Supporting Information

Identification of small-molecule inhibitors of human inositol hexakisphosphate kinases by high throughput screening

Gangling Liao[ζ‡], Wenjuan Ye[#‡], Tyler Heitmann[ζ], Glen Ernst[ζ], Michael DePasquale[ζ], Laiyi Xu[€], Michael Wormald[ζ¥], Xin Hu[#], Marc Ferrer[#], Robert K. Harmel[£!], Dorothea Fiedler[£!], James Barrow[ζ] and Huijun Wei[ζ†*]

Authors affiliations:

National Center for Advancing Translational Sciences, Rockville, MD, USA

ζ Lieber Institute for Brain Development, Baltimore, MD 21205, USA

€ Department of Chemistry, McGill University, Montreal, Quebec H3A 0G4, Canada,

£ Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin, Germany

! Institut für Chemie, Humboldt-Universität zu Berlin , Brook-Taylor-Straße 2 , 12489 Berlin , Germany.

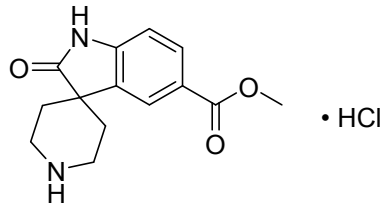
*Corresponding Author: Department of Neurology, pharmacology and Johns Hopkins Drug Discovery, Johns Hopkins School of Medicine, Baltimore, Maryland. Email: Hwei11@jhmi.edu

Table of Contents

General Procedures.

All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on an ISCO CombiFlash Rf™ or Biotage Isolera™ using Biotage, ISCO or Agela Flash cartridges with peak detection at 254 nm. Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a Phenomenex C18 column (150 x 20 mm I.D., S-5 µm). Peak collection was triggered by UV detection at 214 or 254 nm. ^{1}H NMR spectra were recorded on a Bruker 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used as a reference. Analytical LC-MS was performed using Agilent 1260 equipped with autosampler (Agilent Poroshell 120 C18 column (50 x 3.0 mm I.D., 2.7 µm); 0.05% TFA in water/acetonitrile gradient; UV detection at 215 and 254 nm) and electrospray ionization. All final compounds showed purity greater than 95% at 215 and 254 nm using this method. High-resolution mass spectral data was acquired from m/z 50 – 400 using an Agilent 6540 QTOF with a Jet Stream Ion Source in 4 GHz high resolution mode. Test articles were prepared in 0.1% formic acid in methanol and infused at 0.1 mL min-1. The data was analyzed using Agilent Masshunter Qualitative Analysis Software (B.07.00 SP2)

Compound Synthesis and Characterization Data

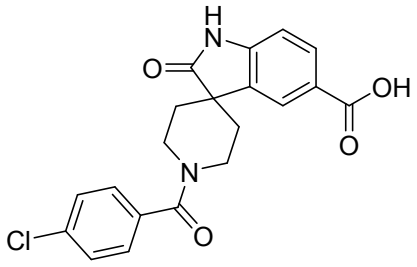


Intermediate A: Methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride

To a suspension of 1'-*tert*-butoxycarbonyl-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (766 mg, 2.21 mmol) in methanol (10 mL) at 0 °C was added thionyl chloride (1.9 mL, 26 mmol). The resulting mixture was allowed to reach room temperature with stirring for 18 h. The solvent was removed in vacuo to give methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (600 mg, 2.02 mmol, 91.4% yield) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.03 (s, 1 H) 8.87 (br. s., 2 H) 7.91 (dd, *J* = 8.08, 1.77 Hz, 1 H) 7.80 (d, *J* = 1.52 Hz, 1 H) 7.01 (d, *J* = 8.34 Hz, 1 H) 3.84 (s, 3 H) 3.44 - 3.54 (m, 2 H) 3.29 (d, *J* = 13.14 Hz, 2 H) 2.11 - 2.21 (m, 2 H) 1.93 (d, *J* = 14.40 Hz, 2 H)

LCMS: [M+1] = 261, rt = 1.40 min.



Example LI-2172: 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

To a suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (52 mg, 0.175 mmol), 4-chlorobenzoic acid (30.2 mg, 0.193 mmol) and HBTU (73.1 mg, 0.193 mmol) in chloroform (5 mL) was added DIPEA (93.8 µL, 0.526 mmol). the resulting mixture was stirred at room temperature for 2.5 h. The solvent was removed with a stream of N_2 and the residue triturated with water/MeOH (3:1) for 1 h. The solid was collected by filtration, washed with water and dried to give a solid which was purified by automated normal-phase chromatography (0-100% EtOAc/heptane, 4 g silica gel cartridge) to give methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (52.4 mg, 0.131 mmol, 74.9% yield) as a white gummy solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.86 (s, 1 H) 8.07 (d, *J* = 1.52 Hz, 1 H) 7.88 (dd, *J* = 8.21, 1.64 Hz, 1 H) 7.56 - 7.60 (m, 2 H) 7.51 - 7.55 (m, 2 H) 6.97 (d, *J* = 8.08 Hz, 1 H) 4.20 (br. s., 1 H) 3.83 (s, 3 H) 3.76 (d, *J* = 9.85 Hz, 2 H) 3.50 (br. s., 1 H) 1.94 - 2.04 (m, 2 H) 1.80 (br. s., 1 H) 1.66 (br. s., 1 H)

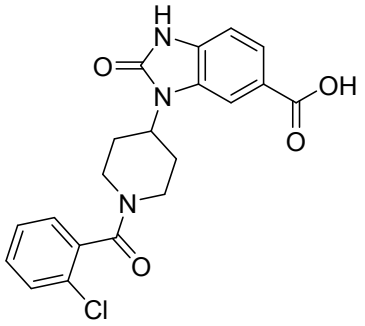
LCMS: [M+1] = 399/401, rt = 2.34 min.

