Supporting Information

Crystal Structures of PI3Kα Complexed with PI103 and Its Derivatives, New Directions for Inhibitor Design

Material and Methods

Expression, purification and crystallization of PI3Kα

The gene templates of full-length human PI3Kα (Accession No. P42336) and human p85 (Accession No. NP_852664) were synthesized at Vivabiotech. Through PCR amplification, the two genes were joined by a linker (GSPGISGGGGG) and then sub-cloned into the pFastBac HtB vector (Invitrogen) for the expression of fusion protein N-6*His tag-TEV site-p85α (318-615)-GSPGISGGGGG-full length PI3Kα. The positive clone containing pFastBac-His–TEV-p85α(318-615)-linker-p110α was verified by sequencing.

pFastBac-His–TEV-p85α(318-615)-linker–p110α was transformed into DH10Bac *Escherichia coli* for transposition into the bacmid, and positive clones were selected on a blue/white LB agar plate. Recombinant bacmid was isolated from positive clones with the PureLink Hipure Plasmid DNA miniprep kit (Invitrogen) and transfected into SF9 cells (Invitrogen) to generate recombinant virus stocks, which were amplified for two cycles before infection of SF9 cells at a multiplicity of infection (MOI) of 2. The SF9 cells, at a density of 2×10^6 cells/mL, were infected with baculovirus stocks at an MOI of 3. The infection was allowed to proceed at 27℃ for 48 hs at 24.5 rocks per minute on an orbital shaker. At the time of harvest, the average cell viability was approximately 75%. The cell suspension was frozen and stored at -80℃ until protein purification.

Cells were lysed by two passes through an Avestin pressure drop homogenizer at 12 kpsi and then centrifuged for 1 h at 15,000 x g. The supernatant was mixed with Ni–NTA agarose (Qiagen) at 4℃ for 3 hrs. The resin was collected and washed with 100 ml of buffer A **(**20 mM Tris–HCl pH 7.8, 250 mM NaCl, 2 mM TCEP, 20 mM imidazole, 5% glycerol**)** and 100 ml of buffer B **(**20 mM Tris–HCl pH 7.8, 250 mM NaCl, 2 mM TCEP, 40 mM imidazole, 5% glycerol**)** and then eluted with buffer C **(**20 mM Tris–HCl pH 7.8, 250 mM NaCl, 2 mM TCEP, 250 mM imidazole, 5% glycerol**)**. The protein was diluted at a ratio of 1:5 with buffer A **(**20 mM Tris-HCL, pH 8.0, 5% glycerol**)** and purified by ion-exchange chromatography on Sepharose Q **(**eluted by a linear gradient from A to B [buffer A=20 mM Tris-HCL, pH 8.0, 5% glycerol; buffer B= 20 mM Tris-HCL, pH 8.0, 1 M NaCl, 5% glycerol]**)**. The protein was then concentrated to 5 ml and purified by size-exclusion chromatography on Superdex 200 (20 mM Tris, 0.2 M NaCl, 5% glycerol, 2 mM TCEP pH 7.8). The resulting protein was approximately 95% pure (as judged by SDS–PAGE).

Crystallization screening was performed by hanging drops of 2 µl (1 µl protein + 1 µl reservoir) at 18°C with 11 mg of fusion protein His–p85α(318-615)-linker–p110α in buffer (20 mM Tris-HCL, 200 mM NaCl, 2 mM TCEP, pH 7.8, 5% glycerol). After screening approximately 1400 conditions, small twin crystals were first identified in a solution (0.2 M lithium sulfate, 0.1 M Tris pH 8.5, 26 % (W/V) PEG 2000 MME). Hair seeding was then used to obtain larger crystals in optimized mother liquor (0.15 M lithium sulfate, 0.1 M Tris PH 8.5, 30 % (W/V) PEG 1000 MME). Diffraction-quality crystals (0.05 mm x 0.1 mm x 0.6 mm in size) were obtained after repeated rounds of macro-seeding. *Apo* crystals were flash-frozen in liquid nitrogen using the reservoir solution plus 10% glycerol as cryoprotectant. Furthermore, *apo* crystals were soaked in 10 mM PI103, **9d** and other compounds for 2 hrs and then mounted according to the above method.

Expression and Purification of Mutated PI3Kα

GST-tagged three mutated PI3Kα proteins (p110α-M772A, p110α-D810A and p110α-Y836A) were co-expressed with full-length p85α using the Bac-to-Bac Baculovirus expression system (Invitrogen). The recombinant proteins were extracted from cells and purified using Glutathione-Sepharose 4B resin (GE Healthcare).

In vitro PI3K Kinase Assays

PI3K inhibitors were dissolved as stock solutions at 10 mM in 100% dimethylsulfoxide (DMSO) and stored in aliquots at -20°C. The compounds were diluted to the desired concentrations immediately before each experiment. The kinase activity of the purified PI3Kα was determined by the PI3-Kinase HTRF Assay (Millipore). The assays were performed according to the manufacturer's protocol. Briefly, the EC_{80} concentration of each enzyme was incubated in the assay buffer, containing 10 µM PIP2, in a white 384-well plate (Perkin Elmer). After incubation at room temperature for 30 min, the reaction was initiated by adding ATP and then terminated by adding the stop solution and the detection mix. The final concentrations of ATP were 5 µM for p110α. The plate was then sealed and incubated at room temperature overnight. The intensity of the light emission was measured by an EnVision Multilabel Reader (PerkinElmer) in TR-FRET mode (excitation at 320 nm and emission at 665 nm). The IC_{50} values were calculated by fitting data to a logistic curve using GraphPad Prism 6 software.

Molecular Modeling

Molecular modeling studies were carried out using AutoDock software version 4.2.[1] Compounds **9a** and **9e** were built using the Sketch module and optimized using the Tripos force field of SYBYL7.3.[2] The protein structure was prepared for docking using AutoDock Tools, which included stripping all water and adding polar hydrogen atoms and Kollman united partial atomic charges for protein. Docking simulations were performed on the crystal structure of PI3Kα–PI103. The grid center was defined at the centroid of PI103, and the number of grid points in the *x*, *y*, and *z* directions was set to 60, 60, and 60 with a spacing value of 0.375 Å using AutoGrid. Fifty conformations were generated using the Lamarckian genetic algorithm. After clustering analysis, the lowest-energy complex in the cluster with the largest number of neighbors was selected for study.

Statistical Analysis

Data are presented as the mean ± SD from at least two independent experiments, and differences were considered significant when $p < 0.05$, as determined by Student's t test.

