Synthesis, in vitro and in vivo evaluation of Phosphoinositide-3-Kinase inhibitors

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Supporting Information

Contents

General experimental procedures.

The compounds and/or intermediates were characterized by high performance liquid chromatography (HPLC) using a Waters Millenium chromatography system with a 2695 Separation Module (Milford, MA). The analytical columns were Alltima C-18 reversed phase, 4.6 x 50 mm, flow 2.5 mL/min, from Alltech (Deerfield, IL). A gradient elution was used, typically starting with 5% acetonitrile/95% water and progressing to 100% acetonitrile over a period of 10 minutes. All solvents contained 0.1% trifluoroacetic acid (TFA). Compounds were detected by ultraviolet light (UV) absorption at either 220 or 254 nm. HPLC solvents were from Burdick and Jackson (Muskegan, MI), or Fisher Scientific (Pittsburgh, PA). In some instances, purity was assessed by thin layer chromatography (TLC) using glass or plastic backed silica gel plates, such as, for example, Baker-Flex Silica Gel 1B2-F flexible sheets. TLC results were readily detected visually under ultraviolet light, or by employing well known iodine vapor and other various staining techniques.

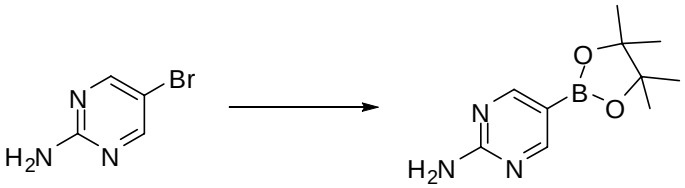
Mass spectrometric analysis was performed on one of two LCMS instruments: a Waters System (Alliance HT HPLC and a Micromass ZQ mass spectrometer; Column: Eclipse XDB-C18, 2.1 x 50 mm; solvent system: 5-95% (or 35-95%, or 65-95% or 95-95%) acetonitrile in water with 0.05% TFA; flow rate 0.8 mL/min; molecular weight range 200-1500; cone Voltage 20 V; column temperature 40 °C) or a Hewlett Packard System (Series 1100 HPLC; Column: Eclipse XDB-C18, 2.1 x 50 mm; solvent system: 1-95% acetonitrile in water with 0.05% TFA; flow rate 0.8 mL/min; molecular weight range 150-850; cone Voltage 50 V; column temperature 30 °C). All masses were reported as those of the protonated parent ions. High resolution mass spectra were obtained on a Q-STAR quadrupole-TOF-MS (Applied Biosystems Inc.) in ESI+ mode or on a Synapt G2 MS (Waters Corp.) in ESI+ mode.

Nuclear magnetic resonance (NMR) analysis was performed on some of the compounds with a Varian 300 MHz NMR (Palo Alto, CA). The spectral reference was either TMS or the known chemical shift of the solvent. Some compound samples were run at elevated temperatures (e.g., 75 °C) to promote increased sample solubility.

Preparative separations were carried out using a Flash 40 chromatography system and KP-Sil, 60A (Biotage, Charlottesville, VA), or by flash column chromatography using silica gel (230-400 mesh) packing material, or by HPLC using a Waters 2767 Sample Manager, C-18 reversed phase column, 30X50 mm, flow 75 mL/min. Typical solvents employed for the Flash 40 Biotage system and flash column chromatography were dichloromethane, methanol, ethyl acetate, hexane, acetone, aqueous ammonia (or ammonium hydroxide), and triethyl amine. Typical solvents employed for the reverse phase HPLC were varying concentrations of acetonitrile and water with 0.1% trifluoroacetic acid.

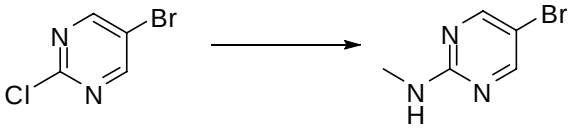
Synthetic experimental procedures.

5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine-2-ylamine



To a dry 500-mL flask was added 2-amino-5-bromopyrimidine (10 g, 57.5 mmol), potassium acetate (16.9 g, 172 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2yl)-1,3,2-dioxaborolane (16.1 g, 63.0 mmol) and dioxane (300 mL). Argon was bubbled through the solution for 15 minutes, at which time dichloro[1,1'-bis(diphenylphosphino)ferrocene] palladium (II) dichloromethane adduct ($Pd(dppf)Cl_2$ CH_2Cl_2) (2.34 g, 2.87 mmol) was added. The reaction mixture was refluxed in a 115 °C oil bath for 4 hours under argon. After cooling to room temperature, EtOAc (500 mL) was added and the resulting slurry was sonicated and filtered. Additional EtOAc (500 mL) was used to wash the solid. The combined organic extracts were washed with H_2O (2x300 mL), $NaCl_{(sat.)}$ (300 mL), dried over Na_2SO_4, and filtered through a 5 cm pad of silica gel. Additional EtOAc was used to flush product. After the solvent was concentrated, the crude was treated with a mixture of 1:3 dichloromethane and hexane (40 mL), filtered and washed with hexane yielding a light yellow solid (8.5 g, 75%). LCMS (*m/z*): 140 (MH^+ of boronic acid, deriving from product hydrolysis on LC). ^1H NMR ($CDCl_3$): δ 8.58 (s, 2H), 5.74 (s, 2H), 1.32 (s, 12H).

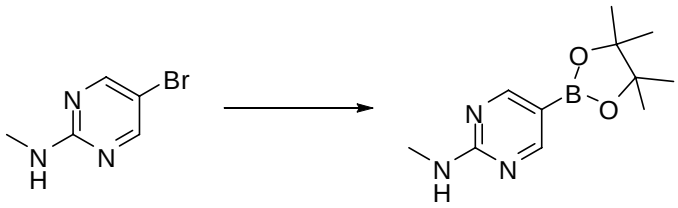
2-Aminomethyl-5-bromopyrimidine



Methylamine (2.0 M in methanol, 40 mL, 80 mmol) was added to 5-bromo-2-chloropyrimidine (5.6 g, 29.0 mmol) in a sealable reaction vessel. After allowing to vent for a few minutes, the vessel was sealed, placed behind a safety shield and heated in a 115 °C oil bath for 48 hours. Upon cooling the volatiles were removed *in vacuo*. The

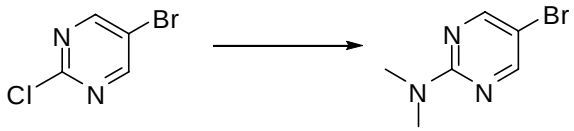
material was dissolved in CH_2Cl_2 (200 mL) and washed with 1M NaOH (40 mL). The aqueous layer was extracted further with CH_2Cl_2 (2x50 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated yielding an off white solid (5.1 g, 93%). LCMS (*m/z*): 188.0/190.0 (MH^+).