1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

A suspension of methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (52.4 mg, 0.131 mmol) and lithium hydroxide monohydrate (5.5 mg, 0.131 mmol) in THF (4 mL) and water (0.4 mL) was stirred at room temperature for 21 h, then at 40 °C for 21 h. The solvent was removed under a stream of N_2 to give a residue which was taken up in water then filtered through a syringe filter. The solution was acidified by addition of 6 N HCl and stirred for 1 h. The solid was collected by filtration, washed with water and air-dried to give a residue which was purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (4.7 mg, 0.012 mmol, 9.3% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.72 (br. s., 1 H) 10.82 (s, 1 H) 8.05 (s, 1 H) 7.86 (d, *J* = 8.08 Hz, 1 H) 7.55 - 7.59 (m, 2 H) 7.51 - 7.55 (m, 2 H) 6.94 (d, *J* = 8.08 Hz, 1 H) 4.17 (br. s., 1 H) 3.78 (t, *J* = 10.61 Hz, 2 H) 3.50 (br. s., 1 H) 1.90 - 2.01 (m, 2 H) 1.80 (br. s., 1 H) 1.67 (br. s., 1 H)

LCMS: [M+1] = 385/387, rt = 2.04 min.



Example LI-2240: 3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylic acid

***tert*-butyl 4-((5-(methoxycarbonyl)-2-nitrophenyl)amino)piperidine-1-carboxylate**

A mixture of methyl 3-fluoro-4-nitrobenzoate (302 mg, 1.52 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate (364 mg, 1.82 mmol) and cesium carbonate (593 mg, 1.82 mmol) in acetonitrile (10 mL) was stirred at room temperature for 90 h. The contents were taken up in EtOAc, washed with water (3x), brine (1x), dried over $MgSO_4$, filtered and the solvent removed in vacuo to give a residue which was purified by automated normal-phase chromatography (0-100% EtOAc/heptane, 20 g silica gel cartridge) to give *tert*-butyl 4-((5-(methoxycarbonyl)-2-nitrophenyl)amino)piperidine-1-carboxylate (440 mg, 1.16 mmol, 76.5% yield) as a yellow-orange solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.19 (d, *J* = 8.84 Hz, 1 H) 7.90 (d, *J* = 8.08 Hz, 1 H) 7.60 (d, *J* = 1.52 Hz, 1 H) 7.17 (dd, *J* = 8.84, 1.77 Hz, 1 H) 3.84 - 3.98 (m, 6 H) 3.02 (br. s., 2 H) 1.94 (d, *J* = 10.36 Hz, 2 H) 1.44 - 1.55 (m, 2 H) 1.42 (s, 9 H)

LCMS: [M-1+23] = 402, rt = 1.90 min (lipophilic method).

tert-butyl 4-((2-amino-5-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate

To a solution of *tert*-butyl 4-((5-(methoxycarbonyl)-2-nitrophenyl)amino)piperidine-1-carboxylate (440 mg, 1.16 mmol) in EtOAc (20 mL) was added 10% Pd/C (40 mg). The resulting suspension was placed under an atmosphere of H_2 via a balloon and stirred at room temperature for 18 h. The contents were filtered through Celite and the solvent removed in vacuo to give *tert*-butyl 4-((2-amino-5-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate (400 mg, 1.145 mmol, 98.7% yield) as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.14 (dd, *J* = 8.08, 1.77 Hz, 1 H) 7.04 (d, *J* = 1.77 Hz, 1 H) 6.55 (d, *J* = 8.08 Hz, 1 H) 5.42 - 5.48 (m, 2 H) 4.41 (d, *J* = 7.58 Hz, 1 H) 3.89 (d, *J* = 10.11 Hz, 2 H) 3.72 (s, 3 H) 3.39 - 3.50 (m, 1 H) 2.94 (br. s., 2 H) 1.91 (d, *J* = 10.11 Hz, 2 H) 1.41 (s, 9 H) 1.21 - 1.33

LCMS: [M-1+23] = 350, rt = 1.14 min (lipophilic method).

