SUPPORTING INFORMATION

Synthesis of a 6-Aza-Isoindolinone based Inhibitor of Phosphoinositide 3-kinase γ via Ruthenium-Catalyzed [2 + 2 + 2] Cyclotrimerization

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Contents of Supporting Information:

1. Description of Biochemical Assays

1a. PI3K Inhibition Assays

The compounds of interest were dissolved in DMSO to make 10 mM initial stock solutions. Serial dilutions in DMSO were then made to obtain the final solutions for the assay. Using a Biomek FX from Beckman Coulter, a 1.5 μL aliquot aliquot of DMSO or inhibitor in DMSO was added to each individual well (hereafter, "test well") in a 96 well polystyrene plate [Corning, Costar Item No. 3697]. Using a Titertek Multidrop, 50 μL of ATP Mix [50 mM HEPES (pH 7.5), 3 mM $MgCl_2$, 100 mM NaCl, 2 mM DTT, 1 mM EGTA, 0.03% CHAPS, and ATP (100 μCi/μmol ^{33}P-ATP) (See Table 1 for ATP concentrations, each equal to Km)] was added to each well. To initiate the reaction, 50 μL of Reaction Mixture [50 mM HEPES (pH 7.5), 3 mM $MgCl_2$, 100 mM NaCl, 2 mM DTT, 1 mM EGTA, 0.03% CHAPS, 20 μM PIP2 (phosphatidylinositol(4,5)-*bis*phosphate diC16 ($PI(4,5)P_2$; Avanti Polar Lipids, Cat. No. 840046P) and PI3K isoform of interest (See Table 1 for isoform concentrations)] was added to each well, followed by incubating the wells for 15 min at room temperature.

Table 1

PI3K Isoform	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
Final ATP concentration (equal to Km)	5 μM	27 μM	10 μM	7 μM
Final enzyme concentration	4 nM	20 nM	6 nM	6 nM

After incubation, the reactions in each well were quenched by addition of 50 μL of stop solution [30% TCA/water, 10 mM ATP]. Each quenched reaction mixture was then transferred to a 96 well glass fiber filter plate [Corning, Costar Item No. 3511]. The plate was vacuum-filtered and washed three times with 150 μL of 5% TCA/water in a modified Bio-Tek Instruments ELX-405 Auto Plate Washer. 50 μL of scintillation fluid was added to each well and the plate read on a Perkin-Elmer TopCount^{TM} NXT liquid scintillation counter to obtain ^{33}P-counts.

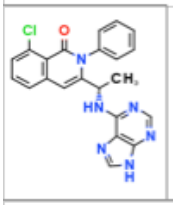
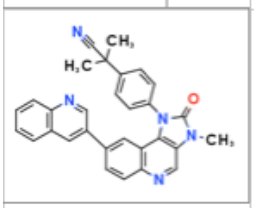
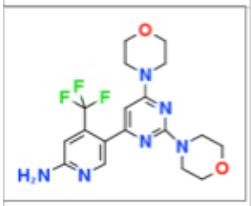
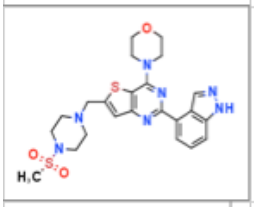
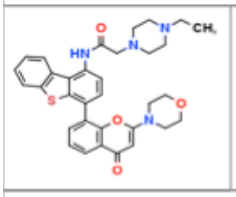
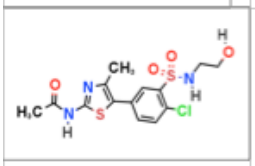
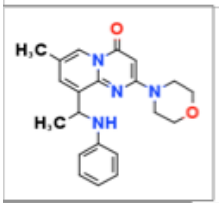
After removing mean background values for all of the data points, Ki(app) data were calculated from non-linear regression analysis of the initial rate data using the Prism software package (GraphPad Prism, GraphPad Software, San Diego California, USA). The data were fit to the Morrison equation for competitive tight binding Ki as described by Copeland (Reference: RA Copeland, *Enzymes*, 2nd edition, Wiley, 2000, Equation 9.6). Compound potencies were determined in singlicate. Average Kis are be reported for compounds that have multiple determinations. The Average of the Robust MSRs for PI3K α, β, γ, δ were determined to be 3.2, 3.0, 3.1, and 2.7 respectively. (References:JBiomolScreen_MSR, AMC_Robust_Statistics, Minimum Significant Ratio – A Statistic to Assess Assay Variability)

