Supporting Information

Thiophene-Pyrazolourea Derivatives as Potent, Orally Bioavailable, and Isoform-Selective JNK3 Inhibitors

Yangbo Feng[1,*], HaJeung Park[2], Luke Bauer[3], Jae Cheon Ryu[3], Sung OK Yoon[3,*]

[1]Reaction Biology Corporation, One Great Valley Parkway, Malvern, PA 19355; [2]Crystallography Core Facility, Scripps Florida, TSRI, 130 Scripps Way, Jupiter, FL 33458; [3]Department of Biological Chemistry & Pharmacology, Ohio State University College of Medicine, Columbus, OH 43210.

[*]To whom corresponding should be addressed: yangbof@gmail.com; sung.yoon@osumc.edu.

1. Experimental Section

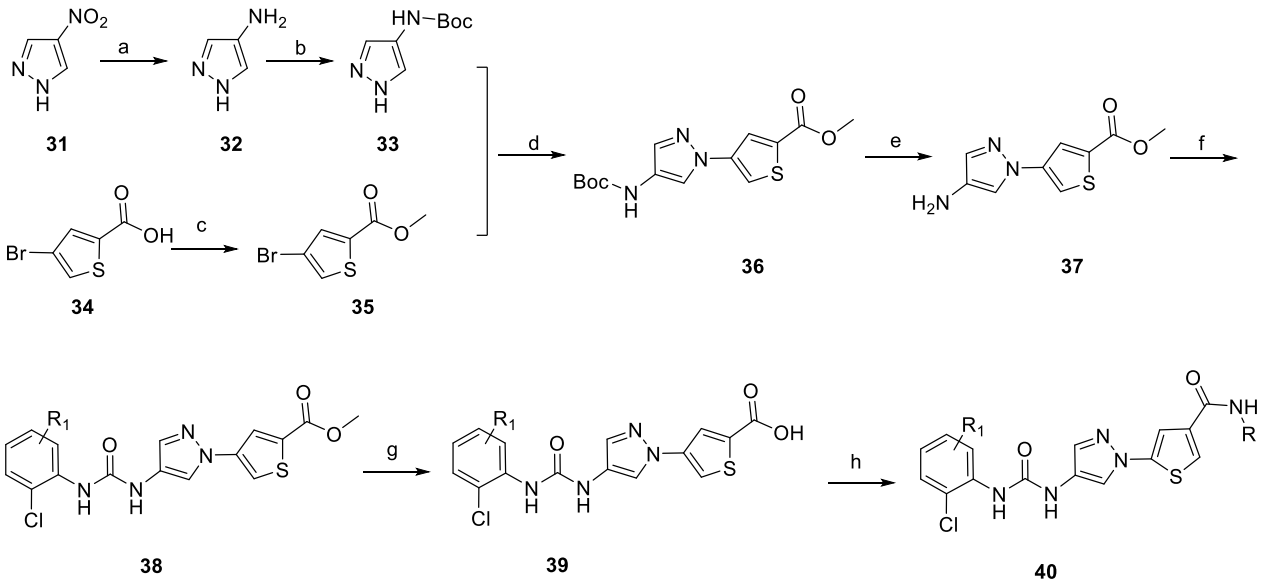
Commercially available reagents and anhydrous solvents were used without further purification unless otherwise specified. Thin layer chromatography (TLC) analyses were performed with precoated silica gel 60 F254. The mass spectra were recorded by LC/MS with Finnigan LCQ Advantage MAX spectrometer of Thermo Electron®. Flash chromatography was performed on prepacked columns of silica gel (230-400 Mesh, 40-63 μm) by CombiFlash® with EtOAc/hexane or MeOH/DCM as eluents. The preparative HPLC was performed on SunFire C_{18} OBD 10 μm (30 x 250 mm) with CH_3CN + 50% MeOH / H_2O + 0.1% TFA as eluents to purify the targeted compounds. Analytic HPLC was performed on agilent technologies 1200 series with CH_3CN (Solvent B) / H_2O + 0.9% CH_3CN + 0.1% TFA (Solvent A) as eluents and the targeted products were detected by UV in the detection range of 215-310 nm. All compounds were determined to be > 95% pure by this method. NMR spectra were recorded with a Bruker® 400 MHz spectrometer at ambient temperature with the residual solvent peaks as internal standards. The line positions of multiplets were given in ppm (δ) and the coupling constants (*J*) were given in Hertz. Calibration was performed with an external calibration mixture immediately prior to analysis.

1.1 General synthetic procedures:

The synthetic route for preparing inhibitors **3-28** is shown in Scheme 1. A mixture of 4-nitro-1*H*-pyrazole **31** (44.3 mmol), Pd/c (500 mg) in anhydrous methanol (50 mL) under a balloon of hydrogen, was stirred at room temperature

for 3h. The mixture was filtered through a celite pad and evaporated to give 4-amino-1*H*-pyrazule **32.** To a solution of 4-amino-1*H*-pyrazole **32** (36.1 mmol) in CH_2Cl_2 (20 mL) were added dropwise Boc_2O(36.1 mmol) in CH_2Cl_2 (10 mL) and TEA (108,4 mmol) at 0°C, then the mixture was stirred at room temperature for 3h.The reaction mixture was washed with HCl (1M, 20 mL × 2), water (20 mL × 2), brine (20 mL), dried over anhydrous Na_2SO_4. The result solution was concentrated to yield a crude product *tert*-butyl 1*H*-pyrazol-4-ylcarbamate **33.**

Scheme 1. Synthetic Scheme for compounds **3-28**[a]



[a]Reagent and Conditions : (a) Pd/C, MeOH, H_2; (b) Boc_2O, TEA, DCM, 0→25°C; (c) H_2SO_4, MeOH, reflux; (d) CuI, *trans*-N,N-dimethylcyclohexane-1,2-diamine, K_3PO_4, DMF, 110°C; (e) HCl/EA; (f) 2-chloroaniline derivative, 4-nitrophenyl carbonochloridate, DIPEA, DCM, 0→25°C; (g) NaOH, $MeOH/H_2O$; (h) amine, PyBoP, DIPEA, DMF, 0→25°C

To a solution of 4-bromothiophene-2-carboxylic acid **34** (24.15 mmol) in Methanol (50 mL) were added concentrated sulfuric acid (2 mL), and stirred at reflux for 3 h. The mixture was pured into water (50 mL) and extracted with EtOAc (50 mL × 3), then washed with $NaHCO_3$ (sat., 50 mL × 2), water (50 mL × 2), brine (50 mL), dried over anhydrous Na_2SO_4. The resulting solution was concentrated in vacuo to yield crude product methyl 4-bromothiophene-2-carboxylate **35**.

A mixture of *tert*-butyl 1*H*-pyrazol-4-ylcarbamate **33** (16.4 mmol), methyl 4-bromothiophene-2-carboxylate **35** (8.2 mmol), CuI (1.64 mmol), K_3PO_4 (24.6 mmol), *trans*-N,N-dimethylcyclohexane-1,2-diamine (3.28 mmol) in DMF (30 mL) was purged with nitrogen, and stirred at 110°C for overnight. The reaction mixture was cooled to room temperature, then pured into water (150 mL). The pH of mixture was adjusted to 3-4 via the addition of citric acid (sat.), then the mixture was extracted with EtOAc (100 mL × 3). The organic phases were washed with water (100 mL × 2), brine (100 mL), dried over anhydrous Na_2SO_4. The resulting solution was concentrated in vacuo and the residue was purified by flash

column chromatography on silica gel to give *methyl 4-(4-(tert-butoxycarbonylamino)-1H-pyrazol-1-yl)thiophene-2-carboxylate* **36**.

To a solution of *methyl 4-(4-(tert-butoxycarbonylamino)-1H-pyrazol-1-yl)thiophene-2-carboxylate* **36** (7.187 mmol) in HCl/EA (3 mol/L, 30mL) were stirred at room temperature for overnight. The reaction mixture was filtered to give crude product methyl 4-(4-amino-1H-pyrazol-1-yl)thiophene-2-carboxylate **37.**

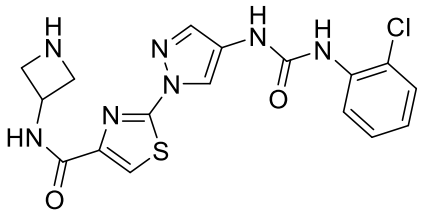
To a solution of 4-Nitrophenyl carbonochloridite (2.77 mmol) in dried CH_2Cl_2 (5 mL) was added a 2-chloroaniline derivative (3.01 mmol) in dried CH_2Cl_2 (2 mL) at room temperature and DIPEA (6.93 mmol) at 0℃,then stirred at room temperature for 1-2 h. The reaction mixture was added methyl 4-(4-amino-1H-pyrazol-1-yl)thiophene-2-carboxylate **37** (2.31 mmol) at room temperature and DIPEA (4.62 mmol) at 0℃,then stirred at room temperature for another 1-2 h. The reaction mixture was diluted with EtOAc (20 mL), washed with $NaHCO_3$ (sat., 20 mL × 3), water (20 mL × 2), brine (20 mL), dried over anhydrous Na_2SO_4. The resulting solution was concentrated in vacuo, and the residue was purified by chromatography on silica gel (dichloromethane/methanol) to give methyl 4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxylate derivative **38.**

To a solution of methyl 4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxylate **38** (0.90 mmol) in methanol (10 mL) were dropwise added NaOH in H_2O (30%, 10 mL), stirred at room temperature for overnight. The reaction mixture was washed with petroleum ether (20 mL × 2), adjusted pH with citric acid (sat.) to 4-5, then extracted with EtOAc (20 mL × 3). The organic phases were washed with water (20 mL × 2), water (20 mL × 2), brine (20 mL), dried over anhydrous Na_2SO_4. The resulting solution was concentrated in vacuo to give crude product 4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxylic acid **39**.

To a solution of 4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxylic acid **39** (0.55 mmol) in anhydrous dimethyl formamide (3 mL) were added PyBop (0.83 mmol), DIPEA (1.66 mmol), and *an amine* (0.83 mmol) at 0℃,then stirred at room temperature for overnight . The reaction mixture was pured into water (20 mL), then extracted with EtOAc (10 mL × 3). The organic phases were washed with water (10 mL × 2), brine (10 mL), dried over anhydrous Na_2SO_4. The resulting solution was concentrated in vacuo and the residue was purified by preparative HPLC to give **final products 6-28.**

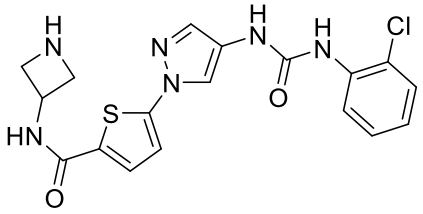
1.2 Characterization of inhibitors 3-28.

N-(azetidin-3-yl)-2-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiazole-4-carboxamide（3）.



