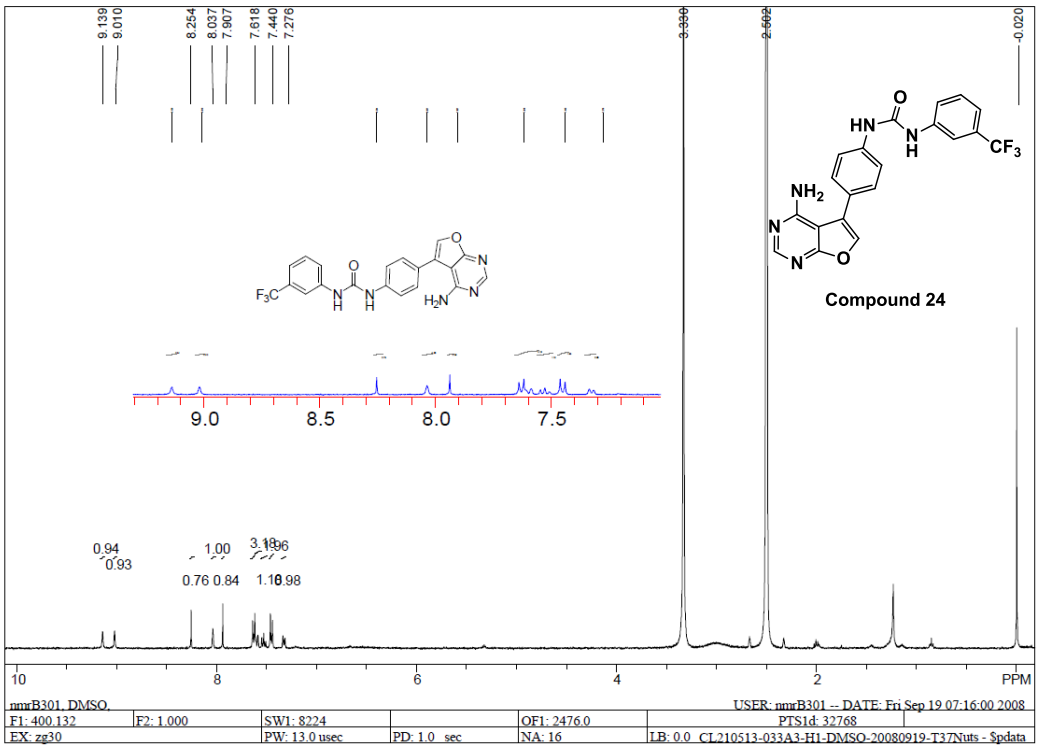
A suspension of N-(3-cyano-4-(4-nitrophenyl)furan-2-yl)formimidamide (0.4 g, 1.56 mmol) in 1,2-dichlorobenzene (5 mL) was microwaved at 200°C for 15 minutes. The reaction mixture was diluted with THF and concentrated. The resulting crude product was purified by chromatography on a silica gel column (eluting with dichloromethane/ methanol 95:5) to afford the product as a yellow solid (0.16 g, 41% yield).

Preparation of 5-(4-aminophenyl)furo[2,3-d]pyrimidin-4-amine

A mixture of 5-(4-nitrophenyl)furo[2,3-d]pyrimidin-4-amine (0.3 g, 1.17 mmol) and ammonium chloride (0.15 g, 2.8 mmol) in 2:1 ethanol/water (20 mL) was heated with stirring to 50^{o}C, treated with iron powder (0.3 g, 5.4 mmol) and heated to 80^{0}C for a further 2 hours. The reaction mixture was cooled to room temperature and filtered through celite and concentrated. The resulting crude product was purified by flash chromatography on a silica gel column (eluting with ethyl acetate / hexanes, 1:2) to afford the product as a brown solid (0.21 g, 70% yield).

Preparation of 1-(4-(4-aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(3,5-bis(trifluoromethyl)phenyl)urea

A suspension of 5-(4-aminophenyl)furo[2,3-d]pyrimidin-4-amine (0.9 g, 0.4 mmol) in dichloromethane (3 mL) was treated with 3,5-bis(trifluoromethyl)phenyl isocyanate (0.1 g, 0.4 mmol), warmed to room temperature, and stirred overnight. The resulting precipitate was collected by vacuum filtration, washed with dichloromethane, and dried to provide the product (0.095 g, 0.19 mmol, 47 % yield) as gray solid.



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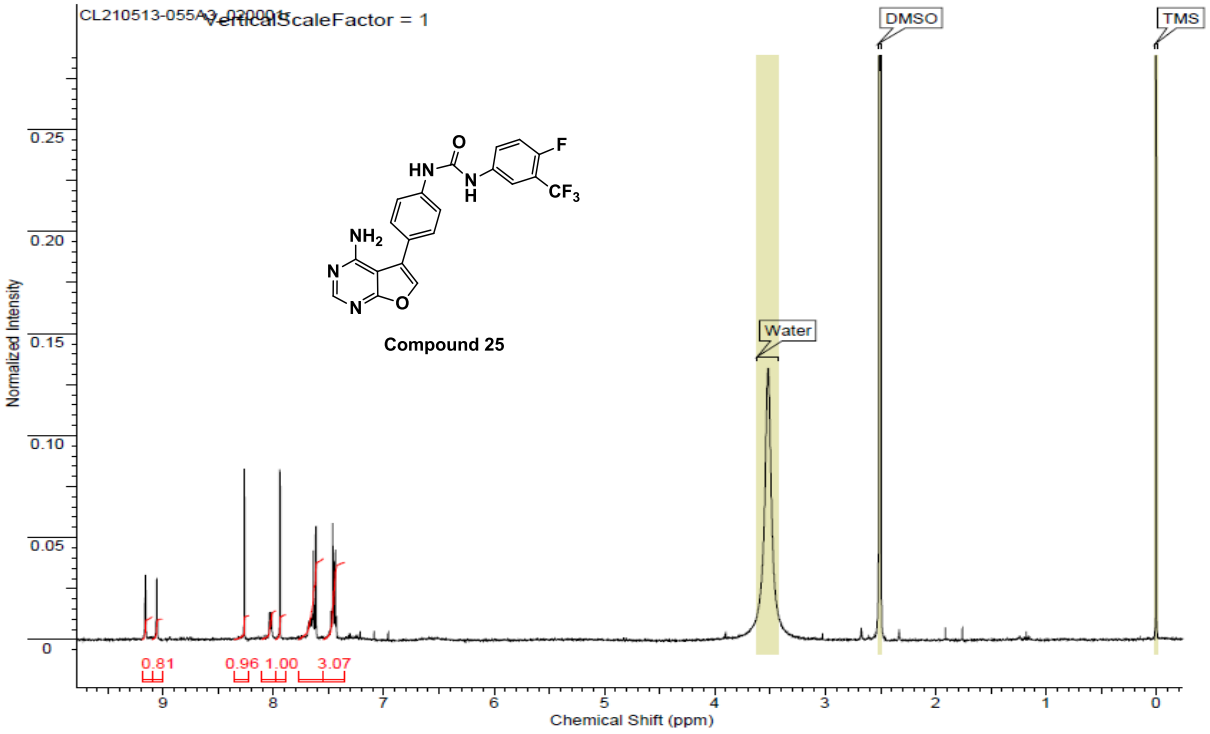

Compound 25

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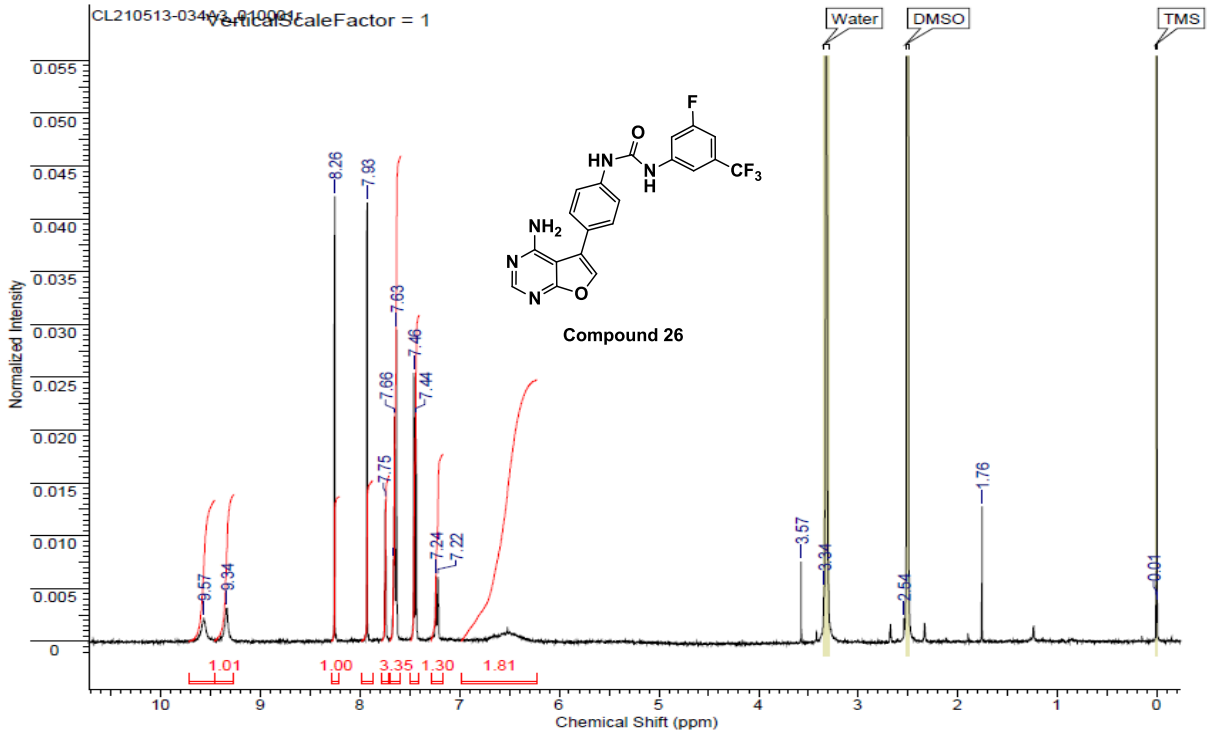
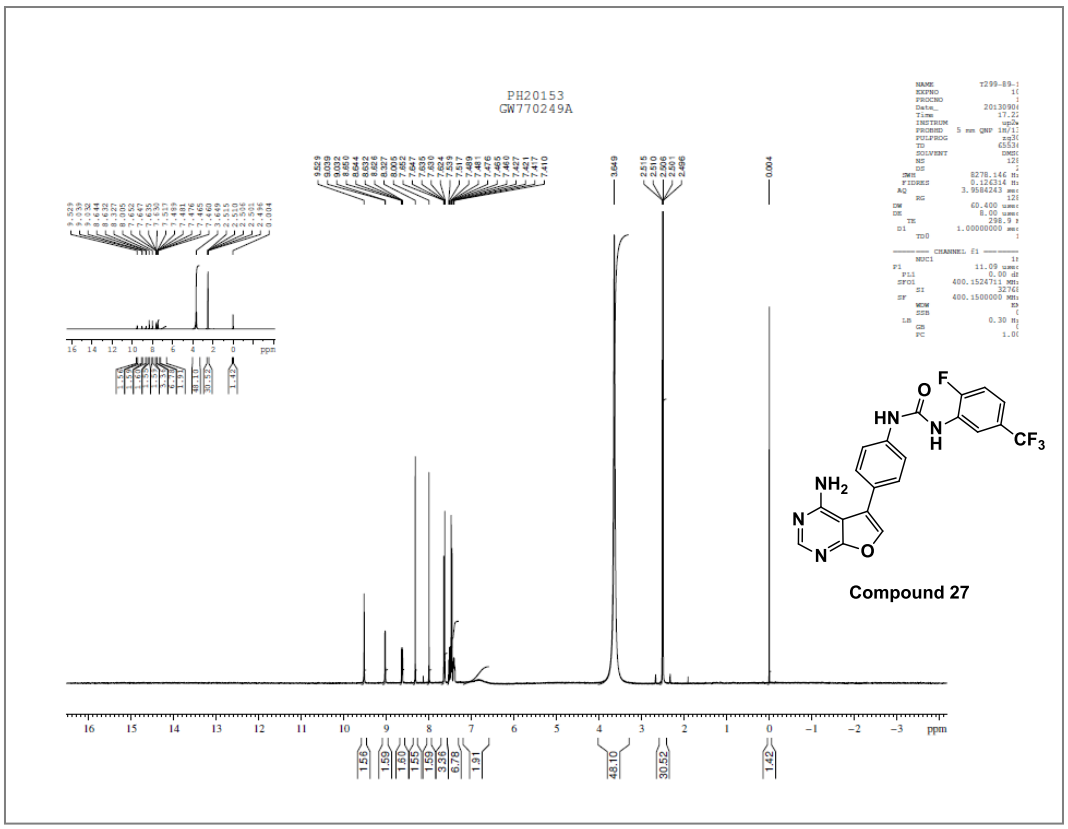