Chemistry

The synthetic approach for the PI103 derivatives, compounds **9a** to **9h**, is outlined in scheme 1. 2-Chloronicotinonitrile (compound **1**) and ethyl glycolate (compound **2**) were treated with DBU in ethanol to give bicyclic acid ester **3**. Then, compounds **5** were synthesized via the coupling of various 3-methoxybenzoic chlorides to bicyclic acid ester **3** under alkaline conditions. A series of acid amides **6** were subsequently obtained through hydrolysis, chlorination and amination. Meanwhile, amides **6** were obtained from analogues **5** via an amino–ester exchange reaction. Subsequently, key intermediates **7** were obtained by cyclizing **6** under previously reported conditions using sodium hydroxide and isopropanol as a solvent in high yield.[3] Compounds **7** were chlorinated with phosphorus oxychloride and immediately reacted with morpholine in toluene under boiling conditions to produce the desired derivatives **8a-8c** and **8e-8g**. Successful synthesis of **8d** from the corresponding **8f** was performed via a reduction with iron. Final compounds **9a-9h** were obtained by demethylation with hydrobromic acid.[4-5]

Synthesis of compounds 3 to 9h:

Ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**). 2-chloronicotinonitrile (40g, 0.301mol), ethyl glycolate (36.08g, 0.357mol), and DBU (66.0ml, 0.433mol) were suspended in 500 mL of ethanol, and the mixture was stirred at 75℃ overnight. After the reaction was completed by TLC,

the solvent was almost evaporated and the brown oil was diluted with ethyl acetate (800 mL) and washed with water (200 mL*2), aqueous $NaHCO_3$ (200 mL*2) and brine (200 mL*2). The organic phase was dried (Na_2SO_4) and concentrated. The crude oil was purified via by flash chromatography (ether: ethyl acetate = 2:1) to afford **3** (13.2g, 21.3%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49-8.51 (m, 1H), 9.94-7.97 (m, 1H), 7.27-7.26 (m, 1H), 5.06 (s, 2H), 4.41-4.46 (m, 2H), 1.42-1.45 (t, 3H). LRMS (ESI+): 206.9 $(M+H)^+$

Ethyl 3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5a**). A solution of 3-fluoro-5-methoxybenzoic acid (0.825g, 4.85mmol) in 10 mL of thionyl chloride (10ml) was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 1.000g, 4.85mmol) and pyridine (0.59mL, 7.27mmol) in 10 mL of tetrahydrofuran while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and kept at room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5a** (1.530g, 88.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.51 (s, 1H), 9.02-9.05 (m, 1H), 8.54-8.56 (m, 1H), 8.35-8.38 (m, 2H), 7.29-7.32 (m, 1H), 6.85-6.88 (m, 1H), 4.49-4.55 (m, 2H), 3.91 (s, 3H), 1.47-1.50 (t, 3H). LRMS (ESI+): 359.0 $(M+H)^+$

Ethyl 3-(3-chloro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5b**). A solution of 3-chloro-5-methoxybenzoic acid (0.905g, 4.85mmol) in 10 mL of thionyl chloride was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 1.000g, 4.85mmol) and pyridine (0.59mL, 7.27 mmol) in 10 mL of tetrahydrofuran while the cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and moved to room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5b** (1.487g, 81.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.48 (s, 1H), 9.00-9.03 (m, 1H), 8.54-8.56 (t, 1H), 7.56-7.57 (t, 1H), 7.45-7.46 (t, 1H), 7.35-7.38 (m, 1H), 7.14-7.15 (t, 1H), 4.50-4.55 (m, 2H), 3.90 (s, 3H), 1.47-1.51 (t, 3H). LRMS (ESI+): 374.9 $(M+H)^+$

Ethyl 3-(3-bromo-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5c**). A solution of 3-bromo-5-methoxybenzoic acid (0.95g, 4.11mmol) in 10 mL of thionyl chloride was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 0.848g, 4.11mmol) and pyridine (0.50mL, 6.17 mmol) in 10 mL of tetrahydrofuran while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and kept at room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5c** (1.543g, 89.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.46 (s, 1H), 9.00-9.02 (m, 1H), 8.54-8.56 (m, 1H), 7.71-7.72 (t, 1H), 7.48-7.49 (t, 1H), 7.35-7.38 (m, 1H), 7.29-7.30 (t, 1H), 4.50-4.55 (m, 2H), 3.90 (s, 3H), 1.47-1.51 (t, 3H). LRMS (ESI+): 419.0 and 421.0 $(M+H)^+$

Ethyl 3-(3,5-dimethoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5e**). A solution of 3,5-dimethoxybenzoic acid (1g, 5.49 mmol) in thionyl chloride (10 mL) was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in tetrahydrofuran (5 mL) and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (1.132 g, 5.49 mmol) and pyridine (0.53 mL, 6.59 mmol) in 10 mL of tetrahydrofuran while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and kept at room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5e** (1.490 g, 73.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.49 (s, 1H), 9.04-9.07 (m, 1H), 8.53-8.55 (m, 1H), 7.34-7.37 (m, 1H), 7.16-7.17 (d, 2H), 6.69-6.70 (t, 1H), 4.48-4.54 (m, 2H), 3.89 (s, 6H), 1.46-1.49 (t, 3H). LRMS (ESI+): 409.0 $(M+K)^+$

Ethyl 3-(3-methoxy-5-nitrobenzamido)furo[2,3-b]pyridine-2-carboxylate (**5f**). A solution of 3-methoxy-5-nitrobenzoic acid (0.956g, 4.85mmol) in 10mL of thionyl chloride was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oil acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 1.000g, 4.85mmol) and pyridine (0.59mL, 7.27 mmol) in 10 mL of tetrahydrofuran whilecooling the reaction mixture to 0~5℃. The reaction was

stirred at the same temperature for about 30 mintues and kept at room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5f** (1.712g, 91.6%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, 1H), 9.00-9.03 (m, 1H), 8.56-8.58 (m, 1H), 8.45-8.46 (t, 1H), 7.97-7.98 (t, 1H), 7.88-7.89 (t, 1H), 7.37-7.40 (m, 1H), 4.51-4.57 (m, 2H), 4.01 (s, 3H), 1.47-1.51 (t, 3H). LRMS (ESI+): 386.0 $(M+H)^+$

Ethyl 3-(3-methoxy-5-methylbenzamido)furo[2,3-b]pyridine-2-carboxylate (5g). A solution of 3-methoxy-5-methylbenzoic acid (0.806g, 4.85mmol) in 10 mL of thionyl chloride was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 1.000g, 4.85mmol) and pyridine (0.59mL, 7.27 mmol) in 10 mL of tetrahydrofuran while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and moved to room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5g** (1.543g, 89.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.47 (s, 1H), 9.04-9.06 (m, 1H), 8.53-8.55 (t, 1H), 7.34-7.39 (m, 3H), 6.97 (s, 1H), 4.48-4.54 (m, 2H), 3.89 (s, 3H), 2.45 (s, 3H), 1.46-1.50 (t, 3H). LRMS (ESI+): 355.0 $(M+H)^+$

