Supporting Information

Discovery of 2,4-difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide (GSK2126458), a Highly Potent Inhibitor of Phosphoinositide 3-Kinase (PI3K) and the Mammalian Target of Rapamycin (mTOR)

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Contents

Biological assays

HTRF *In vitro* Profiling Assays for PI3K Inhibition

The PI3-Kinase profiling assays were developed to measure the compound-dependent inhibition of the alpha, beta, delta, and gamma isoforms of PI3Kin an *in vitro* catalytic assay. This assay was developed and optimized from a kit produced by Upstate (Millipore catalog # 33-017). Briefly, this procedure utilizes a pre-formed HTRF (Homogeneous Time-Resolved Fluorescence energy transfer) complex between four binding partners: 1) biotinylated PIP3, 2) GST tagged pleckstrin homology (PH) domain, 3) Europium labeled anti-GST monoclonal antibody, and 4) Streptavidin-Allophycocyanin (APC). The native PIP3 produced by PI 3-Kinase activity displaces biotin-PIP3 from the PH domain, resulting in the dissociation of the HTRF complex and a decrease in the fluorescence signal. The format of this assay is the same for all 4 isoforms of PI3K; the differences lie in the concentration of enzyme used to achieve the most robust signal. The alpha and delta assays are run at 400pM enzyme; the beta assay is at 200pM enzyme and the gamma assay is run at 1nM enzyme. In addition, the alpha, beta and delta assays are run with 150mM NaCl while the gamma assay is run in the absence of NaCl. The ATP concentration is 100uM in the alpha, beta, and delta assays and 15uM ATP in the gamma assay. All reactions are run at 10uM PIP2.

Assay protocol

Compounds are serially diluted (3-fold in 100% DMSO) across a 384-well polypropylene mother plate from column 1 to column 12 and column 13 to column 24, to yield 11 concentrations for each test compound. Columns 6 and 18 contain only DMSO. Once titrations are made, 0.05µL is transferred to a 384-well low-volume assay plate (Greiner 784076). This assay plate contains three pharmacological controls (known PI3K inhibitors) and 3 assay controls: (1) Enzyme without inhibitor; (2) Buffer minus enzyme, and (3) Buffer minus enzyme plus native PIP3. DMSO is stamped into all wells of columns 6 and 18. PIP3 is added at 40 µM in 1X Reaction buffer (1µL of 200 µM PIP3) to alternating rows of column 18 (wells 18 B, D, F, H, J, L, N, P). The no-enzyme control reactions are run in wells 18 A, C, E, G, I, K, M, O (0.1µL of 100% DMSO).

The PI3-Kinase profiling assay was optimized using the HTRF kit provided by Upstate (Millipore). The assay kit contains seven reagents: 1) 4X Reaction Buffer; 2) native PIP2 (substrate); 3) Stop A (EDTA); 4) Stop B (Biotin-PIP3); 5) Detection Mix A (Streptavidin-APC); 6) Detection Mix B (Eu-labeled Anti-GST plus GST-tagged PH-domain); 7) Detection Mix C (KF). In addition, the following items were obtained or purchased: PI3Kinase (prepared by GSK BR&AD), dithiothreitol (Sigma, D-5545), Adenosine-5'-triphosphate (ATP, Teknova cat. # A0220), native PIP3 (1,2-dioctanoyl-sn-glycero-3-[phosphoinositil-3,4,5-triphosphate] tetraammonium salt (Avanti polar lipids, 850186P), DMSO (Sigma, 472301).

PI3Kinase Reaction Buffer is prepared by diluting the stock 1:4 with de-ionized water. Freshly prepared DTT is added at a final concentration of 5 mM on the day of use. Enzyme addition and compound pre-incubation are initiated by the addition of 2.5µL of

PI3K (at twice its final concentration) in 1X reaction buffer to all wells using a Multidrop Combi. Plates are incubated at room temperature for 15 minutes. Reactions are initiated by addition of 2.5µL of 2X substrate solution (PIP2 and ATP in 1X reaction buffer) using a Multidrop Combi. Plates are incubated at room temperature for one hour. Reactions are quenched by the addition of 2.5µL of stop solution (Stop A and Stop B pre-mixed at a ratio of 5:1, respectively) to all wells using the Multidrop Combi. The quenched reactions are then processed to detect product formation by adding 2.5µL of Detection Solution to all wells using the Mulitdrop Combi (Detection mix C, Detection mix A, and Detection mix B combined together in an 18:1:1 ratio, i.e.: for a 6000 µL total volume, mix 5400 µL Detection mix C, 300µL Detection mix A, and 300 µL Detection mix B. Note: this solution should be prepared 2 hours prior to use). Following a one hour incubation in the dark, the HTRF signal is measured on the Envision plate reader set for 330nm excitation and dual emission detection at 620nm (Eu) and 665nm (APC).