Statistics on compound 2:

Average ± stdev (number of determinations)	
cpd	**PI3Kγ**
2	0.014 ± 0.006 (4)

1b. Reference PI3K inhibitors

In-house versus reported values

Molecule	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	ALIAS	α/ß/γ/δ (μM) http://www.selleckchem.com/PI3K.html	all data from from Selleck Chem site: http://www.selleckchem.com/PI3K.html
	0.14	0.028	0.008	0.005	ABBV-954, Duvelisib, INK-1197, IPI-145,	1.6 / 0.085 / 0.027 / 0.002	Duvelisib is a selectivite p100δ inhibitor with IC_{50} of 2.5 nM, 27.4 nM, 85 nM and 1602 nM for p110δ, P110γ, p110β and p110α, respectively.
	0.004	0.070	0.009	0.004	BEZ-235, Dactolisib, NVP-BEZ-235	0.004 / 0.075 / 0.005 / 0.007	Dactolisib (BEZ235, NVP-BEZ235) is a dual ATP-competitive PI3K and mTOR inhibitor for p110α/γ/δ/β and mTOR(p70S6K) with IC50 of 4 nM /5 nM /7 nM /75 nM /6 nM in cell-free assays, respectively. Inhibits ATR with IC50 of 21 nM in 3T3TopBP1-ER cell.
	0.019	0.15	0.12	0.037	BKM-120, Buparlisib, NVP-BKM120	0.052 / 0.17 / 0.26 / 0.12	Buparlisib (BKM120, NVP-BKM120) is a selective PI3K inhibitor of p110α/β/δ/γ with IC50 of 52 nM/166 nM/116 nM/262 nM in cell-free assays, respectively. Reduced potency against VPS34, mTOR, DNAPK, with little activity to PI4Kβ. Phase 2.
	0.002	0.029	0.013	0.002	GDC-0941, Pictilisib, Pictrelisib, RG-7321	0.003 / ~0.033 / ~0.075 / 0.003	Pictilisib (GDC-0941) is a potent inhibitor of PI3Kα/δ with IC50 of 3 nM in cell-free assays, with modest selectivity against p110β (11-fold) and p110γ (25-fold). Phase 2.
	0.005	0.049	0.037	0.004	KU-0060648	0.004 / 0.0005 / 0.59 / 0.0001	KU-0060648 is a dual inhibitor of DNA-PK and PI3Kα, PI3Kβ, PI3Kδ with IC50 of 8.6 nM and 4 nM, 0.5 nM, 0.1 nM respectively, less inhibition of PI3Kγ with IC50 of 0.59 μM.
	0.096	0.17	0.004	0.008	PIK-93	0.039 / 0.59 / 0.016 / 0.12	PIK-93 inhibits PI3Kγ and PI4KIIIβ, with IC50 values of 16 nM and 19 nM, respectively. PIK-93 also inhibits other members of PI3Ks, including PI3Kα, β, and δ, with IC50 values of 39 nM, 0.59 μM, and 0.12 μM, respectively.
	1.3	0.038	1.7	0.038	TGX-221	na / 0.005 / na / 0.21	The activity of TGX-221 against different isoforms is measured in an in vitro PI3K assay using multiple preparations of recombinant p85/p110. TGX-221 show slow potent to p110δ with IC50 of 211 nM TGX-221 p110β-specific, IC50=5 nM
	0.002	0.012	0.005	0.002	ZSTK-474	0.016 / 0.017 / 0.053 / 0.006	ZSTK474 inhibits the activities of recombinant p110β, -γ, and -δ with IC50 of 17 nM, 53 nM, and 6 nM, respectively.... alpha16

2. Description of Cellular Assay

Materials

THP-1 were obtained from ATCC. 2-mercaptoethanol, Hepes solution 1M, 7.5% BSA were obtained from Invitrogen. Recombinant human MCP-1 from PeproTech; Phospho-Akt antibody (S473) AlexaFluor647 conjugated from Cell SignalingTechnology; Formaldehyde 37% from Sigma; Methanol from JT Baker; 96-well plates, black with clear bottom and 96 well V bottom polypropylene plates from Costar.