Ethyl 3-(3-methoxy-5-(trifluoromethyl)benzamido)furo[2,3-b]pyridine-2-carboxylate (**5h**). A solution of 3-methoxy-5-(trifluoromethyl) benzoic acid (1.068g, 4.85mmol) in 10 mL of thionyl chloride was heated at reflux for 6 hours. The thionyl chloride was almost evaporated and the oily acid chloride was dissolved in 5 mL of tetrahydrofuran and slowly added to a mixture of ethyl 3-aminofuro[2,3-b]pyridine-2-carboxylate (**3**, 1.000g, 4.85mmol) and pyridine (0.59mL, 4.85 mmol) in 10 mL of tetrahydrofuran while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same temperature for about 30 mintues and kept at room temperature overnight. Water was added slowly and the reaction continued stirring for 1 hour. The white solid was formed, filtered, dried and recrystalized to yield the desired product **5h** (1.712g, 86.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.58 (s, 1H), 9.01-9.04 (m, 1H), 8.55-8.57 (t, 1H), 7.85 (s, 1H), 7.73 (s, 1H), 7.36-7.39 (m, 2H), 4.50-4.55 (m, 2H), 3.96 (s, 3H), 1.47-1.50 (t, 3H). LRMS (ESI+): 408.9 $(M+H)^+$

3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6a**) To a mixture of ethyl 3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5a**, 1.512g, 4.22mmol) in 30 mL of methanol was added 20 mL of concentrated ammonium hydroxide solution at room temperature. The reaction mixture was heated at 50℃ for 6 hours. Then the reaction was allowed to cool to room temperature and white a solid precipitated. The reaction mixture was concentrated to half of its original volume, filtered and dried to provide **6a** (1.145g, 82.4%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.67-8.69 (m, 1H), 8.49-8.52 (m, 1H), 8.42 (br, s, 1H), 8.05 (br, s, 1H), 7.45-7.49 (m, 1H), 7.37 (s, 1H), 7.32-7.35 (m, 1H), 7.14-7.18 (m, 1H), 3.86 (s, 3H). LRMS (ESI+): 329.9 $(M+H)^+$

3-(3-chloro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6b**) To a mixture of ethyl 3-(3-chloro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5b**, 1.473g, 3.93mmol) in 30 mL of methanol was added 20 mL of concentrated ammonium hydroxide solution at room temperature. The reaction mixture was heated at 50℃ for 6 hours. Then the reaction was allowed to cool to room temperature and white a solid precipitated. The reaction mixture was concentrated to half of its original volume, filtered and dried to provide **6b** (1.133g, 83.4%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.64-8.67 (m, 1H), 8.50-8.51 (m, 1H), 8.42 (br, s, 1H), 8.05 (br, s, 1H), 7.56-7.57 (t, 1H), 7.45-7.48 (m, 2H), 7.33-7.34 (m, 1H), 3.86 (s, 3H). LRMS (ESI+): 345.9 $(M+H)^+$

3-(3-bromo-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6c**) To a mixture of ethyl 3-(3-bromo-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5c**, 1.529g, 3.65mmol) in 30 mL of methanol was added 20 mL of concentrated ammonium hydroxide solution at room temperature. The reaction mixture was heated at 50℃ for 6 hours. Then the reaction was allowed to cool to room temperature and a white solid precipitated. The reaction mixture was concentrated to half of its original volume, filtered and dried to provide **6c** (1.157g, 81.3%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.64-8.66 (m, 1H), 8.49-8.51 (m, 1H), 8.41 (br, s, 1H), 8.05 (br, s, 1H), 7.69 (s, 1H), 7.43-7.49 (m, 3H), 3.85 (s, 3H). LRMS (ESI+): 389.9 and 391.9 $(M+H)^+$

3-(3,5-dimethoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6e**). To a mixture of ethyl 3-(3,5-dimethoxybenzamido)furo[2,3-b]pyridine-2-carboxylate (**5e**, 1.49 g, 4.02 mmol) in 30 mL of methanol was added 20 mL of concentrated ammonium hydroxide solution at room temperature.

The reaction mixture was heated at 50℃ for 6 hours. Then the reaction was allowed to cool to room temperature and a white solid precipitated. The reaction mixture was concentrated to half of its original volume, filtered and dried to provide **6e** (1.231 g, 89.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68-8.70 (m, 1H), 8.50-8.52 (m, 1H), 8.36 (br, s, 1H), 8.00 (br, s, 1H), 7.47-7.50 (m, 2H), 6.86-6.89 (m, 2H), 3.82 (s, 6H). LRMS (ESI+): 341.9 $(M+H)^+$

3-(3-methoxy-5-nitrobenzamido)furo[2,3-b]pyridine-2-carboxamide (**6f**)**.** To a suspension of ethyl 3-(3-methoxy-5-nitrobenzamido)furo[2,3-b]pyridine-2-carboxylate (**5f**, 1.702g, 4.42mmol) in ethanol (20mL) was added 1.0M aqueous sodium hydroxide (8.84mL) at room temperature and the reaction mixture was stirred at the same condition overnight. The organics were evaporated under reduced pressure. The aqueous layer was neutralized with 1.0M hydrochloric acid solution. The solid was precipitated, filtered and dried to obtain the acid without anyother purification. A solution of acid in thionyl chloride (10mL) was refluxed for 6 hours and the solvent was almost evaporated and the acid chloride was dissolved in 20mL of tetrahydrofuran and slowly added concentrated ammonium hydroxide solution (10mL) while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same condition for about 30 mintues and kept at room temperature overnight. Then water was added slowly and the solid was precipitated. The reaction mixture was filtered and the solid was dried to provide **6f** (1.030g, 65.4%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 8.60-8.62 (m, 1H), 8.50-8.51 (m, 1H), 8.43 (br, s, 1H), 8.36 (s, 1H), 8.05 (br, s, 1H), 7.95 (s, 1H), 7.93 (s, 1H), 7.45-7.48 (m, 1H), 3.96 (s, 3H).LRMS (ESI+): 357.0 $(M+H)^+$

3-(3-methoxy-5-methylbenzamido)furo[2,3-b]pyridine-2-carboxamide (6g). To a mixture of Ethyl 3-(3-methoxy-5-methylbenzamido)furo[2,3-b]pyridine-2-carboxylate (**5g**, 1.530g, 4.32mmol) in 30 mL of methanol was added 20 mL of concentrated ammonium hydroxide solution at room temperature. The reaction mixture was heated at 50℃ for 6 hours. Then the reaction was allowed to cool to room temperature and white a solid precipitated. The reaction mixture was concentrated to half of its original volume, filtered and dried to provide **6g** (1.101g, 78.4%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.19 (s, 1H), 8.76-8.78 (m, 1H), 8.49-8.51 (m, 1H), 8.43 (br, s, 1H), 8.05 (br, s, 1H), 7.45-7.48 (m, 1H), 7.36 (s, 2H), 7.30 (s, 1H), 7.05 (s, 1H), 3.81 (s, 3H). LRMS (ESI+): 325.9 $(M+H)^+$