methyl[5-(4,4,5,5-tetramethyl(1,3,2-dioxaborolan-2-yl))pyrimidin-2-yl]amine



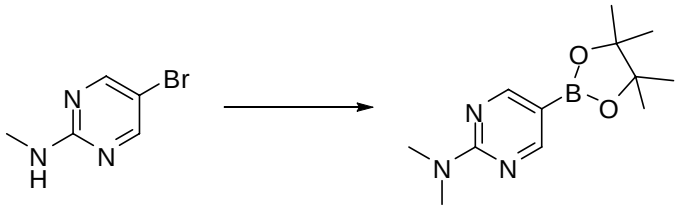
To a dry 500 mL flask was added 2-methylamino-5-bromopyrimidine (9.5 g, 50.5 mmol), potassium acetate (15.1 g, 154.4 mmol), 4,4,5,5,-tetramethyl-2-(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (14.1 g, 55.5 mmol) and dioxane (280 mL). Argon was bubbled through the solution for 15 minutes, at which time 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride dichloromethane adduct (2.05 g, 2.51 mmol) was added. The reaction was refluxed in a 115 °C oil bath for 4 hours under argon. After cooling to room temperature, EtOAc (500 mL) was added and the resulting slurry was sonicated and filtered. Additional EtOAc (500 mL) was used to wash the solid. The combined organics were washed with H_2O (2x300 mL), $NaCl_{(sat.)}$, (300 mL), dried over Na_2SO_4, filtered and the solvents were removed *in vacuo*. Purification by SiO_2 chromatography (50% EtOAc/hexanes) yielded an off white solid (7.66 g, 64%). LCMS (*m/z*): 154 (MH^+ of boronic acid, deriving from *in situ* product hydrolysis on LC). ^1H NMR ($CDCl_3$): δ 8.58 (s, 2H), 5.56 (s, 1H), 3.02 (d, 3H), 1.32 (s, 12H).

5-bromo-N,N-dimethylpyrimidin-2-amine



Dimethylamine (5.6 M in Ethanol, 15 mL, 84 mmol) was added to 5-bromo-2-chloropyrimidine (1.0 g, 5.15 mmol) in a sealable reaction vessel. After allowing to vent for a few minutes, the vessel was sealed, placed behind a safety shield and heated in a 115 °C oil bath for 48 hours. Upon cooling the volatiles were removed *in vacuo*. The material was dissolved in CH_2Cl_2 (200 mL) and washed with 1M NaOH (40 mL). The aqueous layer was extracted further with CH_2Cl_2 (2x50 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated yielding an off white solid (1.01 g, 97%). LCMS (*m/z*): 202.0/204.0 (MH^+).

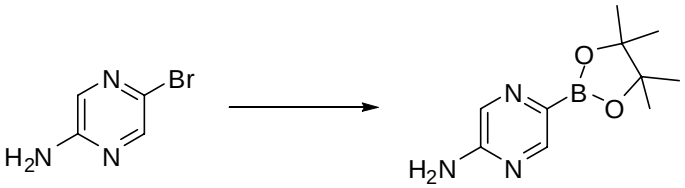
N,N-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine



To a dry 500 mL flask was added 2-methylamino-5-bromopyrimidine (500 mg, 2.5 mmol), potassium acetate (729 mg, 7.4 mmol), 4,4,5,5,-tetramethyl-2-(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (691 mg, 2.7 mmol) and dioxane (15 mL). Argon was bubbled through the solution for 15 minutes, at which time 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride dichloromethane adduct (91 mg, 0.12 mmol) was added. The reaction was refluxed in a 115 °C oil bath for 4 hours under argon. After cooling to room temperature, the volatiles were removed *in vacuo* and the residue was dissolved in EtOAc (200 mL) and washed with H_2O (2x50 mL), $NaCl_{(sat.)}$, (300 mL), dried over Na_2SO_4, filtered and the solvents were removed *in vacuo*. Purification by SiO_2 chromatography (25% EtOAc/hexanes) yielded an off white solid

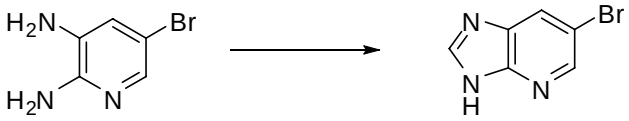
(219 g, 35%). LCMS (*m/z*): 168 (MH^+ of boronic acid, deriving from *in situ* product hydrolysis on LC).

5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazin-2-amine



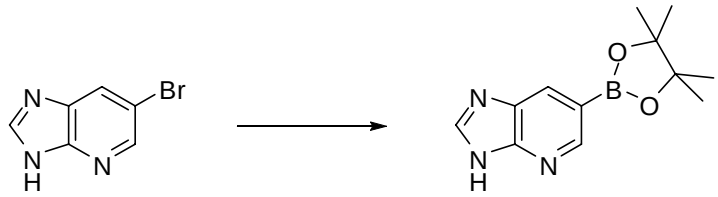
To a mixture of 5-bromopyrazin-2-amine (500 mg, 2.9 mmol), potassium acetate (846 mg, 8.6 mmol), 4,4,5,5,-tetramethyl-2-(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (949 mg, 3.7 mmol) and 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride dichloromethane adduct (117 mg, 0.14 mmol) was added dioxane (4 mL). The mixture was heated in a microwave at 120 °C for 3x20 minutes. After cooling to room temperature, the solids were filtered off and the volatiles were removed *in vacuo* to yield crude boronate ester which was used as is. LCMS (*m/z*): 140.0 (MH^+ of boronic acid, deriving from *in situ* product hydrolysis on LC).

6-bromo-3H-imidazo[4,5-b]pyridine



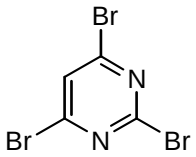
To a slurry of 5-bromopyridine-2,3-diamine (1.0 grams, 5.3 mmoles) in trimethylorthoformate (10 mL) was added $HCl_{(conc.)}$ (1 mL). The resulting solution was refluxed for 16 hours. Upon cooling to rt, H_2O (10 mL) was added, and after adjusting to pH=8 by the addition of 2M NaOH the solution was extracted with EtOAc (2x50 mL) and with CH_2Cl_2 (50 mL). The combined organics were dried over Na_2SO_4, filtered, concentrated and pumped on to yield 6-bromo-3H-imidazo[4,5-b]pyridine (988 mg, 89%). LC/MS (*m/z*): 316.8/318.7 (MH^+).