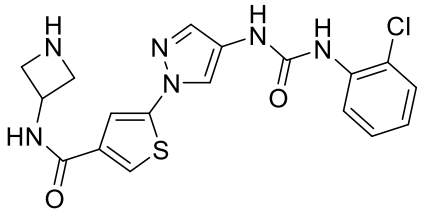
A similar scheme as described in Scheme 1 was used to prepare **3** by replacing the thiophene derivative **34** with a thiozole analog. ^{1}H-NMR (400 MHz, DMSO): δ 9.66 (s, 1 H), 9.13 (d, *J* = 18 Hz, 1 H), 8.76 (s, 1 H), 8.64 (b, 1 H), 8.49 (s, 1 H), 8.14 (d, *J* = 11 Hz, 1 H), 8.09 (s, 1 H), 7.90 (s, 1 H), 7.48 (d, *J* = 20 Hz, 1 H), 7.33 (t, *J* = 20 Hz, 1 H), 7.07 (t, *J* = 19 Hz, 1 H), 4.85 (dd, *J* = 20 Hz, 39 Hz, 1 H), 4.19 (m, 4 H). LC-MS, single peak monitoring at 254 nm. Formula: C17H16ClN7O2S, MS (ESI) m/z: [M + H] calcd: 418; found 418.

N-(azetidin-3-yl)-5-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxamide (4).



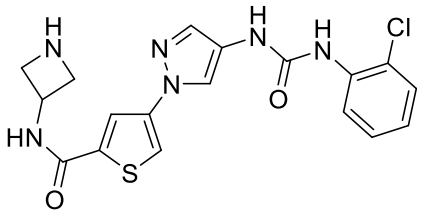
A similar scheme as described in Scheme 1 was used to prepare **4** by replacing the thiophene derivative **34** with the corresponding thiophene analog. ^{1}H-NMR (400 MHz, DMSO): δ 9.46 (s, 1H), 9.09 – 9.07 (m, 1H), 8.71 – 8.65 (m, 1H), 8.47 (s, 1H), 8.38 (s, 1H), 8.17 – 8.15 (m, 1H), 7.80 (s, 1H), 7.68 – 7.66 (m, 1H), 7.47 – 7.45 (m, 1H), 7.38 – 7.37 (m, 1H), 7.32 – 7.28 (m, 1H), 7.06 – 7.02 (m, 1H), 4.76 – 4.71 (m, 1H), 4.18 – 4.08 (m, 4H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H17ClN6O2S, MS (ESI) m/z: [M + H] calcd: 417; found 417.

N-(azetidin-3-yl)-5-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-3-carboxamide (5).



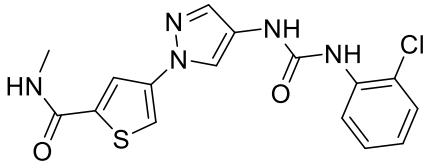
A similar scheme as described in Scheme 1 was used to prepare **5** by replacing the thiophene derivative **34** with a 2-Br thiophene analog. ^{1}H NMR (400 MHz, DMSO): LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H17ClN6O2S, MS (ESI) m/z: [M + H] calcd: 417; found 417.

N-(azetidin-3-yl)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)thiophene-2-carboxamide (6).



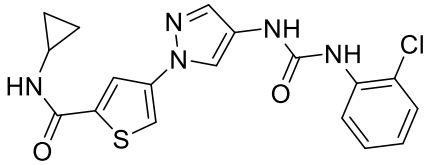
Scheme 1 was used to prepare **6**. ^{1}H-NMR (400 MHz, DMSO): (NMR recorded for the TFA salt form of **6**) δ 9.463 (s, 1 H), 9.303-9.287 (d, *J* = 6.4 Hz, 1 H), 8.719 (s, 2 H), 8.414-8.383 (d, *J* = 12.4 Hz, 2 H), 8.265 (s, 1 H), 8.187-8.166 (d, *J* = 8.4 Hz, 1 H), 7.986 (s, 1 H), 7.741 (s, 1 H), 7.471-7.451 (d, *J* = 8.0 Hz, 1 H), 7.318-7.287 (t, *J* = 16.0 Hz, 1 H), 7.053-7.014 (t, *J* = 15.6 Hz, 1 H), 4.810-4.753 (q, *J* = 7.6 Hz, 1 H), 4.204-4.157 (t, *J* = 18.8 Hz, 2 H), 4.116-4.069 (t, *J* = 18.8 Hz, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H17ClN6O2S, MS (ESI) m/z: [M + H] calcd: 417; found 417.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-methylthiophene-2-carboxamide (7).



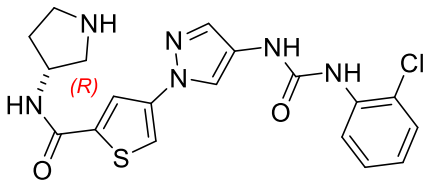
Scheme 1 was used to prepare **7**. ^{1}H-NMR (400 MHz, DMSO): δ 9.40 (s, 1 H), 8.65 (m, 1 H), 8.36 (d, *J* = 20 Hz, 2 H), 8.19 (d, *J* = 20 Hz, 2 H), 7.87 (s, 1 H), 7.72 (s, 1 H), 7.46 (dd, *J* = 20 Hz, J=3 Hz, 1 H), 7.31 (t, *J* = 18 Hz, 1 H), 7.03 (t, *J* = 18 Hz, 1 H), 2.78 (s, 3 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C16H14ClN5O2S, MS (ESI) m/z: [M + H] calcd: 376; found 376.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-cyclopropylthiophene-2-carboxamide (8).



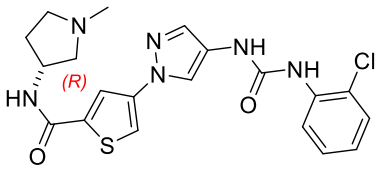
Scheme 1 was used to prepare **8**. ^{1}H-NMR (400 MHz, DMSO): δ 9.395 (s, 1 H), 8.640-8.632 (d, J = 3.2 Hz, 1 H), 8.365-8.350 (d, J = 6 Hz, 2 H), 8.197-8.174 (d, J = 9.2 Hz, 2 H), 7.870 (s, 1 H), 7.709 (s, 1 H), 7.466-7.446 (d, J = 8 Hz, 1 H), 7.315-7.276 (t, J = 8 Hz, 1 H), 7.046-7.008 (t, J = 7.6 Hz, 1 H), 2.827-2.818 (d, J = 3.6 Hz, 1 H), 0.719-0.702 (d, J = 6.8 Hz, 2 H), 0.582 (s, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H16ClN5O2S, MS (ESI) m/z: [M + H] calcd: 402; found 402.

(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(pyrrolidin-3-yl)thiophene-2-carboxamide (9).



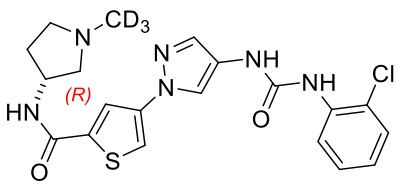
Scheme 1 was used to prepare **9**. ^{1}H-NMR (400 MHz, DMSO): δ 9.443 (s, 1 H), 8.846 (s, 2 H), 8.791-8.776 (d, J = 6 Hz, 1 H), 8.403-8.387 (d, J = 6 Hz, 2 H), 8.275 (s, 1 H), 8.181-8.160 (d, J = 8 Hz, 1 H), 7.952 (s, 1 H), 7.730 (s, 1 H), 7.468-7.449 (d, J = 8 Hz, 1 H), 7.317-7.279 (t, J = 15 Hz, 1 H), 7.051-7.014 (t, J = 15 Hz, 1 H), 4.490-4.450 (m, 1 H), 3.467-3.420 (m, 2 H), 3.202-3.161 (m, 2 H), 2.260-2.171 (m, 1 H), 2.071-1.986 (m, 1 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C19H19ClN6O2S, MS (ESI) m/z: [M + H] calcd: 431; found 431.

(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(1-methylpyrrolidin-3-yl)thiophene-2-carboxamide (10).



Scheme 1 was used to prepare **10**. (NMR recorded for the TFA salt form of **10**) ^{1}H-NMR (400 MHz, DMSO): δ 9.866 (s, 1 H), 9.433 (s, 1 H), 8.915-8.830 (m, 1 H), 8.406-8.379 (d, J = 11 Hz, 2 H), 8.280 (s, 1 H), 8.181-8.161 (d, J = 8 Hz, 1 H), 7.957 (s, 1 H), 7.732 (s, 1 H), 7.468-7.449 (d, J = 8 Hz, 1 H), 7.316-7.279 (t, J = 15 Hz, 1 H), 7.051-7.013 (t, J = 15 Hz, 1 H), 4.530 (s, 1 H), 3.916-3.608 (m, 2 H), 3.252-3.026 (m, 2 H), 2.876 (s, 3 H), 2.328-2.008 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H21ClN6O2S, MS (ESI) m/z: [M + H] calcd: 445; found 445.

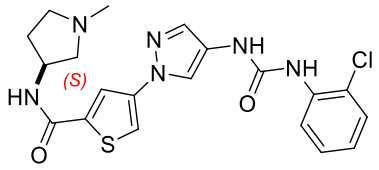
(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(1-(methyl-d3)pyrrolidin-3-yl)thiophene-2-carboxamide (11).



Scheme 1 was used to prepare **11**. (NMR recorded for the TFA salt form of **11**) ^{1}H-NMR (400 MHz, DMSO): δ 9.879 (s, 1 H), 9.442 (s, 1 H), 8.857 (s, 1 H), 8.403-8.385 (d, J = 7 Hz, 2 H), 8.277 (s, 1 H), 8.179-8.158 (d, J = 8 Hz, 1 H), 7.954 (s, 1 H), 7.731 (s, 1 H), 7.467-7.447 (d, J = 8 Hz, 1 H), 7.315-7.276 (d, J = 16 Hz, 1 H), 7.051-7.012 (d, J = 16 Hz, 1 H),

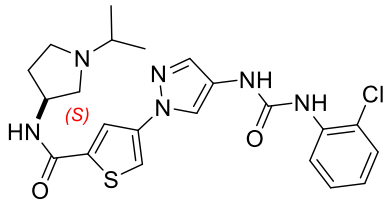
4.530 (s, 1 H), 3.919-3.598 (m, 2 H), 3.275-3.123 (m, 2 H), 2.327-2.121 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H18D3ClN6O2S, MS (ESI) m/z: [M + H] calcd: 448; found 448.

(S)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(1-methylpyrrolidin-3-yl)thiophene-2-carboxamide (12).



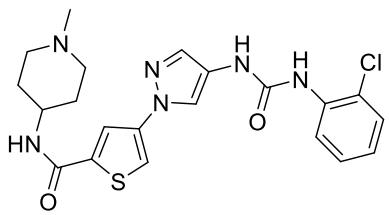
Scheme 1 was used to prepare **12**. (NMR recorded for the TFA salt form of **12**) ^{1}H-NMR (400 MHz, DMSO): δ 9.938 (s, 1 H), 9.449 (s, 1 H), 8.850 (s, 1 H), 8.402-8.385 (d, J = 7 Hz, 2 H), 8.285 (s, 1 H), 8.181-8.160 (d, J = 8 Hz, 2 H), 7.955 (s, 1 H), 7.468-7.448 (d, J = 8 Hz, 1 H), 7.316-7.277 (t, J = 16 Hz, 1 H), 7.051-7.012 (t, J = 16 Hz, 1 H), 4.538 (s, 1 H), 3.733-3.599 (m, 2 H), 3.111-3.098 (m, 2 H), 2.844 (s, 3 H), 2.327-2.052 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H21ClN6O2S, MS (ESI) m/z: [M + H] calcd: 445; found 445.