Methyl 3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate

To a solution of *tert*-butyl 4-((2-amino-5-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate (247 mg, 0.708 mmol) in chloroform (5 mL) was added 1,1'-carbonyldiimidazole (172 mg, 1.06 mmol). The resulting mixture was stirred at 50 °C for 18 h. Added 1,1'-carbonyldiimidazole (86 mg, 0.53 mmol) and stirred at 50 °C for 18 h. The solvent was removed under a stream of N_2, and the residue was purified by automated normal-phase chromatography (0-100% EtOAc/heptane, 4 g silica gel cartridge) to give methyl 3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (220 mg, 0.586 mmol, 82.8% yield) as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.36 (s, 1 H) 7.72 (d, *J* = 1.26 Hz, 1 H) 7.69 (dd, *J* = 8.08, 1.52 Hz, 1 H) 7.09 (d, *J* = 8.08 Hz, 1 H) 4.39 - 4.49 (m, 1 H) 4.01 - 4.14 (m, 2 H) 3.83 (s, 3 H) 2.91 (br. s., 2 H) 2.11 - 2.24 (m, 2 H) 1.71 (d, *J* = 10.11 Hz, 2 H) 1.45 (s, 9 H)

LCMS: [M-1]+23 = 398, rt = 2.44 min

Methyl 2-oxo-3-(piperidin-4-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate hydrochloride

To a suspension of methyl 3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (220 mg, 0.586 mmol) in methanol (5 mL) was added 6 N HCl (977 µL, 5.86 mmol). The resulting mixture was stirred at 60 °C for 4 h. The solvent was removed in vacuo to give methyl 2-oxo-3-(piperidin-4-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate hydrochloride (188 mg,0.605 mmol, 103% yield) as a beige solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.43 (s, 1 H) 9.03 (d, *J* = 11.37 Hz, 1 H) 8.67 (d, *J* = 8.59 Hz, 1 H) 7.90 (s, 1 H) 7.71 (dd, *J* = 8.08, 1.52 Hz, 1 H) 7.11 (d, *J* = 8.08 Hz, 1 H) 4.59 (ddd, *J* =

12.13, 8.08, 3.79 Hz, 1 H) 3.85 (s, 3 H) 3.42 (d, *J* = 11.87 Hz, 2 H) 3.05 - 3.15 (m, 2 H) 2.53 - 2.64 (m, 2 H) 1.89 (d, *J* = 11.87 Hz, 2 H)

LCMS: [M+1] = 276, rt = 1.46 min

Methyl 3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate

A mixture of methyl 2-oxo-3-(piperidin-4-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate hydrochloride (30.0 mg, 0.0962 mmol), 2-chlorobenzoic acid (15.1 mg, 0.0962 mmol), HBTU (36.5 mg, 0.0962 mmol) and DIPEA (17.2 µL, 0.0962 mmol) in chloroform (5 mL) was stirred at room temperature for 41 h. The solvent was removed with a stream of N_2 and the residue was purified by automated normal-phase chromatography (0-100% EtOAc/heptane, 4 g silica gel cartridge) to give methyl 3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (30.1 mg, 0.0727 mmol, 75.6% yield) as an off-white solid. Two amide isomers seen by 1H NMR -- one peak by LCMS

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.35 - 11.40 (m, 1 H) 7.75 - 7.83 (m, 1 H) 7.68 - 7.72 (m, 1 H) 7.54 - 7.62 (m, 1 H) 7.37 - 7.50 (m, 3 H) 7.06 - 7.12 (m, 1 H) 4.52 - 4.76 (m, 2 H) 3.83 - 3.87 (m, 3 H) 3.57 - 3.68 (m, 1 H) 3.22 - 3.31 (m, 1 H) 3.10 - 3.19 (m, 1 H) 2.94 - 3.05 (m, 1 H) 2.14 - 2.43 (m, 3 H) 1.87 (d, *J* = 10.36 Hz, 1 H) 1.64 - 1.75 (m, 1 H)

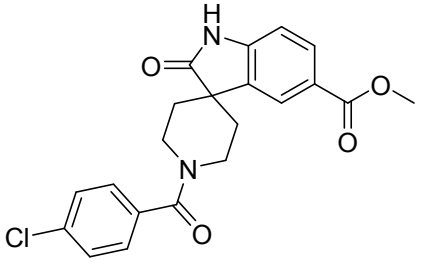
LCMS: [M+1] = 414/416, rt = 2.20 min.

3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylic acid

To a solution of methyl 3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (29.0 mg, 0.0701 mmol) in methanol (5 mL) was added 1 N NaOH (210 µL, 0.210 mmol). The resulting mixture was stirred at 60 °C for 72 h. After cooling, the solvent was removed under a stream of N_2, and the residue taken up in water and filtered through a syringe filter. The solution was acidified by addition of 6 N HCl and stirred for 3 h. The solid was collected by filtration, washed with water and dried under vacuum to give 3-(1-(2-chlorobenzoyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylic acid (12.9 mg, 0.0323 mmol, 46.0% yield) as an off-white solid. Two amide conformers seen by ^{1}H NMR – one peak by LCMS.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.79 (br. s., 1 H) 11.30 - 11.33 (m, 1 H) 7.75 - 7.82 (m, 1 H) 7.67 (dd, *J* = 8.21, 1.39 Hz, 1 H) 7.53 - 7.60 (m, 1 H) 7.35 - 7.50 (m, 3 H) 7.04 - 7.09 (m, 1 H) 4.64 - 4.76 (m, 1 H) 4.51 - 4.64 (m, 1 H) 3.21 - 3.39 (m, 2 H) 2.93 - 3.04 (m, 1 H) 2.15 - 2.43 (m, 2 H) 1.87 (d, *J* = 11.62 Hz, 1 H) 1.69 (br. s., 1 H)

LCMS: [M+1] = 400/402, rt = 1.88 min.