Methods

MCP-1 stimulated pAKT in THP-1 cells

THP-1 cells (human monocytic cell line) were re-suspended at a density of 5 x 10^5 cells/ml in serum-starvation medium (RPMI , 0.1% BSA, 50 U/ml Pen/Strep, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol) for 24 h at 37 ºC, 5% CO_2. Cells were then diluted to a density of 2.5 x 10^6 cells in starvation medium and plated in 96-well plates. Cells were pretreated with compounds for 1 h at 37 ºC, 5% CO_2. Cells were then stimulated by the addition 20 nM MCP-1 for 3 min. This leads to PI3K□-induced phoshorylation of Akt at the Threonin 308 and Serine 473 sites. Cells were fixed rapidly with formaldehyde (4% final concentration). Cells were washed and permeabilized with 90% cold methanol at -20 ^{0}C for 30 min. Cells were washed with PBS and then stained with pAkt antibody for 30 min. at room temperature. Cells were washed and were re-suspended in PBS. Plate was read on a BD FACS Calibur and data was analyzed using FlowJo. IC_{50}'s were calculated using the % pAKT positive values.

3. Synthetic Procedures and Characterization of Compounds

General Information. All of the reagents were obtained commercially without requiring additional purification. The reactions were monitored on a Waters Acquity Classic UPLC-MS coupled to a single quadrupole mass spectrometer (Model SQ2, Waters). Analytical samples were separated using a Waters 1.7 µm CSH column (2.1 x 30 mm) in which a gradient of 5-95% CH_3CN in 0.05% (v/v) TFA in H_2O was applied with a flow rate of 1.2 mL/min over 1.4 min. Compound purifications were done on ISCO Combiflash systems using pre-packed silica or C18 columns. NMR spectra were produced from a Bruker Avance-III (400 MHz) with proton resonances measured in parts per million (ppm) downfield from tetramethylsilane (TMS) at 400 MHz. ^{13}C NMR spectra and ^{19}F NMR spectra were measured on the same instrument, operating at 101 MHz and 376 MHz, respectively. High-resolution mass spectra (HRMS) were collected by direct infusion on a Thermo QExactive. HRMS samples were prepared in CH_3CN and infused with a flow rate of 5 µL/min. Electrospray ionization (ESI) in positive ion mode was employed with a spray voltage of 4.0 kV, with the mass resolution set to 35,000.

6-(5,6-Dimethoxy-3-pyridyl)-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridin-3-one (2). A 2-necked 250 mL round bottom flask was charged with 6-chloro-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridin-3-one (129 mg, 0.390 mmol), 2,3-dimethoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (134 mg, 0.507 mmol), DMF (4 mL), and sodium carbonate (780 µL of 1 M, 0.78 mmol). Nitrogen was bubbled through the solution for 0.5 h, after which $Pd(PPh_3)_4$ (23 mg, 0.0195 mmol). Nitrogen bubbling continued for a further 5 min and the reaction vessel was placed on a heating block set to 110 °C. The reaction was allowed to stir overnight. In the following morning, the mixture was cooled to room temperature. Water was added to the mixture and was extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate (Na_2SO_4) and filtered through a pad of Celite. The layers were concentrated under reduced pressure and purified by silica gel column chromatography (100% ethyl acetate). Yield: (20 mg, 77 %); white solid; ^1H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 2.0 Hz, 1H), 8.35 (s, 1H), 8.02 (s, 1H), 7.90 (d, J = 1.9 Hz, 1H), 7.89 (s, 1H), 5.18 (q, J = 9.0 Hz, 2H), 4.82 (s, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 2.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.7, 156.2, 155.4, 155.3, 152.2, 144.1, 136.2, 125.2 (q, J = 365.7, 282.3, 280.2 Hz), 116.3, 112.5, 55.9, 53.8, 52.1 (q, J = 33.3 Hz), 49.7, 25.4, 20.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{20}H_{18}F_3N_5O_3H]^+$: 434.1435, found: 434.1436.