(S)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(1-isopropylpyrrolidin-3-yl)thiophene-2-carboxamide (13).



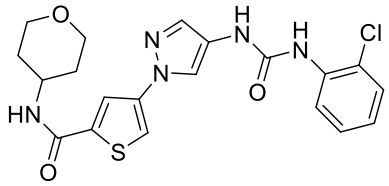
Scheme 1 was used to prepare **13**. ^{1}H-NMR (400 MHz, DMSO): (NMR recorded for the TFA salt form of **13**) δ 9.764 (s, 1 H), 9.447-9.427 (d, J = 8 Hz, 1 H), 8.904-8.793 (m, 1 H), 8.403-8.385 (d, J = 7 Hz, 2 H), 8.304-8.265 (d, J = 16 Hz, 1 H), 8.179-8.159 (d, J = 8 Hz, 1 H), 7.960 (s, 1 H), 7.732 (s, 1 H), 7.468-7.448 (d, J = 8 Hz, 1 H), 7.315-7.277 (t, J = 15 Hz, 1 H), 7.051-7.013 (m, 1 H), 4.515-4.485 (t, J = 12 Hz, 1 H), 3.844-3.830 (m, 1 H), 3.665-3.576 (m, 1 H), 3.493-3.457 (t, J = 14 Hz, 2 H), 3.231-3.070 (m, 1 H), 2.406-2.209 (m, 2 H), 1.284-1.269 (d, J = 6 Hz, 6 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C22H25ClN6O2S, MS (ESI) m/z: [M + H] calcd: 473; found 473.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(1-methylpiperidin-4-yl)thiophene-2-carboxamide (14).



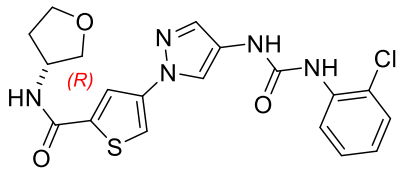
Scheme 1 was used to prepare **14**. (NMR recorded for the TFA salt form of **14**) ^{1}H-NMR (400 MHz, DMSO): δ 9.432 (s, 2 H), 8.685-8.666 (d, J = 8 Hz, 1 H), 8.385 (s, 2 H), 8.290 (s, 1 H), 8.183-8.163 (d, J = 8 Hz, 1 H), 7.911 (s, 1 H), 7.719 (s, 1 H), 7.469-7.449 (d, J = 8 Hz, 1 H), 7.315-7.276 (t, J = 16 Hz, 1 H), 7.051-7.012 (t, J = 16 Hz, 1 H), 3.991-3.975 (d, J = 6 Hz, 1 H), 3.489-3.460 (d, J = 12 Hz, 2 H), 3.112-3.086 (d, J = 10 Hz, 2 H), 2.777 (s, 3 H), 2.067-2.033 (d, J = 16 Hz, 2 H), 1.792-1.703 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C21H23ClN6O2S, MS (ESI) m/z: [M + H] calcd: 459; found 459.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(tetrahydro-2H-pyran-4-yl)thiophene-2-carboxamide (15).



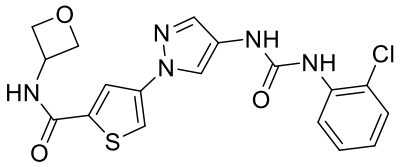
Scheme 1 was used to prepare **15**. ^{1}H-NMR (400 MHz, DMSO): δ 9.398 (s, 1 H), 8.531-8.513 (d, *J*= 7 Hz, 2 H), 8.390-8.356 (d, *J* = 14 Hz, 1 H), 8.296 (s, 1 H), 8.199-8.178 (d, *J* = 8 Hz, 1 H), 7.877 (s, 1 H), 7.716 (s, 1 H), 7.467-7.447 (d, *J* = 8 Hz, 1 H), 7.317-7.279 (t, *J* = 8 Hz, 1 H), 7.047-7.009 (t, *J* = 15 Hz, 1 H), 3.951 (s, 1 H), 3.896-3.872 (d, J = 10 Hz, 2 H), 3.413-3.384 (t, *J* = 12 Hz, 2 H), 1.792-1.766 (d, *J* = 10 Hz, 2 H), 1.606-1.526 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H20ClN5O3S, MS (ESI) m/z: [M + H] calcd: 446; found 446.

(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(tetrahydrofuran-3-yl)thiophene-2-carboxamide (16).



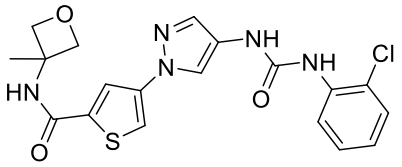
Scheme 1 was used to prepare **16**. ^{1}H-NMR (400 MHz, DMSO): δ 9.392 (s, 1 H), 8.749-8.733 (d, J = 6.4 Hz, 1 H), 8.377 (s, 1 H), 8.347-8.332 (d, J = 6.0 Hz, 2 H), 8.196-8.175 (d, J = 8.4 Hz, 1 H), 7.879 (s, 1 H), 7.714 (s, 1 H), 7.464-7.444 (d, J = 8.0 Hz, 1 H), 7.315-7.276 (t, J = 15.6 Hz, 1 H), 7.046-7.077 (t, J = 15.6 Hz, 1 H), 4.441 (s, 1 H), 3.897-3.824 (m, 2 H), 3.753-3.698 (dd, J = 13.6, 8.0 Hz, 1 H), 3.623-3.590 (dd, J = 9.6, 4.0 Hz, 1 H), 2.211-2.123 (m, 1 H), 1.954-1.894 (m, 1 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C19H18ClN5O3S, MS (ESI) m/z: [M + H] calcd: 432; found 432.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(oxetan-3-yl)thiophene-2-carboxamide (17).



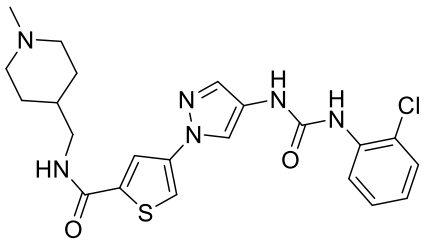
Scheme 1 was used to prepare **17**. ^{1}H-NMR (400 MHz, DMSO): δ 9.407 (s, 1 H), 9.323-9.307 (d, J = 6.4 Hz, 1 H), 8.399 (s, 1 H), 8.358-8.339 (d, J = 7.6 Hz, 2 H), 8.200-8.180 (d, J = 8.0 Hz, 1 H), 7.919 (s, 1 H), 7.731 (s, 1 H), 7.468-7.448 (d, J = 8.0 Hz, 1 H), 7.319-7.280 (t, J = 15.6 Hz, 1 H), 7.048-7.010 (t, J = 15.2 Hz, 1 H), 5.012-4.961 (q, J = 6.8 Hz, 1 H), 4.797-4.763 (t, J = 13.6 Hz, 2 H), 4.609-4.579 (t, J = 12.0 Hz, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H16ClN5O3S, MS (ESI) m/z: [M + H] calcd: 418; found 418.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(3-methyloxetan-3-yl)thiophene-2-carboxamide (18).



Scheme 1 was used to prepare **18**. ^{1}H-NMR (400 MHz, DMSO): δ 9.44 (s, 1 H), 9.10 (s, 1 H), 8.39 (s, 1 H), 8.38 (s, 1 H), 8.26 (d, J = 4.0 Hz, 1 H), 8.19 (dd, J = 4 Hz, 21 Hz, 1H), 7.91 (d, J = 3 Hz, 1 H), 7.73 (s, 1 H), 7.46 (dd, J = 4.0 Hz, 20 Hz, 1 H), 7.30 (t, J = 19 Hz, 1 H), 7.03 (t, J = 19 Hz, 1 H), 4.72 (d, J = 15 Hz, 2 H), 4.38 (t, J = 16 Hz, 2 H), 1.61 (s, 3H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C19H18ClN5O3S, MS (ESI) m/z: [M + H] calcd: 432; found 432.

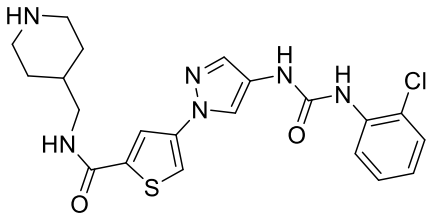
4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-((1-methylpiperidin-4-yl)methyl)thiophene-2-carboxamide (19).



Scheme 1 was used to prepare **19**. ^{1}H-NMR (400 MHz, DMSO): δ 9.449 (s, 1 H), 9.218 (s, 1 H), 8.775-8.748 (t, 1 H), 8.383 (s, 1 H), 8.252 (s, 1 H), 8.180-8.159 (d, J = 8.4 Hz, 1 H), 7.897 (s, 1 H), 7.716 (s, 1 H), 7.467-7.447 (d, J = 8.0 Hz, 1 H), 7.314-7.274 (t, J = 16.0 Hz, 1 H), 7.050-7.011 (t, J = 15.6 Hz, 1 H), 3.192-3.163 (t, J = 11.6 Hz, 2 H), 2.912-2.889 (d, J = 9.2 Hz, 2 H), 2.800-2.669 (m, J = 52.4 Hz, 3 H), 2.005-1.774 (m, J = 92.4 Hz, 4 H), 1.414-1.351 (t, J =25.6 Hz, 2

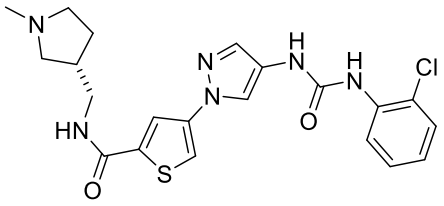
H), 0.863-0.800 (m, J = 25.2 Hz, 1 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C22H25ClN6O2S, MS (ESI) m/z: [M + H] calcd: 473; found 473.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(piperidin-4-ylmethyl)thiophene-2-carboxamide (20).



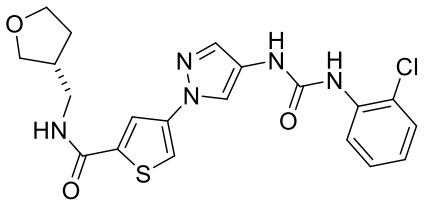
Scheme 1 was used to prepare **20**. ^{1}H-NMR (400 MHz, DMSO): δ 9.438 (s, 1 H), 8.771-8.758 (d, J = 5.2 Hz, 1 H), 8.486 (s, 1 H), 8.385-8.377 (d, J = 3.2 Hz, 2 H), 8.256 (s, 1 H), 8.185-8.165 (d, J = 8.0 Hz, 2 H), 7.898 (s, 1 H), 7.716 (s, 1 H), 7.470-7.450 (d, J = 8.0 Hz, 1 H), 7.319-7.276 (t, J = 17.2 Hz, 1 H), 7.051-7.013 (t, J = 15.2 Hz, 1 H), 3.357-3.296 (d, J = 24.4 Hz, 2 H), 3.184 (s, 1 H), 2.870-2.841 (d, J = 11.6 Hz, 2 H), 1.837-1.806 (m, J = 12.4 Hz, 3 H), 1.349-1.319 (d, J = 12.0 Hz, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C21H23ClN6O2S, MS (ESI) m/z: [M + H] calcd: 459; found 459.

