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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Potent, selective small molecule inhibitors of type III phosphatidylinositol-4-kinase α- but not β- inhibit the phosphatidylinostiol signaling cascade and cancer cell proliferation

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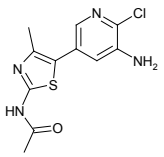
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Supplementary information

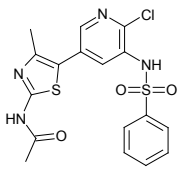
Compound Synthesis

_N_-(5-(5-Amino-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)acetamide



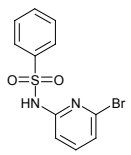
A suspension of _N_-(4-methylthiazol-2-yl)acetamide (1.2 g, 7.68 mmol), CsF (3.49 g, 23 mmol), 5-bromo-2-chloropyridin-3-amine (1.59 g, 7.68 mmol), palladium(II) acetate (90 mg, 0.4 mmol) and tri-_tert_-butylphosphine (162 mg, 0.8 mmol) in dimethylsulfoxide (20 mL) was degassed and purged with nitrogen then immediately heated at 150 °C for 40 minutes, cooled to room temperature and triturated with after (40 mL). The resulting brown solid was collected by filtration, washed with diethyl ether and dried in a vacuum oven at 40 °C for 3 days to afford _N_-(5-(5-amino-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)acetamide (1.75 g, 80%).
m.p. = 226.5-227° C; MS (_m/z_) (ES+) [M+H]+ = 283.0; HRMS (_m/z_) $[M]^+$ calcd. for $C_{11}H_{12}ON_4ClS$, 283.04149; found, 283.04147; ^1H NMR (400 MHz, DMSO-d_6): δ 12.13 (s, 1H), 7.68 (d, J = 2.24 Hz, 1H), 7.22 (d, J = 2.24 Hz, 1H), 5.68 (s, 2H), 2.35 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 168.4, 155.6, 143.1, 141.2, 134.8, 133.6, 128.4, 120.8, 119.7, 22.3, 16.0

_N_-(5-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)acetamide 1



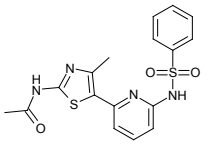
Benzenesulfonyl chloride (6.77 mL, 53.05 mmol) was added in one portion to _N_-(5-(5-amino-6-chloropyridin-3-yl)-4-methylthiazol-2-yl)acetamide (5 g, 17.68 mmol) in pyridine (95 mL) at 20 ºC under nitrogen. The resulting solution was stirred at 45 °C for 20 hours. Water (80 mL) was added and the reaction mixture stirred for 30 minutes. Pyrrolidine (80 mL) was added and the reaction mixture stirred for 20 minutes. The reaction mixture was concentrated and diluted with water (300 mL) and acetic acid to bring the pH to 5. The organic was extracted with ethyl acetate (2 × 300 mL) and washed sequentially with water (300 mL) and saturated brine (150 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 40 to 100% ethyl acetate in heptane. Pure fractions were evaporated to dryness and the brown solid triturated with dichloromethane and filtered, washing with diethyl ether to afford _N_-(5-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-4-methylthiazol-2-yl)acetamide (2.97 g, 40 %) as a solid.
m.p. = 210-210.5 °C; MS (_m/z_) (ES+) [M+H]+ = 283.0; HRMS (_m/z_) $[M]^+$ calcd. for $C_{17}H_{15}O_3N_4ClS_2$, 423.0349; found, 423.03473; ^1H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H), 8.31 (d, J = 2.29 Hz, 1H), 7.76 – 7.81 (m, 2H), 7.64 – 7.72 (m, 2H), 7.61 (m, 3H), 2.28 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 168.6, 156.3, 145.0, 144.5, 143.4, 139.8, 133.7, 133.2, 130.6, 129.4, 128.4, 126.6, 118.4, 22.3, 15.8

_N_-(6-Bromopyridin-2-yl)benzenesulfonamide



Into a 500-mL round-bottom flask was placed a solution of 6-bromopyridin-2-amine (50 g, 289.00 mmol, 1.00 equiv) in pyridine (100 mL). This was followed by the addition of benzenesulfonyl chloride (61 g, 345.37 mmol, 1.20 equiv) dropwise with stirring at 0 °C in 30 min. The resulting solution was stirred for 2 h at room temperature and then diluted with 1 L of water, extracted with ethyl acetate (3 × 1 L). The organic layers were combined and dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was re-crystallized from ethyl acetetate:hexane (1:3). This resulted in 78 g (86%) of _N_-(6-bromopyridin-2-yl)benzenesulfonamide as a solid.
MS (_m/z_) (ES+) [M+H]+ = 313

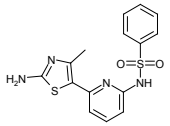
_N_-(4-methyl-5-(6-(phenylsulfonamido)pyridin-2-yl)thiazol-2-yl)acetamide 2



N-(6-bromopyridin-2-yl)benzenesulfonamide (1.0 g, 3.20 mmol), N-(4-methylthiazol-2-yl)acetamide (0.5 g, 3.20 mmol), diacetoxypalladium (0.057 g, 0.26 mmol), cesium fluoride (1.46 g, 9.60 mmol) and tri-tert-butylphosphine (1.24 mL, 0.51 mmol) in DMSO (6 mL) were stirred at 140 °C for 6 hours. After cooling, the mixture was poured into ice/water (50 mL), and the solid was filtered off and dried. The resulting solid was diluted with dichloromethane/methanol mixture (50 mL) and the solid was absorbed onto silica gel and purified by flash chromatography on silica gel eluting with 50 to100% ethyl acetate in heptane. Pure fractions were evaporated to dryness and filtered from a little diethyl ether to afford *N*-(4-methyl-5-(6-(phenylsulfonamido)pyridin-2-yl)thiazol-2-yl)acetamide (0.354 g, 28.5 %) as a solid.

