Discovery of a potent class of PI3Kα inhibitors with unique binding mode via Encoded Library Technology (ELT)

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Supporting Information

General Information

All solvents and reagents were purchased from commercial resource and used without further purification. All $^1$H NMR, and $^{13}$C NMR spectra were recorded either using a Varian Mercury 400 plus (NMR-A) or a Bruker 400 UltraShield Advance (NMR-B) at
ambient temperature. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants (J) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet), m (multiplet), br (broad). High-resolution mass measurement for compound 5e was acquired on a Waters qTOF Premiere. High-resolution mass measurement for the rest of the compounds were obtained on Bruker MicroTOF electrospray mass spectrometer coupled with an Agilent 1100 HPLC system.

**General approach for synthesis of biaryl sulfonamides**

The general route to synthesize desired compounds is described in Scheme S1. A Suzuki cross coupling of aldehyde 6 with a boronic acid or ester followed by reductive amination afforded biaryl 7. Compound 7 can also be made with the order of reductive amination first followed by Suzuki coupling. Saponification of compound 7 gave acid 8, which was further modified via a general coupling reaction to produce amide 5. One of Amide 5 was alkylated to generate tertiary amines.

**Scheme S1**
General procedure for Suzuki cross-coupling

A mixture of a substituted aryl iodide (1.0 equiv), boronic acid or ester (1.5 equiv), Cs$_2$CO$_3$ (1.5 equiv), and Pd(PPh$_3$)$_4$ (0.05 equiv) in DMA was heated at 70 ºC overnight. The reaction was monitored and analyzed by an LC-MS system. The reaction mixture was then diluted with ethyl acetate and washed with H$_2$O (2x) and saturated NaCl (1x). The organic layer was dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography using the Combi-Flash system (Hex:EtOAc) as needed.

General procedure for reductive amination

To a solution of the desired aldehyde (1.0 equiv) in anhydrous DCM, was added amine (1.0 equiv) followed by a few drops of glacial acetic acid. In most cases, 2 to 3 drops of acetic acid is enough. However in some instances a 5-10% acetic acid as co-solvent is necessary to obtain product or to improve the product yield. The reaction was then allowed to stir for 10-20 minutes before addition of Na(OAc)$_3$BH (1.2 equiv). The reaction mixture is then allowed to stir at room temp overnight. The reaction was diluted with ethyl acetate and washed with H$_2$O (2x) and saturated NaCl (1x). The organic layer was dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography using the Combi-Flash system (Hex:EtOAc) as needed.

General procedure for hydrolysis of methyl ester

To a solution of methyl ester (1 equiv) in the mixture of THF/MeOH (4/1) added LiOH water solution (2M, 20 equiv) and the reaction was stirred at rt for 2-6 hours. Upon the completion of the reaction, removed the organic solvent and diluted the residue with water. Filter out any insoluble substance and added 1N HCl to adjust the pH of filtrate to 3–5 so that the final compound can be precipitated out. Either extraction of filtrate using organic solvent or centrifuging the filtrate gave the pure final compound.
**General method for HATU amide coupling**

To a solution of HATU (1.1 equiv) in dry DMF or CH$_2$Cl$_2$/DMF was added carboxylic acid (1.0 equiv) and the desired amine (1.1 equiv) followed by diisopropylethylamine (DIPEA) (5 equiv). The reaction mixture was stirred at rt for 2-18 hours and monitored by LC-MS. Upon the completion of the reaction, the organic solvent was removed. The residue was then redissolved in a mixture of water and DMSO and was purified on reverse-phase preparative HPLC to give desired product.

**Methyl 3-formyl-5-(6-methoxypyridin-3-yl)benzoate (9)**

The title compound 9 was obtained from commercial available starting material 6 and (6-methoxypyridin-3-yl)boronic acid through Suzuki reaction as an orange solid in 56\% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) $\delta$ 10.15 (s, 1H), 8.60 (d, $J = 2.4$ Hz, 1H), 8.43 (m, 2H), 8.40 (m, 1H), 8.15 (dd, $J = 2.4$, 8.4 Hz, 1H), 8.15 (d, $J = 8.8$ Hz, 1H) 3.94 (s, 3H), 3.92 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 193.0, 165.8, 164.2, 145.7, 139.2, 138.4, 137.7, 132.4, 132.2, 131.9, 128.7, 127.8, 111.3, 53.9, 53.1.