Compound 26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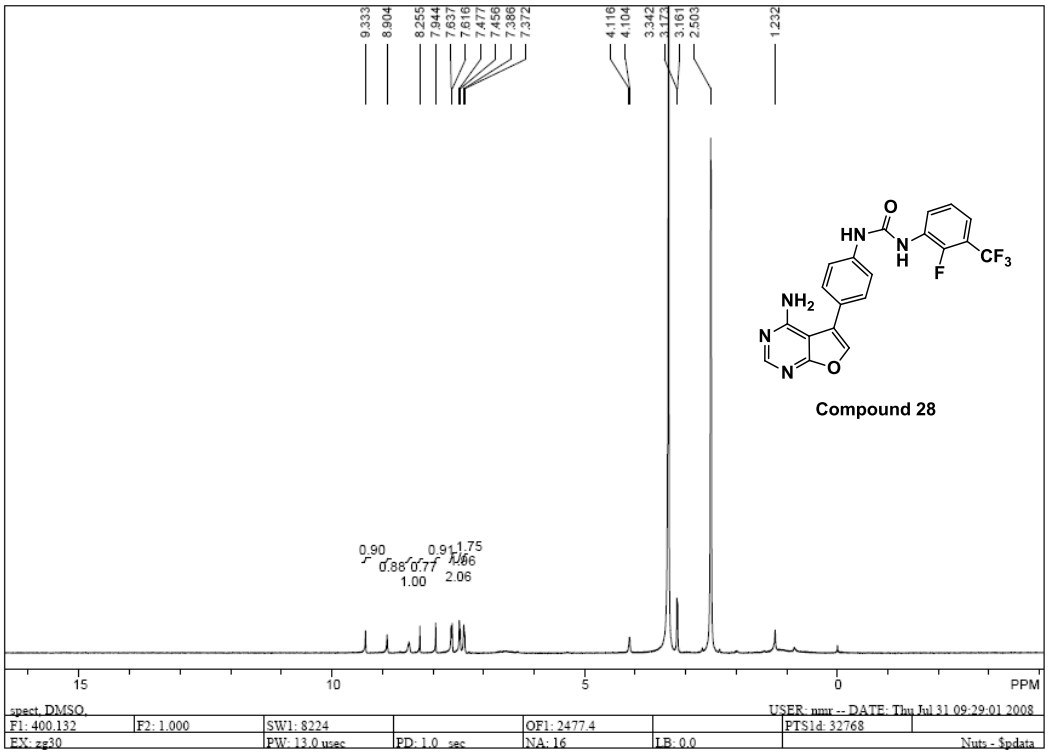
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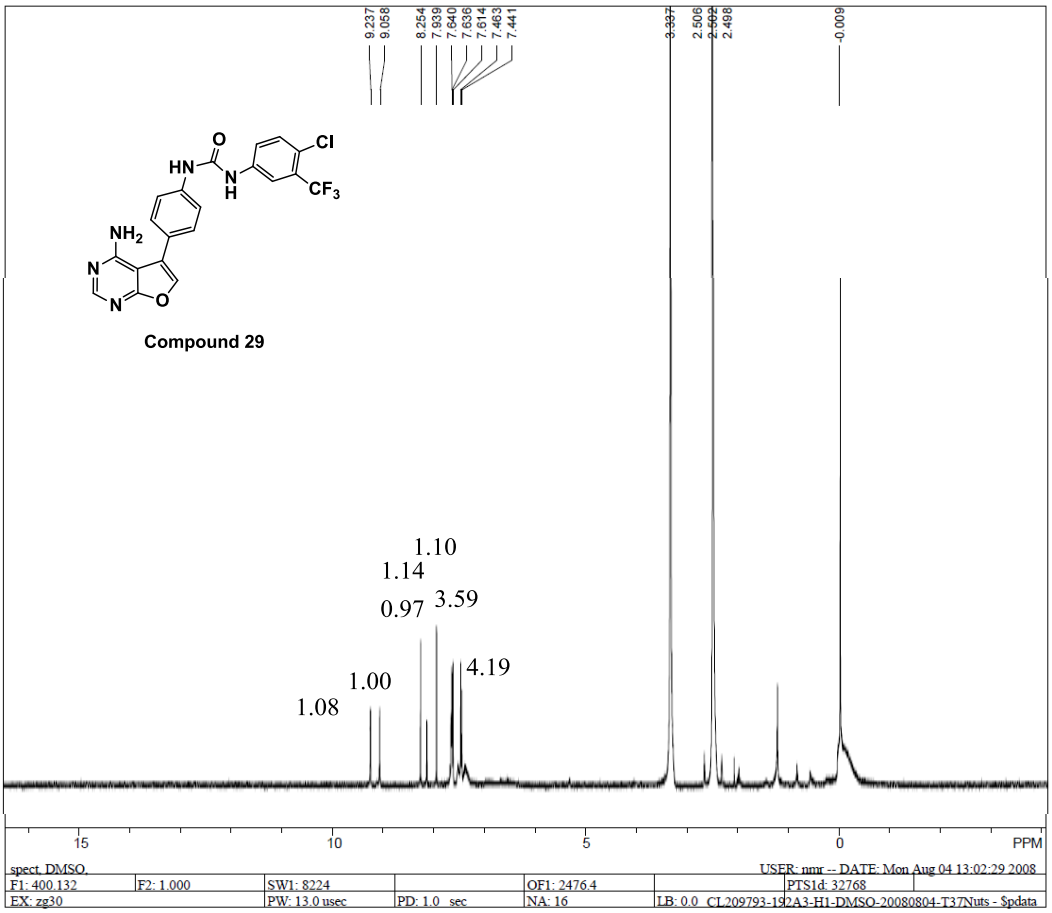

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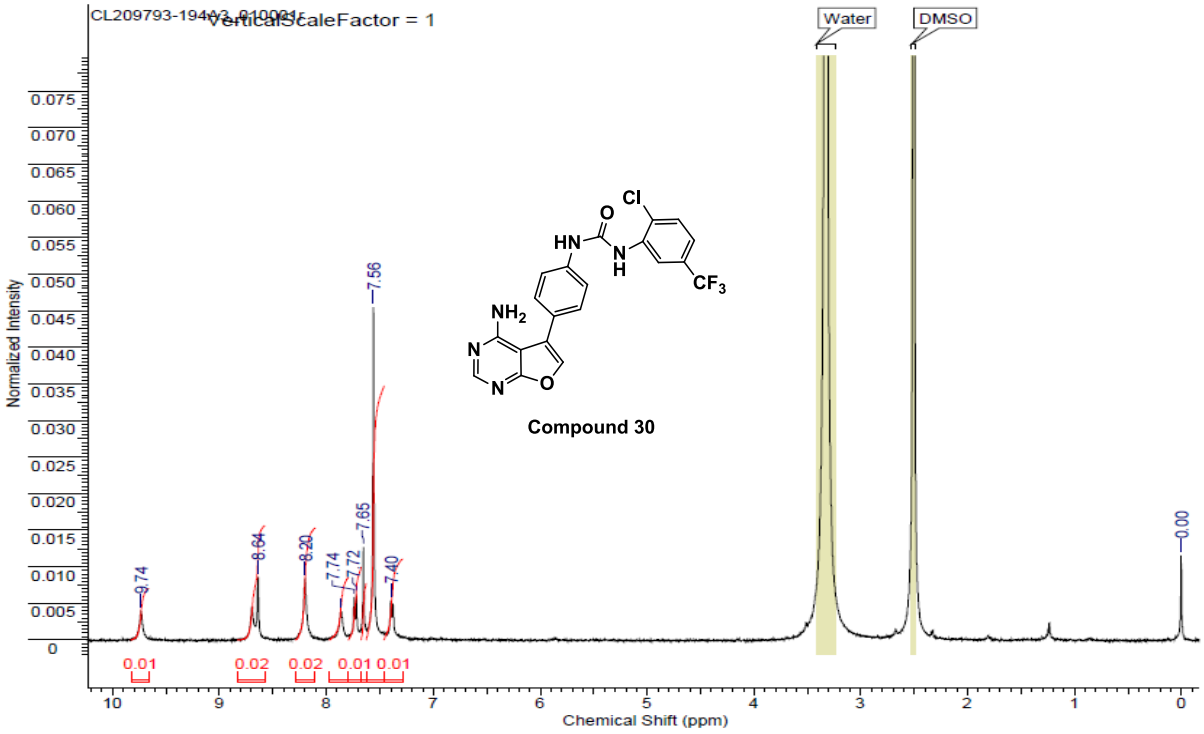
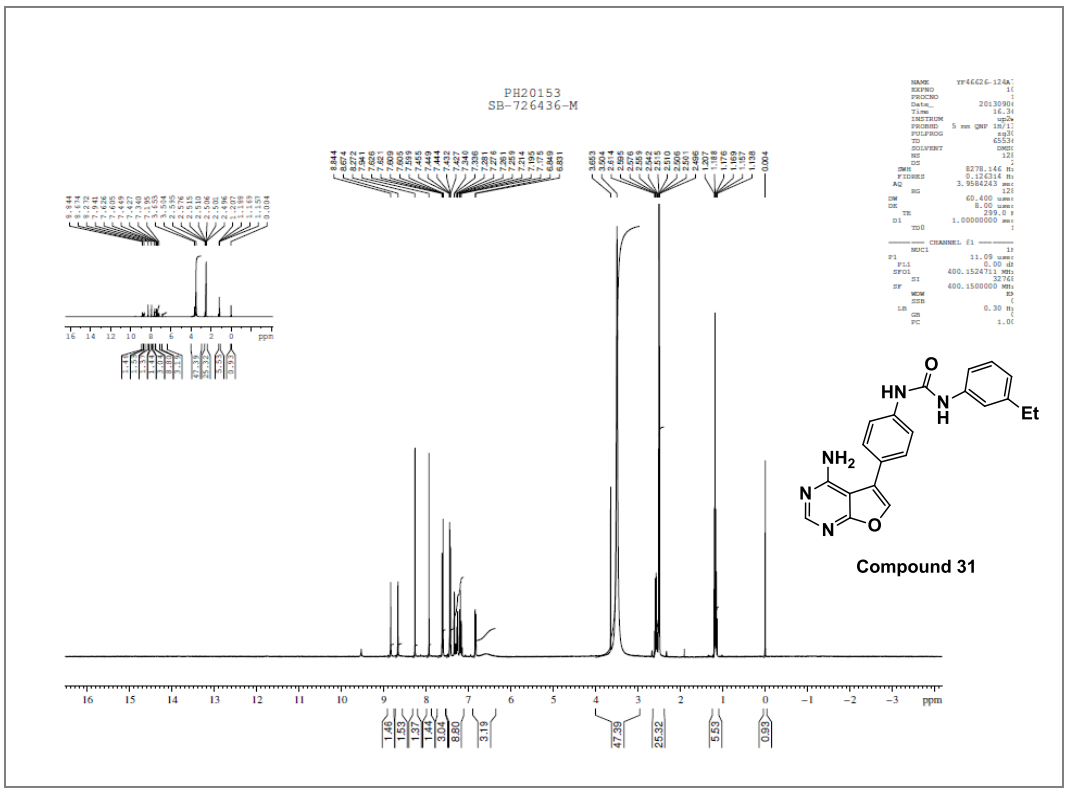