6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-imidazo[4,5-b]pyridine



To a solution of 6-bromo-3H-imidazo[4,5-b]pyridine (88 mg, 0.44 mmoles) in DMF (3 mL) was added potassium acetate (131 mg, 1.3 mmol), 4,4,5,5,-tetramethyl-2-(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (147 mg, 0.59 mmol) and 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride dichloromethane adduct (0.36 mg, 0.04 mmol). The solution was heated in a microwave at 120 ^{o}C for 10 minutes and at 130 ^{o}C for 15 minutes. Analysis by LC/MS indicated formation of boronate ester product along with SM bromide. LCMS (*m/z*): 164.1 (MH^+ of boronic acid, deriving from *in situ* product hydrolysis on LC). The solution was used directly in subsequent Suzuki reactions.

2,4,6-tribromopyrimidine

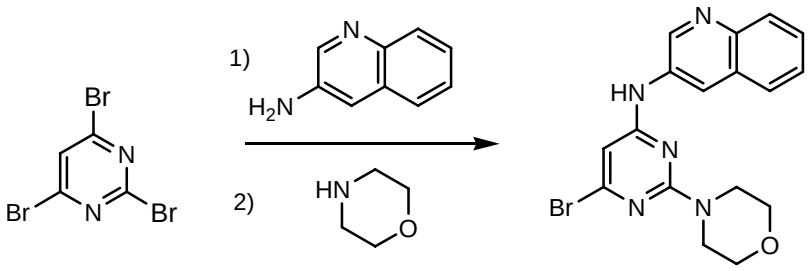


To a mixture of pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (2.66 g, 20.87 mmol) and $POBr_3$ (25 g, 87.2 mmol) in toluene (35 mL) in a 200 mL flask, *N*,*N*-dimethylaniline (4.52 mL, 35.7 mmol) was added. The brick-red slurry was heated to reflux for 3 hours. During the process a biphasic solution formed with a red gum at the bottom of the flask and a clear yellow liquid above. The reaction mixture was cooled to room temperature and the yellow organic layer decanted off. The red gum was rinsed once with EtOAc. The combined organic extracts were washed with saturated $NaHCO_3$ (3x, or until CO_2 evolution ceased), H_2O (3x), brine (2x) and dried over Na_2SO_4. The solution was then concentrated and dried under high vacuum to yield 2,4,6-tribromopyrimidine (5.40 g,

82%), which was used without further purification. LC/MS (*m/z*): 316.8/318.7 (MH^+), R_t 2.78 minutes.

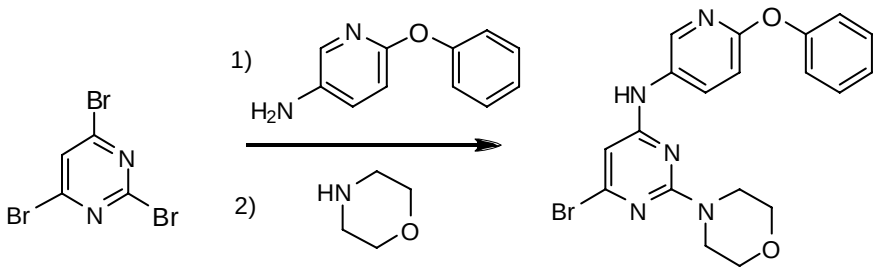
General Procedure 1

N-(6-bromo-2-morpholinopyrimidin-4-yl)quinolin-3-amine



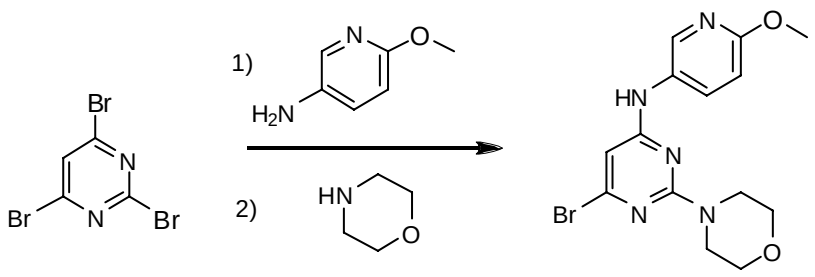
To a solution of 2,4,6-tribromopyrimidine (5.40 g, 17.2 mmol) in acetonitrile (60 mL) was added quinolin-3-amine, followed by DIEA (8.99 mL, 51.6 mmol). The reaction mixture was heated to 45 ºC overnight. Morpholine (1.50 mL, 17.2 mmol) was then added, and the reaction mixture continued heating for 4 h. The reaction mixture was then cooled to room temperature, concentrated and dissolved in EtOAc (about 500 mL), the organic solution was washed with saturated $NaHCO_3$ (3x), H_2O (2x), brine (1x) and dried over Na_2SO_4. The solution was then evaporated in the presence of silica gel and purified by column chromatography (SiO_2, 15-25% EtOAc/Hexanes) to yield *N*-(6-bromo-2-morpholinopyrimidin-4-yl)quinolin-3-amine. LC/MS (*m*/z): 386.1 (MH^+).

6-bromo-2-morpholino-N-(6-phenoxypyridin-3-yl)pyrimidin-4-amine



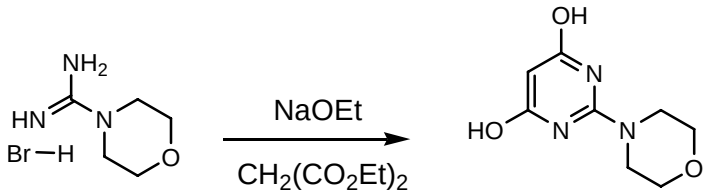
Following General Procedure 1, 6-bromo-2-morpholino-N-(6-phenoxypyridin-3-yl)pyrimidin-4-amine was prepared. LC/MS (*m*/*z*): 428.1 (MH^+).

6-bromo-N-(6-methoxypyridin-3-yl)-2-morpholinopyrimidin-4-amine



Following General Procedure 1, 6-bromo-N-(6-methoxypyridin-3-yl)-2-morpholinopyrimidin-4-amine was prepared. LC/MS (*m*/*z*): 366.1 (MH^+).