m.p. = 235-236 °C; MS (*m/z*) (ES+) [M+H]+ = 389.3; HRMS (*m/z*) $[M]^+$ calcd. for C17H16N4O3S2, 389.07355; found, 389.07355; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (s, 1H), 8.03 (dd, J = 1.81, 7.78 Hz, 2H), 7.70 (t, J = 7.96 Hz, 1H), 7.58 – 7.66 (m, 4H), 7.21 (d, J = 7.76 Hz, 1H), 6.85 (d, J = 8.11 Hz, 1H), 2.45 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 168.5, 156.9, 151.0, 150.1, 145.0, 140.2, 139.0, 132.8, 129.0, 127.3, 124.7, 115.4, 109.2, 22.5, 17.4

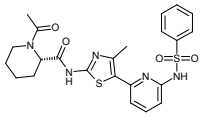
_N_-[6-(2-Amino-4-methyl-1,3-thiazol-5-yl)pyridin-2-yl]benzenesulfonamide hydrochloride



Into a 250-mL round-bottom flask was placed a solution of *N*-[5-(6-benzenesulfonamidopyridin-2-yl)-4-methyl-1,3-thiazol-2-yl]acetamide (3.6 g, 9.27 mmol, 1.00 equiv) in hydrogen chloride (6N) (100 mL). The resulting solution was stirred for 20 h at 80 °C. The solid was collected by filtration and washed with water (3 × 30 mL) to afford *N*-6-(2-amino-4-methyl-1,3-thiazol-5-yl)pyridin-2-yl]benzenesulfonamide hydrochloride (3.2 g, 90%) as a solid.

m.p. = 160-170 °C; MS (*m/z*) (ES+) [M+H]+ = 347; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 9.20 (s, 2H), 7.99 (m, 2H), 7.74 (t, J = 7.98 Hz, 1H), 7.58 – 7.66 (m, 3H), 7.20 (d, J = 7.77 Hz, 1H), 6.88 (d, J = 8.14 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 167.8, 151.0, 147.1, 140.2, 139.4, 134.0, 132.9, 129.2, 127.1, 118.3, 114.7, 110.3, 13.7

_S_-1-Acetyl-_N_-[5-(6-benzenesulfonamidopyridin-2-yl)-4-methyl-1,3-thiazol-2-yl]piperidine-2-carboxamide 3



Into a 250-mL round-bottom flask was placed a solution of *N*-[6-(2-amino-4-methyl-1,3-thiazol-5-yl)pyridin-2-yl]benzenesulfonamide (2 g, 5.77 mmol, 1.00 equiv) in tetrahydrofuran (100 mL), 1-acetylpiperidine-2-carboxylic acid (1.2 g, 7.01 mmol, 1.20 equiv), HATU (3.3 g, 8.68 mmol, 1.50 equiv) and *N*,*N*-diisopropylethylamine (2.25 g, 17.41 mmol, 3.00 equiv). The resulting solution was stirred for 40 h at 40 °C and then concentrated under vacuum. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, CH_3CN/water=0/100 increasing to CH3CN/water=60/40 within 30 min; Detector, UV 254 nm. This resulted in 1.7 g (59%) of 1-acetyl-*N*-[5-(6-benzenesulfonamidopyridin-2-yl)-4-methyl-1,3-thiazol-2-yl]piperidine-2-carboxamide as a solid.

MS (*m/z*) (ES+) [M+H]+ = 500

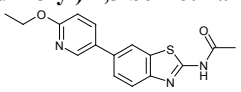
1-acetyl-*N*-[5-(6-benzenesulfonamidopyridin-2-yl)-4-methyl-1,3-thiazol-2-yl]piperidine-2-carboxamide (1.7 g, 3.40 mmol, 1.00 equiv was purified by Prep-SFC with the following conditions : Column, Chiralpak IC0.46*15cm, 5 μm Chiral-A017 Lot No.IC00CD-PL010; mobile phase, DCM(50% to 0%) in ethanol(0.1% diethylamine); detector, UV 254nm. This resulted in 516.7 mg (60%) of the first isomer as a solid and 591.8 mg (69%) of the second as a solid.

$[\alpha]_D^{22}$ -95.59 (c 2 mg/ml in EtOH)

m.p. = 147-149 °C; HRMS (*m/z*) $[M]^+$ calcd. for $C_{23}H_{25}O_4N_5S_2$, 500.14207; found, 500.14203; ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 10.8 (s, 1H), 8.00 (dd, J = 1.56, 8.06 Hz, 2H), 7.68 (t, J = 7.94 Hz, 1H), 7.52 – 7.62 (m, 3H), 7.19 (d, J = 7.67 Hz, 1H), 6.94 (d, J = 8.14 Hz, 1H), 5.13 (s, 1H), 3.91 (s, 1H), 3.36 (s, 1H), 2.55 (m, 1H), 2.47 (s, 3H), 2.21 (m, 1H), 2.08 (s, 3H), 1.69 (m, 3H), 1.48 (m, 2H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 170.7, 170.5, 156.7, 151.5, 149.8, 144.8, 140.8, 138.9, 132.5, 128.9, 127.1, 125.0, 115.0, 109.4, 51.7, 43.8, 26.7, 24.5, 21.6, 19.9, 17.3, 11.1

The absolute configuration of **3** was determined by X-ray crystallography, CCDC code 969955, unit cell parameters: a 7.6067(5) b 16.4862(12) c 43.712(3) P212121.