***N*-prop-2-ynyl-N-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]but-2-ynamide (5)**. A 2-neck 250 mL flask under nitrogen was charged with N-prop-2-ynyl-1-(2,2,2-trifluoroethyl)pyrazol-4-amine (4.41 g, 21.71 mmol, 1.0 eq), but-2-ynoic acid (2.19 g, 26.05 mmol, 1.2 eq), and DMAP (140 mg, 1.146 mmol, 0.05 eq) in DCM (75 mL). DIPEA (11.4 mL, 65.45 mmol, 3.0 eq) was added in one portion to this solution to result in a color change from pale yellow to golden yellow. The solution was then cooled in an ice-water bath, after which EDCI (4 g, 25.77 mmol, 1.2 eq) was added in one portion. The ice-water bath was removed after 5 min, and the solution was allowed to stir overnight at room temperature. Water was added the following morning followed by two additional extractions with DCM. The combined organic layers were passed through a Biotage phase separator and subsequently concentrated under reduced pressure. The residue was purified via reverse phase column chromatography (275g Gold C18Aq column, 10-95% ACN/TFA in aq. TFA). Combined fractions were partially concentrated to remove the acetonitrile, and the resulting water layer was extracted three times with dichloromethane. The organic layers were passed through a Biotage phase separator and concentrated under reduced pressure to afford **5 (**2.6 g, 44% yield). white solid; ^1H NMR (300 MHz, Chloroform-*d*, one rotamer annotated) 8.12 (s, 1H), 7.72 (s, 1H), 4.73 (d, J = 2.5 Hz, 2H), 4.76 - 4.63 (m, 2H), 2.36 (t, J = 2.4 Hz, 1H), 2.07

(s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 154.2 (rot), 151.9 (rot), 138.8 (rot), 132.8 (rot), 128.7 (rot), 127.0 - 118.5 (qd, rot), 91.7 (rot), 91.3 (rot), 78.2 (rot), 77.6 (rot), 73.8 (rot), 73.1 (rot), 72.8 (rot), 72.7 (rot), 53.38 (qd, *J* = 35.2, 15.4 Hz, rot), 40.00 (rot), 37.64 (rot), 4.25 (rot), 3.68 (rot). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -71.6 (rot), -71.9 (rot); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{12}H_{10}F_3N_3OH]^+$: 270.0849, found: 270.0849.

***N*-prop-2-ynyl-1-(2,2,2-trifluoroethyl)pyrazol-4-amine (7)**. A 2-neck 250 mL flask under nitrogen was charged with 1-(2,2,2-trifluoroethyl)pyrazol-4-amine (8.55 g, 51.78 mmol, 1.0 eq) and potassium carbonate (21.5 g, 155.6 mmol, 3 eq) in DMF (100 mL). The stirring solution was cooled to 0 ºC via an ice-water bath, before 3-bromoprop-1-yne in toluene (4.6 mL of 80 %w/w, 41.30 mmol, 0.8 eq) was added in one portion. The ice-water bath was removed after 5 min. After 3 h, water and brine was added to the solution and the crude product was extracted twice with ethyl acetate. The organic layers were combined and dried over sodium sulfate (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified via reverse phase column chromatography (275 g Gold C18Aq column, 10-95% ACN/TFA in aq. TFA). Combined fractions were partially concentrated and the resulting water layer was extracted three times with dichloromethane. The organic layers were passed through a Biotage phase separator and concentrated to afford **7** (4.51 g, 43% yield, white solid). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 (d, *J* = 1.0 Hz, 1H), 7.13 (s, 1H), 4.59 (q, *J* = 8.5 Hz, 2H), 3.77 (q, *J* = 2.5 Hz, 2H), 3.21 (s, 1H), 2.24 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 133. 6, 131. 8, 123.0 (q, *J* = 279.9 Hz), 117.3, 81.0 71.8, 53.3 (q, *J* = 34.9 Hz), 37.0. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -71.8; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_8H_8F_3N_3H]^+$: 204.0743, found: 204.0742

General Procedure for [2+2+2] Cyclotrimerization Reactions (8, 10-22). $RuCp^*(COD)Cl$ (0.05 eq) and the isocyanate (1.0 eq) were mixed with DCE (1 mL) in a 20 mL dram vial while stirring under nitrogen. To this solution was added N-prop-2-ynyl-N-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]but-2-ynamide (100 mg, 1.0 eq) in DCE (2.2 mL) in a dropwise fashion over a 15 minute period at room temperature. Once all of the 1,6-diyne was added, the vial was placed on a heating block set to 60 ºC. The reactions were monitored via LCMS and went to completion within 1 h. The reactions were then cooled to room temperature and concentrated under reduced pressure. The residues were purified by preparative flash chromatography, either via silica gel chromatography (24 g Gold Silica column, 40-100% ethyl acetate in dichloromethane), or reverse phase chromatography (30 g C18 column, 10-95% CH_3CN in 0.05% (v/v) TFA in H_2O), as was appropriate.