3-(3-methoxy-5-(trifluoromethyl)benzamido)furo[2,3-b]pyridine-2-carboxamide (**6h**) To a

suspension of ethyl 3-(3-methoxy-5-nitrobenzamido)furo[2,3-b]pyridine-2-carboxylate (**5h**, 1.698g, 4.16mmol) in ethanol (20mL) was added 1.0M aqueous sodium hydroxide (9.32mL) at room temperature and the reaction mixture was stirred at the same condition overnight. The organics were evaporated under reduced pressure. The aqueous layer was neutralized with 1.0M hydrochloric acid solution. The solid was precipitated, filtered and dried to obtain the acid without anyother purification. A solution of acid in thionyl chloride (10mL) was refluxed for 6 hours. The solvent was almost evaporated and the acid chloride was dissolved in 20mL of tetrahydrofuran and slowly added concentrated ammonium hydroxide solution (10mL) while cooling the reaction mixture to 0~5℃. The reaction was stirred at the same condition for about 30 mintues and kept at room temperature overnight. Then water was added slowly and the solid was precipitated. The reaction mixture was filtered and dried to provide **6h** (1.046g, 66.3%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.25 (s, 1H), 8.62-8.64 (m, 1H), 8.50-8.51 (m, 1H), 8.43 (br, s, 1H), 8.07 (br, s, 1H), 7.86 (s, 1H), 7.79 (s, 1H), 7.54 (s, 1H), 7.45-7.48 (m, 1H), 3.92 (s, 3H). LRMS (ESI+): 379.9 $(M+H)^+$

2-(3-fluoro-5- methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7a**). Within a flask was dissolved the 3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6a**, 560mg, 1.70mmol)in 10 mL of isopropanol. Then was added 3.4mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7a** (383mg, 72.4%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63-8.65 (m, 2H), 7.60- 7.64 (m, 3H), 7.06-7.09 (m, 1H), 3.87 (s, 3H). LRMS (ESI+): 311.9 $(M+H)^+$

2-(3-chloro-5- methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7b**). Within a flask was dissolved the 3-(3-chloro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6b**, 580mg, 1.68mmol) in 10mL of isopropanol. Then was added 3.36mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7b** (414mg, 75.4%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64-8.67 (m, 2H), 7.82 (s, 1H), 7.71 (s, 1H), 7.60-7.63 (m, 1H), 7.25-7.27 (m, 1H), 3.88 (s, 3H). LRMS (ESI+): 327.9 $(M+H)^+$

2-(3-bromo-5-methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7c**). Within a flask was dissolved the 3-(3-bromo-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6c**, 500mg, 1.281mmol) in 5 mL of isopropanol. Then was added 2.56mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7c** (354mg, 74.2%) as a white solid without any purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64-8.68 (m, 2H), 7.95 (s, 1H), 7.74 (s, 1H), 7.60- 7.63 (m, 1H), 7.37-7.40 (m, 1H), 3.87 (s, 3H). LRMS (ESI+): 371.9 and 373.9 $(M+H)^+$

2-(3,5-dimethoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7e**). Within a flask was dissolved the 3-(3,5-dimethoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6e**, 1.221 g, 3.58 mmol) in 20 mL of isopropanol. Then was added 7.14 mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7e** (915mg, 79.1%) as a white solid without any purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61-8.65 (m, 2H), 7.59-7.62 (m, 1H), 7.35 (s, 1H), 7.34 (s, 1H), 6.69-6.71 (m, 1H), 3.83 (s, 6H). LRMS (ESI+): 342.0 $(M+NH_4)^+$

2-(3-methoxy-5-nitrophenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7f**). Within a flask was dissolved the 3-(3-methoxy-5-nitrobenzamido)furo[2,3-b]pyridine-2-carboxamide (**6f**, 600mg, 1.684mmol)in 10 mL of isopropanol. Then was added 3.37mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7f** (438mg, 76.8%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65-8.70 (m, 2H), 8.61 (s, 1H), 8.19 (s, 1H), 7.90-7.91 (t, 1H), 7.61-7.64 (m, 1H), 3.98 (s, 3H). LRMS (ESI+): 338.9 $(M+H)^+$

2-(3-methoxy-5-methylphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7g**). Within a flask was dissolved the 3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6g**, 600mg, 1.84mmol)in 10 mL of isopropanol. Then was added 3.68mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7g** (460mg, 81.1%) as a yellow solid. ^{1}H NMR (400

MHz, DMSO-d_6): δ 8.62-8.65 (m, 2H), 7.59-7.62 (m, 2H), 7.51 (s, 1H), 6.98 (s, 1H), 3.83 (s, 3H), 2.37 (s, 3H). LRMS (ESI+): 308.1 $(M+H)^+$

2-[3-methoxy-5-(trifluoromethyl)phenyl]pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7h**). Within a flask was dissolved 3-(3-fluoro-5-methoxybenzamido)furo[2,3-b]pyridine-2-carboxamide (**6h**, 700mg, 1.85mmol) in 10mL of isopropanol. Then was added 3.7mL of 2N sodium hydroxide solution at room temperature and the mixture was refluxed for 4 hours. The reaction was cooled to room temperature, and adjusted the pH to 3~4 with 2N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7h** (469mg, 70.3%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64-8.68 (m, 2H), 8.10 (s, 1H), 8.02 (s, 1H), 7.60-7.63 (m, 1H), 7.46 (s, 1H), 6.75 (s, 1H), 3.94 (s, 1H). LRMS (ESI+): 362.0 $(M+H)^+$

2-(3-fluoro-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8a**). 2-(3-fluoro-5- methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7a**, 369mg, 1.19mmol) was added 5 mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 10mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8a** (224mg, 49.6%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60-8.62 (m, 2H), 7.86 (s, 1H), 7.77-7.80 (m, 1H), 7.47-7.50 (m, 1H),6.71-6.75 (m, 1H), 4.21-4.24 (t, 4H), 3.91-3.94 (m, 7H). LRMS (ESI+): 381.0 $(M+H)^+$

2-(3-chloro-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8b**). 2-(3-chloro-5- methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7b**, 402mg, 1.23mmol) was added 10 mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 10mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed

with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8b** (290mg, 59.6%) as a white powder. ^1H NMR (400 MHz, $CDCl_3$):8.60-8.62 (m, 2H), 8.06-8.07 (m, 1H), 7.94-7.95 (m, 1H), 7.47-7.50 (m, 1H), 7.00-7.01 (t, 1H), 4.21-4.24 (t, 4H), 3.91-3.94 (m, 7H). LRMS (ESI+): 397.0 $(M+H)^+$