**Methyl 3-iodo-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzoate (5c)**
The title compound 5c was obtained from commercial available starting material 6 and 10 through reductive alkylation reaction as off-white powder in 99% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) δ 8.11 (s, 1H), 8.00 (s, 1H), 7.93 (s, 1H), 7.46 (d, $J$ = 8.8 Hz, 2H), 7.20 (t, $J$ = 6.0 Hz, 1H), 6.63 (d, $J$ = 8.8 Hz, 2H), 4.39 (d, $J$ = 5.6 Hz, 2H), 3.84 (s, 3H), 2.44 (s, 3H). $^{13}$C NMR (NMR-A, 100 MHz, DMSO-d$_6$) δ 165.3, 151.8, 143.4, 140.8, 140.7, 136.4, 132.1, 128.1, 128.0, 127.5, 111.8, 95.4, 52.9, 45.1, 16.5. HRMS (M+H)$^+$ calcd for [C$_{18}$H$_{17}$IN$_4$O$_4$S$_2$+ H] 544.9809; found 544.9823.

Methyl 3-(6-methoxypyridin-3-yl)-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzoate (5d)

The title compound 5d was obtained from compound 9 and commercial available starting material 10 through reductive amination reaction as white solid in 80% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) δ 8.50 (d, $J$ = 2.4 Hz, 1H), 8.05 (m, 2H), 7.94 (m, 2H), 7.46 (d, $J$ = 8.8 Hz, 2H), 7.22 (t, $J$ = 6.4 Hz, 1H), 6.95 (d, $J$ = 8.8 Hz, 1H), 6.67 (d, $J$ = 8.8 Hz, 2H), 4.47 (d, $J$ = 6.0 Hz, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 2.43 (s, 3H). $^{13}$C NMR
3-(6-Methoxypyridin-3-yl)-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzoic acid (5e)

The title compound 5e was hydrolyzed from compound 5d as off-white powder in 90% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) $\delta$ 8.45 (d, $J$ = 2.8 Hz, 1H), 8.00 (m, 2H), 7.87 (m, 2H), 7.38 (d, $J$ = 8.4 Hz, 2H), 6.89 (m 2H), 6.57 (d, $J$ = 5.2 Hz, 2H), 4.39 (d, $J$ = 6.0 Hz, 2H), 3.88 (s, 3H), 2.32 (s, 3H). $^{13}$C NMR (NMR-A, 100 MHz, DMSO-d$_6$) $\delta$ 167.6, 163.8, 154.2, 145.3, 141.4, 138.2, 137.9, 132.3, 130.2, 128.9, 128.3, 128.1, 127.3, 126.1, 111.8, 111.2, 53.8, 46.0, 16.5. HRMS (M+H)$^+$ calcd for [C$_{23}$H$_{21}$N$_5$O$_5$S$_2$+ H] 512.1062; found 512.1064

$N$-Isopentyl-3-(6-methoxypyridin-3-yl)-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzamide (5b)
The title compound 5b was obtained from compound 5e and commercial available starting material 3-methylbutan-1-amine through HATU coupling reaction as white powder in 31% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) δ 8.52 (m, 1H), 8.48 (t, $J$ = 5.2 Hz, 1H), 8.04 (dd, $J$ = 6.0, 2.8 Hz, 1H), 7.97 (s, 1H), 7.80 (s, 1H), 7.77 (s, 1H), 7.45 (d, $J$ = 7.2 Hz, 2H), 6.95 (d, $J$ = 8.4 Hz, 1H), 6.66 (d, $J$ = 6.8 Hz, 2H) 4.42 (s, 2H), 3.90 (s, 3H), 3.29 (dd, $J$ = 5.6, 8.4 Hz, 2H), 2.42 (s 3H), 1.61 (m, 1H), 1.43 (q, $J$ = 7.2 Hz, 2H), 0.91 (s, 3H), 0.90 (s, 3H). $^{13}$C NMR (NMR-A, 100 MHz, DMSO-d$_6$) δ 163.1, 161.2, 152.1, 145.3, 140.9, 138.1, 137.5, 136.1, 129.2, 128.2, 128.1, 128.0, 125.8, 123.8, 113.0, 111.8, 111.1, 53.8, 46.3, 38.6, 38.5, 38.1, 38.0, 25.8, 22.9, 16.5. HRMS (M+H)$^+$ calcd for [C$_{28}$H$_{32}$N$_6$O$_4$S$_2$ + H] 581.1999; found 581.1992.