(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-((1-methylpyrrolidin-3-yl)methyl)thiophene-2-carboxamide (21).



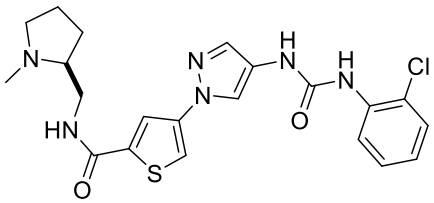
Scheme 1 was used to prepare **21**. ^{1}H-NMR (400 MHz, DMSO): δ 9.755 (s, 1 H), 9.429 (s, 1 H), 8.822 (s, 1 H), 8.387-8.373 (d, *J* = 5.6 Hz, 2 H), 8.245-8.242 (d, *J* = 1.2 Hz, 1 H), 8.185-8.161 (dd, *J* = 8.4, 1.2 Hz, 1 H), 7.917-7.914 (d, *J* = 1.2 Hz, 1 H), 7.721 (s, 1 H), 7.471-7.448 (dd, *J* = 8.0, 1.2 Hz, 2 H), 7.317-7.252 (m, 1 H), 7.054-7.012 (m, 1 H), 3.667-3.565 (d, *J* = 40.8 Hz, 2 H), 3.188-3.022 (m, 2 H), 2.839 (s, 3 H), 2.757 (s, 1 H), 2.208-1.665 (m, 2 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C21H23ClN6O2S, MS (ESI) m/z: [M + H] calcd: 459; found 459.

(R)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-((tetrahydrofuran-3-yl)methyl)thiophene-2-carboxamide (22).



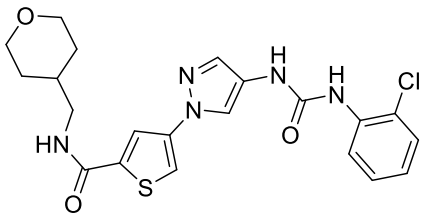
Scheme 1 was used to prepare **22**. ^{1}H-NMR (400 MHz, DMSO): δ 9.393 (s, 1 H), 8.769-8.742 (t, *J* = 10.8 Hz, 1 H), 8.383-8.351 (d, *J* = 12.8 Hz, 2 H), 8.249 (s, 1 H), 8.197-8.176 (d, *J* = 8.4 Hz, 1 H), 7.884 (s, 1 H), 7.718 (s, 1 H), 7.466-7.447 (d, *J* = 7.6 Hz, 1 H), 7.316-7.276 (t, *J* = 16.0 Hz, 1 H), 7.047-7.007 (t, *J* = 16.0 Hz, 1 H), 3.753-3.677 (m, 2 H), 3.650-3.594 (m, 2 H), 3.489-3.456 (m, 2 H), 2.023-1.912 (m, 2 H), 1.641-1.560 (m, 1 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H20ClN5O3S, MS (ESI) m/z: [M + H] calcd: 446; found 446.

(S)-4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-((1-methylpyrrolidin-2-yl)methyl)thiophene-2-carboxamide (23).



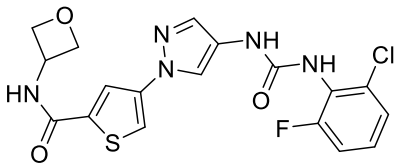
Scheme 1 was used to prepare **23**. LC-MS, single peak monitoring at 254 nm. Chemical Formula: C21H23ClN6O2S, MS (ESI) m/z: [M + H] calcd: 459; found 459.

4-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)thiophene-2-carboxamide (24).



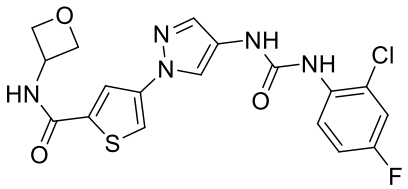
Scheme 1 was used to prepare **24**. ^{1}H-NMR (400 MHz, DMSO): δ 9.393 (s, 1 H), 8.676-8.648 (t, *J* = 11.2 Hz, 1 H), 8.375-8.349 (d, *J* = 10.4 Hz, 2 H), 8.259-8.255 (d, *J* = 1.6 Hz, 1 H), 8.197-8.173 (dd, *J* = 1.2, 8.4 Hz, 1 H), 7.868-7.865 (d, *J* = 1.2 Hz, 1 H), 7.711 (s, 1 H), 7.467-7.444 (dd, *J* = 1.2, 8.0 Hz, 1 H), 7.314-7.275 (m, *J* = 15.6 Hz, 1 H), 7.086-7.007 (m, 1 H), 3.865-3.830 (dd, *J* = 2.4, 11.2 Hz, 2 H), 3.296-3.241 (t, *J* = 22.0 Hz, 2 H), 3.162-3.131 (t, *J* = 12.4 Hz, 2 H), 2.024-1.951 (m, 2 H), 1.620-1.588 (d, *J* = 12.8 Hz, 2 H), 1.468-1.434 (m, 1 H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C21H22ClN5O3S, MS (ESI) m/z: [M + H] calcd: 460; found 460.

4-(4-(3-(2-chloro-6-fluorophenyl)ureido)-1H-pyrazol-1-yl)-N-(oxetan-3-yl)thiophene-2-carboxamide (25).



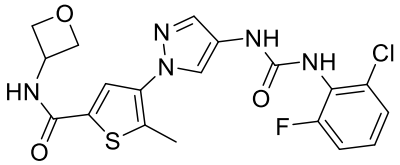
Scheme 1 was used to prepare **25**. ^{1}H-NMR (400 MHz, DMSO): δ 9.27 (d, J = 6.4 Hz, 1H), 8.91 (s, 1H), 8.31 (s, 2H), 8.26 (s, 1H), 7.86 (s, 1H), 7.68 (s, 1H), 7.33 (m, 3H), 4.97 (m, 1H), 4.77 (t, 2H), 4.58 (t, 2H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H15ClFN5O3S, MS (ESI) m/z: [M + H] calcd: 436; found 436.

4-(4-(3-(2-chloro-4-fluorophenyl)ureido)-1H-pyrazol-1-yl)-N-(oxetan-3-yl)thiophene-2-carboxamide (26).



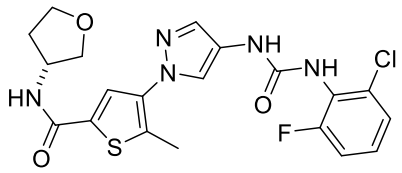
Scheme 1 was used to prepare **26**. ^{1}H-NMR (400 MHz, DMSO): δ 9.31 (s, 2H), 8.33-8.38 (t, 3H), 8.10-8.14 (m, 1H), 7.91 (s, 1H), 7.73 (s, 1H), 7.46-7.49 (m, 1H), 7.19-7.24 (m, 1H), 4.96-5.00 (m, 1H), 4.77-4.80 (t, 2H), 4.58-4.61 (t, 2H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C18H15ClFN5O3S, MS (ESI) m/z: [M + H] calcd: 436; found 436.

4-(4-(3-(2-chloro-6-fluorophenyl)ureido)-1H-pyrazol-1-yl)-5-methyl-N-(oxetan-3-yl)thiophene-2-carboxamide (27).



Scheme 1 was used to prepare **27**. ^{1}H-NMR (400 MHz, DMSO): δ 9.15 (d, J = 6.8 Hz, 1H), 8.90 (s, 1H), 8.24 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 7.71 (s, 1H), 7.26-7.40 (m, 3H), 4.92-4.99 (m, 1H), 4.76 (t, J = 7.0 Hz, 2H), 4.56 (t, J = 6.4 Hz, 2H), 2.52 (s, 3H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C19H17ClFN5O3S, MS (ESI) m/z: [M + H] calcd: 450; found 450.

(R)-4-(4-(3-(2-chloro-6-fluorophenyl)ureido)-1H-pyrazol-1-yl)-5-methyl-N-(tetrahydrofuran-3-yl)thiophene-2-carboxamide (28).



Scheme 1 was used to prepare **28**. ^{1}H-NMR (400 MHz, DMSO): δ 8.90 (s, 1H), 8.59 (d, J = 6.4 Hz, 1H), 8.24 (s, 1H), 8.09 (s, 1H), 8.02 (s, 1H), 7.70 (s, 1H), 7.26-7.39 (m, 3H), 4.37-4.44 (m, 1H), 3.80-3.87 (m, 2H), 3.68-3.73 (m, 1H), 3.55-3.59 (m, 1H), 2.51 (s, 3H), 2.10-2.19 (m, 1H), 1.84-1.91 (m, 1H). LC-MS, single peak monitoring at 254 nm. Chemical Formula: C20H19ClFN5O3S, MS (ESI) m/z: [M + H] calcd: 464; found 464.

2.1 JNK-1/2/3 and p38 assays

Homogeneous Time-resolved Fluorescence Assay-Enzyme inhibition studies were performed in 384-well polystyrene homogeneous time-resolved fluorescence plates (Grainier) for 1hr. at ambient temperature (~22 °C) with 0.5 μM biotinylated FL-ATF-2, 1.25 μM ATP, 0.75 nM activated JNK3α1 (with a control in the absence of kinase for determining the basal signal), JNK2, or JNK1 in 10-μl volumes containing the final concentrations of the following: 50 mM Hepes, pH 7.0, 2.5 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 1 mM DL-dithiothreitol, 0.01% Triton X-100 (all from Sigma-Aldrich), and 5% DMSO (with or without compound). A 10-point titration of all compounds was carried out in 3-fold dilutions from 10 μM to 0.5 nM. After 22 min, the kinase reaction was terminated by addition of 10 μl of quenching solution (1X Lance Buffer, detection reagents, streptavidin-xlAPC (200 nM) and europium cryptate-labeled rabbit polyclonal anti-phospho-ATF-2 (1 nM), were from Cis-Bio. The homogeneous time-resolved fluorescence signal was detected using an EnVision plate reader 1 hr post-quenching. The data from four different experiments were averaged and presented as the mean ± S.D. IC_{50} values were determined by fitting the data to the equation for a four-parameter logistic.

2.2 JNK3 inhibition assays in rat primary neuron cultures: Both hippocampal and cortical neurons were isolated from E18 rat embryos and cultured in NbActive 1 medium that contains B27 (Brainbits, LLC) plus penicillin/streptomycin (Life Technologies). At the 7th day in culture, cells were treated with different concentrations of inhibitors diluted in DMSO and 80 ng/ml Anisomycin for 30 min to activate the endogenous JNK3. At the end of incubation time, whole cell lysates were prepared in RIPA buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.5% deoxycholate supplemented with 10 mM Na pyrophosphate, 1 mM sodium orthovanadate, 25 mM NaF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM β-glycerophosphate, and 2 mM PMSF. The lysates were subjected to Western blotting to detect whether inhibitors block JNK3-dependent phosphorylation of APP at T668 and c-jun at S73. Phospho-APP^{T668}, phosphor-c-jun^{S73}, APP, and c-jun antibodies were from Cell Signaling Technology.