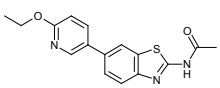
_N_-[6-(6-ethoxypyridin-3-yl)-1,3-benzothiazol-2-yl]acetamide



Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen was placed a solution of *N*-[6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-benzothiazol-2-yl]acetamide (159 mg, 0.50 mmol, 1.00 equiv) in DME/H_2O=4/1 (30 mL), 5-bromo-2-ethoxypyridine (101 mg, 0.50 mmol, 1.00 equiv), potassium carbonate (207 mg, 1.50 mmol, 3.00 equiv), 1,1'-bis(diphenylphosphino)ferrocenedichloropalladium(II) (40.8 mg, 0.05 mmol, 0.10 equiv). The resulting solution was heated to reflux for 80 min in an oil bath. The solvent was removed. The residue was applied onto a silica gel column with petrol:ethyl acetate (1:5). This resulted in 55.5 mg (35%) of *N*-[6-(6-ethoxypyridin-3-yl)-1,3-benzothiazol-2-yl]acetamide as a white solid.

m.p. = 250-253 °C; MS (*m/z*) (ES+) [M+H]+ = 314; ^{1}H NMR (300MHz,$CDCl_3$): δ 8.41 (d, 1H), 7.95 (d, 1H), 7.82-7.86 (m, 2H), 7.61-7.66 (m, 1H), 6.83 (d, 1H), 4.42 (q, 2H), 2.38 (s, 3H), 1.44 (t, 3H)

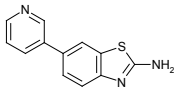
6-(6-Ethoxypyridin-3-yl)benzo[*d*]thiazol-2-amine trifluoroacetate 4



Into a 100-mL round-bottom flask was placed *N*-[6-(6-ethoxypyridin-3-yl)-1,3-benzothiazol-2-yl]acetamide (200 mg, 0.64 mmol, 1.00 equiv), 6N aqueous HCl (10 mL). The resulting solution was stirred for 3 h at 70 °C. The resulting mixture was concentrated under vacuum. The crude product was purified by Flash-Prep-HPLC using the following conditions (1#-Pre-HPLC-016(Waters)): Column, Xbridge Prep C18 5um, 19*150mm;

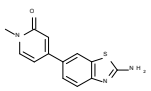
mobile phase, water with 0.05% trifluoroacetic acid and acetonitrile (5.0% acetonitrile up to 42.0% in 10 min, up to 95.0% in 2 min, down to 5.0% in 2 min); Detector, 220/254nm, affording 6-(6-ethoxypyridin-3-yl)-1,3-benzothiazol-2-amine trifluoroacetate (110 mg, 64%) as a solid.
m.p. = 210.5-211.5 °C; HRMS (*m/z*) $[M]^+$ calcd. for $C_{14}H_{13}O_2N_3S$, 272.08521; found, 272.08505; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (d, J = 2.33 Hz, 1H), 7.91 – 8.05 (m, 4H), 7.54 (dd, J = 1.86, 8.36 Hz, 1H), 7.42 (d, J = 8.36 Hz, 1H), 6.87 (d, J = 8.60 Hz, 1H), 4.35 (q, J = 7.03 Hz, 3H), 1.34 (t, J = 7.04 Hz, 3H). ^{13}C NMR (175 MHz, DMSO-d_6): δ 167.81, 162.5, 158.6, 158.4, 146.8, 144.3, 137.3, 131.2, 129.3, 128.8, 124.6, 119.3, 116.6, 61.2, 14.5

6-(Pyridin-3-yl)benzo[*d*]thiazol-2-amine 5



1,1'-Bis(diphenylphosphino)ferrocenedichloropalladium(II) (14.58 mg, 0.02 mmol) was added in one portion to *tert*-butyl (6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]thiazol-2-yl)carbamate (150 mg, 0.40 mmol) and 3-bromopyridine (126 mg, 0.80 mmol) in degassed acetonitrile (3 mL) and 2M aq. potassium carbonate (1 mL) at 20 °C under nitrogen. The resulting mixture was further degassed and stirred in a microwave at 100 °C for 60 minutes. The mixture was well extracted with ethyl acetate. The mixture was concentrated and dissolved in dichloromethane/methanol mixture and was passed through a PL-Thiol MP SPE 500mg/6mL cartridge. The organic filtrate was concentrated. The residue was treated 20% trifluoroacetic acid in dichloromethane (5 ml) and stirred at room temperature for 1.5 hours and concentrated in vacuo. The crude product was purified by preparative HPLC (Waters SunFire column, 5 µm silica, 50 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% ammonia) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness to afford 6-(pyridin-3-yl)benzo[d]thiazol-2-amine (7.00 mg, 7.73 %) as a solid.
HRMS (*m/z*) $[M]^+$ calcd. for $C_{12}H_9N_3S$, 228.05899; found, 228.05896; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.89 (d, J = 2.09 Hz, 1H), 8.52 (d, J = 4.65 Hz, 1H), 8.06 (dd, J = 1.73, 7.72 Hz, 2H), 7.59 (t, J = 4.14 Hz, 3H), 7.46 (dd, J = 4.75, 7.90 Hz, 1H), 7.43 (d, J = 8.31 Hz, 1H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 167.1, 152.9, 143.6, 147.3, 135.6, 133.5, 132.7, 129.7, 124.3, 123.7, 119.1, 118.0