$N$-(2-Fluorophenethyl)-3-(6-methoxypyridin-3-yl)-5-((4-((N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzamide (5a)

The title compound 5a was obtained from compound 5e and 2-(2-fluorophenyl)ethanamine through HATU coupling reaction as white powder in 31% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) δ 8.68 (t, $J$ = 5.2 Hz 1H), 8.51 (d, $J$ = 2.4
Hz, 1H), 8.04 (dd, J = 2.4, 8.8 Hz, 1H), 7.95 (s, 1H), 7.78 (s, 2H), 7.46 (d, J = 8.8 Hz 2H), 7.28 (m, 2H), 7.12 (m, 2H), 6.96 (d, J = 8.8 Hz, 1H), 6.66 (d, J = 8.8 Hz, 2H), 4.42 (s, 2H), 3.91 (s, 3H), 3.50 (dd, J = 6.8, 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 2.42 (s 3H). HRMS (M+H)⁺ calcd for [C₃₁H₂₉FN₆O₄S₂ + H] 633.1749; found 633.1762

3-(Benzo[d][1,3]dioxol-5-yl)-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzoic acid (5h)

The title compound 5h was obtained from compound 5c and benzo[d][1,3]dioxol-5-ylboronic acid through Suzuki coupling reaction as white powder in 93% yield. ¹H NMR (NMR-A, 400 MHz, DMSO-d₆) δ 7.96 (s, 1H), 7.86 (s, 1H), 7.83 (s, 1H), 7.42 (d, J = 9.2 Hz, 2H), 7.25 (d, J = 2 Hz, 1H), 7.14 (m, 2H), 7.01 (d, J = 8.4 Hz, 1H), 6.63 (d, J = 9.2 Hz, 2H), 6.07 (s, 2H), 4.41 (d, J = 5.6 Hz, 2H), 2.39 (s, 3H). ¹³C NMR (NMR-A, 100 MHz, DMSO-d₆) δ 167.7, 152.1, 148.6, 147.7, 141.2, 140.8, 133.9, 132.0, 130.3, 128.3, 128.0, 127.2, 126.9, 126.2, 120.9, 120.6, 111.8, 109.2, 107.6, 101.7, 46.0, 16.5. HRMS (M+H)⁺ calcd for [C₂₄H₂₀N₄O₆S₂ + H] 525.0897; found 525.0907

3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenylamino)methyl)benzoic acid (5g)
The title compound 5g was obtained from compound 5c and (2,3-dihydrobenzo[b][1,4]dioxin-6-yl)boronic acid through Suzuki coupling reaction as white powder in 96% yield. $^1$H NMR (NMR-A, 400 MHz, DMSO-d$_6$) δ 7.96 (s, 1H), 7.84 (d, $J = 9.2$ Hz, 2H), 7.45 (d, $J = 9.2$ Hz, 2H), 7.32 (t, $J = 5.6$ Hz, 1H), 7.14 (m, 2H), 6.95 (d, $J = 8.0$ Hz, 1H), 6.67 (d, $J = 8.8$ Hz, 2H), 4.43 (s, 2H), 4.28 (s, 4H), 2.42 (s, 3H). $^{13}$C NMR (NMR-A, 100 MHz, DMSO-d$_6$) δ 167.7, 152.1, 144.2, 144.0, 141.2, 140.5, 132.9, 132.0, 130.0, 128.3, 128.1, 128.0, 126.8, 125.9, 120.1, 118.1, 115.6, 113.0, 111.8, 64.6, 64.6, 46.0, 16.5. HRMS (M+H)$^+$ calcd for [C$_{25}$H$_{22}$N$_4$O$_6$S$_2$ + H] 539.1054; found 539.1046