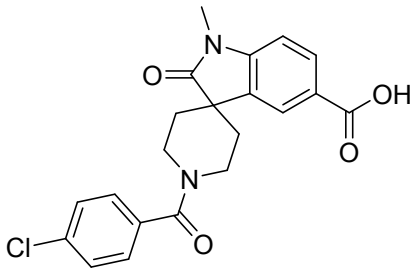


Example LI-2174: Methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

To a suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (51.8 mg, 0.175 mmol), 4-chlorobenzoic acid (30.1 mg, 0.192 mmol) and HBTU (72.8 mg, 0.192 mmol) in chloroform (5 mL) was added DIPEA (93.5 µL, 0.524 mmol). The resulting mixture was stirred at room temperature for 17 h. The solvent was removed with a stream of N_2 and the residue triturated with water/MeOH (3:1) for 18 h. The solid was collected by filtration, washed with water and dried to give methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (63.0 mg, 0.158 mmol, 90.5% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.86 (s, 1 H) 8.07 (s, 1 H) 7.88 (d, *J* = 8.34 Hz, 1 H) 7.56 - 7.59 (m, 2 H) 7.51 - 7.55 (m, 2 H) 6.97 (d, *J* = 8.08 Hz, 1 H) 4.22 (br. s., 1 H) 3.83 (s, 3 H) 3.77 (br. s., 2 H) 3.50 (br. s., 1 H) 1.94 - 2.04 (m, 2 H) 1.77 (br. s., 1 H) 1.67 (br. s., 1 H)

LCMS: [M+1] = 399/401, rt = 2.35 min.

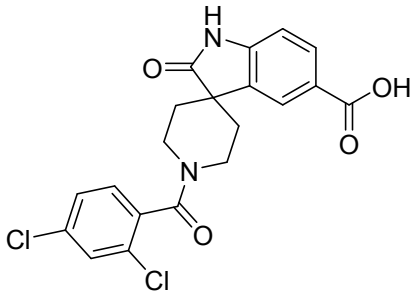


Example LI-2192: 1'-(4-chlorobenzoyl)-1-methyl-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

A suspension of sodium hydride (6.0 mg, 0.15 mmol) in THF (2 mL) was stirred at room temperature for 10 min, then a solution of methyl 1'-(4-chlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (50.0 mg, 0.125 mmol) and iodomethane (9.4 µL, 0.15 mmol) in THF (2 mL) was added portionwise over 5 min at room temperature and the resulting mixture stirred for 18 h. Added sodium hydride (6.0 mg, 0.15 mmol) and stirred an additional 24 h. The solvent was removed under a stream of N_2, and the residue taken up in water and filtered through a syringe filter. The solution was acidified by addition of 6 N HCl and stirred for 2 h. The solid was collected by filtration, washed with water and dried under vacuum to give 1'-(4-chlorobenzoyl)-1-methyl-2-oxo-spiro[indoline-3,4'-piperidine]-5-carboxylic acid (44.3 mg, 0.111 mmol, 88.6% yield) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.76 (br. s., 1 H) 8.09 (br. s., 1 H) 7.95 (d, *J* = 7.23 Hz, 1 H) 7.58 (d, *J* = 8.02 Hz, 2 H) 7.50 - 7.55 (m, 2 H) 7.14 (d, *J* = 8.17 Hz, 1 H) 4.22 (br. s., 1 H) 3.77 (br. s., 2 H) 3.51 (br. s., 1 H) 3.18 (s, 3 H) 1.95 - 2.05 (m, 2 H) 1.77 (br. s., 1 H) 1.65 (br. s., 1 H)

LCMS: [M+1] = 413, rt = 2.22 min.



Example LI-2260: 1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

To a suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (41.0 mg, 0.138 mmol), 2,4-dichlorobenzoic acid (29.0 mg, 0.152 mmol) and HBTU (57.6 mg, 0.152 mmol) in $CHCl_3$ (5 mL) was added DIPEA (74.0 µL, 0.415 mmol). The resulting mixture was stirred at room temperature for 7 days (for convenience). The contents were treated with water and extracted with $CHCl_3$ (3x). The organic layers were filtered through a cotton plug, reduced in volume with a stream of N_2 and then purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give methyl 1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (50.5 mg,0.117 mmol, 84.4% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.85 - 10.91 (m, 1 H) 7.47 - 8.08 (m, 5 H) 6.94 - 7.00 (m, 1 H) 4.13 - 4.33 (m, 1 H) 3.63 - 3.89 (m, 5 H) 3.19 - 3.29 (m, 1 H) 1.59 - 2.10 (m, 4 H)

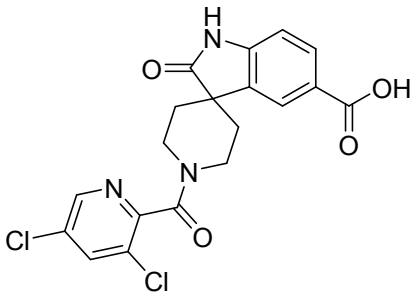
LCMS: [M+1] = 433/435/437, rt = 2.53 min.