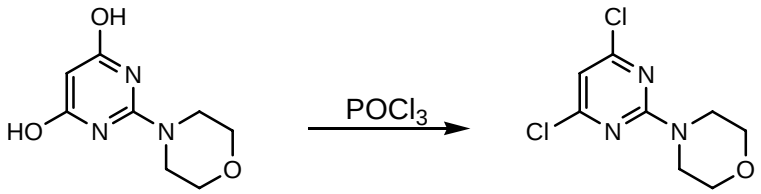
2-morpholinopyrimidine-4,6-diol



Sodium (17.25 g, 150 mmol) was cut into small pieces and slowly added to EtOH (500 mL) in a 1-L round bottom flask under N_2 and cooled with water. After all the sodium was dissolved, morpholinoformamidine hydrobromide (52.5 g, 50 mmol) and diethyl malonate (40 g, 50 mmol) were added. The mixture was heated to reflux for three hours. The reaction mixture was cooled to room temperature, and the ethanol was removed *in vacuo*. Aqueous HCl (1N, 800 mL) was added to the white solid, at room temperature. The solid initially dissolved, giving a clear solution, then the product crashed out as a white solid. After 1 h at room temperature, the solid was filtered,

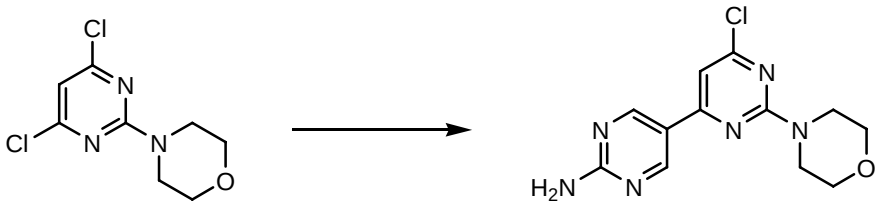
washed with water (3x), dried (air and then over P_2O_5) to give 2-morpholinopyrimidine-4,6-diol (42.5 g, 86%). LC/MS (*m/z*): 198.1 (MH^+), R_t 0.51 minutes.

4,6-dichloro-2-morpholinopyrimidine



A mixture of 2-morpholinopyrimidine-4,6-diol (30 g, 0.15 mol) and $POCl_3$ (150 mL, 1.61 mol) was heated at 120 ºC for 16 h, then cooled to RT. Excess $POCl_3$ was removed to give a semi-solid. The solid was gradually transferred to a stirring solution of water (700 mL) and EtOH (100 mL) occasionally cooled with water. White solid formed and was subsequently filtered, washed with water, 10% EtOH in water, and dried over P_2O_5 to give 4,6-dichloro-2-morpholinopyrimidine (17.82 g, 50%). LC/MS (*m/z*): 233.9 (MH^+), R_t 2.95 minutes.

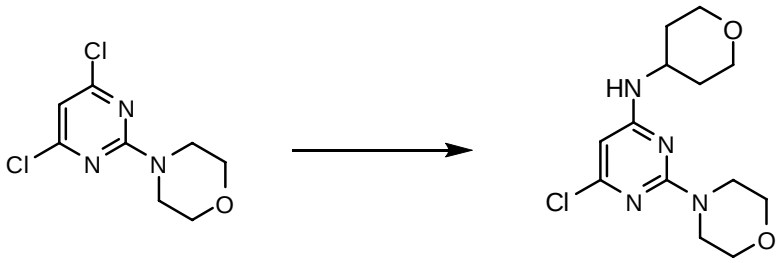
N-(6-(2-aminopyrimidin-5-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine



4,6-Dichloro-2-morpholinopyrimidine (3.0 g, 12.9 mmol) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine (3.43 g, 15.5 mmol) were dissolved in DME (130 mL). Aqueous Na_2CO_3 (2 M, 32 mL, 64 mmol) was then added and the reaction mixture was sparged with N_2 for several minutes. $Pd(OAc)_2$ (145 mg, 0.65 mmol) and PPh_3 (339 mg, 1.29 mmol) were then added and the reaction mixture was

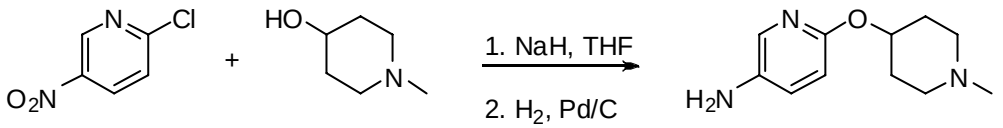
heated at 95 ºC for 1 h. The reaction mixture was allowed to cool to room temperature, the solution was decanted away from the solid residue and concentrated. The solid thus formed was separated from the water phase. The water extracted with EtOAc and this organic layer was combined with the precipitate. Removal of the solvent *in vacuo* gave a solid residue which was triturated with about 20 mL of EtOAc, filtered and evaporated under reduced pressure to give the desired product. Additional product was obtained by concentrating the mother liquor and purifying the solid crash out by trituration with EtOAc. The two crops were combined obtaining 1.98 g (52%) of N-(6-(2-aminopyrimidin-5-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine.
LC/MS (*m/z*): 293.1 (MH^+), R_t 1.92 minutes.

6-chloro-2-morpholino-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine



A solution of 4,6-dichloro-2-morpholinopyrimidine (150 mg, 0.64 mmoles), tetrahydro-2H-pyran-4-amine (128 mg, 1.28 mmoles) and DIEA (0.22 mL, 1.28 mmoles) in NMP (1 mL) was stirred for 5 days. The solution was diluted with EtOAc (100 mL) , washed with $NaHCO_{3\,(sat.)}$ (30 m mL), $NaCl_{(sat.)}$ (30 mL), dried over Na_2SO_4, filtered, concentrated and pumped to yield 6-chloro-2-morpholino-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine (193 mg, 100%) as a yellow solid. The material was used as is. LC/MS (*m/z*): 299.1 (MH^+).

6-(1-methylpiperidin-4-yloxy)pyridin-3-amine



To a mixture of sodium hydride (189 mg, 4.73 mmol) in anhydrous tetrahydrofuran (2 mL) at 0 °C, a solution of 2-chloro-5-nitropyridine (500 mg, 3.16 mmol) and 1-methylpiperidin-4-ol (455 mg, 3.96 mmol) in anhydrous tetrahydrofuran (4 mL) was added dropwise. The reaction was heated reflux for 16 h. The THF was evaporated and water (100 mL) and EtOAc (200 mL) were added. The aqueous layer was extracted with EtOAc (200 mL). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated to give a brown oil. Purification by silica gel column chromatography using 3% methanol/methylene chloride yielded 2-(1-methylpiperidin-4-yloxy)-5-nitropyridine as a yellow solid, (367 mg, 49%). LC/MS (*m*/z): 238.0 (MH^+).