Data analysis

The loss of the HTRF signal is due to the displacement of biotinylated-PIP3 from the PH domain by the PI3K-dependent conversion of PIP2 to PIP3. This loss of signal is nonlinear with respect to both increasing product and time. This non-linear detection will impact accuracy of IC_{50} calculations; therefore, there is a need for a correction factor to obtain more accurate IC_{50} values This correction is derived from the assay standards in the wells of column 6 and 18 of the assay plate. All data were calculated using the ratio of acceptor (APC) to donor (Europium) fluorescence in each well of the assay plate. The percent inhibition for each compound concentration was calculated as follows: %inhibition = 100*(fluorescence ratio – CtrlB)/(CtrlA – CtrlB) where CtrlA= (-) PI3Kinase reaction and CrtlB= PI3Kinase + DMSO. An IC_{50} was then calculated fitting the %inhibition data to the equation: %inhibition = min + (max-min)/(1 + ([inhibitor]/IC_{50})^n) where min is the %inhibition with no inhibitor (typically 0%), max is the signal in the (-) Enzyme control, and n is the Hill slope (typically 1). Finally, the IC_{50} was converted to pIC_{50} (pIC_{50} = -log(IC_{50})), and the pIC_{50} value was corrected by using plate controls and the equation below:
pIC_{50} (corrected) = pIC_{50} (observed) + log10((CtrlA-CtrlB)/(CtrlB-CtrlC)), where CtrlA and CtrlB are as defined above and CrtlC= 10µM PI(3,4,5)P3, 100% displacement of biotinylated PI(3,4,5)P3.

Note: Compounds whose activities approach the tight binding limit in the HTRF assays are retested using 50pM enzyme.

Apparent inhibition constants (Ki's) were determined in a time resolved, fluorescence resonance energy transfer (TR-FRET) assay where the nominal concentration of PI3K alpha was 60 pM. However, the ATP concentration in this assay was fixed at six fold above the apparent Km value giving the assay a theoretical detection limit of 5 pM for ATP competitive inhibitors. IC_{50}'s were determined and then converted to Ki values by means of the Cheng-Prusoff equation:

$$IC_{50} = (1 + \frac{S}{K_m})K_i$$

Each time the assay was run a PIP3 standard curve was included. Assay signal for each well was then converted to the molar amount of product formed. The average reaction velocity for uninhibited controls from Ki determination experiments was 2.2 nM of PIP3 per minute. Each sample was read every 45 seconds for an hour and 15 minutes. Progress curves were linear for at least one hour.

We reported the PI3K enzyme kcat to be 3.1 sec^{-1} with substrates not limiting (Biochem. J. (2008) 409 (519–524). This is in line with reported kcat values for other enzymes.

PI3K alpha Profiling in Leadseeker SPA

Assay principle

SPA imaging beads are microspheres containing scintillant which emit light in response to the transferred energy from a radioactive decay. The Leadseeker beads used in this system are polystyrene beads that have been coupled with polyethyleneimine. When added to the assay mixture, the beads adsorb both the substrate (PIP2) and product (PIP3). [^{33}P]-PIP3 adsorbed to the bead will cause a proximity-dependent increase in signal, measured in the Viewlux as ADUs (analog to digital units). This protocol details the use of the PEI-PS Leadseeker beads for assays using His-p110/p85 PI3K alpha.

Assay protocol

Test compounds dissolved in 100% DMSO are dispensed at 0.1 µl in all wells (except column 6 and 18) of a white 384-well, flat bottom, low volume plate (Greiner 784075). The compounds are serially diluted (3-fold in 100% DMSO) across the plate from column 1 to column 12 and column 13 to column 24 in an 11-point titration. DMSO is added to columns 6 and 18 for assay controls.

The assay buffer contains 50mM MOPS (pH 6.5), 1mM CHAPS, 20mM $MgCl_2$, 2uM ATP, and 1mM DTT. PI3K alpha and PIP2 (L-alpha-D-myo-Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]3-O-phospho linked, D(+)-sn-1,2-di-O-octanoylglyceryl, CellSignals # 901) are mixed and incubated in the plate with compound for 30min prior to starting the reaction with the addition of ^{33}P-ATP and $MgCl_2$. Enzyme-free wells (column 18) are used to determine the low control. PEI-PS Leadseeker beads in PBS/EDTA/CHAPS are added (by Multidrop) to quench the reaction and the plates are allowed to incubate for at least one hour (typically overnight) and then centrifuged at 500xg for 1 minute to separate the beads from the free isotope in solution. The signal is measured using a Viewlux detector and is then imported into curve fitting software (Activity Base) for analysis of concentration response curves. The percent inhibition of activity was calculated relative to high controls (C1, 0.1µl DMSO in column 6, rows A-P)) and low controls (C2, 5 µl of 40 uM PIP2 in buffer in column 18, rows A-P) using, 100*(1-(U1-C2)/(C1-C2)). The concentration of test compound yielding 50% inhibition was determined using the equation, y = ((Vmax*x) / (K+x)) + Y2, where "K" was equal to the IC_{50}. The IC_{50} values were converted to pIC_{50} values, i.e., -log IC_{50} in Molar concentration.