2-(3-bromo-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8c**). 2-(3-bromo-5-methoxyphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7c**, 342mg, 0.92 mmol) was added 10mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 5mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8c** (221mg, 54.6%) as a yellow powder. ^1H NMR (400 MHz, $CDCl_3$): δ 8.60-8.64 (m, 2H), 8.22 (s, 1H), 7.98 (s, 1H), 7.47-7.50 (m, 1H), 7.15-7.16 (t, 1H), 4.21-4.24 (t, 4H), 3.92-3.94 (m, 7H). LRMS (ESI+): 442.9 and 444.0 $(M+H)^+$

3-methoxy-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]aniline (**8d**). To a solution of 2-(3-methoxy-5-nitrophenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8f**, 88mg, 0.216mmol) in a mixture in 10mL of 95% ethanol, ammonium chloride (35mg, 0.648mmol) and iron powder (121mg, 2.16mmol) were added. The reaction was refluxed for 4 hours, then cooled at room temperature and filtered on Celite washing with ethanol. The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate and washed with saturated aq. $NaHCO_3$, water and brine. The organic phase was dried over sodium sulfate, evaporated and purified by column chromatography on silica gel (ether: ethyl acetate=2:1) to obtain **8d** (45mg, 55.2%) as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6): δ 8.61-8.67 (m, 2H), 7.60-7.63 (m, 1H), 7.31-7.33 (s, 1H), 7.18-7.19 (s, 1H), 6.27-6.28 (t, 1H), 5.25 (s, 2H), 4.09-4.11 (t, 4H), 3.83-3.85 (t, 4H), 3.75 (s, 3H). LRMS (ESI+): 378.0 $(M+H)^+$

2-(3,5-dimethoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8e**). 2-(3,5-dimethoxyphenyl)pyrido[3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-one (**7e**, 902mg, 2.79mmol)

was added 10mL of phosphorusoxidetrichloride slowly at ice bath and the contents were heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 20mL of dry toluene and added excess morpholine (8mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq. $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8e** (561 mg, 51.2%) as a yellow powder. ^1H NMR (400 MHz, $CDCl_3$): δ 8.59-8.63(m, 2H), 7.66 (s, 1H), 7.65 (s, 1H), 7.45-7.48 (m, 1H), 6.58-6.59 (t, 1H), 4.20-4.23 (t, 4H), 3.90-3.92 (m, 10H). LRMS (ESI+): 393.0 $(M+H)^+$

2-(3-methoxy-5-nitrophenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8f**). 2-(3-methoxy-5-nitrophenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7f**, 422mg, 1.25mmol) was added 5mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 10mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8f** (273mg, 53.6%) as a yellow powder. ^1H NMR (400 MHz, $CDCl_3$): δ 8.92-8.93 (m, 1H), 8.61-8.64 (m, 2H), 8.37-8.38 (m, 1H), 7.81-7.82 (t, 1H), 7.48-7.52 (m, 2H), 4.23-4.25 (t, 4H), 4.00 (s, 3H), 3.92-3.94 (t, 4H). LRMS (ESI+): 408.0 $(M+H)^+$

2-(3-methoxy-5-methylphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8g**). 2-(3-methoxy-5-methylphenyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7g**, 445mg, 1.45mmol) was added 5mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 10mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently

purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8g** (340mg, 62.4%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60-8.64 (m, 2H), 7.90 (s, 1H), 7.83 (s, 1H), 7.45-7.49 (m, 1H), 6.85 (s, 1H), 4.22-4.24 (t, 4H), 3.92-3.94 (m, 7H), 2.45 (s, 3H). LRMS (ESI+): 377.0 $(M+H)^+$

2-[3-methoxy-5-(trifluoromethyl)phenyl]-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d] pyrimidine (**8h**). 2-[3-methoxy-5-(trifluoromethyl)phenyl]pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-one (**7h**, 454mg, 1.26mmol) was added 5mL of phosphorusoxidetrichloride slowly at ice bath and the contents was heated at 110℃ for about 4 hours. After the removal of the excess reagent, the crude product was dissolved in 10mL of dry toluene and added excess morpholine (4mL) at 0~5℃. Then the reaction was refluxed at 135℃ for about 4 hours. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate =3:1) to afford **8h** (292mg, 53.9%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61-8.63 (m, 2H), 8.34 (s, 1H), 8.02 (s, 1H), 7.47-7.50 (m, 1H), 7.22 (s,1H), 4.21-4.23 (t, 4H), 3.98 (s,3H), 3.95-3.93 (m, 4H). LRMS (ESI+): 431.0 $(M+H)^+$

3-fluoro-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (**9a**). A solution of 2-(3-fluoro-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8a**, 50mg, 0.131mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol = 20:1), to obtain compound **9a** (21mg, 43.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H), 8.64-8.68 (m, 2H), 7.75 (s, 1H), 7.59-7.65 (m, 2H), 6.67-6.70 (d, *J=12*, 1H), 4.10-4.12 (t, 4H), 3.83-3.86 (t, 4H). ^{13}C NMR (100MHz, DMSO-d6) δ162.95, 162.35, 159.42 (*J*=12.9), 157.79, 150.33, 148.86, 146.95, 141.10, 133.50, 132.00, 121.26, 114.86, 111.55, 105.46 (*J*=23.8), 104.55 (J=24), 66.47, 45.91 ppm. LRMS (ESI+): 366.9 $(M+H)^+$, HRMS (ESI+) m/z calcd for

$C_{19}H_{16}FN_4O_3$ $(M+H)^+$ 367.12064, found 367.12076.

3-chloro-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (**9b**). A solution of 2-(3-chloro-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8b**, 50mg, 0.126mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol = 20:1), to obtain compound **9b** (20mg, 41.5%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 8.69 (s, 1H), 8.67 (s, 1H), 7.84-7.86 (m, 2H), 7.62-7.65 (m, 1H), 6.91-6.92 (t, 1H), 4.10-4.12 (t, 4H), 3.84-3.86 (t, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.70, 159.02, 157.61, 150.38, 148.95, 146.98, 141.10, 134.05, 133.53, 132.09, 121.29, 118.70, 117.20, 114.85, 114.19, 66.46, 45.92 ppm. LRMS (ESI+): 383.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{19}H_{16}ClN_4O_3$ $(M+H)^+$ 383.09109, found 383.09152.

3-bromo-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (**9c**). A solution of 2-(3-bromo-5-methoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8c**, 60mg, 0.136mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20 mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol = 20:1), to obtain compound **9c** (27mg, 46.5%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.08 (s, 1H), 8.65 (s, 1H), 8.64 (s, 1H), 7.97 (s, 1H), 7.83 (s, 1H), 7.58-7.61 (t, 1H), 7.03-7.04 (t, 1H), 4.06-4.09 (t, 4H), 3.81-3.83 (t, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.67, 159.08, 157.45, 150.33, 148.89, 146.93, 141.32, 133.50, 132.05, 122.28, 121.59, 121.24, 120.06, 114.82, 114.58, 66.45, 45.89 ppm. LRMS (ESI+): 427.0 and 429.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{19}H_{16}BrN_4O_3$ $(M+H)^+$ 427.04058, found 427.04120.