2.3 MTT cytotoxicity assay: SHSY5Y cells were cultured in DMEM/F12 (Life Technologies) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. A day before the assay, cells were plated at 50,000/well in 96 well plates in the culture medium. Next day, compounds were added at 10 μM final concentration, and cells were incubated for 48 hrs at 37°C. After the incubation, cells were subjected to MTT assay using Vybrant MTT Cell Proliferation kit as directed by the vendor (Life Technologies).

2.4 Kinase panel profiling studies: The wild-type kinase panel of Reaction Biology Corporation (www.reactionbiology.com) (374 kinases which does not including those lipophilic kinases) was used to perform the profiling studies. An inhibitor concentration of ≥100-fold of its JNK3 IC_{50} value was generally used in the profiling. Detailed profiling data for inhibitors **17**, **25** and **27** are shown in the Table below:

	% Enzyme Activity (relative to DMSO controls)					
	Compound 17 (at 5 uM)		Compound 25 (at 5 uM)		Compound 27 (at 10 uM)	
Kinase:	Data 1	Data 2	Data 1	Data 2	Data 1	Data 2
ABL1	83.38	82.39	100.85	99.61	101.96	101.15
ABL2/ARG	92.71	92.27	88.74	84.21	122.68	122.22
ACK1	85.64	85.44	94.57	85.87	106.16	98.53
AKT1	95.44	94.02	105.05	102.94	104.78	101.26
AKT2	89.50	88.19	104.39	103.87	89.76	88.94
AKT3	87.30	84.52	97.63	96.98	94.84	94.19
ALK	101.15	99.78	89.19	84.18	100.18	99.92
ALK1/ACVRL1	107.21	103.64	120.21	119.05	78.71	78.63
ALK2/ACVR1	104.72	104.38	95.68	95.05	107.63	104.44
ALK3/BMPR1A	105.42	104.59	102.62	99.98	92.49	84.86
ALK4/ACVR1B	123.55	112.23	82.02	80.08	78.52	78.37
ALK5/TGFBR1	102.96	101.31	97.42	97.31	104.00	103.56
ALK6/BMPR1B	108.84	107.90	114.21	111.40	103.88	100.94
ARAF	95.08	91.90	94.43	92.64	84.06	83.25
ARK5/NUAK1	97.91	97.71	95.21	94.11	96.93	96.10
ASK1/MAP3K5	91.18	87.11	91.07	89.42	94.02	89.70
Aurora A	105.35	105.00	83.13	82.07	88.44	85.37
Aurora B	93.04	91.41	69.79	68.59	96.38	94.10
Aurora C	104.47	99.98	58.49	58.15	104.19	101.47
AXL	96.72	93.82	73.03	71.80	86.60	85.74
BLK	79.19	77.85	96.11	95.53	111.64	110.69
BMPR2	109.83	99.57	77.18	74.94	84.46	78.97
BMX/ETK	106.00	99.36	96.84	94.77	92.91	91.90
BRAF	93.33	86.38	99.94	97.46	104.84	100.71
BRK	118.11	100.93	85.73	85.37	78.89	77.07
BRSK1	85.45	84.52	99.37	99.31	87.80	84.97
BRSK2	101.84	100.62	100.00	98.75	101.58	99.81
BTK	92.58	91.63	89.33	89.05	87.63	86.96
c-Kit	89.25	88.34	94.05	93.89	64.36	63.84

c-MER	92.67	92.36	77.10	76.05	102.90	100.99
c-MET	101.60	100.21	86.10	84.17	76.98	74.48
c-Src	91.06	85.52	98.44	97.60	96.57	96.02
CAMK1a	87.10	85.37	97.98	97.46	114.97	113.30
CAMK1b	96.26	92.06	102.48	101.77	80.14	74.09
CAMK1d	103.67	101.19	99.58	98.89	104.84	104.05
CAMK1g	94.21	94.01	93.95	91.73	93.14	92.52
CAMK2a	100.41	99.62	99.44	98.52	99.68	98.98
CAMK2b	104.90	103.78	99.09	95.67	100.05	99.77
CAMK2d	102.59	102.33	93.47	92.93	91.34	90.36
CAMK2g	96.39	94.54	90.14	89.86	102.19	99.23
CAMK4	114.38	103.67	98.67	97.56	98.49	96.54
CAMKK1	130.50	128.91	93.74	93.63	121.11	116.36
CAMKK2	91.14	84.08	80.99	78.68	111.01	109.73
CDC7/DBF4	110.97	108.83	84.27	81.54	106.89	99.54
CDK1/cyclin A	95.46	94.64	86.35	85.97	105.30	103.17
CDK1/cyclin B	92.57	91.19	105.47	105.10	92.73	92.47
CDK1/cyclin E	94.72	86.44	98.36	95.05	100.09	97.13
CDK14/cyclin Y (PFTK1)	106.61	105.71	110.11	103.59	100.37	95.26
CDK16/cyclin Y (PCTAIRE)	94.81	93.16	101.27	100.38	101.76	93.30
CDK17/cyclin Y (PCTK2)	106.10	105.77	96.03	94.30	102.18	101.79
CDK18/cyclin Y (PCTK3)	101.21	98.85	91.93	89.49	118.84	117.76
CDK19/cyclin C	104.67	104.15	96.17	93.31	98.09	96.75
CDK2/cyclin A	93.61	92.31	93.81	91.26	98.34	98.06
CDK2/Cyclin A1	105.66	100.91	94.17	91.11	110.76	110.18
CDK2/cyclin E	98.99	97.70	94.59	93.28	106.26	103.08
CDK2/cyclin E2	106.94	101.91	98.88	96.32	90.79	89.27
CDK2/cyclin O	93.24	91.32	94.07	93.54	105.25	105.15
CDK3/cyclin E	106.51	103.23	92.16	90.92	91.20	90.95
CDK3/cyclin E2	95.81	95.18	109.45	102.15	102.60	99.40
CDK4/cyclin D1	92.21	90.56	90.27	90.16	78.01	72.89
CDK4/cyclin D3	98.21	96.07	79.41	78.31	96.53	94.52
CDK5/P25	101.10	101.02	88.15	87.76	97.64	97.00
CDK5/p35	95.38	94.35	94.35	94.17	102.73	101.68
CDK6/cyclin D1	95.43	95.10	105.31	101.02	101.25	95.95
CDK6/cyclin D3	113.72	108.98	92.62	92.38	95.20	93.51
CDK7/cyclin H	99.70	96.56	95.80	94.87	102.77	101.79
CDK8/cyclin C	101.20	100.48	99.05	98.79	92.65	91.81
CDK9/cyclin K	100.31	92.42	120.29	119.71	85.36	85.02
CDK9/cyclin T1	80.17	78.30	96.07	95.78	107.15	106.08
CDK9/cyclin T2	91.08	87.96	92.98	90.35	101.18	97.27
CHK1	98.73	97.38	141.35	138.23	117.85	112.53
CHK2	97.83	94.70	91.47	89.85	95.15	93.80
CK1a1	98.47	93.32	76.62	74.42	81.09	80.23
CK1a1L	109.15	106.14	81.14	80.89	104.98	104.70
CK1d	100.73	100.16	116.74	111.80	111.63	106.74
CK1epsilon	89.59	88.97	88.62	87.18	97.93	97.52
CK1g1	99.02	95.46	102.18	101.70	100.79	97.70
CK1g2	98.47	94.22	90.24	89.00	103.84	99.09

CK1g3	103.19	100.86	92.58	91.24	91.28	90.51
CK2a	95.35	71.10	90.53	87.51	96.99	96.34
CK2a2	97.32	94.89	96.58	95.95	95.49	93.35
CLK1	80.96	80.50	74.91	74.16	98.36	96.78
CLK2	103.48	103.14	112.33	108.85	104.91	99.95
CLK3	120.16	117.28	93.46	90.44	99.57	96.35
CLK4	66.05	63.23	93.23	92.90	101.58	101.37
COT1/MAP3K8	92.74	92.01	93.58	87.64	104.85	104.84
CSK	99.19	98.31	97.48	96.48	97.97	94.91
CTK/MATK	99.69	97.17	100.13	99.12	97.76	97.28
DAPK1	104.08	98.58	98.93	97.01	104.44	101.73
DAPK2	102.82	101.59	101.91	97.72	99.54	97.33
DCAMKL1	99.25	94.99	84.20	83.29	67.21	66.10
DCAMKL2	99.89	97.52	94.31	91.97	106.96	105.46
DDR1	98.38	95.87	88.88	84.28	83.65	83.36
DDR2	99.83	98.92	96.59	94.12	110.69	104.24
DLK/MAP3K12	96.45	92.39	82.16	81.42	nd	nd
DMPK	101.81	101.43	110.50	110.17	95.21	94.17
DMPK2	86.96	85.52	118.65	115.14	117.81	100.83
DRAK1/STK17A	98.39	98.34	89.43	89.42	111.19	107.90
DYRK1/DYRK1A	85.97	85.45	75.09	74.37	95.47	94.26
DYRK1B	87.00	86.59	82.78	82.19	105.31	104.26
DYRK2	88.23	87.06	93.61	91.95	96.34	95.63
DYRK3	93.30	92.61	99.24	99.09	101.28	99.17
DYRK4	94.62	91.60	96.15	93.54	93.69	87.29
EGFR	99.67	98.74	89.44	85.58	98.46	95.17
EPHA1	99.09	98.05	88.36	87.48	88.49	86.42
EPHA2	101.18	95.39	97.87	97.57	101.94	100.00
EPHA3	103.87	102.79	96.14	95.44	109.05	104.71
EPHA4	100.36	98.18	89.09	87.82	116.16	113.09
EPHA5	96.92	93.87	95.00	92.04	105.51	103.31
EPHA6	99.53	99.03	95.69	95.27	80.19	78.80
EPHA7	102.97	100.96	91.24	89.79	98.64	95.88
EPHA8	102.53	101.47	93.83	92.71	91.74	90.38
EPHB1	100.48	99.40	96.08	92.94	100.29	98.70
EPHB2	101.07	99.31	99.86	98.31	109.04	107.17
EPHB3	95.57	84.83	96.46	95.92	97.24	95.82
EPHB4	97.85	97.02	98.09	94.70	93.73	93.67
ERBB2/HER2	102.80	100.91	97.00	95.53	64.09	63.27
ERBB4/HER4	105.02	103.78	101.85	100.85	95.04	94.58
ERK1	92.80	90.11	92.37	92.07	107.04	106.53
ERK2/MAPK1	109.74	104.15	85.88	83.94	90.75	85.53
ERK5/MAPK7	118.72	110.13	75.94	71.15	95.68	95.01
ERK7/MAPK15	140.43	122.73	107.57	104.76	103.00	100.48
ERN1/IRE1	94.29	93.93	95.84	94.61	94.09	93.26
ERN2/IRE2	100.11	100.06	100.33	98.47	80.71	67.84
FAK/PTK2	95.65	95.10	88.95	87.53	104.00	103.33
FER	101.03	100.17	89.79	89.16	89.98	89.54
FES/FPS	99.35	96.09	87.84	83.88	93.73	93.20
FGFR1	98.53	95.93	93.20	91.44	114.41	111.37
FGFR2	95.60	94.71	103.03	102.55	105.57	105.21
FGFR3	97.78	84.94	92.01	90.95	137.06	136.20