$N$-(2-fluorophenethyl)-3-(6-methoxypyridin-3-yl)-5-(((4-(N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)amino)methyl)benzamide (5i)

The title compound 5i was synthesized following the procedures described below in Scheme S2
**Step 1**: preparation of Methyl 3-bromo-5-(hydroxymethyl)benzoate (S3)

To a 250 mL round-bottomed flask under nitrogen (rt) was added dimethyl 5-bromo-1,3-benzenedicarboxylate (S2, 5 g, 18.31 mmol), THF (50 mL) and sodium borohydride (0.693 g, 18.32 mmol) to give a colorless solution. To this mixture was added dropwise 5 mL of MeOH. The reaction mixture was stirred at 50 °C for 1 h. Then cooled and diluted with ethyl acetate and the organic phase was washed with brine and evaporated. The oil residue was purified by silica gel chromatography (0-50% Ethyl acetate / hexanes) to give methyl 3-bromo-5-(hydroxymethyl)benzoate (3.8 g, 15.5 mmol, 42% yield) as a clear oil. MS (ES+) m/e 244.8, 246.8 [M+H]+.

**Step 2**: Preparation of 3-Bromo-N-[2-(2-fluorophenyl)ethyl]-5-(hydroxymethyl)benzamide (S4)

Methyl 3-bromo-5-(hydroxymethyl)benzoate (S3, 1.65 g, 6.73 mmol) and [2-(2-fluorophenyl)ethyl]amine (1.874 g, 13.47 mmol) were added to a microwave vial and heated in the microwave at 175 °C for 1 h. The cooled mixture was dissolved in ethyl acetate and washed sequentially with 1N HCl, saturated bicarbonate solution and 2M LiOH solution. The ethyl acetate solution was dried over sodium sulfate, filtered and concentrated under reduced pressure to give a residue which was triturated with DCM to
give 3-bromo-N-[2-(2-fluorophenyl)ethyl]-5-(hydroxymethyl)benzamide (1.24 g, 3.52 mmol, 52%) as a solid. $^1$H NMR (NMR-B, 400 MHz, DMSO-d6) $\delta$ ppm 2.79 - 3.00 (m, 2 H), 3.42 - 3.57 (m, 2 H), 4.37 - 4.76 (m, 2 H), 5.29 - 5.59 (m, 1 H), 6.99 - 7.22 (m, 2 H), 7.20 - 7.36 (m, 2 H), 7.54 - 7.70 (m, 1 H), 7.73 - 7.80 (m, 1 H), 7.80 - 7.88 (m, 1 H), 8.65 - 8.85 (m, 1 H).

**Step 3**: Preparation of 3-Bromo-N-[2-(2-fluorophenyl)ethyl]-5-formylbenzamide (S5)
3-Bromo-N-[2-(2-fluorophenyl)ethyl]-5-(hydroxymethyl)benzamide (S4, 1.24 g, 3.52 mmol) and manganese dioxide (1.53 g, 17.6 mmol) were dissolved in 30 mL of toluene in a reaction bomb. The bomb was sealed and heated at 90 °C for 20 h. The reaction mixture was cooled, filtered through Celite and the solvent was removed under vacuum to give 3-bromo-N-[2-(2-fluorophenyl)ethyl]-5-formylbenzamide (600 mg, 1.713 mmol, 48%). $^1$H NMR (NMR-B, 400 MHz, DMSO-d6) $\delta$ 2.79 - 3.03 (m, 2 H), 3.42 - 3.73 (m, 2 H), 7.05 - 7.22 (m, 2 H), 7.22 - 7.40 (m, 2 H), 8.09 - 8.48 (m, 3 H), 8.73 - 9.07 (m, 1 H), 9.78 - 10.20 (m, 1 H).