3-amino-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (**9d**). A

solution of 3-methoxy-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]aniline (**8d**, 50mg, 0.132mmol) in 2 mL of acetic acid was added 1 mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20 mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane:methanol = 15:1), to obtain compound **9d** (20mg, 42.1%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.57-8.66 (m, 2H), 7.59-7.62 (m, 1H), 7.18 (s, 1H), 7.09 (s, 1H), 6.13 (s, 1H), 5.09 (s, 2H), 4.09-4.11 (t, 4H), 3.74- 3.94 (t, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.76, 159.97, 158.53, 150.10, 149.81, 148.85, 147.08, 139.84, 133.34, 131.77, 121.11, 115.15, 106.41, 104.61, 103.80, 66.51, 45.95 ppm. LRMS (ESI+): 364.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{19}H_{18}N_5O_3$ $(M+H)^+$ 364.14096, found 364.14014.

5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]benzene-1,3-diol (**9e**). A solution of 2-(3,5-dimethoxyphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-*d*]pyrimidine (**8e**, 50mg, 0.127mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the mixture was extracted with ethyl acetate (20mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol= 20:1), to obtain compound **9e** (21mg, 45.4%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.37 (s, 2H), 8.61-8.67 (m, 2H), 7.61-7.64 (m, 1H), 7.35 (s, 1H), 7.36 (s, 1H), 6.32 (s, 1H), 4.09-4.11 (t, 4H), 3.83-3.85 (t, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.77, 159.40, 158.83, 150.18, 148.91, 147.09, 140.17, 133.42, 131.83, 121.15, 115.09, 106.97, 105.09, 66.49, 45.96 ppm. LRMS (ESI+): 365.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{19}H_{17}N_4O_4$ $(M+H)^+$ 365.12498, found 365.12505.

3-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]-5-nitrophenol (**9f**). A solution of 2-(3-methoxy-5-nitrophenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8f**, 50mg, 0.123mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20 mL*3). The organic phase was concentrated

under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol = 20:1), to obtain compound **9f** (23mg, 47.6%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.64 (s, 1H), 8.68-8.72 (m, 2H), 8.63 (s, 1H), 8.26-8.27 (m, 1H), 7.62-7.66 (m, 2H), 4.12-4.14 (t, 4H), 3.85-3.87 (m, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.66, 158.74, 156.77, 150.33, 149.53, 148.88, 146.92, 140.74, 133.58, 131.93, 121.47, 121,18, 114.69, 113.37, 111.30, 66.42, 45.94 ppm. LRMS (ESI+): 394.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{19}H_{16}N_5O_5$ $(M+H)^+$ 394.11514, found 394.11536.

3-methyl-5-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (**9g**). A solution of 2-(3-methoxy-5-methylphenyl)-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidine (**8g**, 50mg, 0.133mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol = 20:1), to obtain compound **9g** (22mg, 45.7%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.44 (s, 1H), 8.64- 8.67 (m, 2H), 7.70 (s, 1H), 7.68 (s, 1H), 7.60-7.63 (m, 1H), 6.70 (s, 1H), 4.09-4.12 (t, 4H) , 3.83-3.85 (t, 4H), 2.32 (s, 3H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.75, 159.36, 157.86, 150.15, 148.94, 147.10, 139.51, 138.90, 133.39, 131.88, 121.10, 120.00, 118.28, 115.06, 112.78, 66.50, 45.96, 21.61 ppm. LRMS (ESI+): 363.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{20}H_{19}N_4O_3$ $(M+H)^+$ 363.14572, found 363.14630.

3-[4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]-5-(trifluoromethyl)phenol (**9h**). A solution of 2-[3-methoxy-5-(trifluoromethyl)phenyl]-4-(morpholin-4-yl)pyrido[3',2':4,5]furo[3,2-d] pyrimidine (**8h**, 60mg, 0.139mmol) in 2mL of acetic acid was added 1mL of 48% aq. hydrobromic acid at room temperature. Then the mixture of reaction was heated at 80℃ for about 48 hours. The solution was cooled to ambient temperature and basified with concentrated ammonia water, then the product was extracted with ethyl acetate (20mL*3). The organic phase was concentrated under vacuo and the residue was purified by flash chromatography (dichloromethane: methanol =

20:1), to obtain compound **9h** (26mg, 44.8%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 8.68-8.71 (m, 2H), 8.15 (s, 1H), 7.62-7.65 (m, 2H), 7.16 (s, 1H), 4.11-4.13 (t, 4H), 3.85-3.87 (t, 4H). ^{13}C NMR (100MHz, DMSO-d_6)δ162.24, 158.58, 157.39, 150.33, 148.89, 146.92, 140.75, 133.51, 132.05, 130.844 (*J*=62.5), 121.21, 118.96, 115.12. 114.75, 113.51, 66.42, 45.87 ppm. LRMS (ESI+): 417.0 $(M+H)^+$, HRMS (ESI+) m/z calcd for $C_{20}H_{16}F_3N_4O_3$ $(M+H)^+$ 417.11745, found 417.11859

HPLC analysis data of compounds 9a-9h

HPLC analysis data of compounds **9a-9h**. The purities of identified compounds that was essential to the conclusions drawn in the text and determined by one standard instrumentations with one system given in the following table. The peak purity was checked with UV spectra.

	Method	
Equipment	Agilent 1260 with quaternary pump, photodiode array detector (DAD)	
Column	Agilent Zorbax Exlipse Plus C18 (100×4.6 mm, 3.5 μm particle size)	
System condition	a. CH_3CN/H_2O, 20%(v/v) of CH_3CN in the first 3 minutes, then the percentage of CH_3CN was gradually increased to 60% in 1 minutes and the condition was kept at 4-6 mintues. Next, percentage of CH_3CN was slowly increased to 100% in 2 minutes and the condition was maintained at 8-10 minutes, flow rate: 1.5mL/min, calculated the relative purity of each compound at 254nM.	b. CH_3CN/H_2O, 20%(v/v) of CH_3CN in the first 3 minutes, then the percentage of CH_3CN was gradually increased to 60% in 1 minutes and the condition was kept at 4-6 mintues. Next, percentage of CH_3CN was slowly increased to 100% in 2 minutes and the condition was maintained at 8-10 minutes, flow rate: 1.5mL/min, calculated the relative purity of each compound at 280nM.