4-(2-Aminobenzo[*d*]thiazol-6-yl)-1-methylpyridin-2(1*H*)-one 6



1,1'-Bis(diphenylphosphino)ferrocenedichloropalladium(II) (14.58 mg, 0.02 mmol) was added in one portion to the mixture of *tert*-butyl (6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]thiazol-2-yl)carbamate (150 mg, 0.40 mmol) and 4-bromo-1-methylpyridin-2(*1H*)-one (94 mg, 0.50 mmol) in degassed acetonitrile (3 mL) and 2M aq. potassium carbonate (1 mL) at 20°C under nitrogen. The resulting mixture was further degassed and stirred within a microwave reactor at 100 °C for 60 minutes. The mixture was extracted with ethyl acetate. The mixture was concentrated and disolved in dichloromethane / methanol mixture and was passed through a PL-Thiol MP SPE 500mg/6mL cartridge. The fractions containing product were concentrated *in vacuo*. The residue was treated 20% TFA in DCM (5 ml) and stirred at room temperature for 2.5 hours and concentrated *in vacuo*. The crude product was purified by preparative HPLC (Waters SunFire column, 5µ silica, 50 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% ammonia) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(2-aminobenzo[*d*]thiazol-6-yl)-1-methylpyridin-2(1*H*)-one (44.1 mg, 43.0 %) as a solid.
m.p. = 315 °C (decomposition); HRMS (*m/z*) $[M]^+$ calcd. for $C_{13}H_{11}ON_3S$, 258.06956; found, 258.06955; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.09 (d, J = 1.92 Hz, 1H), 7.73 (d, J = 7.12 Hz, 1H), 7.66 (s, 2H), 7.58 (dd, J = 2.04, 8.43 Hz, 1H), 7.39 (d, J = 8.32 Hz, 1H), 6.66 (d, J = 2.05 Hz, 1H), 6.59 (dd, J = 2.13, 7.09 Hz, 1H), 3.44 (s, 3H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 167.8, 162.0, 154.0, 150.4, 139.43, 131.9, 124.2, 119.2, 117.7, 113.7, 103.7, 36.2

4-(2-Aminobenzo[*d*]thiazol-6-yl)-1-(cyclopropylmethyl)pyridin-2(1*H*)-one 7

Into a 100-mL round-bottom flask purged with nitrogen, was placed a solution of *N*-[6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-benzothiazol-2-yl]acetamide (636 mg, 2.00 mmol) in DME/water (20/5 mL), 4-bromo-1-(cyclopropylmethyl)-1,2-dihydropyridin-2-one (547 mg, 2.40 mmol, 1.20 equiv), $Pd(dppf)Cl_2.CH_2Cl_2$ (163 mg, 0.20 mmol, 0.10 equiv), sodium carbonate (636 mg, 6.00 mmol, 3.00 equiv). The resulting solution was stirred for 20 h at 100 °C. The resulting solution was diluted with 30 mL of water and extracted with ethyl acetate (3 × 30 mL). The organic layers combined and dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with dichloromethane/methanol (from 40:1 to 10:1). This resulted in 280 mg (47%) of 4-(2-aminobenzo[*d*]thiazol-6-yl)-1-(cyclopropylmethyl)pyridin-2(1*H*)-one as a solid.
m.p. = 271.5-272 °C; HRMS (*m/z*) $[M]^+$ calcd. for $C_{16}H_{15}ON_3S$, 298.10086; found, 298.10071; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.47 - 0.53 (m, 4H), 1.18 - 1.31 (m, 1H), 3.76 (d, 2H), 6.61 (dd, 1H), 6.66 (d, 1H), 7.40 (d, 1H), 7.60 (dd, 1H), 7.69 (s, 2H), 7.78 (d, 1H), 8.10 (d, 1H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 167.8, 161.6, 154.0, 150.3, 138.6, 131.9, 129.1, 124.2, 119.2, 114.1, 103.8, 51.8, 10.7, 3.2

Biology

Compound Handling

All compounds or DMSO for the PI4Kα, PI4Kβ, PIP5Kγ and PI3Kα biochemical, and IP1 cell-based, assays were dispensed from source plates containing compounds at 10 mM in 100% (v/v) DMSO or 100% DMSO, directly into assay plates using an ECHO 555 Acoustic dispenser (Labcyte Inc™, Sunnyvale, CA, USA).