1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

To a suspension of methyl 1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (50.5 mg, 0.117 mmol) in methanol (5 mL) was added 1 N NaOH (583 µL, 0.583 mmol). The resulting mixture was stirred at 50 °C for 24 h. The solvent was removed under a stream of N_2, and the residue taken up in water and filtered through a syringe filter. The solution was acidified by addition of 6 N HCl and stirred for 3 days. The solid was collected by filtration, washed with water and dried under vacuum to give 1'-(2,4-dichlorobenzoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (33.8 mg, 0.0806 mmol, 69.2% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.70 (br. s., 1 H) 10.79 - 10.87 (m, 1 H) 7.46 - 8.06 (m, 5 H) 6.90 - 6.98 (m, 1 H) 4.11 - 4.29 (m, 1 H) 3.62 - 3.92 (m, 2 H) 3.20 - 3.29 (m, 1 H) 1.60 - 2.06 (m, 4 H)

LCMS: [M+1] = 419/421/423, rt = 2.18 min.



Example LI-2180: 1'-(3,5-dichloropicolinoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-(3,5-dichloropicolinoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

To a suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (40.0 mg, 0.135 mmol), 3,5-dichloropyridine-2-carboxylic acid (28.5 mg, 0.148 mmol) and HBTU (56.2 mg, 0.148 mmol) in chloroform (5 mL) was added DIPEA (72.2 µL, 0.404 mmol). The resulting mixture was stirred at room temperature for 21 h. The solvent was removed with a stream of N_2 and the residue triturated with water/MeOH (5:1) for 18 h. The solid was collected by filtration, washed with water and dried to give methyl 1'-(3,5-dichloropicolinoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (29.2 mg, 0.0672 mmol, 49.9% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.93 (s, 1 H) 8.71 (d, *J* = 2.02 Hz, 1 H) 8.44 (d, *J* = 2.02 Hz, 1 H) 7.87 - 7.93 (m, 2 H) 6.99 (d, *J* = 8.08 Hz, 1 H) 4.10 - 4.22 (m, 1 H) 3.79 - 3.92 (m, 4 H) 3.63 - 3.74 (m, 1 H) 3.23 - 3.32 (m, 1 H) 1.69 - 1.96 (m, 4 H)

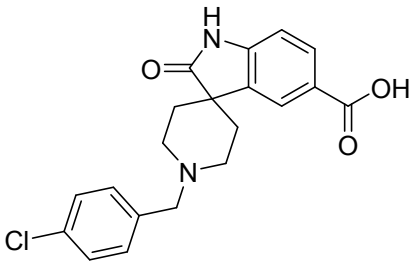
LCMS: [M+1] = 434/436, rt = 2.22 min.

1'-(3,5-dichloropicolinoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

To a solution of methyl 1'-(3,5-dichloropyridine-2-carbonyl)-2-oxo-spiro[indoline-3,4'-piperidine]-5-carboxylate (29.2 mg, 0.0672 mmol) in methanol (5 mL) was added 1 N NaOH (135 µL, 0.135 mmol). The resulting mixture was stirred at room temperature for 22 h, then at 50 °C for 48 h. The solvent was removed with a stream of N_2 to give a residue which was purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give 1'-(3,5-dichloropicolinoyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (18.0 mg, 0.0428 mmol, 63.7% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.76 (br. s., 1 H) 10.89 (s, 1 H) 8.71 (d, *J* = 2.02 Hz, 1 H) 8.44 (d, *J* = 2.02 Hz, 1 H) 7.84 - 7.90 (m, 2 H) 6.96 (d, *J* = 8.34 Hz, 1 H) 4.09 - 4.20 (m, 1 H) 3.84 - 3.93 (m, 1 H) 3.63 - 3.73 (m, 1 H) 3.28 (d, *J* = 13.89 Hz, 1 H) 1.86 (d, *J* = 4.80 Hz, 2 H) 1.71 - 1.80 (m, 2 H)

LCMS: [M+1] = 420/422/424, rt = 1.90 min.



Example LI-2178: 1'-(4-chlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-(4-chlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

A suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (56.5 mg, 0.190 mmol), 4-chlorobenzaldehyde (29.4 mg, 0.209 mmol) and sodium triacetoxyborohydride (121 mg, 0.571 mmol) in chloroform (5 mL) was stirred at room temperature for 26 h. The solvent was removed with a stream of N_2 and the residue was partitioned between water and EtOAc. The solvent was removed from the organic layer with a stream of N_2 to give a residue which was purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give methyl 1'-[(4-chlorophenyl)methyl]-2-oxo-spiro[indoline-3,4'-piperidine]-5-carboxylate acetate (72.0 mg, 0.162 mmol, 85.0% yield) as a colorless gum.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.02 (br. s., 1 H) 10.82 (s, 1 H) 7.96 (s, 1 H) 7.87 (d, *J* = 8.08 Hz, 1 H) 7.42 (s, 4 H) 6.96 (d, *J* = 8.08 Hz, 1 H) 3.83 (s, 3 H) 3.63 (s, 2 H) 2.83 (br. s., 2 H) 2.56 (br. s., 2 H) 1.92 (s, 3 H) 1.81 (br. s., 2 H) 1.71 (br. s., 2 H)

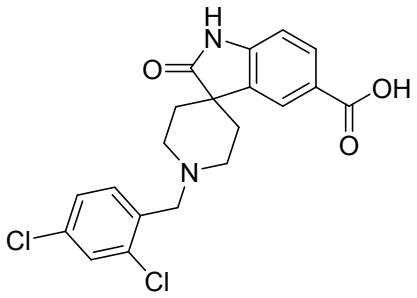
LCMS: [M+1] = 385/387, rt = 2.01 min.