**Step 4**: Preparation of N-[2-(2-Fluorophenyl)ethyl]-3-formyl-5-[6-(methyloxy)-3-pyridinyl]benzamide (S6)
3-Bromo-N-[2-(2-fluorophenyl)ethyl]-5-formylbenzamide (S5, 1.22 g, 3.5 mmol), 2-(methyloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (905 mg, 3.85 mmol) and PdCl$_2$(dppf)-CH$_2$Cl$_2$ adduct (143 mg, 0.175 mmol) were added to a 50 mL flask followed by dioxane (17 mL) and saturated sodium bicarbonate (7 mL). The reaction mixture was heated at 100 °C for 2 h. The dioxane was evaporated and the residue was purified by silica gel chromatography (40 g column eluting with chloroform / methanol 0-5% gradient) to give N-[2-(2-fluorophenyl)ethyl]-3-formyl-5-[6-(methyloxy)-3-pyridinyl]benzamide (610 mg, 1.612 mmol, 46%). $^1$H NMR (NMR-B, 400 MHz, DMSO-d6) $\delta$ 2.9 (m, 2 H), 3.5 ( m, 2 H), 3.8 (s, 3 H), 6.6 (m, 1 H), 7.1 - 7.2 (m, 2 H), 7.3 - 7.4 (m, 2 H), 7.8 (s, 1H), 7.91 - 7.97 (m, 2 H), 8.7 - 8.8 (m, 2 H), 8.59 (m, 1 H), 10 (s, 1 H).
Step 5: Preparation of 4-Amino-N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (S8)

4-Amino-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (S7, 541 mg, 2 mmol) was dissolved in DMF (30 mL). Then iodomethane (541 mg, 2.1 mmol) and potassium carbonate (304 mg, 2.1 mmol) were added. The reaction mixture was stirred overnight at room temperature. Water was added to the reaction mixture and the product was extracted into DCM which was back washed with water containing several drops of 1N HCl. The organic phase was dried and concentrated in vacuo. Purification of the residue by silica gel chromatography (DCM / methanol gradient, 0-2%) gave 4-amino-N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (200 mg, 0.703 mmol, 35%). \(^1\)H NMR (NMR-B, 400 MHz, DMSO-d6) δ 2.45 (s, 3 H), 3.6 (s, 3 H), 5.95 (s, 2 H), 6.60 (d, 2 H), 7.45 (d, 2H).

Step 6: Preparation of N-(2-Fluorophenethyl)-3-(6-methoxypyridin-3-yl)-5-(((4-(N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)amino)methyl)benzamide (5i)

To a mixture of N-[2-(2-Fluorophenyl)ethyl]-3-formyl-5-[6-(methyloxy)-3-pyridinyl]benzamide (S6, 100 mg, 0.264 mmol) and 4-amino-N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (S8, 75 mg, 0.264 mmol) in THF (2.5 mL), added and acetic acid (47.6 mg, 0.793 mmol) and zinc chloride (2M in THF, 0.529 mmol). The reaction mixture was stirred at 55 °C for two days. Sodium triacetoxyborohydride (440 mg, 0.661 mmol) was added and the reaction mixture was stirred at 55 °C for three hours. The THF was evaporated and the residue was dissolved in ethyl acetate, washed with water and dried with sodium sulfate. The residue was purified by silica gel chromatography (DCM / methanol gradient 0-2%) to give N-(2-fluorophenethyl)-3-(6-methoxypyridin-3-yl)-5-(((4-(N-methyl-N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)amino)methyl)benzamide (50 mg, 0.077 mmol, 29% yield). \(^1\)H NMR (NMR-B, 400 MHz, DMSO-d6) δ 2.44 (s, 3 H), 2.89 (t, \(J = 7.20\) Hz, 2 H), 3.50 (q, \(J = 6.57\) Hz, 2 H), 3.56 (s, 3 H), 3.91 (s, 3 H), 4.42 (d, \(J = 6.06\) Hz, 2 H), 6.67 (m, \(J = 9.09\) Hz, 2 H), 6.96 (d, \(J = 8.59\) Hz, 1 H), 7.08 - 7.17 (m, 2 H), 7.19 - 7.33 (m, 3 H), 7.49 (m, \(J = 8.84\) Hz, 2 H), 7.79 (s, 2 H), 7.95 (s, 1 H), 8.04 (dd, \(J = 8.59, 2.53\) Hz, 1
H), 8.52 (d, \( J = 2.53 \) Hz, 1 H), 8.69 (t, \( J = 5.56 \) Hz, 1 H). \(^{13}\)C NMR (NMR-A, 100 MHz, DMSO-d\(_6\)) \( \delta \) 166.4, 164.0 (d, \( J = 42.7 \) Hz), 162.4, 160.0, 152.6 (d, \( J = 79.2 \) Hz), 145.3, 141.1, 137.8 (d, \( J = 57.1 \) Hz), 135.9, 131.7, 129.0 (d, \( J = 32.8 \) Hz), 128.7, 128.2, 128.0, 126.5 (d, \( J = 15.2 \) Hz), 125.8, 124.8, 123.8, 115.6 (d, \( J = 21.3 \) Hz), 111.8, 111.1, 53.8, 46.2, 37.1, 29.1, 16.4. HRMS (M+H)\(^+\) calcd for \([C_{32}H_{31}FN_6O_4S_2+H]\) 647.1905; found 647.1901.