PI4Kα and PI4Kβ ADP-Glo Assays

Assays were performed with the ADP-Glo™ Kinase Assay Kit (Promega, Madison, WI, USA), in Greiner 384-well white low volume plates in a 5 µL reaction volume consisting of 20 mM Tris-Bis Propane (pH 7.5), 10 mM $MgCl_2$, 0.5 mM EGTA, 1 mM DTT, 0.075 mM Triton X100, 1.5% DMSO with or without an inhibitor at varying concentrations, D-myo-Phosphatidylinositol (PI) substrate (Echelon Biosciences, Salt Lake City, UT, USA), ATP and purified enzyme. The PI4Kα assay was performed with

1 nM PI4KCA (Millipore, Dundee, UK), 72 µM ATP (K_{Mapp}^{ATP}) and 25 µM PI (K_{Mapp}^{PI}). The PI4Kβ assay was performed with 2 nM PI4KCB (SignalChem, UK), 220 µM ATP (K_{Mapp}^{ATP}) and 50 µM PI ($<K_{Mapp}^{PI}$). The assay was allowed to proceed for 45 min at ambient temperature before stopping the reaction by the addition of 5 µl of ADP-Glo reagent. Plates were then covered and incubated for 40 min at ambient temperature. 10 µl Kinase Detection Reagent was then added and plates were incubated for 30 min, before the luminescence signal was read with a PHERAstar plate reader (BMG Labtech GmbH, Offenburg, Germany).

PIP5Kγ ADP-Glo Assay

The PIP5Kγ assay was performed with the ADP-Glo™ Kinase Assay Kit (Promega, Madison, WI, USA), in Greiner 384-well white low volume plates in a 5 µL reaction volume consisting of 20 mM Tris-Bis Propane (pH 7.0), 10 mM $MgCl_2$, 0.5 mM EGTA, 1 mM DTT, 0.024 mM Triton X100, 1.5% DMSO with or without an inhibitor at varying concentrations, 14 µM D-*myo*-Phosphatidylinositol 4-phosphate (PIP) substrate ($<K_{Mapp}^{PI(4)P}$) (Echelon Biosciences, Salt Lake City, UT, USA), 20 µM ATP (K_{Mapp}^{ATP}) and PIP5K1C (expressed, purified and used at a 1:750 dilution, 4 nM total concentration). Assay incubations, additions and reads were performed as described for the PI4Kα and PI4Kβ ADP-Glo assays.

PI3Kα ATP-Glo Assay

The PI3Kα assay was performed using the Kinase-Glo® Plus Luminescence Assay Kit (Promega, Madison, WI, USA) in Greiner 384-well white low volume plates. 20 nM PIK3CA (expressed and purified by AZ) in phosphorylation buffer consisting of 50 mM Tris (pH7.4), 0.05% CHAPSO, 10 mM $MgCl_2$ and 2.1 mM DTT was pre-incubated with or without an inhibitor at varying concentrations for 20 min, before adding 80 µM phosphatidylinositol 4, 5-bisphosphate (PIP2) substrate (Cayman Chemicals, Ann Arbor, MI, USA) and 8 µM ($<K_{Mapp}^{ATP}$) ATP, to give a final assay volume of 6 µl (2% v/v DMSO assay final). The assay was allowed to proceed for 80 min at ambient temperature, before the assay was stopped with the addition of 4 µl Kinase Glo® Plus reagent. Plates were then covered and incubated for 20 min before the luminescence signal was read with a PHERAstar plate reader (BMG Labtech GmbH, Offenburg, Germany).

IP1 Cellular Assay

The IP1 cell-based assay was performed using the HTRF IP-One Tb kit (CisBio, Bedford, MA, USA) in Greiner 384-well TC-treated white low volume plates. NIH3T3 cells, stably transfected using SV40 with the PDGFRβ receptor, were cultured in DMEM, 10% Fetal Bovine Serum, 1% Glutamax and 2 ng/ml Puromycin. Cells were starved 24 hr prior to use in DMEM, 1% Charcoal Stripped Fetal Bovine Serum and 1% Glutamax. After harvesting, cells were resuspended at a final concentration of 1.25 x 10^6 cells/ml in IP1 Cell Stimulation Buffer, and 8 µl was dispensed into each well of the assay plate pre-dosed with compound or DMSO. After incubation for 30 min at 37 °C, cells were then stimulated with 150 nM PDGF (Sigma-Aldrich, St Louis, MO, USA) dispensed using an ECHO 555. A standard curve IP1 calibration plate was prepared according to the manufacturer's instructions. After incubation for 30 min at 37 °C, cells were lysed with the addition of 3 µl Lysis Buffer with IP-One d2 (1:20 dilution), and 3 µl Lysis Buffer with anti-IP-One Tb cryptate (1:20 dilution). Plates were covered and incubated for 2 hr at ambient temperature, before a HTRF read at 615 nm and 665 nm on an EnVision plate reader (Perkin Elmer, Waltham, MA, USA). Assay values were then normalised to the IP1 calibration curve.