1'-(4-chlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

To a solution of methyl 1'-[(4-chlorophenyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate acetate (72.0 mg, 0.162 mmol) in methanol (5 mL) was added 1 N NaOH (485 µL, 0.485 mmol). The resulting mixture was stirred at room temperature for 18 h, then at 50 °C for 31 h. After cooling, 6 N HCl (27 µL, 1 eq.) was added and the solvent removed with a stream of N_2 to give a residue which was purified by automated normal-phase chromatography (0-40% MeOH/DCM, 4 g silica gel cartridge) to give 1'-[(4-chlorophenyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (29.1 mg, 0.0785 mmol, 48.5% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.79 (s, 1 H) 7.98 (s, 1 H) 7.86 (dd, *J* = 8.21, 1.64 Hz, 1 H) 7.37 - 7.46 (m, 4 H) 6.94 (d, *J* = 8.08 Hz, 1 H) 3.63 (br. s., 2 H) 2.84 (br. s., 2 H) 2.56 (br. s., 2 H) 1.78 - 1.88 (m, 2 H) 1.71 (br. s., 2 H)

LCMS: [M+1] = 371/373, rt = 1.99 min.



Example LI-2242: 1'-(2,4-dichlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-(2,4-dichlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

A suspension of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (41.4 mg, 0.140 mmol), 2,4-dichlorobenzaldehyde (26.9 mg, 0.154 mmol) and sodium triacetoxyborohydride (88.7 mg, 0.419 mmol) in $CHCl_3$ (5 mL) was stirred at room temperature for 4 days (for convenience). The contents were treated with 5% Na_2CO_3 and extracted with $CHCl_3$ (3x) and passed through a cotton plug. The solvent was removed with a stream of N_2 to give a residue which was purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give methyl 1'-[(2,4-dichlorophenyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (44.8 mg, 0.107 mmol, 76.6% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.83 (s, 1 H) 7.98 (d, *J* = 1.52 Hz, 1 H) 7.88 (dd, *J* = 8.08, 1.52 Hz, 1 H) 7.62 - 7.65 (m, 2 H) 7.45 (dd, *J* = 8.34, 2.27 Hz, 1 H) 6.97 (d, *J* = 8.08 Hz, 1 H) 3.83 (s, 3 H) 3.70 (s, 2 H) 2.90 (td, *J* = 7.58, 3.54 Hz, 2 H) 2.63 (dt, *J* = 7.39, 3.76 Hz, 2 H) 1.80 - 1.87 (m, 2 H) 1.71 - 1.79 (m, 2 H)

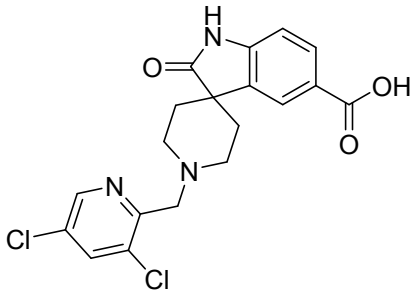
LCMS: [M+1] = 419/421/423, rt = 2.07 min.

1'-(2,4-dichlorobenzyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

To a suspension of methyl 1'-[(2,4-dichlorophenyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (44.8 mg, 0.107 mmol) in methanol (5 mL) was added 1 N NaOH (534 µL, 0.534 mmol). The resulting mixtures was stirred at 50 °C for 22 h. After cooling, the solvent was removed under a stream of N_2, and the residue taken up in water and filtered through a syringe filter. The solution was carefully neutralized by addition of 2 N HCl and stirred for 3 h. The solid was collected by filtration, washed with water and dried under vacuum to give 1'-[(2,4-dichlorophenyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid (38.0 mg, 0.0938 mmol, 87.8% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.72 (br. s., 1 H) 10.79 (s, 1 H) 7.99 (s, 1 H) 7.86 (dd, *J* = 8.08, 1.52 Hz, 1 H) 7.61 - 7.66 (m, 2 H) 7.45 (dd, *J* = 8.34, 2.27 Hz, 1 H) 6.94 (d, *J* = 8.08 Hz, 1 H) 3.70 (s, 2 H) 2.89 (d, *J* = 7.83 Hz, 2 H) 2.63 (br. s., 2 H) 1.83 (d, *J* = 8.08 Hz, 2 H) 1.74 (br. s., 2 H)

LCMS: [M+1] = 405/407/409, rt = 1.88 min.