Cellular assays:

DAY 1

- Plate cells before noon
  - 10K cells/well in clear flat-bottomed 96-well plates (*f.v. 105ul*)
  - Last four wells in last column receive media only
  - Place in 37degC incubator overnight
- Compound plate
  - Prepare in polypropylene round-bottomed 96-well plates; 8 compounds per plate, 11-pt titrations of each (3x serial dilution), DMSO in last column (0.15% f.c. on cells)
  - 15ul in first well, 10ul DMSO in the rest; take 5ul from first well and mix in next, continue across plate (*excluding last column*); seal with foil lid and place at 4degC

DAY 2

- Take out Lysis buffer inhibitors (4degC/-20degC) and compound plates (4degC), thaw on bench top; make 1x Tris wash buffer (WB) to fill reservoir on plate washer and top off bench supply (use MiliQ), turn on centrifuge to allow it to cool
- Block MSD plates
  - Make 20ml 3% blocking solution/plate (600mg blocker A in 20ml WB), add 150ul/well and incubate at RT for at least 1 hr
- Add compound (while blocking)
  - Add 300ul growth media (RPMI w/ Q, 10% FBS) per well (682x dil of compound) to each compound plate
  - Add 5ul compound dilution into each well (*f.v. 110ul*) on duplicate plates
  - Place in 37degC incubator for 30min
- Make lysates
  - Prepare MSD Lysis buffer; for 10ml add 200ul protease inhibitor solution, and 100ul each of Phosphatase inhibitors I & II (*Keep on ice until ready for use*)
  - Remove plates post-incubation, aspirate media with plate washer, wash 1x with cold PBS, and add 80ul MSD Lysis buffer per well; incubate on shaker at 4degC for ≥30min
  - Spin cold at 2500rpm for 10min; leave plates in 4degC centrifuge until ready for use
- AKT duplex assay
  - Wash plates (4x with 200ul/well WB in plate washer); tap plates on paper towel to blot
  - Add 60ul of lysates/well, incubate on shaker at RT for 1hr
  - During incubation prepare detection Ab (3ml/plate; 2ml WB and 1ml blocking solution w/ Ab at 10nM); repeat wash step as above
  - Add 25ul of Ab/well, incubate on shaker at RT for 1hr; repeat wash step as above
  - Add 150ul/well 1x Read Buffer (dilute 4x stock in ddH2O, 20ml/plate), read immediately
- Analysis

- Observe all the data points at each compound concentration.
- The data point from highest inhibitor concentration must be equal or greater than 70% of DMSO control.
- IC50 for duplicate runs must be within 2-fold of each other (not flagged in summary template).
- Y min must be greater than zero; if both mins are red flagged (>35) then compound is listed as inactive (IC50= > highest dose). If only one min is red flagged, but still ≤50 then call IC50 as listed.
- Any data points equal or greater than 30% off the curve will not be considered.

Cell Growth/Death Assay:

BT474, HCC1954 and T-47D (human breast) were cultured in RPMI-1640 containing 10% fetal bovine serum at 37^0C in 5% CO_2 incubator. Cells were split into T75 flask (Falcon #353136) two to three days prior to assay set up at density which yields approximately 70-80% confluence at time of harvest for assay. Cells were harvested using 0.25% trypsin-EDTA (Sigma #4049). Cell counts were performed on cell suspension using Trypan Blue exclusion staining. Cells were then plated in 384 well black flat bottom polystyrene (Greiner #781086) in 48 µl of culture media per well at 1,000 cells/well. All plates were placed at 5% CO_2, 37^0C overnight and test compounds were added the following day. One plate was treated with CellTiter-Glo (Promega #G7573) for a day 0 (t=0) measurement and read as described below. The test compounds were prepared in clear bottom polypropylene 384 well plates (Greiner#781280) with consecutive two fold dilutions. 4 µl of these dilutions were added to 105 µl culture media, after mixing the solution, 2 µl of these dilutions were added into each well of the cell plates. The final concentration of DMSO in all wells was 0.15%. Cells were incubated at 37^0C, 5% CO_2 for 72 hours. Following 72 hours of incubation with compounds each plate was developed and read. CellTiter-Glo reagent was added to assay plates using a volume equivalent to the cell culture volume in the wells. Plates were shaken for approximately two minutes and incubated at room temperature for approximately 30 minutes and chemiluminescent signal was read on the Analyst GT (Molecular Devices) reader. Results were expressed as a percent of the t=0 and plotted against the compound concentration. Cell growth inhibition was determined for each compound by fitting the dose response with a 4 or 6 parameter curve fit using XLfit

software and determining the concentration that inhibited 50% of the cell growth (gIC50) with the Y min as the t=0 and Y max as the DMSO control. Value from wells with no cells was subtracted from all samples for background correction.