Compound 30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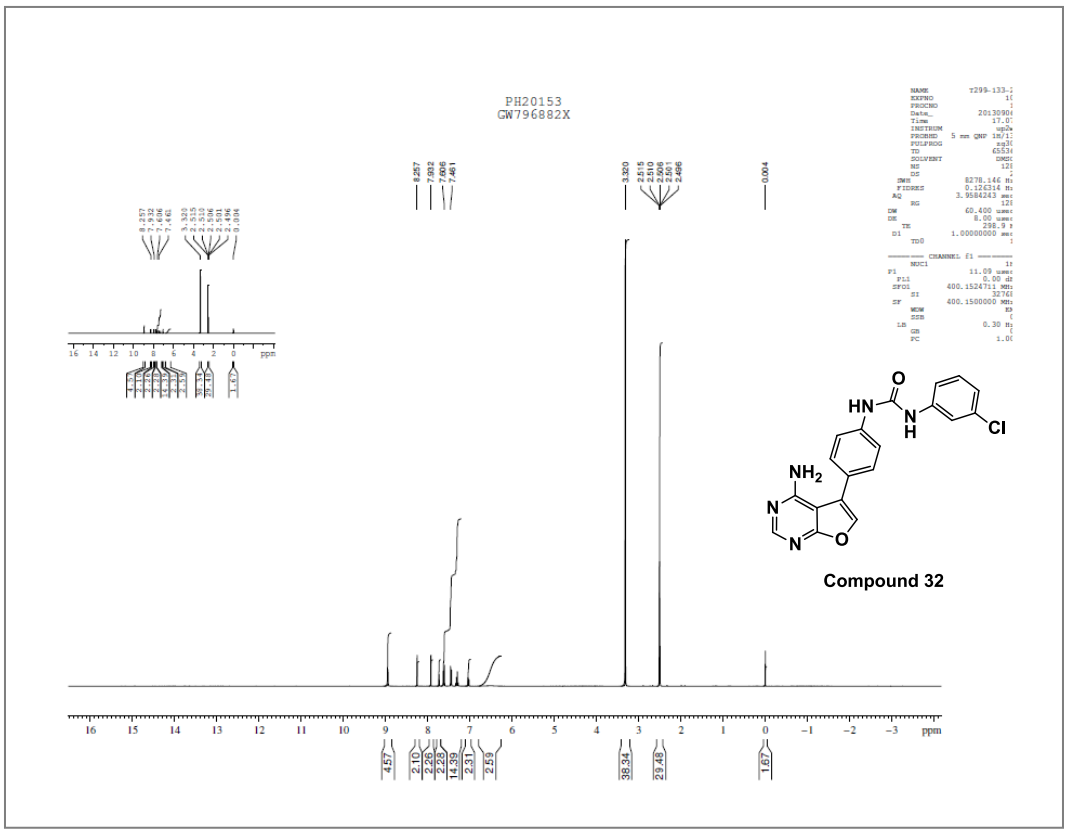
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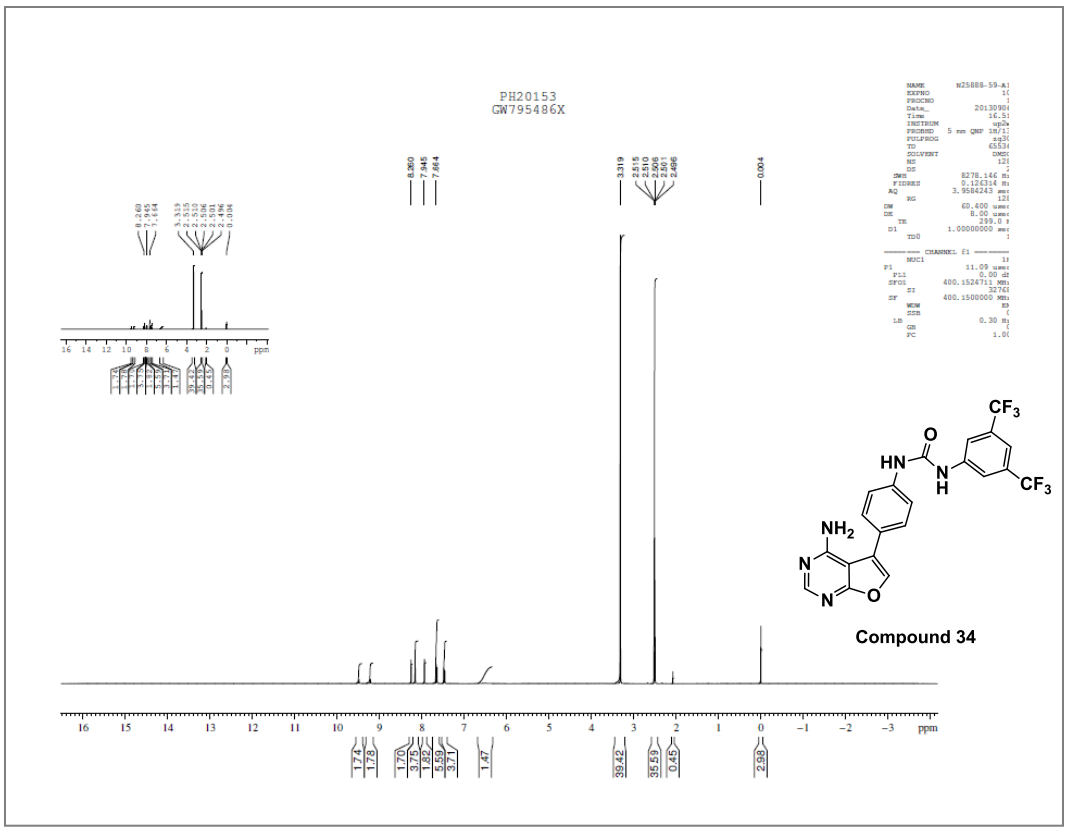

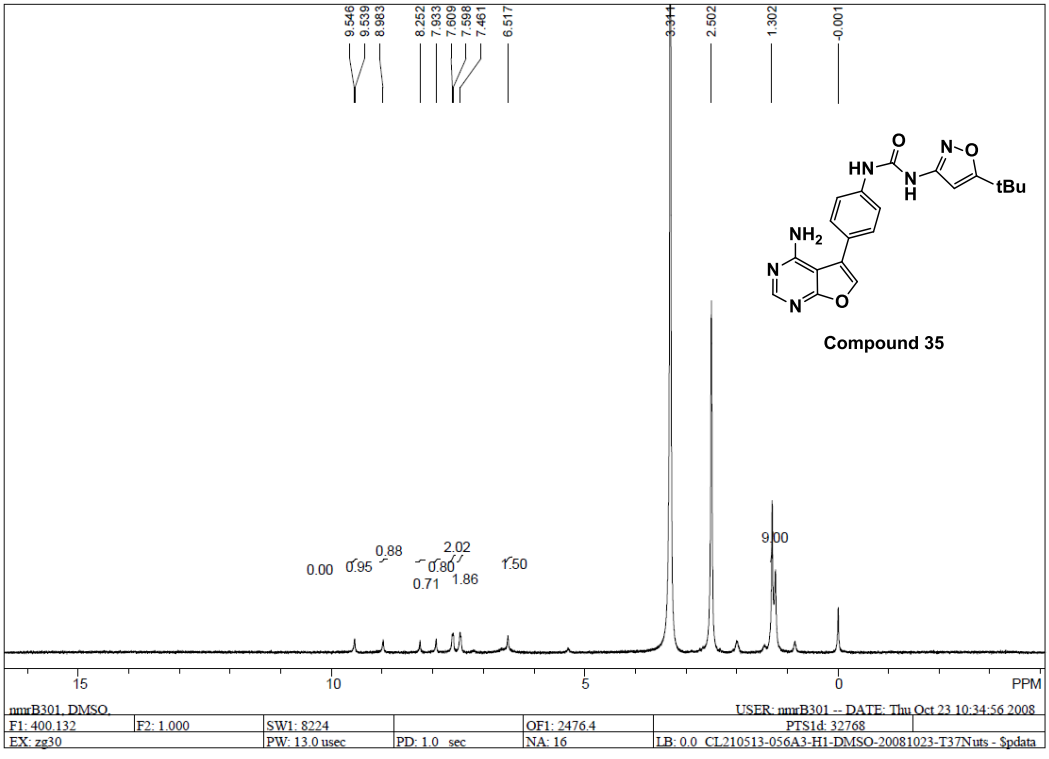

Biological *in vitro* biochemical assay

A fluorescent polarization based binding assay was developed to quantitate interaction of novel test compounds at the ATP binding pocket of RIP1, by competition with a fluorescently labeled ATP competitive ligand. GST-RipK1(1-375) was purified from a Baculovirus expression system and was used at a final assay concentration of 10nM . A fluorescent labeled ligand (14-(2-{[3-({2-{[4-(cyanomethyl)phenyl]amino}-6-[(5-cyclopropyl-1H-pyrazol-3-yl)amino]-4-pyrimidinyl}amino)propyl]amino}-2-oxoethyl)-16,16,18,18-tetramethyl-6,7,7a,8a,9,10,16,18-octahydrobenzo[2'',3'']indolizino[8'',7'':5',6']pyrano[3',2':3,4]pyrido[1,2-a]indol-5-ium-2-sulfonate was used at a final assay concentration of 5nM. Both the enzyme and ligand were prepared in solutions in 50mM HEPES pH7.5, 10mM NaCl, 50mM MgCl2, 0.5mM DTT, and 0.02% CHAPS. Test compounds were prepared in neat DMSO and 100nL was dispensed to individual wells of a multiwell plate. Next, 5ul GST-RipK1(1-375) was added to the test compounds at twice the final assay concentration, and incubated at room temperature for 10 minutes. Following the incubation, 5ul of the fluorescent labeled ligand solution, was added to each reaction, at twice the final assay concentration, and incubated at room temperature for at least 15 minutes. Finally, samples were read on an instrument capable of measuring fluorescent polarization. Test compound inhibition was expressed as percent (%) inhibition of internal assay controls. For concentration response experiments, normalized data were fit and pIC_{50}s determined using conventional techniques. The pIC_{50}s are averaged to determine a mean value, for a minimum of 2 experiments.