Kinases tested with 3 and 7 in Milipore panel

ABL1, ABL2, ACVR1B, AKT1, AKT2, AKT3, ALK, AURKA, AURKB, AURKC, AXL, BLK, BMX, BRAF, BRSK1, BRSK2, BTK, CaMK1, CAMK1d, CAMK2B, CAMK2d, CAMK2g, CAMK4, CDC42BPa, CDC42BPb, CDK1/CCNB1, CDK2/CCNA2, CDK2:CE, CDK3, CDK5:p25, CDK5:p35, CDK6/CCND3, CDK7/CCNH/MNAT1, CDK9/CCNT1, CHEK1, CHK2, CHUK, CK2, CLK1, CLK2, CLK3, CLK4, CSF1R, CSK, CSNK1d, CSNK1G1, CSNK1g2, CSNK1g3, CSNK2A2, DAPK1, DAPK2, DCAMKL2, DDR2, DMPK, DYRK2, EEF2K, EGFR T790M L858R, EGFR, EIF2AK3, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA7, EPHA8,EPHB1, EPHB2, EPHB3, EPHB4, ERBB4, FER, FES, FGFR1 V561M, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRAP1 FKBP12, FYN, FYN, GRK5, GRK6, GRK7, GSG2, GSK3A, GSK3B, HCK, HCK, HIPK1, HIPK2, HIPK3, IGF1R, IGF1R, IKBKB, IKBKE, INSR, INSR, INSRR, IRAK1, IRAK4, ITK, JAK1, JAK2, JAK3, JNK3, KDR, KIT, LCK, LCK, LIMK1, LRRK2, LYN, MAP2K1, MAP2K6, MAP2K7, MAP3K5, MAP3K7, MAP3K9, MAP4K2, MAPK1, MAPK13, MAPK14, MAPK3, MAPK8, MAPK9, MAPKAP3, MAPKAPK1B, MAPKAPK2, MARK1, MARK2, MELK, MERTK, MET, MINK1, MKNK2, MST1R, MTOR, MuSK, MYLK, NEK11, NEK2, NEK3, NEK6, NEK7, NLK, NTRK1, NTRK3, NUAK1, p38b, p38g, PAK1, PAK2, PAK4, PAK6, PAK7, PASK, PDGFRa, PDGFRB, PDPK1, PHKG2, PIK3C2A, PIK3C2G, PIK3CA p85a, PIK3CB p85a, PIK3CD p85a,PIK3CG p120, PIM1, PIM2, PIM3, PIP4K2A, PIP5K1A, PIP5K1C, PKCdelta, PKC mu, PKCb, PKCb, PKCg, PKCi, PKCRK2, PKD2, PKG1, PKX, PLK1, PLK2, PLK3, PRAK, PRKAA1 (a1,b1,g1), PRKAA2 (a2,b1,g1), PRKACA, PRKCA, PRKCE, PRKCH, PRKCH, PRKCQ, PRKG1, PTK2, PTK2b, PTK6, RAF1, RET V804L, RET V804M, RET, RIPK2, ROCK1, ROCK2, ROS1, RPS6KA1, RPS6KA2, RPS6KA4, RPS6KA5, RPS6KA6, RPS6KB1, SGK1, SGK2, SGK3, SIK1, SIK1, SRC 1-530, SRC, SRPK1, SRPK2, STE20, STK10, STK10, STK11, STK17A, STK23, STK24, STK33, STK4, SYK, TAO1, TAO2, TAO3, TBK1, TEC, TGFBR1, TIE2, TLK2, TNK2, TSSK1B, TSSK2, TXK, TYK2, TYRO3, ULK2, ULK3, VRK2, WEE1, WNK2, WNK3, YES1, ZAP70, ZIPK.

Lipid mass spectrometry analysis

The Lipid extraction and derivitisation was carried out as per Clark J et al, Nature Methods 2011, B, 267.

Analysis of the derivatised lipids was performed on an API4000 mass spectrometer (ABSciex), connected to an Ultimate 3000 RS pump (Thermo Fisher Scientific) with 20ul of sample injected. The RS pump was connected to the API4000 flowing at 300ul/minute, 45% Buffer B. Buffer A was 0.1% formic acid and Buffer B was100% acetonitrile 0.1% formic acid. A 45% to 100 % B gradient over 10 minutes was used with a Waters ACQUITY UPLC BEH C4 (1.7um 2.1 x 100mm) column.

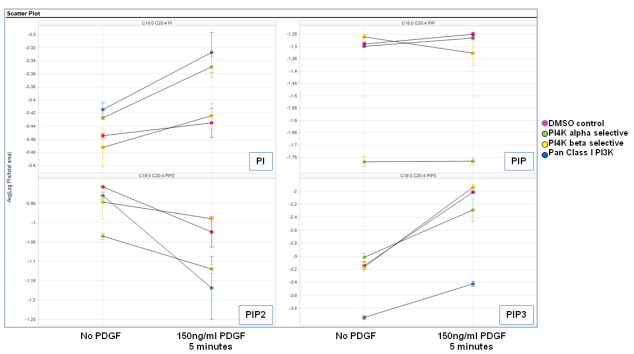
The API400 was set up with the Turbo Ion source working in

positive ion mode. The following settings were used: Curtain gas 10, GS1 30, GS2 50, TEM 150oC, ISV 4500V, Q1 resolution UNIT, Q2 resolution LOW, Collision Energy 40.

The following MRM method was used which consisted of transitions for the methylated species of PI, PIP, PIP2 and PIP3 (Internal standard and C18:0 20:4 species shown)

Q1 mass	Q3 mass	Dwell time (msec)	
1225.57	627.60	50.00	C18:0C20:4 PtdInsP3
1117.22	627.60	50.00	C18:0C20:4 PtdInsP2
901.57	627.60	50.00	C18:0C20:4 PtdIns
1009.57	627.60	50.00	C18:0C20:4 PtdInsP
1165.6	565.6	50.00	C16:0C17:0 PIP_3 Internal standard

Following analysis the raw data files were transferred to MultiQuant V2.0.2 software (ABSciex) here the peak integration was manually verified. The peak areas were transferred to Excel for further analysis. The %CV was calculated for the pooled QC sample to assess the technical reproducibility; this is was less than 10%. To normalise the peak areas of the samples, the median of the internal standard peak area was determined and this was then converted into a normalisation factor for each individual sample. This factor was then multiplied with the peak areas for every PI transition within the sample. This figure was then divided by the total load for the sample and the Log value taken. The data was then visualised in Spotfire.