		c. CH_3CN/H_2O, 20%(v/v) of CH_3CN in the first 3 minutes, then the percentage of CH_3CN was gradually increased to 40% in 1 minutes and the condition was kept at 4-6 mintues. Next, percentage of CH_3CN was slowly increased to 100% in 2 minutes and the condition was maintained at 8-10 minutes, flow rate: 1.5mL/min, calculated the relative purity of each compound at 254nM.	d. CH_3CN/H_2O, 20%(v/v) of CH_3CN in the first 3 minutes, then the percentage of CH_3CN was gradually increased to 40% in 1 minutes and the condition was kept at 4-6 mintues. Next, percentage of CH_3CN was slowly increased to 100% in 2 minutes and the condition was maintained at 8-10 minutes, flow rate: 1.5mL/min, calculated the relative purity of each compound at 280nM.	
result	compd	Retention time (min)	Relactive purity (%)	
			254nM	280nM
	9a	5.944[ab]	96.7	99.2
	9b	6.421 [ab]	95.5	98.3
	9c	6.786 [ab]	95.3	96.3
	9d	5.096[ab]	95.4	95.6
	9e	5.469[cd]	95.3	96.9
	9f	6.296 [ab]	96.0	97.8
	9g	5.852 [ab]	98.9	99.4
	9h	7.107[ab]	96.4	98.7

Figure S1. Binding mode of PI103 and key residues in the pocket of PI3Kα. (A) Superimposition of the crystal structures of *apo* PI3Kα (cyan) and the PI3Kα-PI103 complex (green). Residues Met772 and Trp780 as well as PI103 are depicted by stick. Met772 is in an 'up' conformation in the *apo* PI3Kα and a 'down' conformation in the PI3Kα-PI103 complex. Hydrogen atoms are omitted for clarity. (B) The hydrophobic interactions between Met772 and PI103. Met772 and PI103 are depicted by dots.

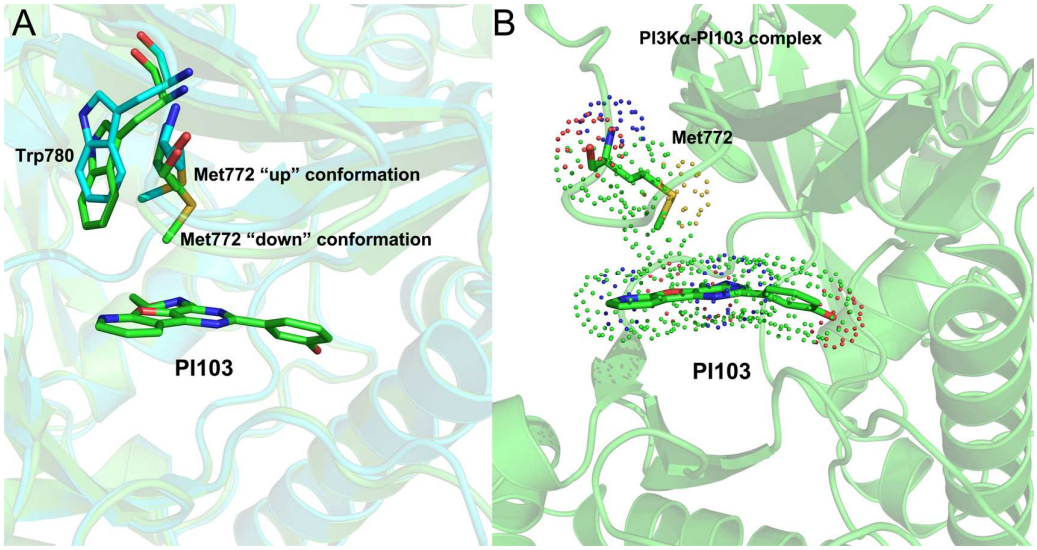
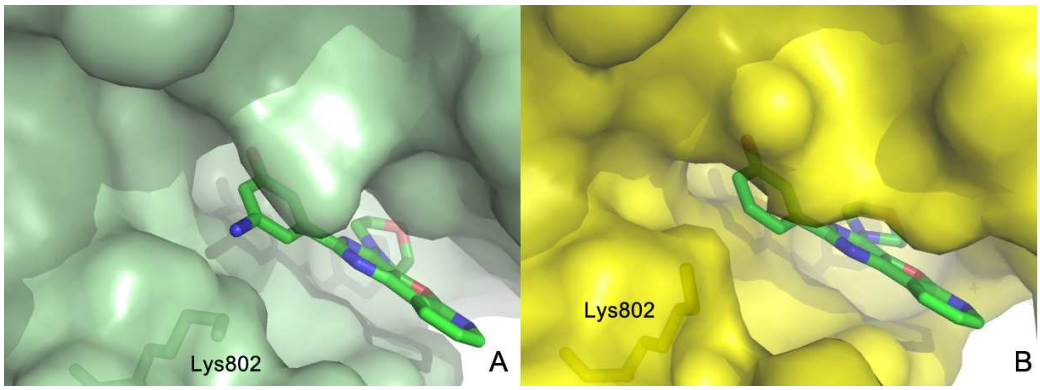


Figure S2. Side-by-side comparison of the cavity of PI3Kα induced by (A) compound **9d** and (B) PI103. The residue Lys802 gating the cavity is shown as sticks and PI3Kα is represented by the molecular surface.



Scheme S1. Synthesis of PI103 derivatives 9a to 9h. Reagents and conditions: (a) $HOCH_2CO_2Et$ **2**, DBU, EtOH; (b) (i) RCOOH **4**, $SOCl_2$; (ii) TEA, THF; (c) (i) 1N NaOH, EtOH; (ii) $SOCl_2$; (iii) NH_4OH, THF; (d) NH_4OH, MeOH; (e) 2N NaOH, 2-PrOH; (f) (i) $POCl_3$, (ii) morpholine, toluene; (g) HBr, AcOH; (h) Fe, NH_4Cl, 95% EtOH