Compound potency against RIP1 kinase activity was determined using an ADP-Glo luminescence assay, which measures the conversion of ATP to ADP. Test compounds were prepared in neat DMSO and 100nL was dispensed to each well of a multiwell plate. The primary reaction consist of 10nM GST-RIPK1 (1-375) and 50uM ATP in 50mM HEPES pH 7.5, 50mM NaCl, 30mM MgCl2, 1mM DTT, 0.5mg/mL BSA, and 0.02% CHAPS. 5uL of enzyme and 5uL of ATP were added to the plate at twice the final assay concentration and incubated at room temperature for 4 hours. Following this reaction, 5uL of Promega's ADP-Glo reagent was added to each well and incubated for 1 hour at room temperature. This stops the kinase reaction and depletes any remaining ATP. 5uL of ADP-Glo detection reagent is then added to each well and incubated at room temperature for at least 30minutes. The detection reagent converts ADP to ATP and introduces luciferase and luciferin to detect ATP. The luminescence is then measured on a plate reader. Test compound inhibition was expressed as percent inhibition of internal assay controls. For concentration response curves, normalized data is fit and pIC_{50}s determined using conventional techniques. The pIC_{50}s are averaged to determine a mean value, for a minimum of 2 experiments.

GST-RipK1 Preparation

His.GST.TEV.RIPK1 1-375

The RIPK1 gene [receptor (TNFRSF)-interacting serine-threonine kinase 1] was cloned from human adrenal gland cDNA. Primers were designed from the reference sequence NM_003804.3 with an added CACC Kozak directional tag for cloning into pENTR/TEV/D-TOPO. Gateway® LR cloning was used to site-specifically recombine RIPK1 downstream to an N-terminal HisGST contained within the destination vector pDEST8-His.GST according to the protocol described by Invitrogen. A stop codon was inserted after amino acid 375 using Quikchange Stratagene mutagenesis kit according to manufacturer's protocol and resulted in pDEST8.His.GST.TEV.human RIPK1 1-375. His.GST.Tev.human RIPK1 1-375 baculovirus

was generated using the bac to bac system (Invitrogen) according to manufacturer's specifications. Transfection of *Spodoptera frugiperda*(Sf9) insect cells was performed using Fugene 6 (Roche), according to the manufacturer's protocol. His.GST.TEV.human RIPK1 1-375 baculovirus infected insect cells (BIICs) were prepared during the baculovirus generation according to David Wasilko and S Edward Lee, TIPS: Titerless Infected Cells Preservation and Scale up, BioProcessing Journal Fall 2006 p29-32. 20L Sf9 cells were grown in serum free Hyclone, SFX media (HyClone Laboratories, 925 West 1800 South Logan, Utah 84321) at 27°C in wave bags seeded at a density of.8X10^6cell/ml with a rock rate of 25rpm, airflow of .18 to .22. in wave reactor (WAVE Bioreactor, System 20/50EH). Cells were grown ON at 27C. His.GST.TEV.human RIPK1 1-375 baculovirus infected insect cells (BIICs) were used to infect Sf9s at a cell density of 1.7 to 2.4 X10^6. 2ml of BIIC (1 X10^7cells/mL) were added to 20L cells. Rock rate is increased to 25rom at infection. Harvest 72 hrs post infection using the Viafuge. Weigh pellets, seal wave bags and freeze at -80.

A 50g cell pellet was re-suspended in 250ml lysis buffer (50mM Tris pH 7.5, 250mM NaCl, 1mM DTT and Complete Protease Inhibitor tablets (1/50ml, from Roche Diagnostics). The cells were lysed by sonication on ice, 3x30" at power level 4 using the large probe on a Branson Sonicator. The suspension was then clarified by centrifugation at 15,000g for 30 minutes, at 4°C. The lysate was decanted from the insoluble pellet and batch bound to 10ml of Glutathione Agarose (Pierce) for 2h at 4C with gentle end over end rotation. The beads were then packed into a column and washed to baseline with lysis buffer (no protease inhibitors) and then eluted with 20mM reduced glutathione in 50mM Tris, pH8.

Fractions identified by SDS-PAGE as containing protein of interest were pooled (10ml total volume), concentrated to about 5ml and loaded onto a 300ml SDX200 SEC column (GE Healthcare) which had been equilibrated in 50mM Tris, pH7.5, 150mM NaCl, 1mM DTT and 10% Glycerol. The Rip1 protein eluted as a dimer off the SEC column.

The protein concentration was determined by Bradford assay using BSA as a standard. The yield was 12.5mg at 0.63mg/ml. The purity was >95% as determined by scanning a Coomassie stained SDS-PAGE gel.

LCMS analysis showed that the major species had lost the N-terminal methionine, was acetylated and had one phosphorylated site. The protein was aliquoted and frozen at -80C for use as needed.

Biological *in vitro* cell assay

The efficacy of RIP1 inhibitors were tested in mice *in vitro* using a human monocytic leukemia U937 cells in a necroptosis assay (He S.; Wang L.; Miao L.; Wang T.; Du F.; Zhao L.; Wang X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell*, **2009**, 137, 1100). Cells were maintained in RPMI supplemented with 10% fetal bovine serum 100U/ml penicillin, 100ug/ml streptomycin. For the assay, cells were suspended at 5e5 cells/ml in phenol red free RPMI supplemented with 1% fetal bovine serum, 100U/ml penicillin, 100ug/ml streptomycin. Thirty-five (35) ul of the cell suspension was aliquotted into a white, half area assay plate. Five (5) ul each of QVD (final concentration 50uM) or compound was added to the cells and incubated at 37°C for 30 min to 1h. Following the incubation, 5ul TNFα (final concentration 100ng/ml) was added to the cells and the samples were incubated overnight. The next day, cellular levels of ATP was determined using the Cell Titer-Glo Luminescent Cell Viability kit (available from Promega Corporation, Madison, Wisconsin, USA). All data are shown as means ± standard deviation of the mean.

FlagRip1(1-324) Preparation

Cloning and Expression. The RIPK1 gene [receptor (TNFRSF)-interacting serine-threonine kinase 1] was cloned from human adrenal gland cDNA. Primers were designed from the reference sequence NM_003804.3 with an added CACC Kozak and FLAG tag for cloning into pFASTBac. Infusion was used to site-specifically (BamHI/XhoI) recombine RIPK1 1-324 to a pFASTBac vector according to the protocol described by Clontech resulting in pFASTBacFlag humanRIPK1 1-324. Baculovirus was generated using the bac to bac system (Invitrogen) according to manufacturer's specifications. Transfection of *Spodoptera frugiperda*(Sf9) insect cells was performed using Fugene 6 (Roche), according to the manufacturer's protocol. pFASTBacFlag.human RIPK1 1-324 baculovirus infected insect cells (BIICs) were prepared during the baculovirus generation according to David Wasilko and S Edward Lee, TIPS: Titerless Infected Cells Preservation and Scale up, BioProcessing Journal Fall 2006 p29-32. 20L Sf9 cells were grown in serum free Hyclone, SFX media (HyClone Laboratories, 925 West 1800 South Logan, Utah 84321) at 27°C in wave bags seeded at a density of.8X10^6cell/ml with a rock rate of 25rpm, airflow of .18 to .22. in wave reactor (WAVE Bioreactor, System 20/50EH). Cells were grown ON at 27C. pFASTBacFlag.human RIPK1 1-324 baculovirus infected insect cells (BIICs) were used to infect Sf9s at a cell density of 1.7 to 2.4 X10^6. 2ml of BIIC (1 X10^7cells/mL) were added to 20L cells. Rock rate is increased to 25rom at infection. Harvest 72 hrs post infection using the Viafuge. Weigh pellets, seal wave bags and freeze at -80.

Purification. 60 grams of cells were lysed in 300 mL of lysis buffer (50 mM Tris pH = 7.5, 150 mM NaCl, 10 % glycerol) with a Dounce homogenizer, 10 strokes with a B pestle per 100 mL of lysate. The lysate was spun at 100,000xg for 90 minutes in a 30.25 rotor. The supernatant was batch bound to 10 mL of FLAG resin at 4C for 2 hours. The beads were spun and washed extensively and eluted in a drip column with 200 ug / mL Flag peptide in lysis buffer. Fractions Containing the protein were pooled, concentrated and run on a sizing column (SDX200) equilibrated with 25 mM Tris pH=7.5, 150 mM NaCl, 1mM DTT and 5 % glycerol. Fractions with Rip1 protein were pooled and used for incubating compound and co-crystallization**.**

Crystallization. Compound **8** was incubated over night with protein concentration of 0.5 mgs / ml and concentrated to 16mgs /mL. Concentrated complex was screened with Hampton Index Screen with sitting drop vapor diffusion with 200 nL protein plus 200 nL reservoir solution. Crystals grew from condition, E8 (0.2 M Potasium Chloride, 0.05 M HEPES pH=7.5, 35 % v/v Pentaerythritol Propoxylate (5/4 PO/OH). After optimization, single crystals suitable for x-ray data collection grew from 0.2 M Potasium Chloride, 0.05 M Bis Tris pH 6.5, 40 % v/v Pentaerythritol Propoxylate (5/4 PO/OH). Crystals were mounted in loops and plunged into liquid nitrogen for data collection.

Structure Determination and refinement. X-ray diffraction data was collected from a single crystal at 100˚K at a wavelength of 0.97872 Å using a Rayonix225 detector on LS-CAT beamline 21IDF located at the Advanced Photon Source (Argonne, IL). HKL2000[1] was used to integrate and scale the diffraction data. The Rip1 structure was solved using data to 3.0Å with the phaser.brunett[2] program. A number of models were tried in molecular replacement, with the best results obtained using a model based on the LIMK1 structure (PDB code 3S95) from residues 337-612 in which several significant stretches of residues had been deleted (346:351, 371:375, 405-408, 428-432, 485:514, 522:526, 546:569). Two molecules of RIP1 were identified in the asymmetric unit of the R32 cell. Molecular replacement phases were further improved by several rounds of Autobuster[3] refinement at the full resolution of the data 2.6

Å. The RIP1 structure was built using the Coot[4] molecular graphics program. Initial model refinement was carried out using phenix.refine[5] alternating with model rebuilding in coot. Final refinement steps were carried out using Refmac5.6[6]. The crystallographic data and refinement statistics are summarized in Table S1.

1. Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data. *Methods enzymol* 1997, *276*, 307-326.

2. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser crystallographic software. *Journal of applied crystallography* 2007, *40*, 658-674.

3. Bricogne, G.; Blanc E.; Brandl M.; Flensburg C.; Keller P.; Paciorek W.; Roversi P.; Sharff A.; Smart O.S.; Vonrhein C.; Womack, T. O. BUSTER. [2.11.5]. 2013. Cambridge, United Kingdom, Global Phasing Ltd. Ref Type: Computer Program

4. Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallographica Section D: Biological Crystallography* 2004, *60*, 2126-2132.

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6. Murshudov, G.N.; Vagin, A.A; Dodson, E.J. Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Crystallographica Section D: Biological Crystallography* 1997, **53**, 240-255.

Table S1. Data collection and refinement statistics.

X-Ray Data	
Source	AdvancedPhoton Source, LS-CAT; 21IDf
Wavelength (Å)	0.97872
Resolution range (Å)	74.62 - 2.57 (2.71 - 2.57)
Space group	R 3 2 :H
Unit cell (Å and degrees)	149.25 149.25 187.66
	90 90 120
Total reflections	90008 (13076)
Unique reflections	25546 (3723)
Multiplicity	3.5 (3.5)
Completeness (%)	99.4 (99.9)

Mean I/sigma(I)	16.1 (3.1)
Wilson B-factor	56.5
R-merge	0.065 (0.485)

Refinement Statistics

R-work	0.181 (0.2091)
R-free	0.242 (0.312)
Number of atoms	4629
Macromolecules	4390
Ligands	61
Water	221
Protein residues	561
RMS(bonds)	0.009
RMS(angles)	1.280
Ramachandran favored (%)	98
Ramachandran outliers (%)	0
Clashscore	4.6
Average B-factor	60.50
Macromolecules	60.80
Ligands	45.30
Solvent	58.70

Statistics for the highest-resolution shell are shown in parentheses.

Rmerge = Σ (|Ihl - <Ih>|)/ Σ (<Ih>),

R-work = Σ |Fp – F calc | /ΣFp, where Fp and Fcalc are observed and calculated structure factors. R-free is calculated from a randomly chosen 5 % of reflections that are never used in refinement

Biological *in vivo* assay

The efficacy of RIP1 inhibitors can be tested in mice *in vivo* using a TNF-driven systemic inflammatory response syndrome model (Duprez L.; Takahashi N.; Van Hauwermeiren F.; Vandendriessche B.; Goossens V.; Vanden Berghe T.; Declercq W.; Libert C.; Cauwels A.; Vandenabeele, P. RIP Kinase-Dependent Necrosis Drives Lethal Systemic Inflammatory Response Syndrome. *Immunity* **2011**, 35, 908). A total of 7 mice per dose group were orally pre-dosed with vehicle or compound **27** at doses of 0.2, 2.0 and 20 mg/kg 15 minutes before i.v. administration of mouse TNF (30 mg/mouse). Temperature loss in the mice was measured by rectal probe. The study was terminated after ~7 hours when the control group lost 7 degrees. All data are shown as means ± standard error of the mean.

All studies involving the use of animals were conducted after review by the GlaxoSmithKline (GSK) Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Kinome selectivity

Compounds **8**, **27** and **17** were tested against 300 kinases using a P33 radiolabled assay at Reaction Corp Biology.[22] Compounds were tested in single dose duplicate mode at 1 µM. Reactions were carried out at 10 µM ATP. Data is reported as % enzyme activity (relative to DMSO controls).

Kinase	Compound 8			Compound 27			Compound 17	
	Data 1	Data 2		Data 1	Data 2		Data 1	Data 2
ABL1	64.25	65.37		90.02	87.25		92.63	99.96
ABL2/ARG	89.80	89.65		87.56	85.99		93.20	94.11
ACK1	87.44	91.93		90.46	98.00		91.39	94.92
AKT1	102.91	102.19		94.13	93.16		83.67	92.32
AKT2	93.36	97.22		85.31	88.44		88.42	83.94
AKT3	91.26	97.90		77.94	73.61		80.86	74.78
ALK	97.38	103.55		83.32	84.71		100.88	100.68
ALK1/ACVRL1	88.82	95.04		91.14	91.06		110.51	120.18
ALK2/ACVR1	108.94	105.73		103.00	98.22		100.25	99.85
ALK4/ACVR1B	119.66	112.03		103.26	101.43		97.85	104.49
ALK5/TGFBR1	97.32	100.00		96.58	94.40		97.42	95.46
ARAF	58.27	58.58		99.61	101.72		102.09	101.85
ARK5/NUAK1	117.43	106.45		112.34	99.31		84.90	89.58
ASK1/MAP3K5	105.65	106.89		103.98	100.97		97.59	99.61
Aurora A	77.01	75.91		67.13	63.48		80.84	64.99
Aurora B	8.02	9.23		29.48	26.80		89.78	83.07
Aurora C	64.51	65.17		75.16	80.56		83.59	80.53
AXL	35.58	36.59		84.78	83.71		102.16	98.10
BLK	101.93	97.45		79.74	92.72		102.12	113.58
BMX/ETK	109.85	108.93		72.56	70.52		105.90	109.53
BRAF	60.91	62.67		97.08	88.27		94.34	94.17
BRK	63.60	62.24		106.82	97.72		103.02	91.47
BRSK1	106.45	109.35		94.34	78.06		84.23	98.15
BRSK2	93.10	104.88		97.62	95.74		94.23	95.43
BTK	111.62	107.87		96.64	95.60		98.68	100.82
c-Kit	9.61	7.35		33.72	32.90		97.19	99.29
c-MER	62.64	65.32		76.15	76.31		98.55	88.28
c-MET	34.28	28.67		96.19	82.00		89.48	99.82

c-Src	84.63	95.99		100.24	94.57		95.58	94.21
CAMK1a	107.73	107.15		102.20	102.91		106.02	111.41
CAMK1b	100.91	99.26		93.51	106.52		94.23	103.07
CAMK1d	111.53	113.25		114.70	113.65		117.04	117.85
CAMK1g	89.02	88.48		85.87	81.90		91.82	92.89
CAMK2a	111.23	111.05		95.97	105.40		95.14	123.57
CAMK2b	100.95	103.58		87.05	88.15		96.58	86.67
CAMK2d	109.32	113.42		118.04	117.02		75.51	83.43
CAMK2g	111.33	113.30		113.86	122.03		109.09	99.88
CAMK4	100.49	99.63		93.55	94.29		100.22	94.28
CAMKK1	84.10	89.20		109.90	99.47		93.93	91.91
CAMKK2	92.10	92.44		104.28	102.30		99.20	106.96
CDK1/cyclin A	107.33	105.68		83.25	84.48		83.32	79.06
CDK1/cyclin B	103.10	109.78		108.01	86.15		97.30	85.53
CDK2/cyclin A	103.23	100.02		89.65	84.98		88.52	81.58
CDK2/cyclin E	92.94	92.58		96.65	87.40		105.71	91.25
CDK3/cyclin E	99.93	96.11		78.84	82.06		86.23	87.75
CDK4/cyclin D1	89.33	96.91		95.98	91.98		94.14	95.46
CDK4/cyclin D3	84.56	79.22		106.12	104.66		103.47	102.63
CDK5/p25	98.60	103.35		91.79	83.81		89.99	87.83
CDK5/p35	93.49	93.41		104.41	117.64		92.31	95.48
CDK6/cyclin D1	115.89	119.41		97.76	97.05		96.64	96.70
CDK6/cyclin D3	107.65	98.51		106.16	104.97		103.25	99.17
CDK7/cyclin H	91.07	88.38		118.32	111.83		121.39	103.28
CDK9/cyclin K	62.74	60.13		92.37	98.70		114.46	122.82
CDK9/cyclin T1	67.12	66.01		92.07	93.84		97.22	98.44
CHK1	116.82	104.16		95.62	92.77		94.42	92.22
CHK2	92.99	92.62		96.17	96.92		100.06	98.99
CK1a1	104.33	102.41		81.11	92.10		79.96	79.83
CK1d	88.66	86.60		93.55	96.16		92.42	99.69
CK1epsilon	92.47	99.10		101.06	99.40		98.36	97.75
CK1g1	102.38	107.59		100.22	104.51		103.19	113.87
CK1g2	95.72	95.28		98.39	96.53		99.50	98.65
CK1g3	98.59	99.41		98.53	96.23		94.05	100.42
CK2a	79.75	74.90		114.67	116.27		112.26	113.03
CK2a2	104.05	100.18		94.30	102.00		103.22	112.28
CLK1	47.51	48.61		101.54	102.46		105.86	101.54
CLK2	61.64	63.69		89.96	88.87		87.73	89.63
CLK3	83.49	84.93		105.96	110.24		116.88	113.54
CLK4	75.08	76.17		111.07	133.81		121.80	106.93
COT1/MAP3K8	99.88	101.08		86.13	100.35		98.20	95.04
CSK	93.79	89.77		87.24	88.37		86.05	77.46
CTK/MATK	108.05	107.47		88.29	82.95		72.46	69.40
DAPK1	73.95	75.10		98.47	101.92		96.66	93.38
DAPK2	119.29	128.46		110.99	97.92		89.01	89.45
DCAMKL2	101.97	98.51		100.73	99.05		98.93	98.97
DDR2	1.45	1.84		9.87	9.40		109.43	100.89
DMPK	76.43	97.20		108.58	84.94		93.89	92.90
DRAK1/STK17A	99.05	102.64		88.59	96.87		103.78	90.45