Example LI-2263: 1'-((3,5-dichloropyridin-2-yl)methyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

Methyl 1'-((3,5-dichloropyridin-2-yl)methyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate

A mixture of methyl 2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate hydrochloride (48.8 mg, 0.164 mmol), 3,5-dichloropyridine-2-carboxaldehyde (17.5 µL, 0.181 mmol) and sodium triacetoxyborohydride (104.6 mg, 0.493 mmol) in $CHCl_3$ (5 mL) was stirred at room temperature for 6 days (for convenience) The contents were treated with 10% Na_2CO_3 and extracted with $CHCl_3$ (3x) and passed through a cotton plug. The solvent was removed with a stream of N_2 to give a residue which was purified by automated normal-phase chromatography (0-20% MeOH/DCM, 4 g silica gel cartridge) to give methyl 1'-[(3,5-dichloro-2-pyridyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (25.0 mg, 0.0595 mmol, 36.2% yield) as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.80 (s, 1 H) 8.63 (d, *J* = 2.20 Hz, 1 H) 8.25 (d, *J* = 2.20 Hz, 1 H) 7.98 (s, 1 H) 7.87 (dd, *J* = 8.17, 1.73 Hz, 1 H) 6.96 (d, *J* = 8.17 Hz, 1 H) 3.87 (s, 2 H) 3.83 (s, 3 H) 2.90 - 2.96 (m, 2 H) 2.72 - 2.80 (m, 2 H) 1.76 - 1.84 (m, 2 H) 1.61 - 1.69 (m, 2 H)

LCMS: [M+1] = 434/436/438, rt = 1.94 min.

1'-((3,5-dichloropyridin-2-yl)methyl)-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid

To a suspension of methyl 1'-[(3,5-dichloro-2-pyridyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylate (25.0 mg, 0.0595 mmol) in methanol (5 mL) was added 1 N NaOH (297 µL, 0.297 mmol). The resulting mixture was stirred at 60 °C for 24 h. The solvent was removed under a stream of N_2, and the residue taken up in water and filtered through a syringe filter. The solution was acidified by addition of 6 N HCl and stirred for 18 h. The solid was collected by filtration, washed with water and dried under vacuum to give 1'-[(3,5-dichloro-2-pyridyl)methyl]-2-oxospiro[indoline-3,4'-piperidine]-5-carboxylic acid hydrochloride (12.1mg,0.0273mmol, 45.948% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.77 (br. s., 1 H) 10.90 - 11.10 (m, 1 H) 10.08 - 10.52 (m, 1 H) 8.76 - 8.89 (m, 1 H) 8.36 - 8.49 (m, 2 H) 7.86 - 7.96 (m, 1 H) 7.81 (s, 1 H) 6.94 - 7.05 (m, 1 H) 4.77 - 5.03 (m, 2 H) 3.55 - 3.86 (m, 4 H) 1.92 - 2.30 (m, 2 H)

LCMS: [M+1] = 406/408/410, rt = 1.71 min.

Supplemental Table 1. Summary of IC_{50} for 123 hits tested in 10 point-dose-response assay.

FORMATTED_ID	COMMENTS	IC50 (μM) qHTS	IC50 (μM) 384 wells
LI-0001737	NCGC00016107-09	9.2	5.9
LI-0001736	NCGC00021152-06	3.7	6.6
LI-0001739	NCGC00025170-13	5.2	179.4
LI-0001742	NCGC00115156-01	7.3	23.8
LI-0001743	NCGC00119360-01	18.4	72.9
LI-0001745	NCGC00123043-01	7.3	177.4
LI-0001747	NCGC00137614-01	4.6	31.8
LI-0001748	NCGC00139237-01	9.2	24.9
LI-0001749	NCGC00139422-01	10.3	34.9
LI-0001750	NCGC00140605-02	10.3	47.8
LI-0001752	NCGC00165880-06	5.8	28.1
LI-0001753	NCGC00165913-03	18.4	2.2
LI-0001754	NCGC00167761-04	1.5	7.6
LI-0001755	NCGC00168759-10	9.2	13.4
LI-0001751	NCGC00182059-03	6.5	15.2
LI-0001757	NCGC00241455-08	10.3	76.0
LI-0001759	NCGC00244250-02	10.3	136.4
LI-0001756	NCGC00346436-02	3.3	2.8
LI-0001815	NCGC00346488-06	10.3	164.6
LI-0001761	NCGC00346678-04	9.2	176.8
LI-0001762	NCGC00346950-03	4.1	79.1
LI-0001766	NCGC00373874-01	16.4	188.0
LI-0001768	NCGC00374143-01	10.6	28.5
LI-0001769	NCGC00374177-01	13.3	33.8
LI-0001770	NCGC00374926-01	16.4	60.1
LI-0001772	NCGC00374945-01	4.6	5.7
LI-0001773	NCGC00374956-01	5.2	5.9
LI-0001774	NCGC00375051-01	5.8	19.8
LI-0001775	NCGC00375073-01	1.3	8.9
LI-0001771	NCGC00375083-01	5.8	178.4
LI-0001776	NCGC00375309-01	12.8	44.3
LI-0001777	NCGC00375726-01	16.4	107.0
LI-0001780	NCGC00379223-01	10.3	57.9
LI-0001782	NCGC00386401-02	8.2	101.9
LI-0001783	NCGC00386412-04	3.7	4.0
LI-0001784	NCGC00387229-03	3.3	135.4
LI-0001785	NCGC00387768-01	6.5	65.7
LI-0001786	NCGC00387872-01	9.2	109.7
LI-0001787	NCGC00391735-01	4.6	35.3