4-((3-(6-methoxypyridin-3-yl)benzyl)amino)-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (5f)

\[
\text{I} \quad + \quad \text{N} \quad \text{S} \quad \text{S} \\
\text{N} \quad \text{N} \quad \text{S} \\
\text{O} \quad \text{B} \quad \text{O} \\
\text{H} \quad \text{H} \quad \text{H}
\]

The title compound \(5f\) was obtained from compound 7 (synthesized in house with same method to generate compounds \(5c\)) and (6-methoxypyridin-3-yl)boronic acid through Suzuki coupling reaction as white powder in 67% yield. \(^1\)H NMR (NMR-A, 400 MHz, DMSO-d\(_6\)) \( \delta \) 8.43 (s, 1 H), 7.84 (d, \( J = 8.4 \) Hz, 1 H), 7.61 (s, 1 H), 7.51 (d, \( J = 7.2 \) Hz, 1 H), 7.44 (dd, \( J = 8.8, 1.6 \) Hz, 2 H), 7.39 (t, \( J = 8.4 \) Hz, 1 H), 7.30 (d, \( J = 7.2 \) Hz, 1 H), 6.90 (d, \( J = 8.4 \) Hz, 1 H), 6.65 (d, \( J = 7.6 \) Hz, 2 H), 4.36 (s, 2 H), 3.87 (s, 3 H), 2.40 (s, 3 H). \(^{13}\)C NMR (NMR-A, 100 MHz, DMSO-d\(_6\)) \( \delta \) 167.4, 163.5, 154.2, 152.2, 145.0, 140.5, 138.1, 137.5, 129.7, 129.6, 128.1, 128.0, 126.7, 125.8, 125.4, 111.8, 111.1, 53.8, 46.4, 16.5. HRMS (M+H)\(^+\) calcd for \([C_{22}H_{21}N_5O_3S_2+H]\) 468.1159; found 468.1143

N-(1,3-dihydroxypropan-2-yl)-3-(6-methoxypyridin-3-yl)-5-(((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)amino)methyl)benzamide (5j)
The title compound 5j was obtained from compound 5e and 2-aminopropane-1,3-diol through HATU coupling reaction as white powder in 20% yield. $^1$H NMR (NMR-B, 400 MHz, DMSO-$d_6$) $\delta$ 8.53 (d, $J = 2.02$ Hz, 1 H), 8.02 - 8.11 (m, 2 H), 7.99 (s, 1 H), 7.77 - 7.84 (m, 2 H), 7.37 (d, $J = 8.59$ Hz, 2 H), 6.94 (d, $J = 8.59$ Hz, 1 H), 6.74 (t, $J = 5.68$ Hz, 1 H), 6.55 (d, $J = 8.59$ Hz, 2 H), 4.68 (t, $J = 5.68$ Hz, 2 H), 4.37 (d, $J = 5.56$ Hz, 2 H), 3.95 - 4.02 (m, 1 H), 3.91 (s, 3 H), 3.53 (t, $J = 5.31$ Hz, 4 H), 2.31 (s, 3 H). $^{13}$C NMR (NMR-A, 100 MHz, DMSO-$d_6$) $\delta$ 169.4, 166.6, 163.7, 153.1, 150.3, 145.4, 141.3, 138.1, 137.3, 136.2, 133.0, 129.3, 128.2, 127.8, 126.1, 124.1, 111.4, 111.1, 60.9, 54.4, 53.8, 46.6, 16.1. HRMS (M+H)$^+$ calcd for $[C_{26}H_{28}N_6O_6S_2+ H]^+$ 585.1585; found 585.1586.