DYRK1/DYRK1A	101.05	102.69		101.90	105.95		94.28	92.97
DYRK1B	101.82	105.00		99.54	103.33		99.02	103.66
DYRK2	34.47	30.74		105.68	101.95		58.51	68.99
DYRK3	49.62	51.15		96.59	92.54		100.12	97.32
DYRK4	119.68	111.98		106.94	98.23		98.66	98.40
EGFR	96.16	104.04		90.41	89.82		85.60	75.97
EPHA1	60.22	60.77		77.30	69.39		110.31	111.85
EPHA2	11.60	12.01		43.86	39.47		93.36	88.27
EPHA3	50.84	44.39		61.83	62.66		92.54	92.43
EPHA4	46.44	46.48		53.83	53.28		94.82	91.32
EPHA5	47.58	45.39		81.17	82.99		110.81	98.93
EPHA6	6.89	3.60		7.48	8.66		99.09	93.37
EPHA7	8.84	8.59		3.12	3.15		33.14	30.92
EPHA8	68.90	68.66		74.71	70.53		95.49	101.24
EPHB1	45.20	44.53		56.31	53.61		99.86	94.08
EPHB2	19.74	20.41		23.37	22.38		104.47	90.92
EPHB3	42.20	39.48		38.82	42.12		69.91	68.38
EPHB4	50.62	53.75		33.51	31.46		91.49	80.89
ERBB2/HER2	116.26	104.49		98.40	109.89		102.02	94.56
ERBB4/HER4	93.92	95.90		101.41	101.43		107.08	118.48
ERK1	87.99	87.91		105.48	94.63		103.08	102.29
ERK2/MAPK1	95.77	87.80		93.35	101.66		93.87	101.49
FAK/PTK2	94.22	96.24		97.99	102.03		103.31	107.97
FER	111.95	112.74		107.16	106.45		104.52	106.67
FES/FPS	91.77	91.45		90.72	88.31		93.91	96.17
FGFR1	63.54	60.13		78.32	80.78		94.16	98.33
FGFR2	57.39	56.51		57.52	61.89		80.89	99.54
FGFR3	61.99	60.97		81.37	88.92		93.69	94.36
FGFR4	86.41	79.69		77.17	82.18		101.12	98.55
FGR	79.17	84.71		91.89	85.93		97.11	93.04
FLT1/VEGFR1	17.65	17.54		67.55	66.13		106.53	97.59
FLT3	-0.13	0.57		4.52	3.49		30.63	32.75
FLT4/VEGFR3	13.89	14.60		61.82	67.01		122.67	124.20
FMS	8.57	6.81		13.27	14.39		104.05	103.99
FRK/PTK5	11.84	10.27		51.79	44.83		96.57	99.06
FYN	92.74	95.98		112.56	107.80		105.80	101.42
GCK/MAP4K2	78.20	86.40		41.56	42.09		17.36	17.72
GRK2	102.19	101.96		99.38	95.13		97.82	96.10
GRK3	92.52	94.51		84.42	98.18		80.64	77.29
GRK4	96.00	102.76		78.36	75.38		78.34	75.67
GRK5	91.98	104.34		110.59	105.00		82.61	83.55
GRK6	105.08	105.65		84.15	96.50		81.38	90.43
GRK7	92.48	90.99		118.48	114.37		106.18	118.42
GSK3a	112.43	99.41		124.73	122.16		98.08	84.60
GSK3b	139.66	153.59		111.17	101.13		104.32	100.02
Haspin	96.36	102.92		63.13	50.74		64.96	71.43
HCK	76.42	80.44		77.04	78.49		87.59	84.97
HGK/MAP4K4	23.21	26.95		93.15	93.65		73.04	78.11

HIPK1	72.95	76.29		94.84	93.40		94.81	94.81
HIPK2	92.98	98.82		60.96	55.88		87.31	94.77
HIPK3	87.50	96.87		76.37	77.47		83.86	85.71
HIPK4	2.43	2.49		8.71	8.89		23.16	20.15
IGF1R	112.19	92.05		92.52	93.84		96.27	93.26
IKKa/CHUK	100.14	111.52		99.26	99.63		94.19	104.28
IKKb/IKBKB	94.70	102.59		92.84	76.41		105.43	110.20
IKKe/IKBKE	100.61	107.22		89.27	83.08		92.87	88.47
IR	102.94	103.89		95.35	100.87		92.93	92.11
IRAK1	1.91	2.16		95.64	95.00		91.97	92.94
IRAK4	87.85	83.71		81.43	79.26		78.74	82.04
IRR/INSRR	83.30	87.37		92.70	87.15		84.57	90.78
ITK	100.73	99.77		114.60	107.65		119.33	114.65
JAK1	123.45	118.83		100.90	98.36		102.70	115.58
JAK2	119.24	121.55		88.36	97.66		128.35	108.93
JAK3	118.91	117.41		82.10	82.54		85.50	83.05
JNK1	108.76	106.36		103.66	101.20		84.44	85.69
JNK2	83.37	85.53		92.61	112.03		108.93	110.29
JNK3	90.57	99.66		109.80	104.41		116.31	103.96
KDR/VEGFR2	15.93	17.64		56.63	49.01		75.25	68.93
KHS/MAP4K5	26.45	23.36		21.58	25.04		49.73	50.75
LCK	47.44	47.78		69.28	67.28		85.27	85.45
LIMK1	61.55	66.03		90.99	94.65		96.03	98.19
LKB1	121.94	121.44		91.78	90.74		99.42	99.94
LOK/STK10	11.24	10.77		55.64	58.29		88.44	92.67
LRRK2	39.40	41.76		49.09	51.17		73.90	69.61
LYN	51.62	58.53		77.72	83.64		107.63	102.64
LYN B	73.13	73.61		95.11	104.62		110.06	113.23
MAPKAPK2	113.45	112.58		84.97	95.09		96.42	89.34
MAPKAPK3	102.83	105.42		103.71	98.74		94.40	107.23
MAPKAPK5/PRAK	108.64	112.74		97.78	84.16		100.79	100.06
MARK1	77.83	93.22		136.10	130.88		132.76	129.51
MARK2/PAR-1Ba	107.77	96.43		130.68	127.37		138.58	134.53
MARK3	106.05	89.21		93.27	92.15		86.23	88.62
MARK4	106.32	110.95		96.52	100.26		74.16	99.39
MEK1	71.76	83.09		106.75	107.71		90.39	89.77
MEK2	42.07	43.18		74.15	77.56		102.18	99.95
MEKK2	103.86	103.29		113.27	112.04		109.54	118.72
MEKK3	103.31	102.47		116.05	109.09		103.79	108.02
MELK	91.58	102.36		89.90	95.54		86.92	86.64
MINK/MINK1	11.07	10.51		105.93	108.47		102.19	100.86
MKK6	86.56	85.62		99.26	102.09		99.74	104.15
MLCK/MYLK	87.83	91.46		85.53	85.91		93.05	86.38
MLCK2/MYLK2	33.83	32.34		85.75	85.68		100.01	105.37
MLK1/MAP3K9	98.93	98.84		95.09	96.47		107.16	109.77
MLK2/MAP3K10	85.37	89.18		68.50	66.31		84.85	85.34
MLK3/MAP3K11	57.05	70.99		57.40	60.06		76.61	78.99

MNK1	4.25	4.82		77.43	78.82		89.02	94.51
MNK2	6.56	7.72		70.63	73.22		91.54	91.10
MRCKa/CDC42 BPA	100.31	102.21		105.09	122.00		117.72	118.76
MRCKb/CDC42 BPB	91.43	78.67		87.34	97.47		85.75	98.40
MSK1/RPS6KA5	93.86	88.15		72.26	80.58		85.20	86.54
MSK2/RPS6KA4	72.13	69.98		95.01	82.86		89.55	93.70
MSSK1/STK23	75.07	67.91		70.32	65.26		105.81	107.51
MST1/STK4	116.98	118.47		102.15	104.85		99.34	95.76
MST2/STK3	108.98	108.71		86.77	86.01		85.79	85.25
MST3/STK24	142.84	129.15		262.55	257.28		114.52	113.33
MST4	125.29	126.96		115.21	121.27		108.58	113.86
MUSK	80.57	79.38		98.11	101.89		97.44	99.56
MYO3b	97.99	100.77		76.94	75.02		70.07	68.98
NEK1	46.92	55.60		54.92	54.25		59.11	64.29
NEK11	93.08	78.99		89.28	81.32		102.09	91.75
NEK2	111.23	109.65		117.02	114.52		102.92	108.77
NEK3	82.72	96.86		84.01	94.46		84.91	97.85
NEK4	70.81	72.14		101.69	95.09		98.67	101.84
NEK6	114.84	109.68		96.95	94.72		95.52	94.55
NEK7	127.50	123.60		141.38	145.91		101.02	103.09
NEK9	96.20	88.77		93.23	91.06		103.19	108.83
NIK/MAP3K14	99.53	107.19		115.85	136.44		123.40	136.87
NLK	85.98	79.27		110.40	113.20		104.14	109.58
OSR1/OXSR1	110.03	108.87		96.80	90.06		98.77	100.36
P38a/MAPK14	31.63	32.45		80.51	78.52		92.57	93.41
P38b/MAPK11	22.11	20.98		89.04	84.18		102.46	98.32
P38d/MAPK13	50.38	51.78		85.51	85.42		90.77	91.35
P38g	30.78	28.40		51.65	51.57		50.65	62.83
p70S6K/RPS6KB1	65.43	63.87		28.98	33.99		119.11	105.69
p70S6Kb/RPS6KB2	104.26	108.38		72.61	72.54		88.15	96.18
PAK1	104.36	104.16		117.95	118.58		112.99	117.22
PAK2	108.68	105.92		98.65	100.70		101.17	99.56
PAK3	106.37	107.18		97.63	94.41		96.54	92.25
PAK4	92.27	96.80		103.04	97.77		97.44	100.66
PAK5	106.91	104.10		102.07	92.27		101.06	116.80
PAK6	81.12	88.78		94.16	86.86		98.01	103.43
PASK	106.50	103.88		102.82	84.39		89.68	95.61
PBK/TOPK	100.78	100.27		94.28	106.79		96.63	94.23
PDGFRa	5.61	5.35		17.32	15.18		92.88	92.97
PDGFRb	8.07	7.86		37.29	36.72		95.22	91.38
PDK1/PDPK1	100.26	97.15		85.67	92.52		85.23	71.80
PHKg1	50.99	51.88		87.53	84.73		79.18	87.75
PHKg2	114.30	115.99		84.57	97.62		97.52	88.28
PIM1	98.98	100.15		97.17	105.38		99.78	101.58
PIM2	109.96	109.28		98.86	96.63		98.04	104.43
PIM3	132.55	136.18		91.10	88.65		86.75	109.19

PKA	89.06	104.54		99.15	90.96		101.33	111.90
PKAcg	66.38	58.95		138.19	128.96		122.80	115.90
PKCa	118.21	98.10		69.29	67.61		75.18	76.62
PKCb1	94.19	108.14		97.81	91.02		99.36	96.21
PKCb2	90.73	95.49		98.31	89.84		98.81	91.26
PKCd	112.50	105.83		93.89	88.16		92.37	88.60
PKCepsilon	97.88	98.47		95.77	113.74		103.72	101.29
PKCeta	100.63	89.61		89.68	89.26		95.50	89.41
PKCg	116.97	119.84		108.28	98.20		87.34	81.40
PKCiota	103.00	102.43		88.88	100.44		104.36	100.38
PKCmu/PRKD1	98.97	87.16		104.21	110.65		95.44	106.40
PKCnu/PRKD3	75.96	74.80		89.44	88.09		88.56	90.11
PKCtheta	89.93	75.26		91.18	106.51		98.17	87.55
PKCzeta	101.20	101.67		86.11	97.22		65.86	54.64
PKD2/PRKD2	77.64	74.28		100.02	90.98		86.04	92.19
PKG1a	78.87	77.53		87.99	86.04		110.53	95.46
PKG1b	62.51	58.26		100.65	97.75		86.22	93.21
PKG2/PRKG2	101.15	105.27		102.65	98.61		88.59	93.28
PKN1/PRK1	101.25	100.27		120.24	104.35		113.76	96.93
PKN2/PRK2	90.27	96.37		89.97	72.50		99.69	100.78
PLK1	94.02	99.29		99.36	92.99		96.54	99.99
PLK2	106.41	97.94		48.79	42.49		108.29	100.73
PLK3	99.76	91.96		102.68	104.83		107.93	105.90
PRKX	104.20	107.77		106.73	108.59		97.99	85.44
PYK2	99.41	98.63		97.71	95.94		96.87	95.93
RAF1	7.29	7.25		46.61	40.79		93.89	89.52
RET	5.91	7.07		2.80	2.99		89.11	98.17
RIPK2	28.58	28.65		94.49	104.57		101.34	104.16
RIPK5	105.28	116.14		105.03	115.97		105.48	93.95
ROCK1	76.10	75.93		105.14	123.56		92.09	83.00
ROCK2	73.95	62.90		78.15	77.05		102.46	105.06
RON/MST1R	30.82	19.12		95.95	97.67		96.30	94.10
ROS/ROS1	59.72	66.33		86.75	72.98		85.34	94.65
RSK1	76.44	80.16		65.96	67.72		105.50	106.27
RSK2	99.86	85.19		93.45	89.77		103.59	94.56
RSK3	69.60	71.32		71.61	64.96		93.24	94.61
RSK4	105.45	107.39		95.18	104.36		100.18	103.77
SGK1	92.57	86.07		98.23	106.77		104.07	102.51
SGK2	110.11	105.07		94.36	94.80		87.25	96.03
SGK3/SGKL	98.74	107.18		87.92	90.68		99.33	85.59
SIK2	113.37	112.28		97.69	95.09		98.13	101.66
SLK/STK2	31.11	33.91		55.13	53.02		72.07	89.02
SNARK/NUAK2	118.93	119.89		101.16	108.61		108.56	100.47
SRMS	128.71	124.44		91.80	90.67		86.88	86.09
SRPK1	73.86	96.57		98.08	98.08		101.37	98.75
SRPK2	103.85	106.41		103.89	85.32		114.43	83.44
STK16	109.41	107.73		98.04	95.04		97.78	83.11
STK22D/TSSK1	106.63	107.45		90.22	98.30		93.81	94.65
STK25/YSK1	106.18	110.89		115.40	114.46		109.47	101.67