Kinase Selectivity

Selectivity against protein kinases was determined from evaluation of compounds in our in-house assays, as well as data obtained from Millipore's full panel.

Additional references:

The compounds of the present invention can also be tested to determine their inhibitory activity at PI3Kα, PI3Kδ, PI3Kβ and PI3Kγ according to the assays in the following references:

For all PI3K isoforms:

1. Cloning, expression, purification, and characterization of the human Class Ia phosphoinositide 3-kinase isoforms: Meier, T.I.; Cook, J.A.; Thomas, J.E.; Radding, J.A.; Horn, C.; Lingaraj, T.; Smith, M.C. Protein Expr. Purif., **2004**, 35(2), 218.
2. Competitive fluorescence polarization assays for the detection of phosphoinositide kinase and phosphatase activity: Drees, B.E.; Weipert, A.; Hudson, H.; Ferguson, C.G.; Chakravarty, L.; Prestwich, G.D. Comb. Chem. High Throughput.Screen., **2003**, 6(4), 321.

For PI3Kγ: WO 2005/011686 A1

Biological Data with Statistical Significance

All data reported as mean ± range unless otherwise noted.

Table 1. Thiazolidinedione replacement SAR

Cmpd	PI3Kα IC_{50} (nM)	pAKT IC_{50} (nM)
2	2 ± 0.7*	40 ± 38*
6a	1800	8080 ± 6420
6b	258 ± 28	>29,300
6c	73 ± 1	2700 ± 290
6d	7 ± 1.4*	76 ± 19
6e	10	49 ± 5

* mean ± standard deviation.

Table 2. Selected pyridylsulfonamide analog SAR

Cmpd	IC_{50} (nM)		Rat Oral
	PI3Kα	pAKT	DNAUC
6e	10	49 ± 5	NQ
6f	1.2	63 ± 13	250 ± 110*
6g	1 ± 0.47	45 ± 24*	920 ± 220*
6h	0.1 ± 0.008*	7 ± 3*	1100 ± 190*

* mean ± standard deviation.

Units: DNAUC (ng h mL^{-1} mg^{-1} kg^{-1}); NQ = not quantifiable.

Table 3. Biochemical activity of **1**

p110 Isoform app K_i (nM)				p110α Mutant app K_i (nM)		
α	β	δ	γ	E542K	E545K	H1047R
0.019 ± 0.01	0.13 ± 0.03	0.024 ± 0.01	0.06 ± 0.008	0.008 ± 0.03	0.008 ± 0.03	0.009 ± 0.03

Table 4. mTOR Complex activity of GSK2126458 (**1**)
Data reported as mean ± range.

mTORC1 app K_i (nM)	mTORC2 app K_i (nM)
0.18 ± 0.01	0.3 ± 0.1

Table 5. Mechanistic and functional cellular activity of **1**

pAKT-S473 IC_{50} (nM)		Growth IC_{50} (nM)	
T47D	BT474	T47D	BT474

0.41 ± 0.27	0.18 ± 0.05*	3.0 ± 0.8*	2.35 ± 0.15

Compound	PI3Kα IC_{50} (nM)
GSK2126458 (**1**)	0.04 ± 0.02*
BEZ235	6.2 ± 4
GDC-0941	9.2 ± 4.2*
LY294002	470 ± 99*
wortmannin	2.4
ZSTK-474	7.4 ± 0.7*
PI-103	12

Table 6. Pre-clinical PK profile of **1**

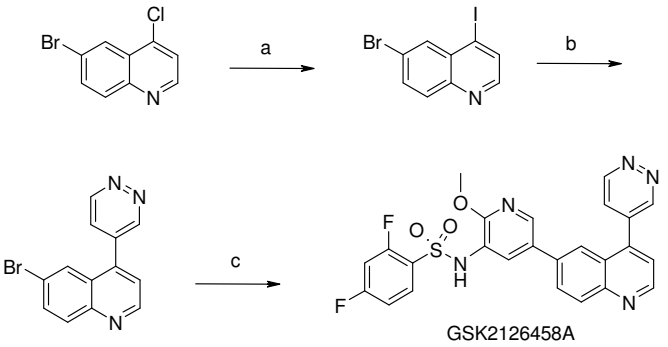
The pharmacokinetics of **1** (free base) were studied following single intravenous and/or oral administration to the male mouse, rat, dog and monkey. The IV and PO solution formulations contained 40% (v/v) PEG-400, 16% (w/v) encapsin in saline and water, respectively. The pH was adjusted to within 3.0-4.0 for the mouse, rat, dog and monkey solutions. Oral bioavailability was estimated using a cross-over study design for the dog and monkey (n = 3). Oral bioavailability in the rat was estimated using crossover (n = 1) and non-crossover (n = 2) designs and a non-crossover serial design was employed in the mouse (n = 2 IV and n = 3 PO). Blood samples were assayed for GSK2126458 using protein precipitation with acetonitrile followed by HPLC/MS/MS analysis employing positive-ion Turbo IonSpray ionization. Blood concentration-time data were analyzed by non-compartmental methods. Mouse and rat data reported as mean ± range. Dog and monkey data reported as mean ± standard deviation.