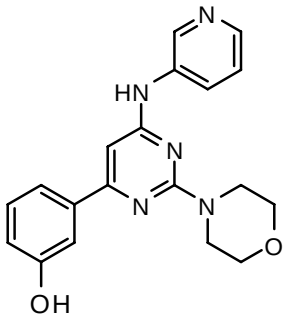
2-(1-Methylpiperidin-4-yloxy)-5-nitropyridine (100 mg, 0.42 mmol) was dissolved in 5 mL of methanol and placed under a nitrogen atmosphere. A catalytic amount of 10% palladium on carbon was added and a hydrogen balloon connected to the reaction flask. The flask was flushed five times with hydrogen and stirred at room temperature under hydrogen atmosphere. The solid was filtered and washed with methanol. The filtrate was evaporated under reduced pressure to yield 6-(1-methylpiperidin-4-yloxy)pyridin-3-amine as a brown solid (85 mg, 98%). LC/MS (*m*/z): 208.2 (MH^+).

General Suzuki procedure for preparation of compounds 1-19

A solution of N-(6-bromo-2-morpholinopyrimidin-4-yl)quinolin-3-amine (150 mg, 0.41 mmole, 1 equiv), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine-2-ylamine (272 mg, 1.23 mmole, 3 equiv) and 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride dichloromethane adduct (43 mg, 0.053 mmol, 0.13 equiv) in 4 mL of 3:1 Dimethoxyethane/2M Na_2CO_3 was heated in a microwave at 120 ^oC for 10

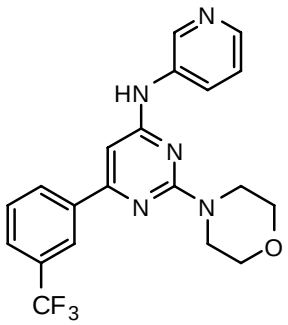
minutes. The mixture was diluted with EtOAc (100 mL), washed with H_20 (3x25 mL) with $NaCl_{(sat.)}$ (25 mL), dried over $MgSO_4$, filtered, concentrated, purified by RP-HPLC and lyophilized directly to yield N-(2-morpholino-4,5'-bipyrimidin-6-yl)quinolin-3-amine as the TFA salt (85%). LC/MS (*m/z*): 381.4 (MH^+). HRMS: $[M+H]^+$ calculated 381.1782, found 381.1777. bis HCl salt, ^1H NMR ($DMSO_{d6}$): δ 10.30 (bs, 1H), 8.84 (s, 2H), 8.44 (d, J=2.7, 1H), 7.96 (dd, J=9.0, 2.7, 1H), 6.88 (d, J=9.0, 1H), 6.51 (s, 1H), 4.20 (bs, 2H), 3.85(s, 3H), 3.69-3.75(m, 8 H).

Compound 1



3-(2-morpholino-6-(pyridin-3-ylamino)pyrimidin-4-yl)phenol was prepared by the general Suzuki procedure. LC/MS (*m/z*): 350.1 (MH^+). HRMS: $[M+H]^+$ calculated 350.1612, found 350.1609.

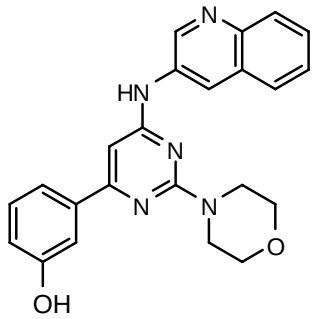
Compound 2



2-morpholino-N-(pyridin-3-yl)-6-(3 (trifluoromethyl)phenyl)pyrimidin-4-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 402.1 (MH^+). HRMS:

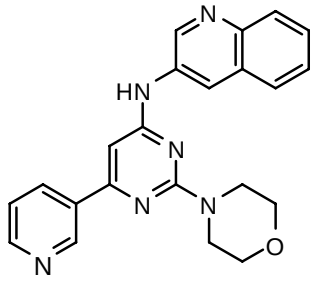
$[M+H]^+$ calculated 402.1536, found 402.1531. free base, ^1H NMR ($DMSO_{d6}$): δ 10.93 (s, 1H), 9.45 (d, J=2.4, 1H), 8.59 (dd, J=8.8, 2.4, 1H), 8.51 (d, J=4.8, 1H), 8.28 (d, J=9.2, 1H), 8.26 (s, 1H), 7.98 (dd, J=8.8, 5.6, 1H), 7.87 (d, J=8.4, 1H), 7.76 (t, J=8.0, 1H), 6.86 (s, 1H), 3.78 (m, 4 H), 3.72 (m, 4 H).

Compound 3



3-(2-morpholino-6-(quinolin-3-ylamino)pyrimidin-4-yl)phenol was prepared by the general Suzuki procedure. LC/MS (*m/z*): 400.1 (MH^+). HRMS: $[M+H]^+$ calculated 400.1768, found 400.1764.

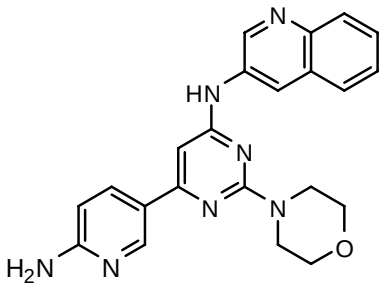
Compound 4



N-(2-morpholino-6-(pyridin-3-yl)pyrimidin-4-yl)quinolin-3-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 385.1 (MH^+). HRMS: $[M+H]^+$ calculated 385.1771, found 385.1765. HCl salt, ^1H NMR ($DMSO_{d6}$): δ 10.74 (s, 1H), 9.42 (d, J=2.8, 1H), 9.36 (d, J=2.0, 1H), 8.96 (d, J=2.4, 1H), 8.91 (dd, J=6.8, 1.2, 1H), 8.87 (dd, J=8.0, 1.6, 1H), 8.10 (d, J=8.4, 1H), 8.09 (d, J=6.2, 1H), 7.99 (dd, J=8.0, 5.6,

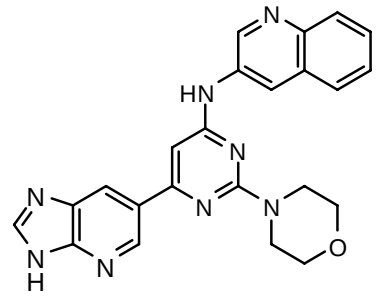
1H), 7.79 (ddd, J=8.0, 6.8, 1.2, 1H), 7.72 (ddd, J=8.8, 8.0, 0.8, 1H), 6.90 (s, 1H), 3.83 (m, 4 H), 3.73 (m, 4 H).