STK33	61.17	64.38		100.25	98.99		108.82	108.41
STK38/NDR1	94.86	100.89		80.97	78.52		94.82	98.33
STK39/STLK3	103.12	103.97		73.61	73.54		92.35	92.79
SYK	111.09	113.52		107.49	106.47		100.52	115.11
TAK1	59.83	58.93		60.94	57.61		45.44	44.05
TAOK1	56.32	57.77		99.82	94.95		91.31	101.17
TAOK2/TAO1	22.06	22.24		80.34	75.72		84.25	81.95
TAOK3/JIK	69.75	76.91		104.03	102.38		106.72	97.47
TBK1	103.73	100.17		98.03	101.26		98.79	91.39
TEC	99.25	101.80		105.63	100.71		102.21	104.77
TGFBR2	110.22	107.83		89.15	87.46		93.36	90.31
TIE2/TEK	65.91	67.03		33.78	35.02		97.73	95.80
TLK2	100.18	97.60		89.06	88.58		96.76	90.74
TRKA	11.51	11.64		25.13	28.67		29.46	25.50
TRKB	13.47	13.39		38.14	39.17		62.64	64.48
TRKC	6.30	4.80		16.57	15.93		31.56	29.58
TSSK2	98.31	105.87		86.45	83.83		92.32	85.78
TTK	77.82	84.83		102.71	82.25		111.12	96.48
TXK	104.23	109.05		101.86	110.14		101.74	110.74
TYK1/LTK	109.75	108.24		69.80	75.12		90.47	98.78
TYK2	107.72	114.93		93.05	99.20		105.29	104.10
TYRO3/SKY	59.41	61.89		97.13	94.56		102.78	90.51
ULK1	121.06	120.87		104.67	93.92		100.70	100.92
ULK2	93.07	92.71		108.08	107.33		102.87	100.20
ULK3	86.23	97.25		89.75	92.72		92.16	96.59
VRK1	97.92	97.56		103.16	106.51		95.20	99.04
WEE1	109.29	113.84		116.19	122.51		111.00	113.20
WNK2	95.91	101.40		93.20	93.10		96.25	90.63
WNK3	101.40	103.27		99.59	109.97		103.22	104.71
YES/YES1	83.84	86.46		70.06	65.97		96.29	91.86
ZAK/MLTK	57.62	61.64		82.43	83.60		99.00	96.42
ZAP70	100.37	108.58		98.86	103.78		125.04	107.28
ZIPK/DAPK3	99.20	94.35		104.01	109.90		104.39	95.28

Compounds **1** and **18** were tested against 317 kinases using a P33 radiolabled assay at Reaction Corp Biology.[20] Compounds were tested in single dose duplicate mode at 1 µM. Reactions were carried out at 10 µM ATP. Data is reported as % enzyme activity (relative to DMSO controls).

	Compound 1			**Compound 18**	
Kinase:	**Data 1**	**Data 2**		**Data 1**	**Data 2**
ABL1	100.03	81.57		91.31	84.56
ABL2/ARG	95.30	95.85		95.80	80.83
ACK1	97.30	92.73		90.05	90.14
AKT1	104.81	100.50		101.60	98.72
AKT2	101.29	109.26		97.85	99.22
AKT3	103.70	95.87		92.46	95.95
ALK	105.87	105.29		77.23	78.42
ALK1/ACVRL1	99.73	99.73		101.34	102.99

ALK2/ACVR1	108.50	90.83		101.07	95.33
ALK4/ACVR1B	103.40	92.32		96.75	91.14
ALK5/TGFBR1	94.67	97.63		95.26	96.47
ALK6/BMPR1B	88.29	104.28		92.15	100.84
ARAF	94.68	93.16		97.39	95.08
ARK5/NUAK1	100.88	102.28		94.33	91.73
ASK1/MAP3K5	102.40	98.47		103.04	100.61
Aurora A	82.84	82.22		88.19	79.41
Aurora B	8.72	10.31		87.77	90.05
Aurora C	81.25	67.04		97.90	102.84
AXL	84.54	73.70		101.23	97.54
BLK	89.33	89.29		86.27	80.58
BMPR2	83.12	79.19		91.94	98.33
BMX/ETK	103.16	101.02		83.01	78.83
BRAF	70.08	66.14		76.54	89.77
BRK	82.57	77.55		93.65	93.55
BRSK1	94.65	92.31		92.74	93.97
BRSK2	97.84	82.79		107.82	98.79
BTK	81.34	78.37		74.80	79.48
c-Kit	12.73	11.93		78.32	78.02
c-MER	86.45	88.06		95.76	100.67
c-MET	51.28	51.51		86.89	89.46
c-Src	78.04	74.26		89.33	89.45
CAMK1a	114.87	108.53		105.44	88.03
CAMK1b	101.09	103.47		98.90	94.51
CAMK1d	92.49	97.09		72.68	63.09
CAMK1g	96.48	88.27		92.05	86.08
CAMK2a	125.45	113.67		94.68	99.54
CAMK2b	101.53	96.26		103.38	89.35
CAMK2d	107.30	106.44		107.86	102.40
CAMK2g	122.89	105.66		107.32	104.49
CAMK4	92.94	82.45		85.96	87.56
CAMKK1	100.67	83.27		94.68	79.99
CAMKK2	106.28	120.41		94.10	101.68
CDK1/cyclin A	85.95	80.01		105.48	98.38
CDK1/cyclin B	83.10	80.09		99.96	91.20
CDK1/cyclin E	111.98	101.67		97.58	91.97
CDK2/cyclin A	95.68	93.65		95.41	92.24
CDK2/cyclin E	95.89	95.45		84.29	78.43
CDK3/cyclin E	105.80	93.58		97.58	90.42
CDK4/cyclin D1	105.61	100.06		98.56	97.74
CDK4/cyclin D3	78.68	85.73		96.26	100.05
CDK5/p25	93.79	90.48		64.33	59.21
CDK5/p35	100.72	92.16		87.61	91.21
CDK6/cyclin D1	102.11	99.38		96.21	92.10
CDK6/cyclin D3	83.43	95.72		78.64	87.46
CDK7/cyclin H	97.56	89.37		89.03	80.77
CDK9/cyclin K	107.69	96.50		100.39	88.95
CDK9/cyclin T1	105.38	91.23		91.20	112.02

CHK1	100.20	100.44		102.44	96.49
CHK2	104.61	98.18		101.31	96.64
CK1a1	92.43	93.37		102.36	95.20
CK1d	104.04	105.68		91.82	97.19
CK1epsilon	104.23	90.17		83.77	76.75
CK1g1	111.64	100.36		115.58	103.60
CK1g2	61.97	73.09		99.84	101.93
CK1g3	108.13	102.42		105.10	93.28
CK2a	120.94	126.57		116.45	102.88
CK2a2	102.68	107.39		107.00	100.41
CLK1	82.61	76.67		60.30	62.57
CLK2	91.98	89.02		72.96	71.69
CLK3	92.81	87.97		98.48	96.99
CLK4	90.94	85.17		81.31	71.10
COT1/MAP3K8	103.66	98.99		99.65	105.95
CSK	96.38	97.20		100.54	96.12
CTK/MATK	95.72	95.46		100.41	99.87
DAPK1	77.28	64.26		89.87	87.24
DAPK2	96.37	106.93		97.23	101.10
DCAMKL1	107.70	93.55		127.30	121.52
DCAMKL2	102.32	98.13		120.01	124.05
DDR1	0.24	-0.53		57.57	49.67
DDR2	2.13	2.25		106.72	103.69
DMPK	113.22	111.35		106.39	106.44
DRAK1/STK17A	103.55	106.19		92.09	77.34
DYRK1/DYRK1A	91.62	98.06		68.37	61.58
DYRK1B	91.55	96.03		65.69	65.11
DYRK2	118.27	112.07		92.82	95.46
DYRK3	65.85	80.82		83.63	92.50
DYRK4	98.32	96.36		91.26	89.10
EGFR	116.63	100.17		108.97	101.76
EPHA1	117.34	127.28		86.49	113.24
EPHA2	17.10	17.30		105.83	106.94
EPHA3	73.39	62.53		93.63	90.14
EPHA4	47.76	48.20		89.75	90.92
EPHA5	49.57	53.58		75.96	90.83
EPHA6	3.88	3.18		47.30	43.80
EPHA7	13.82	15.33		17.45	21.06
EPHA8	69.26	69.37		98.20	97.26
EPHB1	45.38	49.56		79.62	97.30
EPHB2	24.62	22.80		97.28	101.11
EPHB3	52.86	41.70		93.89	93.50
EPHB4	38.81	29.15		98.37	89.69
ERBB2/HER2	99.56	97.41		99.12	98.00
ERBB4/HER4	96.97	99.38		93.85	96.58
ERK1	98.75	96.75		118.02	98.38
ERK2/MAPK1	100.13	100.01		107.12	105.11
ERK5/MAPK7	105.75	104.27		108.96	99.31
FAK/PTK2	106.09	104.50		82.54	82.33