FGFR4	88.21	88.01	99.57	98.51	96.76	94.67
FGR	90.00	89.22	95.53	95.52	80.17	70.96
FLT1/VEGFR1	101.29	96.75	86.06	82.27	84.13	82.63
FLT3	89.66	88.39	99.15	98.71	69.13	68.41
FLT4/VEGFR3	93.81	93.50	86.58	86.16	81.92	79.20
FMS	105.23	104.80	98.87	97.36	103.26	100.68
FRK/PTK5	97.37	96.66	95.63	95.58	87.35	86.33
FYN	97.83	91.46	90.28	88.53	99.85	97.10
GCK/MAP4K2	104.91	101.55	95.33	90.96	90.19	88.53
GLK/MAP4K3	97.50	96.25	100.27	99.15	117.79	117.10
GRK1	91.88	91.85	92.91	92.72	112.87	108.66
GRK2	96.08	96.04	106.16	104.29	106.27	106.21
GRK3	102.23	102.06	101.19	100.13	106.77	104.98
GRK4	97.03	93.20	93.61	93.46	112.87	107.95
GRK5	97.97	97.86	101.75	100.70	112.82	110.81
GRK6	97.93	97.75	102.38	97.97	91.45	89.66
GRK7	96.38	94.88	94.04	92.57	99.89	98.35
GSK3a	97.42	97.03	93.53	93.48	128.22	128.04
GSK3b	94.97	94.45	95.66	94.78	94.86	94.56
Haspin	111.79	109.87	110.88	108.04	92.41	92.33
HCK	94.03	89.72	91.33	90.95	95.04	92.55
HGK/MAP4K4	81.09	80.14	90.41	81.60	93.42	92.65
HIPK1	96.26	92.79	91.66	91.33	115.54	114.53
HIPK2	101.63	100.28	92.56	92.50	102.68	98.63
HIPK3	97.62	96.89	98.77	96.59	88.47	84.89
HIPK4	100.37	97.20	110.76	103.92	98.39	97.39
HPK1/MAP4K1	132.29	127.20	92.66	89.08	99.22	95.40
IGF1R	95.83	94.87	94.86	94.40	101.94	101.66
IKKa/CHUK	167.10	144.34	107.75	105.68	100.80	93.70
IKKb/IKBKB	95.58	95.22	94.67	94.13	86.76	82.06
IKKe/IKBKE	110.26	108.28	96.80	95.05	115.84	114.63
IR	98.37	95.58	101.73	97.94	85.46	84.44
IRAK1	112.23	107.37	107.05	105.55	79.94	78.52
IRAK4	92.12	88.09	97.83	97.00	103.45	102.26
IRR/INSRR	95.98	93.76	75.90	70.29	71.87	70.84
ITK	106.70	99.31	92.77	92.29	94.41	91.41
JAK1	98.67	94.40	107.40	103.94	72.22	71.47
JAK2	98.19	95.41	96.46	88.12	95.04	94.99
JAK3	102.01	100.23	98.66	96.18	96.73	95.21
JNK1	**57.60**	**55.85**	**49.13**	**47.91**	76.30	74.25
JNK2	**34.12**	**33.90**	**22.70**	**21.84**	**51.87**	**50.09**
JNK3	**7.81**	**6.08**	**8.85**	**8.76**	**10.47**	**9.63**
KDR/VEGFR2	95.19	94.57	88.29	87.45	88.22	87.45
KHS/MAP4K5	99.13	94.61	102.78	102.05	98.39	96.58
KSR1	100.82	100.22	104.82	100.19	102.95	102.74
KSR2	106.91	104.96	92.66	91.89	99.22	94.21
LATS1	105.98	105.37	93.53	92.23	97.22	96.57
LATS2	95.97	92.68	103.57	101.69	94.69	94.42
LCK	99.10	91.26	94.20	92.42	46.53	44.32
LCK2/ICK	112.41	110.26	100.84	96.59	100.39	100.15
LIMK1	96.16	96.01	104.47	98.61	104.69	104.17
LIMK2	96.76	96.21	93.98	93.07	112.92	98.20

LKB1	118.26	115.31	94.65	94.10	97.61	96.97
LOK/STK10	87.86	87.02	88.98	87.45	96.39	95.76
LRRK2	88.23	87.94	91.27	90.24	101.06	100.86
LYN	96.75	94.36	95.30	93.32	105.59	103.36
LYN B	94.68	93.09	87.31	82.84	88.78	86.83
MAK	98.82	98.63	97.56	96.43	119.88	110.01
MAPKAPK2	97.08	95.77	99.85	92.89	93.57	89.94
MAPKAPK3	83.54	77.32	95.64	92.60	109.92	108.41
MAPKAPK5/PRAK	92.82	91.04	99.73	96.33	96.47	95.33
MARK1	96.35	93.65	108.08	107.68	95.34	95.19
MARK2/PAR-1Ba	104.31	104.12	90.71	89.37	99.82	97.94
MARK3	98.23	97.73	99.34	98.43	101.51	100.16
MARK4	107.82	101.29	97.91	97.87	108.32	107.61
MAST3	nd	nd	108.27	104.14	96.70	95.18
MASTL	96.74	96.59	93.69	93.53	110.07	108.24
MEK1	103.88	101.93	107.16	106.60	95.52	92.97
MEK2	92.15	91.56	90.56	87.91	99.54	97.50
MEK3	103.66	103.65	110.84	98.78	88.77	81.98
MEK5	98.20	98.01	103.04	99.33	96.85	89.58
MEKK1	103.43	103.38	98.70	98.46	94.54	91.66
MEKK2	110.44	103.97	80.13	78.60	88.07	88.06
MEKK3	112.66	105.89	81.92	80.44	113.33	108.54
MEKK6	98.85	94.33	92.50	90.69	79.33	77.68
MELK	48.70	48.18	61.40	59.61	102.99	100.16
MINK/MINK1	79.36	78.64	62.22	57.78	102.17	101.98
MKK4	113.08	111.42	133.47	128.10	93.03	93.02
MKK6	96.44	95.10	94.41	84.96	86.38	85.38
MKK7	112.18	109.33	135.29	134.99	119.77	115.05
MLCK/MYLK	97.51	94.38	95.87	92.85	102.60	102.42
MLCK2/MYLK2	89.90	89.22	83.54	79.44	78.65	78.49
MLK1/MAP3K9	95.06	94.25	88.47	88.03	87.52	87.12
MLK2/MAP3K10	98.47	98.09	94.31	92.29	90.80	88.47
MLK3/MAP3K11	93.45	92.56	84.69	83.36	90.67	86.28
MLK4	93.15	92.52	94.32	94.11	85.24	83.89
MNK1	94.27	94.24	103.28	103.05	94.15	93.25
MNK2	99.35	96.75	90.79	90.66	83.39	79.46
MRCKa/CDC42BPA	99.34	96.17	98.97	97.51	104.07	101.90
MRCKb/CDC42BPB	97.10	95.66	89.02	84.42	111.73	111.02
MSK1/RPS6KA5	89.48	87.65	104.50	101.01	111.30	103.83
MSK2/RPS6KA4	90.68	90.63	99.85	99.35	104.68	104.36
MSSK1/STK23	91.94	90.93	89.79	85.70	98.87	92.65
MST1/STK4	99.29	94.51	96.62	92.82	107.06	105.79
MST2/STK3	119.51	116.20	93.18	93.01	93.94	92.76
MST3/STK24	113.14	110.47	81.61	81.18	67.27	63.25
MST4	98.26	94.12	71.40	69.65	72.41	71.35
MUSK	94.45	91.22	86.81	86.23	103.41	102.42
MYLK3	95.09	90.49	101.81	92.13	111.63	109.69
MYLK4	85.24	83.36	91.73	90.80	108.71	108.51
MYO3A	94.53	91.46	82.83	75.76	91.09	88.15
MYO3b	128.21	125.72	100.86	95.64	78.67	73.50
NEK1	115.21	111.73	77.17	68.70	90.41	89.34
NEK11	144.68	136.40	89.55	88.71	70.00	63.48

NEK2	104.16	102.20	96.24	93.32	88.02	84.41
NEK3	102.58	98.96	87.25	82.26	87.45	86.55
NEK4	108.95	106.86	90.82	89.09	75.55	75.28
NEK5	110.33	107.26	88.09	87.92	105.32	102.25
NEK6	102.77	99.56	87.15	86.75	93.27	92.66
NEK7	101.79	101.22	88.11	87.60	100.04	95.94
NEK8	107.28	106.90	nd	nd	nd	nd
NEK9	104.35	104.34	88.36	85.75	111.28	110.53
NIM1	101.39	101.29	70.72	69.78	107.17	107.12
NLK	99.47	96.41	87.41	87.33	85.15	83.86
OSR1/OXSR1	92.58	92.43	96.51	91.54	126.78	118.37
P38a/MAPK14	141.98	140.87	104.85	103.81	93.16	85.97
P38b/MAPK11	100.86	98.03	91.71	88.84	94.33	88.02
P38d/MAPK13	122.26	119.83	98.42	91.95	89.20	85.65
P38g	99.37	94.52	98.27	97.49	90.47	80.85
p70S6K/RPS6KB1	92.11	91.11	97.74	97.56	92.12	91.12
p70S6Kb/RPS6KB2	94.17	94.02	101.38	99.90	98.93	97.40
PAK1	106.01	103.66	81.20	76.08	103.67	98.15
PAK2	90.11	86.23	95.31	92.21	105.80	103.94
PAK3	97.39	96.80	64.23	62.40	113.75	108.53
PAK4	98.87	92.17	106.73	104.75	106.39	103.46
PAK5	92.12	90.70	92.86	92.78	103.47	103.05
PAK6	130.58	127.00	92.92	88.33	99.92	99.07
PASK	96.31	94.19	99.72	93.30	96.14	92.92
PBK/TOPK	114.88	111.94	98.16	95.69	88.48	86.39
PDGFRa	95.25	91.67	106.99	105.80	114.34	101.61
PDGFRb	107.18	98.53	100.99	92.40	97.93	88.31
PDK1/PDPK1	100.87	94.26	99.58	99.08	99.18	95.19
PEAK1	91.36	90.97	nd	nd	nd	nd
PHKg1	101.60	100.36	91.24	90.46	83.95	83.21
PHKg2	100.57	98.24	131.01	127.31	88.50	80.45
PIM1	64.61	64.36	73.33	72.49	95.09	93.11
PIM2	96.37	96.02	128.59	126.93	96.51	95.53
PIM3	61.24	60.47	73.91	73.07	128.82	126.62
PKA	99.81	96.77	107.72	102.18	94.54	92.47
PKAcb	98.97	97.74	98.29	86.39	104.28	104.11
PKAcg	104.45	101.81	97.57	97.05	99.03	97.39
PKCa	101.32	100.41	90.65	90.27	95.91	92.54
PKCb1	101.55	100.64	100.22	98.69	110.62	104.62
PKCb2	102.73	102.48	98.95	98.60	98.35	96.76
PKCd	112.31	107.07	101.26	100.19	98.01	95.12
PKCepsilon	101.98	98.35	94.25	92.54	101.17	99.42
PKCeta	102.59	100.01	99.05	97.40	91.56	84.37
PKCg	97.98	97.24	98.35	95.74	101.69	100.79
PKCiota	95.54	94.16	87.37	81.40	90.83	90.48
PKCmu/PRKD1	113.86	105.64	90.68	88.67	93.35	93.19
PKCnu/PRKD3	104.21	100.90	104.98	102.96	104.52	102.17
PKCtheta	113.89	100.22	94.74	92.16	96.88	96.03
PKCzeta	105.39	101.79	93.62	92.76	103.63	103.55
PKD2/PRKD2	94.93	92.84	102.17	98.13	95.68	95.36
PKG1a	110.14	107.44	93.23	93.09	120.40	109.05
PKG1b	87.55	86.19	99.99	98.34	87.31	84.74