Ethyl 4-methyl-3,6-dioxo-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1, 2, 3, 6-tetrahydro-5H-pyrrolo [3, 4-c]pyridine-5-carboxylate (**8**). Yield: (179 mg, 47%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.84 (s, 1H), 6.51 (s, 1H), 5.18 (q, J = 9.1 Hz, 2H), 4.74 (d, *J* = 1.5 Hz, 2H), 4.51 (q, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.3, 160.7, 153.3, 152.0, 145.3, 131. 3, 124.1 (q, *J* = 279.7 Hz), 123.5, 121.8, 110.2, 109.8, 66.9, 52.1 (q, *J* = 33.4 Hz), 49.1, 14.0, 13.3; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{16}H_{15}F_3N_4O_4H]^+$: 385.1118, found: 385.1115.

4-Methyl-5-((tetrahydrofuran-2-yl)methyl)-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**10**). Yield: (84 mg, 58 %); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 – 8.25 (m, 1H), 7.82 (d, *J* = 0.7 Hz, 1H), 6.42 (s, 1H), 5.17 (q, J = 9.1 Hz, 2H), 4.67 (d, *J* = 1.4 Hz, 2H), 4.21 (dd, *J* = 13.7, 2.9 Hz, 1H), 4.11 (dtd, *J* = 9.6, 6.9, 2.8 Hz, 1H), 3.95 (dd, *J* = 13.6, 8.8 Hz, 1H), 3.78 (dt, *J* = 8.3, 6.7 Hz, 1H), 3.61 (td, *J* = 7.8, 6.1 Hz, 1H), 2.87 (s, 3H), 2.07 – 1.96 (m, 1H), 1.96 – 1.86 (m, 1H), 1.86 – 1.76 (m, 1H), 1.62 (ddt, *J* = 12.0, 8.4, 7.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.4, 162.3, 151.3 (d, *J* = 2.7 Hz), 131.3, 124.1 (q, *J* = 279.8 Hz), 123.8, 121.6, 109.1, 109.0, 76.6, 67.8, 52.1 (q, *J* = 33.4 Hz),

48.5, 48.06, 29., 25.7, 14.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{18}H_{19}F_3N_4O_3H]^+$: 397.1482, found: 397.1484.

5-(2-Chloroethyl)-7-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-3H-pyrrolo[3,4-c]pyridine-1,6-dione and 5-(2-chloroethyl)-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**11**). (*Mixture of inseparable regioisomers):* Yield: (71 mg, 58%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.29 (s, 1H), 7.94 (s, 1H), 7.84 - 7.81 (m, 1H), 7.80 (s, 1H), 6.46 (s, 1H), 5.21 (ddd, J = 15.2, 9.1, 6.5 Hz, 6H), 5.14 (d, J = 3.8 Hz, 2H), 4.94 (t, J = 9.0 Hz, 2H), 4.70 (d, J = 1.5 Hz, 2H), 4.35 (t, J = 6.6 Hz, 2H), 3.91 (t, J = 6.6 Hz, 2H), 3.02 (s, 3H), 2.91 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.2, 162.1 (d, J = 6.2 Hz), 161.8, 161.3, 151.6, 150.7, 150.0, 131.7, 131.3, 128.4 – 119.8 (m), 123.7, 122.9, 122.5, 121.7, 121.3, 109.6, 109.3, 102.4, 72.7, 52.7 – 51.6 (m), 50.5, 49.1, 48.6, 45.3, 41.2, 14.4, 14.2; ^{19}F NMR (376 MHz, DMSO- d_6) δ -70.2, -70.2; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{15}H_{14}ClF_3N_4O_2H]^+$: 375.0830, found: 375.0831.