LI-0001788	NCGC00395836-01	10.3	109.7
LI-0001794	NCGC00397895-01	16.8	101.9
LI-0001792	NCGC00411983-01	0.9	11.6
LI-0001793	NCGC00411987-01	3.3	11.5
LI-0001795	NCGC00411993-01	11.9	123.3
LI-0001796	NCGC00412056-01	15.8	138.8
LI-0001797	NCGC00412066-01	12.6	86.9
LI-0001798	NCGC00412087-01	15.8	65.6
LI-0001799	NCGC00412153-01	17.8	69.0
LI-0001801	NCGC00412164-01	12.6	76.7
LI-0001802	NCGC00412172-01	14.9	180.1
LI-0001803	NCGC00412203-01	4.2	105.3
LI-0001804	NCGC00412222-01	9.4	62.0
LI-0001806	NCGC00412275-01	15.8	90.7
LI-0001807	NCGC00412293-01	14.9	149.9
LI-0001847	NCGC00412442-01	14.9	79.6
LI-0001858	NCGC00412595-01	14.9	65.4
LI-0001758	NCGC00412658-01	6.7	29.6
LI-0001810	NCGC00416618-01	4.2	16.0
LI-0001811	NCGC00416629-01	26.6	35.8
LI-0001812	NCGC00417466-01	14.9	57.2
LI-0001856	NCGC00417481-01	14.9	103.4
LI-0001857	NCGC00417519-01	14.9	29.8
LI-0001813	NCGC00419079-01	3.5	15.9
LI-0001814	NCGC00419100-01	2.7	80.6
LI-0001817	NCGC00419103-01	14.9	18.1
LI-0001819	NCGC00419114-01	10.6	25.5
LI-0001821	NCGC00419125-01	1.9	27.1
LI-0001822	NCGC00419140-01	13.3	35.2
LI-0001824	NCGC00419160-01	11.9	140.0
LI-0001826	NCGC00419173-01	7.5	111.8
LI-0001828	NCGC00419210-01	1.9	8.1
LI-0001832	NCGC00421835-01	10.3	69.9
LI-0001850	NCGC00422007-01	20.6	2.2
LI-0001851	NCGC00422009-01	9.2	1.5
LI-0001852	NCGC00422010-01	9.2	2.9
LI-0001853	NCGC00422011-01	16.4	4.5
LI-0001849	NCGC00422014-01	16.4	2.2
LI-0001833	NCGC00432183-01	2.9	148.7
LI-0001834	NCGC00435449-01	10.3	22.8
LI-0001837	NCGC00476381-01	4.2	5.3

LI-0001838	NCGC00480624-01	8.2	16.4
LI-0001839	NCGC00480633-01	13.3	63.6
LI-0001848	NCGC00480634-01	5.9	37.5
LI-0001840	NCGC00480636-01	9.2	54.9
LI-0001841	NCGC00480642-01	11.6	88.7
LI-0001842	NCGC00480646-01	4.1	6.8
LI-0001843	NCGC00482115-01	7.3	80.1
LI-0001844	NCGC00485900-01	7.3	30.6
LI-0001845	NCGC00500370-01	8.2	56.5
LI-0001846	NCGC00501004-01	16.8	8.7

Supplemental Table 2. Inhibition against 58 kinases by 10 μM LI-2172 and LI-2242

Percentage inhibition by compounds (KinaseProfiler by Eurofins Discovery)

	LI-2172 / 3 @ 10 μM	LI-2242 / 2 @ 10 μM
Abl(h)	-4	-21
ALK(h)	-12	2
AMPKα1(h)	7	6
ASK1(h)	-10	-3
Aurora-A(h)	-9	15
CaMKI(h)	13	20
CDK1/cyclinB(h)	8	5
CDK2/cyclinA(h)	-1	-25
CDK6/cyclinD3(h)	25	1
CDK7/cyclinH/MAT1(h)	-17	3
CDK9/cyclin T1(h)	-28	4
CHK1(h)	-19	-25
CK1γ1(h)	-19	27
CK2α2(h)	20	24
c-RAF(h)	-1	25
DRAK1(h)	-1	-9
eEF-2K(h)	9	9
EGFR(h)	-7	-11
EphA5(h)	27	6
EphB4(h)	-9	-17
Fyn(h)	10	10
GSK3β(h)	-4	-7
IGF-1R(h)	-16	1
IKKα(h)	6	13
IRAK4(h)	10	26
JAK2(h)	19	16
KDR(h)	9	16
LOK(h)	13	4
Lyn(h)	-1	-2
MAPKAP-K2(h)	-7	20
MEK1(h)	-7	-9
MLK1(h)	3	11
Mnk2(h)	-24	-9
MSK2(h)	-9	-11
MST1(h)	-1	7
mTOR(h)	8	6
NEK2(h)	-4	6
p70S6K(h)	20	17
PAK2(h)	1	-11
PDGFRβ(h)	-11	-3
Pim-1(h)	2	-2
PKA(h)	3	11
PKBα(h)	4	-1
PKCα(h)	19	4

PKCθ(h)	-16	6
PKG1α(h)	10	5
Plk3(h)	-12	-11
PRAK(h)	7	-29
ROCK-I(h)	3	7
Rse(h)	-7	-10
Rsk1(h)	8	7
SAPK2a(h)	1	-5
SRPK1(h)	-1	18
TAK1(h)	9	3
PI3 Kinase (p110β/p85α)(h)	0	0
PI3 Kinase (p120γ)(h)	0	0
PI3 Kinase (p110δ/p85α)(h)	3	1
PI3 Kinase (p110α/p85α)(h)	1	8