PKG2/PRKG2	100.92	97.41	100.85	99.42	100.48	96.50
PKMYT1	86.96	86.80	89.61	89.25	nd	nd
PKN1/PRK1	109.51	108.34	96.50	94.16	76.85	73.93
PKN2/PRK2	111.95	109.14	105.38	104.85	87.07	86.46
PKN3/PRK3	92.46	91.58	76.19	74.34	98.61	97.90
PLK1	97.01	94.28	95.55	92.77	103.87	102.40
PLK2	86.05	85.33	100.13	99.51	95.21	93.24
PLK3	96.08	93.64	96.04	95.88	101.94	100.40
PLK4/SAK	88.18	87.95	84.29	83.82	100.36	99.38
PRKX	103.95	102.07	91.49	88.11	87.49	79.11
PYK2	93.53	92.71	91.04	89.71	103.87	101.80
RAF1	86.96	82.02	92.48	88.10	92.60	85.53
RET	97.80	95.48	98.13	98.08	113.39	110.76
RIPK2	120.34	113.72	80.24	75.68	89.29	87.55
RIPK3	100.98	98.09	97.55	92.71	nd	nd
RIPK4	89.19	77.00	96.69	92.28	102.82	98.45
RIPK5	102.02	101.13	95.51	94.86	96.59	95.70
ROCK1	108.96	108.68	78.18	75.70	96.81	96.41
ROCK2	100.38	99.37	107.03	102.48	93.57	91.72
RON/MST1R	108.15	94.20	99.98	96.90	96.89	88.05
ROS/ROS1	102.99	101.59	85.55	85.00	102.30	102.23
RSK1	92.36	91.37	96.32	95.98	93.18	90.97
RSK2	100.61	95.11	79.87	78.52	73.04	71.41
RSK3	102.78	102.31	90.06	89.38	100.27	94.06
RSK4	107.17	102.38	92.19	91.27	115.69	115.36
SBK1	99.08	98.84	97.70	95.71	87.45	80.58
SGK1	93.72	92.96	95.38	91.71	115.37	114.27
SGK2	101.25	100.07	88.57	88.54	97.24	94.05
SGK3/SGKL	93.77	86.54	95.34	86.29	90.11	89.10
SIK1	102.09	100.71	98.34	96.55	99.79	95.43
SIK2	97.01	96.84	95.77	95.38	89.82	88.74
SIK3	92.41	91.64	94.50	93.35	86.93	81.60
SLK/STK2	89.71	87.93	94.99	94.62	99.70	99.11
SNARK/NUAK2	99.78	97.60	97.80	95.90	111.83	111.42
SNRK	96.83	90.60	98.76	94.52	102.32	100.60
SRMS	99.78	99.25	104.82	102.21	103.66	102.62
SRPK1	98.71	94.40	103.79	99.01	96.92	96.82
SRPK2	95.64	94.96	94.00	93.75	113.80	111.56
SSTK/TSSK6	103.45	102.43	102.90	92.58	94.01	87.61
STK16	98.61	94.14	106.54	100.13	94.36	94.26
STK21/CIT	90.14	87.64	nd	nd	98.81	97.63
STK22D/TSSK1	101.70	97.48	106.56	101.81	102.88	102.11
STK25/YSK1	106.06	105.80	96.11	94.22	70.55	69.19
STK32B/YANK2	122.29	122.14	102.97	101.16	89.81	81.71
STK32C/YANK3	113.00	105.00	90.70	85.47	112.12	111.17
STK33	99.34	95.39	81.81	80.29	87.62	87.51
STK38/NDR1	99.16	98.55	95.53	95.29	91.50	88.33
STK38L/NDR2	102.63	100.37	94.75	93.00	95.14	93.75
STK39/STLK3	87.51	83.27	82.30	74.78	64.55	62.58
SYK	103.79	100.71	90.34	86.91	88.53	85.46
TAK1	98.00	97.75	109.47	109.41	98.42	97.70
TAOK1	122.31	121.33	90.64	89.61	109.94	108.33

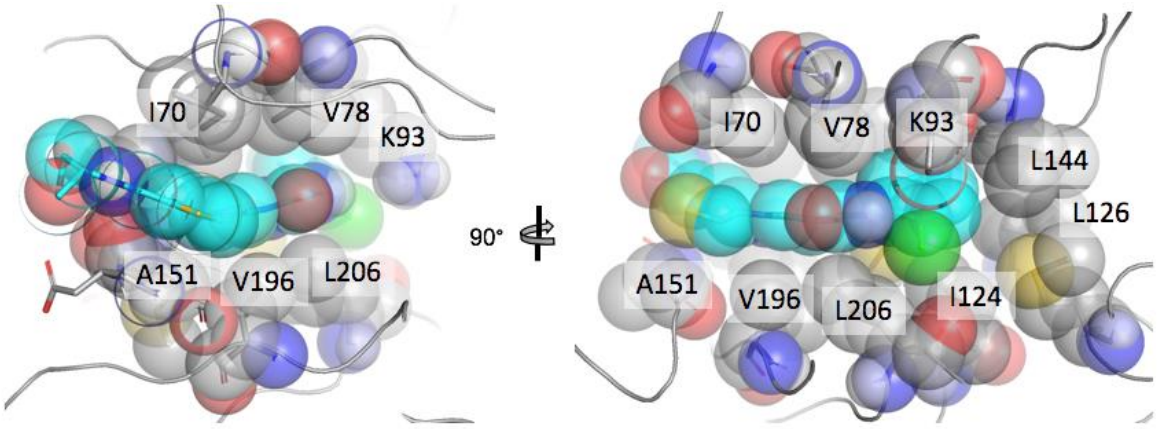
TAOK2/TAO1	101.21	101.17	88.17	84.66	93.26	88.63
TAOK3/JIK	103.32	102.95	90.15	88.00	97.96	94.36
TBK1	102.03	99.45	98.79	98.48	97.79	95.84
TEC	97.16	95.04	97.65	97.28	91.08	90.91
TESK1	100.67	100.49	91.00	84.69	88.70	87.96
TESK2	93.34	91.78	80.12	78.64	99.22	97.37
TGFBR2	113.33	104.08	82.58	82.14	86.85	86.82
TIE2/TEK	100.42	97.21	105.44	104.23	86.64	85.92
TLK1	104.55	102.03	53.36	48.17	105.12	102.81
TLK2	99.08	98.73	109.31	106.34	97.16	94.14
TNIK	53.25	51.88	84.33	83.10	94.47	93.91
TNK1	100.42	98.27	91.54	90.74	105.21	102.24
TRKA	99.96	95.41	87.82	87.48	80.99	76.75
TRKB	84.15	83.82	91.45	88.25	92.37	82.95
TRKC	64.31	64.04	38.61	36.52	85.97	85.08
TSSK2	91.55	90.03	95.14	95.11	84.68	84.23
TSSK3/STK22C	97.71	97.63	101.08	96.70	100.00	99.06
TTBK1	97.25	92.57	82.32	80.80	113.33	101.61
TTBK2	106.96	105.69	103.68	102.18	97.61	92.08
TXK	89.75	87.45	90.81	90.15	76.68	73.77
TYK1/LTK	99.18	94.47	92.19	91.35	114.12	109.99
TYK2	95.87	91.08	89.26	88.73	114.21	109.10
TYRO3/SKY	75.40	75.37	68.40	65.60	98.80	96.81
ULK1	133.53	131.26	93.93	91.13	77.26	70.77
ULK2	196.10	194.58	105.22	90.15	74.92	69.83
ULK3	111.12	103.36	76.58	74.98	105.03	99.50
VRK1	109.03	98.12	91.87	91.08	108.94	104.53
VRK2	103.33	102.70	102.23	101.89	101.73	100.17
WEE1	124.04	121.51	85.22	72.39	97.27	91.19
WNK1	99.70	97.40	93.27	92.88	88.89	87.30
WNK2	96.35	95.59	91.94	88.67	91.10	90.51
WNK3	99.55	96.88	91.80	87.30	102.15	100.53
YES/YES1	96.53	91.46	91.00	90.52	81.42	81.19
YSK4/MAP3K19	105.00	104.03	126.35	97.32	95.47	92.17
ZAK/MLTK	100.61	98.53	90.44	88.52	89.88	85.41
ZAP70	101.56	98.40	103.97	103.52	103.24	98.81
ZIPK/DAPK3	100.30	95.77	107.76	100.23	100.51	99.46

3.1 Crystallization: Purification of JNK3 39-402 and its crystallization with ATP was done following previously published procedure.[2-5] The JNK3 inhibitor was soaked into JNK3-ATP crystals by adding 1 mM of the compound into the crystallization drop and incubating for 24 hrs. The crystals were then transferred to mounting loops and excess drop solutions were removed and flash frozen by plunging into liquid nitrogen.

3.2 Data collection and refinement: Diffraction datasets were collected on Rayonix MX300 at the APS beamline 21-ID-F. The datasets were processed with HKL2000 (HKL Research, Inc) and were phased using Phaser (Phenix suite)[6] with PDB ID 1JNK as the serarch model for molecular replacements. Initial phased map showed positive density for the target compounds in the ATP pocket. Restraints and coordintates for the compounds were generated using eLBOW

(Phenix suite)[6] and incorporated into JNK3 coordinate. The models were refined using BUSTER (Global Phasing Ltd.) or phenix.refine (Phenix suite)[6]. The models were manually inspected and adjusted after each refinement cycle using Coot[7]. Data processing and refinement statistics are given in Table S1. Structural analysis and figure preparations were done with PyMol (Schrodinger, Inc.). The final coordinates were deposited with PDB IDs 7KSK, 7KSJ and 7KSI.

3.3 The Co-crystal structure of compound 17 in human JNK3: Transparent sphere and wire model of residues involved in hydrophobic pocket formation. The residues, I70 and A151 sandwich the thiopene ring. V78, A91(not visible), and V196 VdW contact with pyrazole ring. The residues K93, I124, L126 and L144 wraps around Cl-phenyl ring. **17** is colored blue and the protein residues are colored grey.



3.4 The Co-crystal structures of compound 17 (cyan) and 25 (orange) in human JNK3: The stick models of residues involved in H-bond/Halogen bond interactions. The hinge residue M149 make two H-bonds. K93 and D150 are involved in water-mediated H-bonds. Appearance of the water mediated H-bonds in both structures indicate they are stable H-bonds. K93 also makes halogen bond with Cl of Cl-phenyl.