Species	iv Dosing				Oral Dosing		
	Dose	Cl_b	Vd_{ss}	$T_{½}$	Dose	DNAUC	% F
mouse	0.6	10 ± 0.7	1.0 ± 0.05	2.1 ± 0.3	2.6	1100 ± 400	100
rat	0.4	2.3 ± 0.3	1.1 ± 0.05	6.2 ± 0.6	2.1	6100 ± 1400	81 ± 19
dog	0.1	5.8 ± 0.5	0.7 ± 0.1	1.3 ± 0.1	0.5	2400 ± 680	80 ± 24
monkey	0.1	3.6 ± 0.4	0.8 ± 0.2	3.5 ± 0.4	0.6	2300 ± 740	49 ± 12

Units: Dose (mg kg^{-1}); Cl_b (mL min^{-1} kg^{-1}); Vd_{ss} (L kg^{-1}); $T_{½}$ (h); DNAUC (ng h mL^{-1} mg^{-1} kg^{-1}).

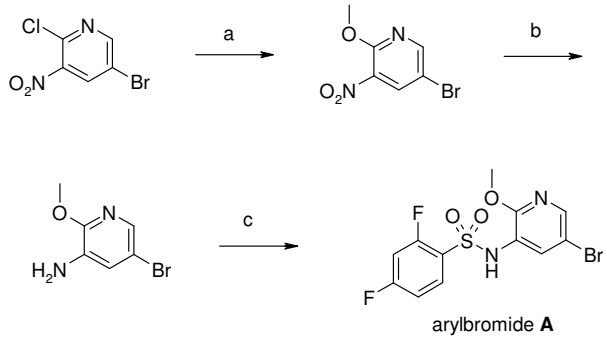
Preparation and Characterization of GSK2126458

Scheme 1



Conditions: a) 2 M HCl/ether, THF, rt; then NaI, EtCN, reflux; b) 4-(tributylstannanyl)pyridazine, $PdCl_2(dppf)_2$-CH_2Cl_2, dioxane, heat; c) bis(pinacolato)diboron, $PdCl_2(dppf)_2$-CH_2Cl_2, potassium acetate, dioxane, heat; then arylbromide **A**, $PdCl_2(dppf)_2$-CH_2Cl_2, saturated aqueous Na_2CO_3, heat.

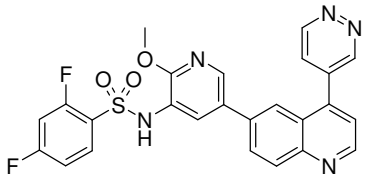
Scheme 2



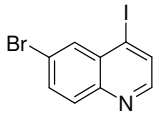
Conditions: a) NaOMe, MeOH, 0 °C to rt; b) $SnCl_2$, EtOAc, reflux; c) 2,4-difluorosulfonylchloride, pyridine, 0 °C to rt.

Experimental

2,4-difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide (GSK2126458)



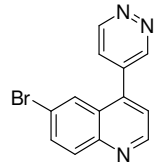
a) 6-bromo-4-iodoquinoline



Following the general procedure of Wolf, Christian et al. (*SynLett* **2003** *12*, 1801-1804), to a solution of 6-bromo-4-chloroquinoline (30 g, 0.124 mol) in anhydrous THF (500 mL) was added 2 M HCl in diethylether (74 mL, 0.148 mol). A white precipitate formed immediately. After stirring for 30 min, the suspension was concentrated *in vacuo* and dried under vacuum to provide 6-bromo-4-chloroquinoline hydrochloride as an off-white solid (34.6 g, quantitative yield).