Mass spectrometry data of arachidonyl species of PI, PIP, PIP_2 and PIP_3 (C18:0 C20:4). Data plotted as Log PIx/total load area

Compound 3 cell lines and pGI_{50} values

SK-HEP-1, 6.1; Molm 13, 5.9; IM-9, 5.7; AMO-1, 5.7; JJN-3, 5.5; OCI-LY-19, 5.4; RS411, 5.4; Hep3B, 5.4; MV-4-11, 5.4; HCC1954, 5.2; OCI-AML2, 5.2; MDA-MB-231, 5.2; L-363, 5.2; SNU-5, 5.2; JVM-3, 5.2; MOLP-8, 5.2; KG-1, 5.0; ARH-77, 5.0; T47D, <5.0; SK-CO-1, <5.0; DMS 114, <5.0; PANC-1, <5.0; NCI-H1703, <5.0; SNU-620, <5.0; SNU-878, <5.0; NCI-H526, <5.0; SW48, <5.0; JIMT-1, <5.0; NCI-H1869, <5.0; MDA-MB-468, <5.0; HCC1806, <5.0; RKO, <5.0; NCI-H2286, <5.0; LUDLU-1, <5.0; SNU-354, <5.0; NIH:OVCAR-3, <5.0; HCC1569, <5.0; GTL-16, <5.0; AZ521, <5.0; NCI-H23, <5.0; KATO III, <5.0; CCK-81, <5.0; MDA-MB-157, <5.0; Hel92.1.7, <5.0; MGH-U3, <5.0; SNU-668, <5.0; BEL7405, <5.0; RERF-LC-Sq1, <5.0; HCC1187, <5.0; C-99, <5.0; MHCC97L, <5.0; HCC95, <5.0; SNU-601, <5.0; RPMI-8226, <5.0; AGS, <5.0; BT-20, <5.0; NCI-H522, <5.0; SW480, <5.0; PC9, <5.0; SNU-449, <5.0; PC-3, <5.0; SK-MES-1, <5.0; HX147, <5.0; NCI-H322, <5.0; LS 180, <5.0; SW403, <5.0; HRA-19, <5.0; SW780, <5.0; SNU-398, <5.0; NCI-H647, <5.0; MIA PaCa-2, <5.0; LOVO, <5.0; BFTC-905, <5.0; SW620, <5.0; NCl-N87, <5.0; 1A6 [PTA-556], <5.0; NCI-H520, <5.0; MDA-MB-436, <5.0; HuH-7, <5.0; QGY7703, <5.0; HCCC9810, <5.0; EBC-1, <5.0; NCI-H2126, <5.0; SNU-484, <5.0; SNU-1, <5.0; DU 145, <5.0; SW948, <5.0; CMK, <5.0; A2058, <5.0; Jurkat, <5.0; SNU-638, <5.0; HT-29, <5.0; HCC1419, <5.0; NCI-H1299, <5.0; LNCap-CasRes(AZ), <5.0; OE33, <5.0; BT-549, <5.0; 23132/87, <5.0; Calu-6, <5.0; COLO 320DM, <5.0; HCC1937, <5.0; HuH-1, <5.0; 22Rv1, <5.0; 5637, <5.0; 647V, <5.0; 97-7, <5.0; A549, <5.0; BEL7404, <5.0; Calu-1, <5.0; Calu-3, <5.0; CAMA-1, <5.0; CC20, <5.0; COLO 205, <5.0; HARA, <5.0; HCA-7, <5.0; HCC1395, <5.0; HCC-15, <5.0; HCT 116, <5.0; HCT-15, <5.0; HCT-8, <5.0; HepG2, <5.0; HGC27, <5.0; HLE, <5.0; HLF, <5.0; HS746T, <5.0; HT1197, <5.0; HT1376, <5.0; IM95m, <5.0; J82, <5.0; JEKO-1, <5.0; K-562, <5.0; KU-19-19, <5.0; LK-2, <5.0; LNCaP, <5.0; MCF7, <5.0; MCF7/mdr+, <5.0; MDA-MB-453, <5.0; MEC-1, <5.0; MKN1, <5.0; MKN74, <5.0; Molm 16, <5.0; MonoMac6, <5.0; NAMALWA, <5.0; NCI-H1437, <5.0; NCI-H1793, <5.0; NCI-H1975, <5.0; NCI-H2085, <5.0; NCI-H2170, <5.0; NCI-H226, <5.0; NCI-H2291, <5.0; NCI-H358, <5.0; NCI-H460, <5.0; NCI-H460 DNp53, <5.0; NCI-H596, <5.0; NCI-H838, <5.0; Nomo-1, <5.0; NUGC-3, <5.0; NUGC-4, <5.0; OCUM-1, <5.0; OE19, <5.0; PAMC82, <5.0; PNT1A, <5.0; Raji, <5.0; Ramos, <5.0; Reh, <5.0; RERF-LC-AI, <5.0; RT112/84, <5.0; RT4, <5.0; SC-1, <5.0; SCaBER, <5.0; SK-BR-3, <5.0; SMMC7721, <5.0; SNU-16, <5.0; SNU-216, <5.0; SNU-368, <5.0; SNU-739, <5.0; SNU-761, <5.0; SNU-886, <5.0; SUM52PE, <5.0; SW, <5.0; SW1710, <5.0; T24, <5.0; TCC-SUP, <5.0.