**Materials and Methods for Crystallography:**

Recombinant purified$^{51}$ PI3Kalpha protein was concentrated to 32 mg/ml and stored at -80°C. The protein was incubated with 20 µM PIP2 (Phosphatidylinositol 4,5-bisphosphate) and 1 mM inhibitor on ice at 4°C for 24 hours. Protein was centrifuged in a microcentrifuge for 10 min at 14,000g. Seed stock for crystallization experiments was prepared from an apo crystal that was crushed and diluted with 25% ethylene glycol, 0.1 M HEPES, pH 7.5. Dilutions of 1:50 and 1:500 produced the best crystals for this inhibitor. Reagents for the crystallization drop were dispensed onto 96-well hanging drop plates using the Mosquito liquid handler at 4°C in the following order: 200 nl protein, 50 nl seed dilution, 15 nl well solution. Plates were incubated for growth at 4°C. Crystals were mounted from a croprotectant solution of 30% ethylene glycol, 70% well solution.
Data were collected from a single crystal at the 21idG LS-CAT beamline at Argonne National Laboratory. The dataset was indexed and scaled using HKL2000\textsuperscript{S2}. The structure was solved by molecular replacement using CCP4i\textsuperscript{S3} Phaser\textsuperscript{S4} with an unpublished structure of apo Pi3Kalpha as the model. Model building was done with the program Coot\textsuperscript{S5} and the structure was refined with Phenix\textsuperscript{S6}. The structure was validated with MolProbity\textsuperscript{S7}.

Residue numbering of model:

Figure 3 uses standard p110a numbering. Structure deposited in the RCSB Protein Data Bank (4YKN) of construct (p85a (308-615) – GSPGISGGGGG-p110a) was numbered with the standard p110a residue numbers incremented by 1000.

Structure results:

The structure of this fusion protein is highly similar to the structures of previously published structures of Pi3K alpha. Electron density for the backbone atoms of the p110a residues is generally good but density for p85a residues is disordered and difficult to fit in many places. There is excellent density for the ligand in the ATP binding site. Two additional copies of the ligand are bound in a non-relevant surface pocket far from the ATP binding site.

References:

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S2) Otwinowski, Z.; Minor, W. Methods in Enzymology 1997, 276, 307-326.
S3) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicolas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S.; Acta. Cryst. 2011, D67, 235-242.
Table S1. Statistics for data collection and structure refinement

|                          |                |
|--------------------------|----------------|
| PDB Code                 |                |
| X-ray Source             | LS-CAT 21idG   |
| Wavelength, Å            | 0.97856        |
| Space group              | P2_12_12_1     |
| No. Molecules in the asymmetric unit | 1              |
| Unit cell, a, b, c, in Å (α, β, γ in º) | 84.7, 118.2, 190.3, 90, 90, 90 |
| Resolution Range, (last shell) Å | 29.9-2.9 (3.0-2.9) |
| Rsym, (last shell)       | 0.096 (0.549)  |
| Competence, % (last shell %) | 100.0 (100.0) |
| No. Of Unique Reflections (last shell) | 43067 (4217) |
| Multiplicity (last shell) | 7.4 (7.6)      |
| Avg, I/σ (last shell)    | 17.3 (4.4)     |
| Refinement Statistics    |                |
| Total No. Of Atoms       | 9587/105       |
| Protein/Inhibitor        |                |
| Rfree, Rfactor           | 24.7, 20.2     |
| RMS deviation bond lengths, Å, angles, º | 0.003, 0.55 |
| Avg.B-factor             |                |
| Protein/Inhibitor/Solvent | 47/51/30       |
| Ramachandran             |                |
| Favored, Allowed, Outliers, % | 97.7, 2.21, 0.08 |
| Rotamer outliers, %      | 0.41           |