Compound 5



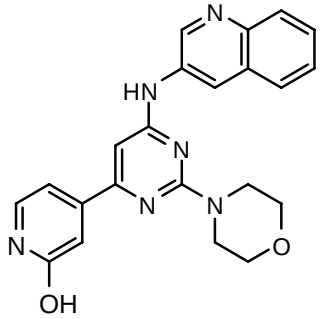
N-(6-(6-aminopyridin-3-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine was prepared by the general Suzuki procedure . LC/MS (*m/z*): 400.4 (MH^+). HRMS: $[M+H]^+$ calculated 400.188, found 400.1876. HCl salt, ^1H NMR ($DMSO_{d6}$): δ 10.20 (s, 1H), 9.09 (d, J=2.4, 1H), 8.69 (d, J=2.8, 1H), 8.56 (d, J=2.8, 1H), 8.39 (d, J=2.4, 1H), 8.36 (d, J=2.0, 1H), 7.91 (d, J=8.0, 1H), 7.87 (dd, J=6.4, 1.6, 1H), 7.51-7.60 (m, 2H), 7.07 (d, J=9.6, 1H), 6.61 (s, 1H), 3.76 (m, 4 H), 3.68 (m, 4 H).

Compound 6



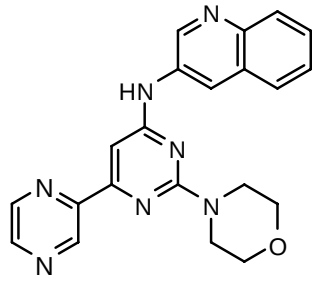
N-(6-(3H-imidazo[4,5-b]pyridin-6-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 425.3 (MH^+). HRMS: $[M+H]^+$ calculated 425.1838, found 425.1835.

Compound 7



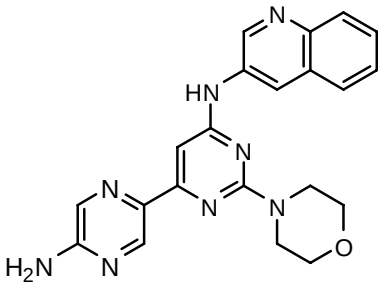
A solution of N-(6-(2-fluoropyridin-4-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine (15 mg, prepared by the general Suzuki procedure using 2-fluoropyridin-4-ylboronic acid) in dioxane (3 mL), H_20 (1 mL) and conc. HCl (0.25 mL) was refluxed for 15 hours. After removal of the volatiles *in vacuo* and purification by RP-HPLC, 4-(2-morpholino-6-(quinolin-3-ylamino)pyrimidin-4-yl)pyridin-2-ol was obtained (11 mg, 58 %) as the TFA salt. LC/MS (*m/z*): 401.1 (MH^+). HRMS: $[M+H]^+$ calculated 401.1721, found 401.1717.

Compound 8



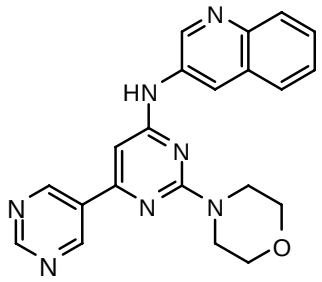
N-(2-morpholino-6-(pyrazin-2-yl)pyrimidin-4-yl)quinolin-3-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 386.1 (MH^+). HRMS: $[M+H]^+$ calculated 386.1724, found 386.172.

Compound 9



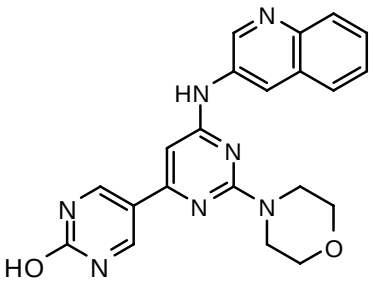
N-(6-(5-aminopyrazin-2-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 401.3 (MH^+). HRMS: $[M+H]^+$ calculated 401.1838, found 401.1838.

Compound 10



N-(2-morpholino-4,5'-bipyrimidin-6-yl)quinolin-3-amine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 386.1 (MH^+). HRMS: $[M+H]^+$ calculated 386.1724, found 386.1717.

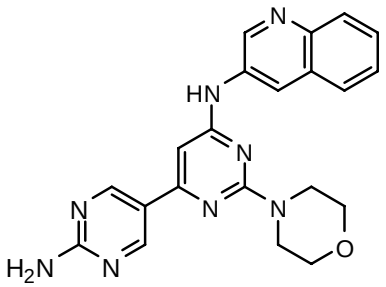
Compound 11



A solution of N-(2'-methoxy-2-morpholino-4,5'-bipyrimidin-6-yl)quinolin-3-amine (30 mg, prepared by the general Suzuki procedure using 2-methoxypyrimidin-5-

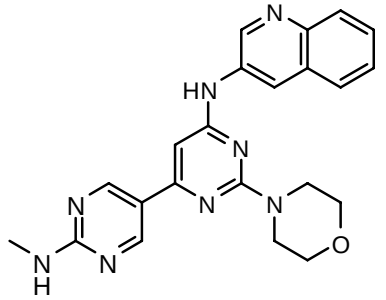
ylboronic acid) in dioxane (3 mL), H_20 (1 mL) and conc. HCl (0.25 mL) was refluxed for 15 hours. After removal of the volatiles *in vacuo* and purification by RP-HPLC, 4-(2-morpholino-6-(quinolin-3-ylamino)pyrimidin-4-yl)pyridin-2-ol was obtained (12 mg, 26 %) as the TFA salt. LC/MS (*m/z*): 402.1 (MH^+). HRMS: $[M+H]^+$ calculated 402.1673, found 402.1671.

Compound 12



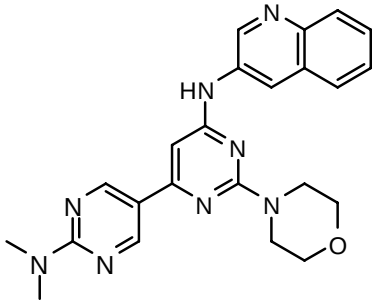
2-morpholino-N6-(quinolin-3-yl)-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 401.4 (MH^+). HRMS: $[M+H]^+$ calculated 401.1833, found 401.1826. HCl salt, ^1H NMR ($DMSO_{d6}$): δ 10.58 (s, 1H), 9.40 (d, J=2.4, 1H), 8.93 (s, 2H), 8.91 (d, J=2.8, 1H), 8.07- 8.10 (m, 2H), 7.70-7.79 (m, 2H), 6.65 (s, 1H), 3.79 (m, 4 H), 3.71 (m, 4 H).