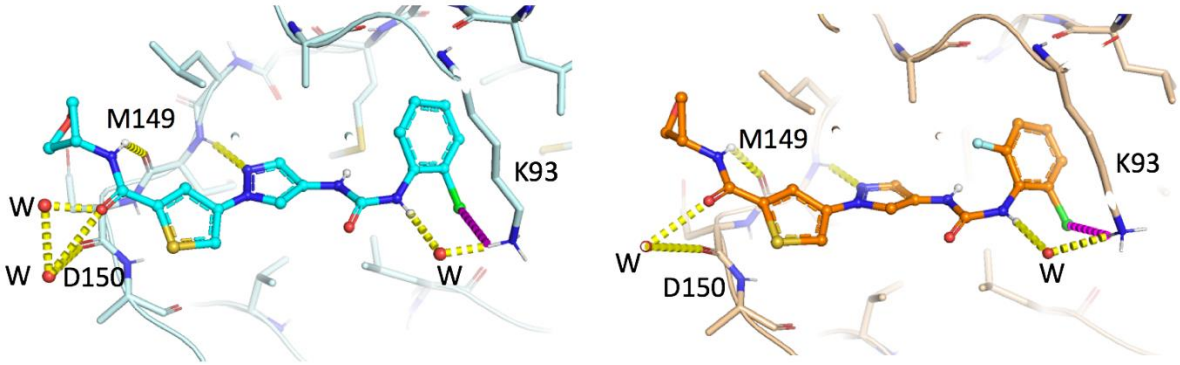


Table S1. Data processing and refinement statistics

Protein	JNK3:17 (7KSK)	JNK3:25 (7KSJ)	JNK3:27 (7KSI)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Cell constants a, b, c, α, β, γ	52.14Å, 71.22Å, 107.60Å, 90.00°, 90.00°, 90.00°	51.45Å, 71.24Å, 107.80Å, 90.00°, 90.00°, 90.00°	52.13Å, 71.09Å, 107.40Å, 90.00°, 90.00°, 90.00°
Resolution (Å)	53.80 – 1.84	41.71 – 2.06	53.70 – 1.73
% Data completeness (in resolution range)	86.9 (53.80-1.84)	77.6 (41.71-2.06)	62.1 (53.70-1.73)
R_{merge} (in resolution range)	0.042 (53.80-1.84)	0.063 (41.71-2.06)	0.051(53.70-1.73)
Total unique reflections (in resolution range)	31286 (53.80-1.84)	23626 (41.71-2.06)	28032 (53.70-1.73)
$< I/\sigma(I) >$ [1]	2.65 (at 1.83Å)	3.42 (at 2.06Å)	1.52 (at 1.73Å)
Refinement program	PHENIX dev_3965	BUSTER 2.10.3	BUSTER 2.10.3
R, R_{free}	0.247, 0.290	0.230, 0.292	0.220, 0.255
R_{free} test set	1557 reflections (5.00%)	978 reflections (5.00%)	1328 reflections (5.01%)
Wilson B-factor ($Å^2$)	27.2	21.6	23.3
Anisotropy	0.832	1.003	0.084
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.34, 37.5	0.27, 47.3	0.35, 39.0
L-test for twinning[2]	<|L|>=0.49, $<L^2>$=0.32	<|L|>=0.44, $<L^2>$=0.27	<|L|>=0.48, $<L^2>$=0.31
Estimated twinning fraction	No twinning to report.	No twinning to report.	No twinning to report.
F_o, F_c correlation	0.94	0.89	0.93
Total number of atoms	5657	3018	5959
Average B, all atoms ($Å^2$)	50.0	45.0	33.0

[1]Intensities estimated from amplitudes.
[2]Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets

4. Pharmacology experiment

4.1 Pharmacokinetics

Pharmacokinetics studies were conducted in C57Bl/6J mice. The compound was formulated in a generic formulation at 1 mg/mL (e.g. 10:40:4:46, DMSO:PEG 400: tween 80: PBS, v:v:v:v) or other specified formulations and dosed at 1 mg/kg intravenous into the femoral vein or 1-10 mg/kg by oral gavage, or 5-20 mg ip. Blood was obtained at t=5 min, 15 min, 30 min, 1hr, 2hr, 4hr, 6hr, and 8hr. Blood was collected by heart puncture into EDTA containing tubes and plasma was generated by standard centrifugation methods. Brains were collected following perfusion with ice-cold saline, and snap frozen in liquid N2. Both plasma and brains were stored at -80°C until processing. All procedures and handling were according to standard operating procedures approved by IAPUC at Scripps Florida and/or Ohio State University. In order to assess in vivo pharmacokinetic parameters an LC-MS/MS bioanalytical method was developed where 25 Tl of plasma was treated with 125 Tl of acetonitrile containing an internal standard in a Millipore Multscreen Slovinter 0.45 micron low binding PTFE hydrophilic filter plate (#MSRLN0450) and allowed to shake at room temperature for five minute. The plate was then centrifuged for 5 minutes at 4000 rpm in a tabletop centrifuge and the filtrate was collected in a polypro-pylene capture plate. The filtrate (10 Tl) is injected using an Agilent 1200 HPLC equipped with a Thermo Betasil C18 HPLC column 5 T (50×2.1 mm) #70105-052130. Mobile Phase A was water with 0.1% formic acid. Mobile phase B was acetonitrile with 0.1% formic acid. Flow rate was 375 Tl/min using a gradient of 90%A/10% B from, 0-0.5 min, ramped to 5%A/95%B at 2 min, held at 5%A/95%B until 3.0 min, ramped to 90% A/10% B at 4 min, and held at 90%A/10%B until

7 min. An API Sciex 4000 equipped with a turbo ion spray source was used for all analytical measurement. MRM methods were developed in position ion mode. Peak areas of the analyte ion were measured against the peak areas of the internal standard. Data was fit using WinNonLin using an IV bolus model.[8-13]

4.2 P450s inhibition

P450s inhibition for four major isoforms are evaluated using a cocktail inhibition assay where the metabolism of specific marker substrate (CYP1A2 phenaceten demethylation to acetaminophen); CYP2C9, tolbutamide hydroxylation to hydrocytolbutamide; CYP2D6, bufuralol hydroxylation to 4'-Hydroxybufuralol; and CYP3A4, midazolam hydroxylation to 1'-Hydroxymidazolam) in the presence or absence of 10 TM probe compound is evaluated. The concentration of each marker substrate is approximately its Km. Conditions were similar to those described by Tesino and Patonay[14] except 2C19 was not evaluated as we found that stock solution of the 2C19 probe substrate, omeprazole, had poor stability. Specific inhibitors for each isoform are included in each run to validate the system.

References:

1. Yoon, S. O.; Park, D. J.; Ryu, J. C.; Ozer, H. G.; Tep, C.; Shin, Y. J.; Lim, T. H.; Pastorino, L.; Kunwar, A. J.; Walton, J. C.; Nagahara, A. H.; Lu, K. P.; Nelson, R. J.; Tuszynski, M. H.; Huang, K. JNK3 perpetuates metabolic stress induced by Abeta peptides. *Neuron* **2012,** 75, 824-37.
2. Kamenecka, T.; Habel, J.; Duckett, D.; Chen, W. M.; Ling, Y. Y.; Frackowiak, B.; Jiang, R.; Shin, Y. S.; Song, X. Y.; LoGrasso, P. Structure-Activity Relationships and X-ray Structures Describing the Selectivity of Aminopyrazole Inhibitors for c-Jun N-terminal Kinase 3 (JNK3) over p38. *J. Biol. Chem.* **2009,** 284, 12853-12861.
3. Kamenecka, T.; Jiang, R.; Song, X. Y.; Duckett, D.; Chen, W. M.; Ling, Y. Y.; Habel, J.; Laughlin, J. D.; Chambers, J.; Figuera-Losada, M.; Cameron, M. D.; Lin, L.; Ruiz, C. H.; LoGrasso, P. V. Synthesis, Biological Evaluation, X-ray Structure, and Pharmacokinetics of Aminopyrimidine c-jun-N-terminal Kinase (JNK) Inhibitors. *Journal of Medicinal Chemistry* **2010,** 53, 419-431.
4. Chambers, J. W.; Howard, S.; LoGrasso, P. V. Blocking c-Jun N-terminal kinase (JNK) translocation to the mitochondria prevents 6-hydroxydopamine-induced toxicity in vitro and in vivo. *J. Biol. Chem.* **2013,** 288, 1079-1088.
5. Chambers, J. W.; Pachori, A.; Howard, S.; Iqbal, S.; LoGrasso, P. V. Inhibition of JNK mitochondrial localization and signaling is protective against ischemia/reperfusion injury in rats. *J. Biol. Chem.* **2013,** 288, 4000-11.
6. Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **2010,** 66, 213-21.
7. Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **2004,** 60, 2126-32.
8. Feng, Y.; Yin, Y.; Weiser, A.; Griffin, E.; Cameron, M. D.; Lin, L.; Ruiz, C.; Schurer, S. C.; Inoue, T.; Rao, P. V.; Schroter, T.; Lograsso, P. Discovery of substituted 4-(pyrazol-4-yl)-phenylbenzodioxane-2-carboxamides as potent and highly selective Rho kinase (ROCK-II) inhibitors. *J Med Chem* **2008,** 51, 6642-5.
9. Sessions, E. H.; Yin, Y.; Bannister, T. D.; Weiser, A.; Griffin, E.; Pocas, J.; Cameron, M. D.; Ruiz, C.; Lin, L.; Schurer, S. C.; Schroter, T.; LoGrasso, P.; Feng, Y. Benzimidazole- and benzoxazole-based inhibitors of Rho kinase. *Bioorg. Med. Chem. Lett.* **2008,** 18, 6390-6393.

10. Chowdhury, S.; Sessions, E. H.; Pocas, J. R.; Grant, W.; Schroter, T.; Lin, L.; Ruiz, C.; Cameron, M. D.; Schurer, S.; LoGrasso, P.; Bannister, T. D.; Feng, Y. Discovery and optimization of indoles and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-I). *Bioorg. Med. Chem. Lett.* **2011,** 21, 7107-7112.
11. Fang, X.; Chen, Y. T.; Sessions, E. H.; Chowdhury, S.; Vojkovsky, T.; Yin, Y.; Pocas, J. R.; Grant, W.; Schroter, T.; Lin, L.; Ruiz, C.; Cameron, M. D.; LoGrasso, P.; Bannister, T. D.; Feng, Y. Synthesis and biological evaluation of 4-quinazolinones as Rho kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2011,** 21, 1844-1848.
12. Sessions, E. H.; Chowdhury, S.; Yin, Y.; Pocas, J. R.; Grant, W.; Schroter, T.; Lin, L.; Ruiz, C.; Cameron, M. D.; LoGrasso, P.; Bannister, T. D.; Feng, Y. Discovery and optimization of indole and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-II). *Bioorg. Med. Chem. Lett.* **2011,** 21, 7113-7118.
13. Yin, Y.; Lin, L.; Ruiz, C.; Khan, S.; Cameron, M. D.; Grant, W.; Pocas, J.; Eid, N.; Park, H.; Schroter, T.; Lograsso, P. V.; Feng, Y. Synthesis and biological evaluation of urea derivatives as highly potent and selective rho kinase inhibitors. *J. Med. Chem.* **2013,** 56, 3568-3581.
14. Testino, S. A., Jr.; Patonay, G. High-throughput inhibition screening of major human cytochrome P450 enzymes using an in vitro cocktail and liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* **2003,** 30, 1459-67.