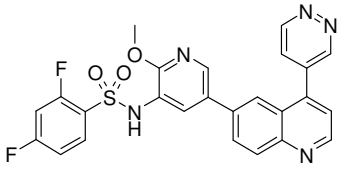
A 3-neck roundbottom flask equipped with a reflux condenser and mechanical stirrer was charged with 6-bromo-4-chloroquinoline hydrochloride (34.6 g, 0.124 mol), anhydrous sodium iodide (92.93 g, 0.62 mol) and propionitrile (1 L). The resulting slurry was stirred vigorously at reflux for 96 hrs. The solution was cooled to room temperature and 500 mL of 10% K_2CO_3 solution was added, followed by a 200 mL of a 5% sodium sulfite solution. The reaction mixture was extracted with CH_2Cl_2 (600 mL x 4). The combined organic extracts were dried (Na_2SO_4), filtered and conc. *in vacuo* to provide the title compound as an off-white solid (41.8 g, >quantitative yield), which was used without further purification. LCMS $[M]^+$ = 333.8, 334.8, 336.0 and 337.0; H^1 NMR (400 MHz, *d*-DMSO) δ (ppm) = 7.98-7.96 (2 H, m), 8.14-8.16 (1 H, m), 8.23 (1 H, d), 8.53 (1 H, d).

b) 6-bromo-4-(4-pyridazinyl)quinoline



Dissolved 6-bromo-4-iodoquinoline (17.43 g, 52.2 mmol), 4-(tributylstannanyl)pyridazine (19.27 g, 52.2 mmol), and $PdCl_2$(dppf)-CH_2Cl_2 (2.132 g, 2.61 mmol) in 1,4-dioxane (200 mL) and heated to 105 °C. After 3 h, added more palladium catalyst and heated for 6 h. Concentrated and dissolved in methylene chloride/methanol. Purified by column chromatography (combiflash) with 2% MeOH/EtOAc to 5% MeOH/EtOAc to give the crude title compound. Trituration with EtOAc furnished 6-bromo-4-(4-pyridazinyl)quinoline (5.8 g, 20.27 mmol, 38.8 % yield). MS(ES)+ m/e 285.9, 287.9 $[M+H]^+$.

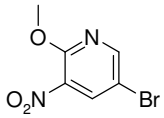
c) 2,4-difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide



A slurry of 6-bromo-4-(4-pyridazinyl)quinoline (4.8 g, 16.78 mmol), bis(pinacolato)diboron (4.69 g, 18.45 mmol) , $PdCl_2$(dppf)-CH_2Cl_2 (530 mg, 0.649 mmol) and potassium acetate (3.29 g, 33.6 mmol) in anhydrous 1,4-dioxane (120 ml) was heated at 100 °C for 3 h. The complete disappearance of the starting bromide was observed by LCMS. The reaction was then treated with N-[5-bromo-2-(methyloxy)-3-pyridinyl]-2,4-difluorobenzenesulfonamide (6.68 g, 17.61 mmol), aqueous Na_2CO_3 (30 mL) and another portion of $PdCl_2$(dppf)-CH_2Cl_2 (550 mg, 0.673 mmol), then heated at 110 °C for 16 h. The reaction was allowed to cool to room temperature, filtered, and concentrated. Purification of the residue by chromatography (Analogix; 5% MeOH / 5% CH_2Cl_2 / 90% EtOAC) gave 6.5 g (76%) desired product. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.65 (s, 3 H), 7.19 (td, *J*=8.46, 2.02 Hz, 1 H), 7.51 - 7.61 (m, 1 H), 7.68 (d, *J*=4.29 Hz, 1 H), 7.73 (td, *J*=8.53, 6.44 Hz, 1 H), 7.95 (t, *J*=2.53 Hz, 2 H), 8.08 (dd, *J*=5.31, 2.27 Hz, 1 H), 8.13 (dd, *J*=8.72, 1.89 Hz, 1 H), 8.26 (d, *J*=8.59 Hz, 1 H), 8.42 (d, *J*=2.27 Hz, 1 H), 9.06 (d, *J*=4.55 Hz, 1 H), 9.48 (dd, *J*=5.31, 1.26 Hz, 1 H), 9.57 (t, *J*=1.77 Hz, 1 H), 10.35 (s, 1 H). Anal. ($C_{25}H_{17}F_2N_5O_3S$) Theory C: 59.40, H: 3.39, N: 13.85; Found C: 59.30, H: 3.19, N: 13.57. 99.41% pure by HPLC A% at 240 nm. MS *m/z* 505.9 $[M+H]^+$. mp 187-189 °C.