FER	106.80	93.87		94.21	87.87
FES/FPS	79.62	83.12		73.04	81.35
FGFR1	65.17	62.72		72.79	73.69
FGFR2	72.45	65.14		76.80	77.25
FGFR3	65.92	68.38		53.37	63.85
FGFR4	119.90	116.72		106.77	102.82
FGR	91.23	90.83		103.08	101.48
FLT1/VEGFR1	57.40	57.90		103.54	95.30
FLT3	5.67	5.71		32.79	30.58
FLT4/VEGFR3	53.30	55.97		76.58	82.76
FMS	15.95	14.15		102.29	108.28
FRK/PTK5	41.15	43.24		86.09	86.93
FYN	102.80	97.86		93.20	90.43
GCK/MAP4K2	98.82	75.41		6.45	7.06
GRK1	89.77	88.00		98.29	93.53
GRK2	104.57	104.12		108.40	103.77
GRK3	112.42	102.85		112.65	119.62
GRK4	100.23	99.78		103.47	102.17
GRK5	95.13	84.40		98.27	83.32
GRK6	100.21	89.02		108.87	109.66
GRK7	87.13	89.49		97.82	99.23
GSK3a	66.07	67.86		61.15	57.73
GSK3b	97.58	102.93		69.33	77.06
Haspin	99.58	104.22		57.86	60.96
HCK	104.84	96.05		95.22	93.56
HGK/MAP4K4	71.98	78.75		19.79	18.62
HIPK1	100.15	104.08		57.27	62.04
HIPK2	69.63	62.45		69.28	66.69
HIPK3	87.86	74.38		68.17	69.23
HIPK4	2.56	2.61		18.74	18.59
HPK1/MAP4K1	83.44	80.33		34.16	33.96
IGF1R	100.90	97.06		104.03	109.16
IKKa/CHUK	109.92	107.21		89.54	88.11
IKKb/IKBKB	98.82	101.41		70.53	88.00
IKKe/IKBKE	98.50	99.79		93.72	92.43
IR	88.11	85.22		76.23	74.78
IRAK1	78.50	74.52		106.86	103.86
IRAK4	95.98	104.94		137.44	116.25
IRR/INSRR	92.57	81.14		78.29	93.60
ITK	104.42	103.22		101.39	98.79
JAK1	99.07	76.72		97.11	98.27
JAK2	67.70	64.73		94.63	89.83
JAK3	105.05	107.48		98.88	98.60
JNK1	81.15	85.90		103.16	92.69
JNK2	107.58	105.25		93.35	91.60
JNK3	98.20	103.59		99.44	96.87
KDR/VEGFR2	22.39	25.64		79.02	79.19
KHS/MAP4K5	17.38	15.11		36.19	31.07
LATS2	83.97	82.80		86.24	85.22

LCK	96.25	99.16		92.86	97.09
LIMK1	132.07	101.53		100.50	96.35
LKB1	92.32	94.22		99.78	96.51
LOK/STK10	31.00	25.14		90.81	79.74
LRRK2	98.72	91.85		76.87	76.64
LYN	86.56	79.54		95.09	87.08
LYN B	90.23	75.55		93.42	79.97
MAPKAPK2	110.75	100.39		79.24	89.73
MAPKAPK3	109.38	101.13		110.64	98.08
MAPKAPK5/PRAK	109.07	100.77		116.28	102.89
MARK1	88.03	98.09		95.22	90.13
MARK2/PAR-1Ba	89.66	90.17		87.31	86.02
MARK3	116.95	114.25		71.46	76.66
MARK4	100.34	99.08		98.40	89.73
MEK1	101.90	89.45		108.96	81.58
MEK2	96.14	97.85		97.02	105.81
MEKK1	102.14	96.83		82.64	86.12
MEKK2	108.89	96.83		131.53	112.60
MEKK3	104.81	102.21		126.53	101.38
MELK	89.08	85.02		86.87	79.27
MINK/MINK1	92.64	88.76		38.60	39.59
MKK6	109.51	110.41		102.46	105.67
MLCK/MYLK	100.76	95.26		101.93	98.42
MLCK2/MYLK2	68.16	67.41		60.49	60.89
MLK1/MAP3K9	95.93	89.48		76.39	73.49
MLK2/MAP3K10	72.30	64.86		81.18	73.20
MLK3/MAP3K11	74.53	74.07		63.75	64.02
MNK1	15.98	16.23		103.22	102.23
MNK2	14.92	12.46		93.76	90.99
MRCKa/CDC42BPA	106.01	102.04		93.65	95.22
MRCKb/CDC42BPB	96.72	105.78		94.12	95.62
MSK1/RPS6KA5	102.84	109.39		109.55	93.08
MSK2/RPS6KA4	92.57	90.53		97.89	83.39
MSSK1/STK23	68.55	67.36		96.23	95.29
MST1/STK4	111.50	112.89		88.97	81.23
MST2/STK3	95.70	95.66		97.79	93.57
MST3/STK24	100.93	100.52		185.62	184.43
MST4	101.25	103.56		96.33	102.09
MUSK	93.93	86.56		84.49	81.79
MYLK3	100.27	98.41		95.19	95.60
MYO3b	105.03	101.97		71.20	68.96
NEK1	83.69	73.28		89.11	90.04
NEK11	108.92	111.14		105.77	109.40
NEK2	107.08	111.08		92.40	90.15
NEK3	81.72	85.91		93.03	92.66
NEK4	82.04	88.71		102.02	98.73
NEK6	107.57	105.57		97.04	102.59

NEK7	101.83	95.44		99.26	95.04
NEK9	96.75	91.63		107.01	102.80
NIK/MAP3K14	102.93	116.19		104.28	115.68
NLK	106.04	100.71		102.19	98.19
OSR1/OXSR1	79.16	77.89		82.22	85.13
P38a/MAPK14	56.78	68.07		95.51	100.65
P38b/MAPK11	60.26	52.39		99.07	91.91
P38d/MAPK13	113.42	114.63		81.61	81.55
P38g	78.98	75.69		30.27	29.26
p70S6K/RPS6KB1	75.72	70.75		92.50	91.52
p70S6Kb/RPS6KB2	97.99	95.56		94.18	99.05
PAK1	105.27	102.29		109.26	102.73
PAK2	94.13	97.28		98.08	82.04
PAK3	90.94	95.90		150.13	155.68
PAK4	105.86	109.70		101.67	102.82
PAK5	100.88	95.53		99.67	91.35
PAK6	98.41	93.52		100.11	85.67
PASK	104.38	91.25		76.27	84.51
PBK/TOPK	93.13	97.51		83.79	89.39
PDGFRa	20.88	20.79		85.96	83.97
PDGFRb	29.04	27.60		84.40	90.65
PDK1/PDPK1	98.02	97.41		89.47	87.69
PHKg1	92.34	79.59		87.87	80.19
PHKg2	107.17	112.60		101.50	98.54
PIM1	100.77	100.85		91.22	94.60
PIM2	67.44	63.89		101.86	113.70
PIM3	86.72	97.78		69.83	91.30
PKA	104.11	98.01		103.94	96.68
PKAcg	77.03	85.32		102.79	108.86
PKCa	109.68	77.60		93.85	102.59
PKCb1	88.64	85.95		102.45	104.05
PKCb2	98.29	101.18		100.92	95.10
PKCd	104.52	106.21		88.36	100.92
PKCepsilon	97.41	92.71		100.64	91.39
PKCeta	97.10	94.56		95.28	90.85
PKCg	94.42	88.26		85.16	80.22
PKCiota	98.84	96.39		106.27	102.91
PKCmu/PRKD1	87.66	100.85		90.96	86.84
PKCnu/PRKD3	92.59	83.66		90.75	89.09
PKCtheta	104.67	107.71		89.31	84.15
PKCzeta	100.59	109.09		100.86	104.65
PKD2/PRKD2	86.78	88.35		89.63	95.05
PKG1a	113.95	113.54		145.48	146.90
PKG1b	95.98	96.81		95.71	106.83
PKG2/PRKG2	85.38	89.44		83.83	79.80
PKN1/PRK1	91.11	106.65		98.99	91.92
PKN2/PRK2	113.09	111.68		122.31	112.52
PLK1	107.78	113.60		102.45	98.96

PLK2	72.90	82.65		96.46	96.61
PLK3	97.63	100.90		96.63	94.91
PLK4/SAK	76.88	61.00		111.62	95.80
PRKX	103.75	100.22		94.51	98.69
PYK2	95.73	96.06		88.72	88.85
RAF1	21.09	17.76		111.17	102.98
RET	3.02	2.45		79.82	55.26
RIPK2	46.11	48.39		83.86	87.03
RIPK5	99.12	97.26		104.97	101.48
ROCK1	105.60	83.38		104.28	94.17
ROCK2	64.60	62.57		107.01	102.86
RON/MST1R	60.46	58.23		83.67	83.02
ROS/ROS1	95.36	83.18		80.38	77.29
RSK1	76.49	83.12		74.91	80.15
RSK2	89.49	91.75		79.66	88.38
RSK3	93.39	82.69		98.32	99.44
RSK4	87.89	94.11		85.99	87.16
SGK1	90.25	94.03		87.78	91.76
SGK2	99.44	106.12		97.65	99.26
SGK3/SGKL	102.99	101.55		97.03	87.45
SIK1	98.49	101.75		93.33	99.05
SIK2	99.58	96.91		92.89	94.82
SLK/STK2	76.12	71.40		76.59	76.03
SNARK/NUAK2	101.25	97.94		136.12	137.23
SRMS	114.53	106.85		108.05	104.67
SRPK1	97.84	89.99		92.63	101.71
SRPK2	55.97	70.26		62.33	79.92
STK16	104.14	104.28		97.91	93.79
STK22D/TSSK1	106.73	100.29		92.27	86.22
STK25/YSK1	79.78	79.40		96.37	91.26
STK33	75.24	75.36		92.66	91.72
STK38/NDR1	104.40	95.54		103.53	99.08
STK38L/NDR2	73.85	73.76		90.33	89.11
STK39/STLK3	99.03	84.63		93.67	86.09
SYK	95.14	98.30		86.48	87.74
TAK1	124.58	112.33		30.22	26.24
TAOK1	91.47	90.74		125.48	124.42
TAOK2/TAO1	39.51	36.38		49.99	46.98
TAOK3/JIK	98.66	85.13		95.25	98.61
TBK1	101.16	106.68		100.94	111.66
TEC	83.05	92.32		107.47	90.28
TGFBR2	87.07	89.27		115.96	113.06
TIE2/TEK	66.31	57.26		104.64	99.16
TLK1	112.02	104.48		112.76	115.31
TLK2	97.94	102.98		105.85	103.68
TNK1	59.16	54.11		68.33	62.16
TRKA	13.68	12.48		3.09	4.86
TRKB	33.63	34.82		11.63	9.40
TRKC	14.47	12.78		7.85	5.51

TSSK2	85.07	84.01		83.58	88.62
TSSK3/STK22C	114.32	108.19		84.39	93.17
TTK	92.68	88.84		87.08	126.90
TXK	102.65	97.58		101.50	104.62
TYK1/LTK	84.17	87.25		94.84	89.21
TYK2	91.88	96.85		88.38	94.19
TYRO3/SKY	85.98	80.43		93.72	92.51
ULK1	104.31	109.71		116.83	111.20
ULK2	80.09	82.21		94.88	79.23
ULK3	88.67	79.77		79.26	79.65
VRK1	109.67	106.76		114.74	115.04
VRK2	79.89	105.34		81.00	79.70
WEE1	79.84	83.99		98.41	91.23
WNK1	88.17	81.23		87.49	89.62
WNK2	97.68	97.65		87.74	88.72
WNK3	100.45	98.37		103.62	100.95
YES/YES1	100.09	94.40		99.97	97.29
ZAK/MLTK	83.93	71.09		105.47	92.50
ZAP70	99.77	90.02		113.02	113.28
ZIPK/DAPK3	84.86	82.76		164.90	83.38