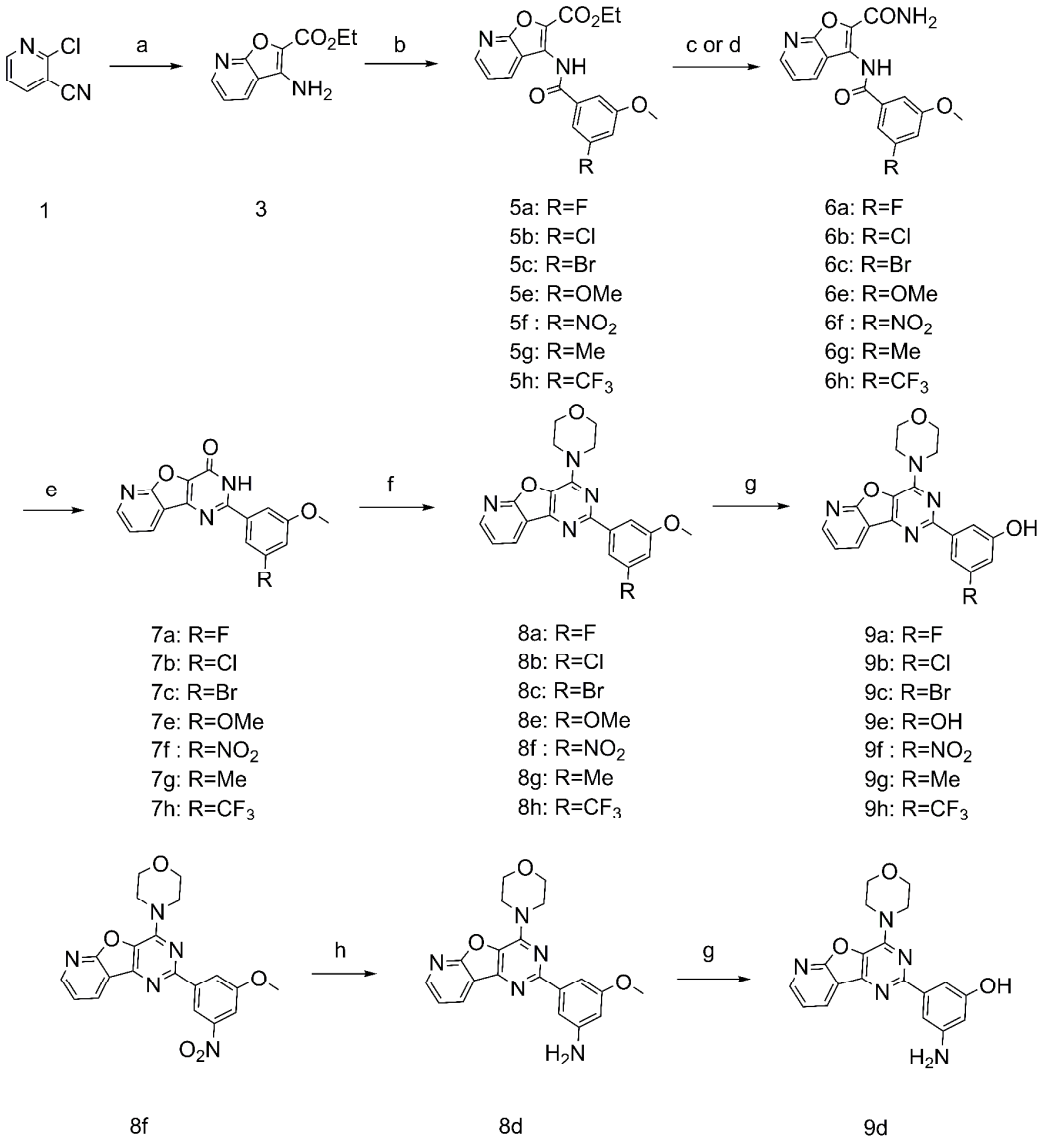


Table S1. The solved crystal structures of PI3K-inhibitors complexes currently deposited in Protein Data Bank

Isoforms	Total numbers	PDB ID
PI3Kα	3	4A55 3ZIM 3HHM
PI3Kβ	2	2Y3A 4AJW
PI3Kδ	13	2WXF 2WXG 2WXH 2WXJ 2WXK 2WXL 2WXM 2WXO 2WXQ 2WXI 2WXN 2WXP 2X38
PI3Kγ	74	4KZO 4KZC 3T8M 3L54 4FJY 4FJZ 3LO8 3APC 3APD 3APF 4EZJ 3QJZ 3QKO 3PRZ 3OAW 4EZK 4EZL 3PRE 3PS6 3S2A 3DPD 4G11 3ML8 3ML9 4F1S 3DBS 3TL5 3ENE 4FHK 3CSF 3CST 4GB9 4FLH 4FHJ 3QAQ 3QAR 3P2B 3SD5 3TJP 4DK5 3L13 3L16 3L17 3LJ3 3IBE 2V4L 4FUL 3MJW 4FA6 4FAD 4HLE 3NZS 3NZU 3R7Q 3R7R 3ZVV 3ZW3 4J6I 1E8Z 1E7V 1E8W 1E8X 1E90 1E7U 4ANU 4ANV 4AOF 2A4Z 2A5U 2CHW 2CHX 2CHZ 4ANW 4ANX

Table S2. Data Collection and Refinement Statistics[a]

	p110α/p85α	p110α/p85α-PI103	P110α/p85α-**9d**
Diffraction Data			
Resolution (Å)*	50 - 2.58 (2.67 - 2.58)	50 - 2.5 (2.59 - 2.5)	55 - 2.8 (2.9 - 2.8)
Space group	P212121	P212121	P212121
Unit cell parameters			
a (Å)	70.561	71.628	70.94
b (Å)	136.901	136.377	136.971
c (Å)	150.448	151.487	150.484
α (˚)	90.00	90.00	90.00
β (˚)	90.00	90.00	90.00
γ (˚)	90.00	90.00	90.00
Total reflections	221963	341951	175623

Unique reflections	46123	51036	35587
Completeness (%)*	99.44 (95.56)	98.43 (86.38)	96.29 (97.57)
Multiplicity*	4.8 (4.4)	6.7 (3.7)	4.9 (5.0)
Average I/σ(I)*	8.98 (2.42)	16.93 (2.32)	27.35 (5.83)
Rmerge (%)*	13.5 (61.1)	7.7 (50.5)	8.2 (43.4)
Refinement			
Resolution (Å)*	47.09 – 2.59 (2.63 – 2.59)	38.99 - 2.50 (2.54 - 2.50)	101.29 - 2.80 (2.87 – 2.80)
Completeness (%)*	99 (100)	98 (83)	96 (97)
Rwork (%)	21.7 (28.6)	21.5 (28.9)	21.5 (29.5)
Rfree (%)	27.4 (33.3)	27.3 (32.2)	27.1 (40.1)
RMSD in Bond Lengths (Å)	0.004	0.003	0.005
RMSD in Bond Angles (°)	0.85	0.85	0.961
Number of Atoms			
Total	10528	10928	10533
Protein	10486	10634	10473
Ligand	5	42	43
Water	37	252	17
B factor Statistics			
Average B Value (Å²)	70.80	52.60	85.90
Protein Mean B Value (Å²)	70.90	52.90	86.00
Ligand Mean B Value (Å²)	67.40	43.10	71.20
Water Mean B Value (Å²)	57.00	42.70	51.40
Ramachandran Statistics			
Most favored regions	90.0%	93.3%	92.4%
Additional allowed regions	9.9%	6.5%	7.5%
Generously allowed regions	0.1%	0.3%	0.1%
Disallowed regions	0.0%	0.0%	0.0%

[a]Values in parentheses are for the highest resolution bin.

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