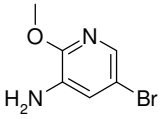
N-[5-bromo-2-(methyloxy)-3-pyridinyl]-2,4-difluorobenzenesulfonamide (arylbromide **A**)

a) 5-bromo-2-(methyloxy)-3-nitropyridine



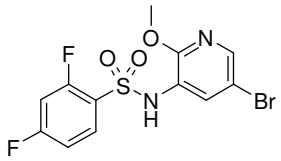
To a cooled (0 °C) solution of 5-bromo-2-chloro-3-nitropyridine (50 g, 211 mmol) in methanol (200 mL) was added dropwise over 10 minutes 20% sodium methoxide (50 mL, 211 mmol) solution. The reaction, which quickly became heterogeneous, was allowed to warm to ambient temperature and stirred for 16 h. The reaction was filtered and the precipitate diluted with water (200 mL) and stirred for 1 h. The solids were filtered, washed with water (3 x 100 mL) and dried in a vac oven (40 °C) to give 5-bromo-2-(methyloxy)-3-nitropyridine (36 g, 154 mmol, 73.4 % yield) as a pale yellow powder. The original filtrate was concentrated in vacuo and diluted with water (150 mL). Saturated ammonium chloride (25 mL) was added and the mixture stirred for 1 h. The solids were filtered, washed with water, and dried in a vac oven (40 °C) to give a second crop of 5-bromo-2-(methyloxy)-3-nitropyridine (9 g, 38.6 mmol, 18.34 % yield). Total yield = 90%. MS(ES)+ m/e 232.8, 234.7 $[M+H]^+$.

b) 5-bromo-2-(methyloxy)-3-pyridinamine



To a solution of 5-bromo-2-(methyloxy)-3-nitropyridine (45 g, 193 mmol) in ethyl acetate (1 L) was added tin(II) chloride dihydrate (174 g, 772 mmol). The reaction mixture was heated at reflux for 4 h. LC/MS indicated some starting material remained, so added 20 mol% tin (II) chloride dihydrate and continued to heat at reflux. After 2 h, the reaction was allowed to cool to ambient temperature and concentrated in vacuo. The residue was treated with 2 N sodium hydroxide and the mixture stirred for 1 h. The mixture was then with methylene chloride (1 L), filtered through Celite, and washed with methylene chloride (500 mL). The layers were separated and the organics dried over magnesium sulfate and concentrated to give 5-bromo-2-(methyloxy)-3-pyridinamine (23 g, 113 mmol, 58.7 % yield). The product was used crude in subsequent reactions. MS(ES)+ m/e 201.9, 203.9 $[M+H]^+$.

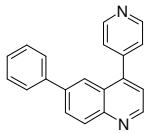
c) *N*-[5-bromo-2-(methyloxy)-3-pyridinyl]-2,4-difluorobenzenesulfonamide



To a cooled (0 °C) solution of 5-bromo-2-(methyloxy)-3-pyridinamine (20.3 g, 100 mmol) in pyridine (200 mL) was added slowly 2,4-difluorobenzenesulfonyl chloride (21.3 g, 100 mmol) over 15 min (reaction became heterogeneous). The ice bath was removed and the reaction was stirred at ambient temperature for 16 h, at which time the reaction was diluted with water (500 mL) and the solids filtered off and washed with copious amounts of water. The precipitate was dried in a vacuum oven at 50 °C to give *N*-[5-bromo-2-(methyloxy)-3-pyridinyl]-2,4-difluorobenzenesulfonamide (12 g, 31.6 mmol, 31.7 % yield) MS(ES)+ m/e 379.0, 380.9 $[M+H]^+$.

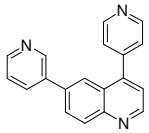
NMR Characterization of Compounds 6a – 6h

6-phenyl-4-(4-pyridinyl)quinoline (**6a**)



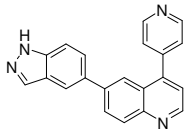
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J* = 4.29 Hz, 1H), 8.76 - 8.84 (m, 2H), 8.22 - 8.27 (m, 1H), 8.14 - 8.19 (m, 1H), 7.99 (d, *J* = 1.77 Hz, 1H), 7.67 - 7.73 (m, 4H), 7.57 (d, *J* = 4.29 Hz, 1H), 7.50 (t, *J* = 7.45 Hz, 2H), 7.38 - 7.45 (m, 1H).

6-(3-pyridinyl)-4-(4-pyridinyl)quinoline (**6b**)



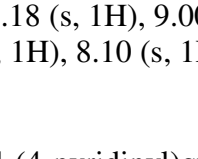
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, *J* = 4.29 Hz, 1H), 8.95 (d, *J* = 2.02 Hz, 1H), 8.78 - 8.83 (m, 2H), 8.62 (dd, *J* = 1.52, 4.55 Hz, 1H), 8.25 - 8.31 (m, 1H), 8.19 - 8.24 (m, 1H), 8.14 (dt, *J* = 2.02, 7.83 Hz, 1H), 8.06 (d, *J* = 2.02 Hz, 1H), 7.67 - 7.74 (m, 2H), 7.60 (d, *J* = 4.29 Hz, 1H), 7.52 (dd, *J* = 4.80, 7.83 Hz, 1H).