Compound 13



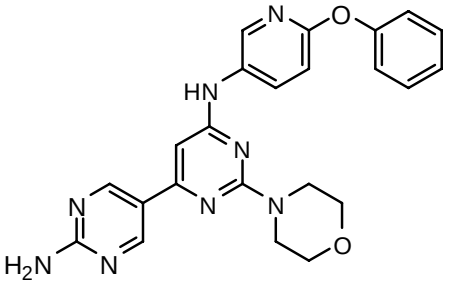
N2'-methyl-2-morpholino-N6-(quinolin-3-yl)-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 415.3 (MH^+). HRMS: $[M+H]^+$ calculated 415.1995, found 415.1989.

Compound 14



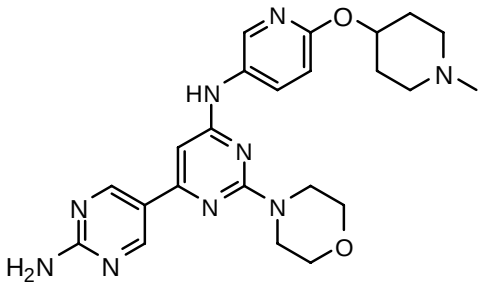
N2',N2'-dimethyl-2-morpholino-N6-(quinolin-3-yl)-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 429.1 (MH^+). HRMS: $[M+H]^+$ calculated 429.2146, found 429.2138.

Compound 15



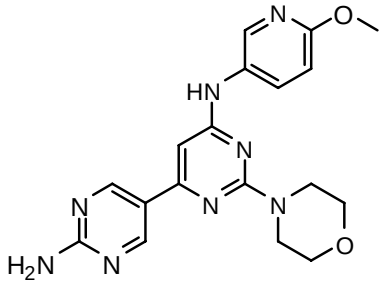
2-morpholino-N6-(6-phenoxypyridin-3-yl)-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 443.4 (MH^+). HRMS: $[M+H]^+$ calculated 443.1938, found 443.1931.

Compound 16



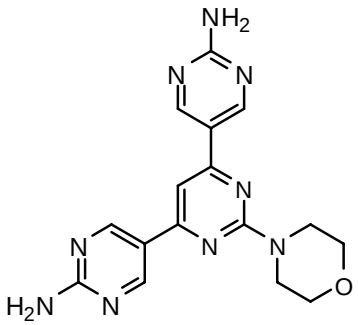
A mixture of $Pd(OAc)_2$ (0.2 equivalents), BINAP (0.25 equivalents), cesium carbonate (1.5 equivalents), 5-(6-chloro-2-morpholinopyrimidin-4-yl)pyrimidin-2-amine (1 eq) and 6-(1-methylpiperidin-4-yloxy)pyridin-3-amine (2 eq) in THF was heated under microwave irradiation for 10 minutes at 110 ºC. The solution was filtered, concentrated under reduced pressure and purified by RP-HPLC to yield N6-(6-(1-methylpiperidin-4-yloxy)pyridin-3-yl)-2-morpholino-4,5'-bipyrimidine-2',6-diamine as te TFA salt. LC/MS (*m/z*): 464.4 (MH^+). HRMS: $[M+H]^+$ calculated 464.2517, found 464.2510.

Compound 17



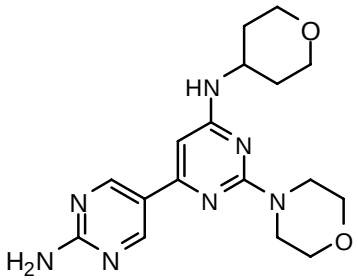
N6-(6-methoxypyridin-3-yl)-2-morpholino-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 381.4 (MH^+). HRMS: $[M+H]^+$ calculated 381.1782, found 381.1777. bis HCl salt, ^1H NMR ($DMSO_{d6}$): δ 10.30 (bs, 1H), 8.84 (s, 2H), 8.44 (d, J=2.7, 1H), 7.96 (dd, J=9.0, 2.7, 1H), 6.88 (d, J=9.0, 1H), 6.51 (s, 1H), 4.20 (bs, 2H), 3.85(s, 3H), 3.69-3.75(m, 8 H).

Compound 18



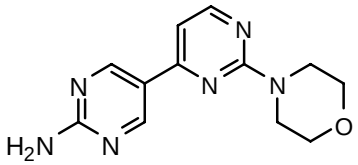
5,5'-(2-morpholinopyrimidine-4,6-diyl)dipyrimidin-2-amine was prepared using the general Suzuki procedure. LC/MS (*m/z*): 352.3 (MH^+). HRMS: $[M+H]^+$ calculated 352.1629, found 352.1629.

Compound 19



2-morpholino-N6-(tetrahydro-2H-pyran-4-yl)-4,5'-bipyrimidine-2',6-diamine was prepared by the general Suzuki procedure. LC/MS (*m/z*): 358.3 (MH^+). HRMS: $[M+H]^+$ calculated 358.1991, found 358.1988.

Compound 20



To a degassed solution of N-(6-(2-aminopyrimidin-5-yl)-2-morpholinopyrimidin-4-yl)quinolin-3-amine (1.0 equiv.) and diisopropylethylamine (15.0 equiv.) in EtOH and EtOAc (1:1, 0.004 M) was added Pd/C (0.5 equiv.) and the reaction was stirred under a hydrogen balloon for 18 hrs. The solution was filtered through a pad of Celite, the Celite was washed with ethyl acetate. the filtrate was concentrated under vacuo and the residue was purified directly by RP-HPLC to afford 2-morpholino-4,5'-bipyrimidin-2'-amine. LC/MS (*m/z*): 259.2 (MH^+). HRMS: $[M+H]^+$ calculated 259.1307, found 259.1307.

Enzymatic PI3K Assays

Assay 1: Homogenous solution phase assay

Compounds to be tested are dissolved in DMSO and directly distributed into 384-well flashplates at 1.25 µL per well. To start the reaction, 20 µL of 6 nM PI3 kinase are added into each well followed by 20 µL of 400 nM ATP containing a trace of radiolabeled ATP and 900 nM 1-alpha-phosphatidylinositol (PI). The plates are briefly centrifuged to remove any air gap. The reaction is performed for 15 minutes and then stopped by the addition of 20 µL of 100 mM EDTA. The stopped reaction is incubated overnight at RT to allow the lipid substrate to bind by hydrophobic interaction to the surface of the flashplate. The liquid in the wells is then washed away, and the labeled substrate is detected with scintillation counting.