Compound 7 cell lines and pGI_{50}s

SK-HEP-1, <5.0; Molm 13, 5.2; IM-9, <5.0; AMO-1, <5.0; JJN-3, <5.0; OCI-LY-19, <5.0; RS411, <5.0; Hep3B, 5.1; MV-4-11, 5.1; HCC1954, <5.0; OCI-AML2, <5.0; MDA-MB-231, 5.4; L-363, 5.4; SNU-5, <5.0; JVM-3, <5.0; MOLP-8, 5.3; KG-1, 5.2; ARH-77, <5.0; T47D, 5.8; SK-CO-1, 5.8; DMS 114, 5.7; PANC-1, 5.7; NCI-H1703, 5.6; SNU-620, 5.6; SNU-878, 5.5; NCI-H526, 5.5; SW48, 5.5; JIMT-1, 5.5; NCI-H1869, 5.5; MDA-MB-468, 5.4; HCC1806, 5.4; RKO, 5.4; NCI-H2286, 5.4; LUDLU-1, 5.4; SNU-354, 5.4; NIH:OVCAR-3, 5.4; HCC1569, 5.4; GTL-16, 5.3; AZ521, 5.3; NCI-H23, 5.3; KATO III, 5.3; CCK-81, 5.3; MDA-MB-157, 5.3; Hel92.1.7, 5.3; MGH-U3, 5.3; SNU-668, 5.3; BEL7405, 5.3; RERF-LC-Sq1, 5.3; HCC1187, 5.2; C-99, 5.2; MHCC97L, 5.2; HCC95, 5.2; SNU-601, 5.2; RPMI-8226, 5.2; AGS, 5.2; BT-20, 5.2; NCI-H522, 5.2; SW480, 5.2; PC9, 5.2; SNU-449, 5.2; PC-3, 5.1; SK-MES-1, 5.1; HX147, 5.1; NCI-H322, 5.1; LS 180, 5.1; SW403, 5.1; HRA-19, 5.1; SW780, 5.1; SNU-398, 5.1; NCI-H647, 5.1; MIA PaCa-2, 5.1; LOVO, 5.1; BFTC-905, 5.1; SW620, 5.1; NCl-N87, 5.1; 1A6 [PTA-556], 5.1; NCI-H520, 5.1; MDA-MB-436, 5.1; HuH-7, 5.1; QGY7703, 5.1; HCCC9810, 5.1; EBC-1, 5.1; NCI-H2126, 5.1; SNU-484, 5.1; SNU-1, 5.1; DU 145, 5.1; SW948, 5.1; CMK, 5.1; A2058, 5.1; Jurkat, 5.0; SNU-638, 5.0; HT-29, 5.0; HCC1419, 5.0; NCI-H1299, 5.0; LNCap-CasRes(AZ), 5.0; OE33, 5.0; BT-549, 5.0;

23132/87, 5.0; Calu-6, 5.0; COLO 320DM, 5.0; HCC1937, 5.0; HuH-1, 5.0; 22Rv1, <5.0; 5637, <5.0; 647V, <5.0; 97-7, <5.0; A549, <5.0; BEL7404, <5.0; Calu-1, <5.0; Calu-3, <5.0; CAMA-1, <5.0; CC20, <5.0; COLO 205, <5.0; HARA, <5.0; HCA-7, <5.0; HCC1395, <5.0; HCC-15, <5.0; HCT 116, <5.0; HCT-15, <5.0; HCT-8, <5.0; HepG2, <5.0; HGC27, <5.0; HLE, <5.0; HLF, <5.0; HS746T, <5.0; HT1197, <5.0; HT1376, <5.0; IM95m, <5.0; J82, <5.0; JEKO-1, <5.0; K-562, <5.0; KU-19-19, <5.0; LK-2, <5.0; LNCaP, <5.0; MCF7, <5.0; MCF7/mdr+, <5.0; MDA-MB-453, <5.0; MEC-1, <5.0; MKN1, <5.0; MKN74, <5.0; Molm 16, <5.0; MonoMac6, <5.0; NAMALWA, <5.0; NCI-H1437, <5.0; NCI-H1793, <5.0; NCI-H1975, <5.0; NCI-H2085, <5.0; NCI-H2170, <5.0; NCI-H226, <5.0; NCI-H2291, <5.0; NCI-H358, <5.0; NCI-H460, <5.0; NCI-H460 DNp53, <5.0; NCI-H596, <5.0; NCI-H838, <5.0; Nomo-1, <5.0; NUGC-3, <5.0; NUGC-4, <5.0; OCUM-1, <5.0; OE19, <5.0; PAMC82, <5.0; PNT1A, <5.0; Raji, <5.0; Ramos, <5.0; Reh, <5.0; RERF-LC-AI, <5.0; RT112/84, <5.0; RT4, <5.0; SC-1, <5.0; SCaBER, <5.0; SK-BR-3, <5.0; SMMC7721, <5.0; SNU-16, <5.0; SNU-216, <5.0; SNU-368, <5.0; SNU-739, <5.0; SNU-761, <5.0; SNU-886, <5.0; SUM52PE, <5.0; SW, <5.0; SW1710, <5.0; T24, <5.0; TCC-SUP, <5.0.