6-(1*H*-indazol-5-yl)-4-(4-pyridinyl)quinoline (**6c**)



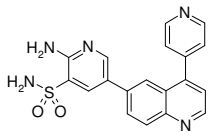
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 9.00 (d, *J* = 4.29 Hz, 1H), 8.69 - 8.88 (m, 2H), 8.19 - 8.28 (m, 2H), 8.15 (s, 1H), 8.10 (s, 1H), 8.01 (s, 1H), 7.62 - 7.74 (m, 4H), 7.56 (d, *J* = 4.29 Hz, 1H).

6-(1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)-4-(4-pyridinyl)quinoline (**6d**)



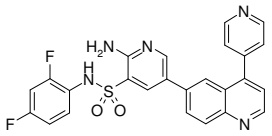
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.80 (br. s., 1H), 9.03 (d, *J* = 4.29 Hz, 1H), 8.89 (d, *J* = 2.02 Hz, 1H), 8.81 (d, *J* = 5.81 Hz, 2H), 8.56 (d, *J* = 2.02 Hz, 1H), 8.19 - 8.35 (m, 3H), 8.08 (s, 1H), 7.73 (d, *J* = 5.81 Hz, 2H), 7.59 (d, 1H).

2-amino-5-[4-(4-pyridinyl)-6-quinolinyl]-3-pyridinesulfonamide (**6e**)



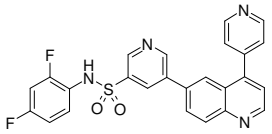
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, *J* = 4.29 Hz, 1H), 8.81 (d, *J* = 5.81 Hz, 2H), 8.57 (d, *J* = 2.27 Hz, 1H), 8.23 (d, *J* = 8.84 Hz, 1H), 8.13 (d, *J* = 2.27 Hz, 1H), 8.08 - 8.12 (m, 1H), 7.92 (d, *J* = 1.77 Hz, 1H), 7.67 - 7.71 (m, 2H), 7.57 (s, 2H), 7.56 (br. s., 1H), 6.77 (br. s., 2H).

2-amino-*N*-(2,4-difluorophenyl)-5-[4-(4-pyridinyl)-6-quinolinyl]-3-pyridinesulfonamide (**6f**)



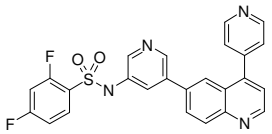
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.99 (d, *J* = 4.29 Hz, 1H), 8.79 - 8.84 (m, 2H), 8.59 (d, *J* = 2.27 Hz, 1H), 8.20 (d, *J* = 8.59 Hz, 1H), 8.01 (dd, *J* = 2.02, 8.84 Hz, 1H), 7.91 (d, *J* = 2.53 Hz, 1H), 7.80 (d, *J* = 2.02 Hz, 1H), 7.63 - 7.70 (m, 2H), 7.55 (d, *J* = 4.29 Hz, 1H), 7.31 (td, *J* = 6.06, 9.09 Hz, 1H), 7.18 - 7.27 (m, 1H), 6.85 - 6.99 (m, 3H).

N-(2,4-difluorophenyl)-5-[4-(4-pyridinyl)-6-quinolinyl]-3-pyridinesulfonamide (**6g**)



^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 9.23 (d, *J* = 2.02 Hz, 1H), 9.08 (d, *J* = 4.55 Hz, 1H), 8.69 - 8.87 (m, 3H), 8.34 (t, *J* = 2.02 Hz, 1H), 8.32 (d, *J* = 8.84 Hz, 1H), 8.18 (dd, *J* = 2.02, 8.84 Hz, 1H), 8.10 (d, *J* = 1.52 Hz, 1H), 7.71 (d, *J* = 5.81 Hz, 2H), 7.63 (d, *J* = 4.55 Hz, 1H), 7.30 (td, *J* = 6.06, 8.97 Hz, 1H), 7.24 (ddd, 1H), 6.96 - 7.09 (m, 1H). 100% by LCMS: *m/z* 475.0 $[M+H]^+$.

2,4-difluoro-*N*-{5-[4-(4-pyridinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide (**6h**)



^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (br. s., 1H), 9.05 (d, *J* = 4.55 Hz, 1H), 8.83 (d, *J* = 5.81 Hz, 2H), 8.67 (d, *J* = 1.52 Hz, 1H), 8.35 (d, *J* = 2.02 Hz, 1H), 8.27 (d, *J* = 8.84 Hz,

1H), 8.09 (dd, *J* = 1.89, 8.72 Hz, 1H), 7.85 - 7.98 (m, 2H), 7.75 (s, 1H), 7.69 (d, *J* = 5.81 Hz, 2H), 7.60 (d, *J* = 4.29 Hz, 1H), 7.47 - 7.58 (m, 1H), 7.14 - 7.29 (m, 1H). 97% by LCMS: *m/z* 475.0 $[M+H]^+$.

Syntheses of BEZ235, GDC-0941, LY294002, ZSTK-474, and PI-103 were accomplished using literature methods. Each compound was isolated in >98% purity by HPLC.