Assay 2: One step solid phase assay

This method is similar to Assay 1 except that the lipid substrate (1-alpha-phosphatidylinositol (PIP)) is first dissolved in a coating buffer and incubated on flashplate at room temperature overnight to allow the lipid substrate to bind by hydrophobic interaction to the surface of the flashplate. Unbound substrate is then washed away. On the day of assay, 20 µL of 6 nM PI3 kinase are added into each well followed by 20 µL of 400 nM ATP containing trace of radiolabeled ATP. Compounds

are added together with enzyme and ATP to the lipid-coated plates. The plates are briefly centrifuged to remove any air gap. The reaction is performed for two to three hours. The reaction is stopped by addition of 20 µL of 100 mM EDTA or by immediate plate washing. Phosphorylated lipid substrate is detected by scintillation counting.

Assay 3: ATP depletion assay

Compounds to be tested are dissolved in DMSO and directly distributed into a black 384-well plate at 1.25 µL per well. To start the reaction, 25 µL of 10 nM PI3 kinase and 5 µg/mL 1-alpha-phosphatidylinositol (PI) are added into each well followed by 25 µL of 2 µM ATP. The reaction is performed until approx 50% of the ATP is depleted, and then stopped by the addition of 25 µL of KinaseGlo solution. The stopped reaction is incubated for 5 minutes and the remaining ATP is then detected via luminescence.

pSer473 Akt Assays to Monitor PI3K Pathway

In this method, an assay for measuring the PI3K-mediated pSer473-Akt status after treatment with representative inhibitor compounds of the invention is described.

A2780 cells were cultured in DMEM supplemented with 10% FBS. L-glutamine, sodium pyruvate, and antibiotics. Cells were plated in the same medium at a density of 15,000 cells per well into 96 well tissue culture plates, with outside wells vacant, and allowed to adhere overnight.

Test compounds supplied in DMSO were diluted further into DMSO at 500 times the desired final concentrations before dilution into culture media to 2 times the final concentrations. Equal volumes of 2x compounds were added to the cells in 96 well plates and incubated at 37 ºC for one hour. The media and compounds were then removed, the plates chilled and cells lysed in a lysis buffer (150 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100) supplemented with phosphatase and protease inhibitors. After thorough mixing, lysates were transferred to both pSer473Akt and total Akt assay plates from Meso Scale Discovery (MSD), and incubated overnight with

shaking at 4 ºC. The plates were washed with 1 x MSD wash buffer and the captured analytes detected with secondary antibodies. After incubation with the secondary antibody at room temperature for 1-2 hours, the plates were washed again and 1.5x concentration of Read Buffer T (MSD) was added to the wells.

The assays were read on a SECTOR Imager 6000 instrument (Meso Scale Discovery). Ratios of the signal from pSer473Akt and total Akt assays were used to correct for any variability and the percent inhibition of pSer473Akt from the total signal seen in cells treated with compound versus DMSO alone was calculated and used to determine EC_{50} values for each compound.

Pharmacology Target Modulation and Efficacy Study
in Ovarian Cancer Xenograft Model

A2780 ovarian cancer cells obtained from George Coukos (Fox Chase Cancer Center, University of Pennsylvania, Philadelphia, PA) were maintained in DMEM (Invitrogen, Inc.) supplemented with 10% heat-inactivated fetal bovine serum with 1% glutamine. Cells were propagated as recommended by the Dr. Coukos and colleagues.. Female nu/nu mice (8-12 weeks old, 20-25 g, Charles River) were used for all *in vivo* pharmacology studies. The mice were housed and maintained in accordance with state and federal guidelines for the humane treatment and care of laboratory animals and received food and water *ad libitum*. Cancer cells were harvested from mid-log phase cultures using trypsin-EDTA (Invitrogen, Inc.). Five million cells were subcutaneously injected into the right flank of each mouse. Compound treatment was initiated when tumor size reached to 300–400 mm^3 for PK/PD studies and 200-300 mm^3 for efficacy studies. All compound treatment was administrated orally. Tumor volumes were determined by using StudyDirector software.

For *in vivo* target modulation PK/PD time-course studies, tumor tissues were resected from individual mice at different time points ranging from 30 min to 36 hr after

a single dose of compound (60 or 100 mg/kg) or vehicle was administrated orally. For PK/PD dose-dependent studies, tumor-bearing mice were given a single oral dose of compound at different concentrations (10, 30, 60 and 100 mg/kg or vehicle) and tumors were resected at 10 hr or 24 hr after dosing. Blood samples were taken by cardiac puncture using a syringe primed with heparin sulfate. Resected tumors were snap frozen on dry ice and pulverized using a liquid nitrogen-cooled cryomortar and pestle, and lysed in cold cell extraction buffer (Biosource) containing protease inhibitor tablet (Complete; EDTA-free, Amersham). Supernatants were taken after centrifugation of tumor lysates at 300xg for 10 min at 4 ºC and the protein concentration in each supernatant was determined by BCA (BioRad). An equal amount of protein from each tumor lysate was loaded onto 10% Tris-glycine gels (Invitrogen), for sodiumdoceylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after which proteins were transferred from the gel onto PVDF membrane. Membranes were probed with antibodies that recognize phosphoAkt^{Ser473} or phosphoAkt^{Thr308} (Cell Signaling) followed by secondary goat anti-rabbit IgG conjugated to HRP(Amersham). Positive bands were visualized by enhanced chemiluminescence with X-ray film. Similar procedures were used to determine total AKT in the same tumor lysates to serve as normalization for total protein in each determination. The density of the positive band on the X-ray film was scanned and the target modulation for each compound was expressed as percentage inhibition by each compound compared to vehicle treatment. A rank order (<50%, 50-75%, >75%, as compared to vehicle treatment) of target inhibition is used to present compound target modulation activity.

For efficacy studies, A2780 cancer cells (5 x 10^6 in 100 µl of DMEM culture medium) were injected subcutaneously into the right flank of each nu/nu mouse. When average tumor sizes reached about 200 mm^3, mice were dosed orally daily (q.d.) or twice a day (b.i.d.) at three different compound concentrations (typically at 10, 30 and 100 mg/kg) in 100 µl incipient. Tumor growth and animal body weight was measured

twice weekly with daily clinical observation to monitor potential toxicities related to the treatment. Typically, studies were terminated when tumors in vehicle-treated group reached 2500 mm^3 or adverse clinical effects were observed. **A**ctivation of the PI3K signaling pathway results in the phosphorylation of the downstream signaling molecule Akt at Ser^{473} and/or Thr^{308}. Compound modulation of Akt^{Ser473} phosphorylation was examined in A2780 xenograft tumors at time points ranging from 30 min to 24 hr after a single compound dose at 100 mg/kg.