5-(2,2-Dimethoxyethyl)-4-methyl-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**12**). Yield: (149 mg, 65%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.82 (s, 1H), 6.43 (s, 1H), 5.17 (q, J = 9.1 Hz, 2H), 4.68 (d, J = 1.4 Hz, 2H), 4.60 (t, J = 5.4 Hz, 1H), 4.11 (d, J = 5.4 Hz, 2H), 3.32 (s, 6H), 2.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.3, 162.4, 151.4 (d, J = 18.0 Hz), 131.3, 124.1 (q, J = 279.8 Hz), 123.8, 121.7, 109.4, 109.0, 102.5, 55.5, 52.1 (q, J = 33.3 Hz), 48.6, 46.4, 14.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{17}H_{19}F_3N_4O_4H]^+$: 401.1431, found: 401.1430.

5-(2,2-Difluoroethyl)-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**13**). Yield: (68 mg, 48%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 – 8.26 (m, 1H), 7.83 (d, J = 0.7 Hz, 1H), 6.51 (s, 1H), 6.31 (tt, J = 55.8, 4.2 Hz, 1H), 5.17 (q, J = 9.1 Hz, 2H), 4.71 (d, J = 1.5 Hz, 2H), 4.52 (td, J = 14.1, 4.2 Hz, 2H), 2.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.0, 162.3, 151.9, 150.6, 131.3, 124.1 (q, J = 279.8 Hz), 123.6, 121.7, 114.2 (t, J = 241.5 Hz), 110.0, 109.3, 52.1 (q, J = 33.5 Hz), 48.6, 45.6 (t, J = 28.2 Hz), 14.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3, -120.7; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{15}H_{13}F_5N_4O_2H]^+$: 377.1031, found: 377.1031.

Ethyl 2-[4-methyl-3,6-dioxo-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridin-5-yl]acetate (**14**). Yield: (79 mg, 65%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.90 – 7.84 (m, 1H), 6.52 (s, 1H), 5.23 (q, J = 9.1 Hz, 2H), 4.93 (s, 2H), 4.78 (d, J = 1.4 Hz, 2H), 4.22 (q, J = 7.1 Hz, 2H), 2.81 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.3, 164.1, 162.1, 151.7, 150.4, 131.3, 124.1 (q, J = 283.6, 278.3, 274.2 Hz), 109.5, 109.0, 61.8, 52.1 (q, J = 33.4 Hz), 48.7, 45.6, 14.5, 14.0; ^{19}F NMR (282 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{17}H_{17}F_3N_4O_4H]^+$: 399.1275, found: 399.1276.

4-Methyl-5-(2-(methylthio)ethyl)-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**15**). Yield: (66 mg, 67%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 0.7 Hz, 1H), 7.82 (d, J = 0.7 Hz, 1H), 6.44 – 6.40 (m, 1H), 5.17 (q, J = 9.1 Hz, 2H), 4.68 (d, J = 1.5 Hz, 2H), 4.25 – 4.17 (m, 2H), 2.90 (s, 3H), 2.78 – 2.71 (m, 2H), 2.16 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.3, 162.1, 151.3, 150.4, 131.3, 124.1(q, J = 279.7 Hz), 123.8, 121.6, 109.3, 109.2, 52.1 (q, J = 33.2 Hz), 48.6, 43.5, 30.9, 15.3, 13.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.2; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{16}H_{17}F_3N_4O_2SH]^+$: 387.1103, found: 387.1096.

4-Methyl-5-tetrahydropyran-4-yl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**16**). Yield: (93 mg, 42%); off-white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.81 (s, 1H), 6.32 (s, 1H), 5.17 (q, J = 9.1 Hz, 2H), 4.63 (s, 2H), 4.44 (t, J = 10.1

Hz, 1H), 4.32 (s, 1H), 3.82 (ddd, *J* = 20.8, 7.8, 3.4 Hz, 2H), 3.31 (td, *J* = 10.7, 5.5 Hz, 1H), 2.90 (s, 3H), 2.80 (d, *J* = 13.0 Hz, 1H), 1.75 (tdd, *J* = 12.1, 9.4, 7.2, 3.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.3, 163.2, 150.9, 131.3, 124.1 (q, *J* = 279.8 Hz), 123.8, 121.6, 110.9, 109.8, 67.5, 67.2, 56.3, 52.2 (q, *J* = 33.5 Hz), 48.2, 26.6, 25.1, 14.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{18}H_{19}F_3N_4O_3H]^+$: 397.1482, found: 397.1480

4-Methyl-5-((methylsulfonyl)methyl)-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**17**) Yield: (83 mg, 59%); white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.86 – 7.81 (m, 1H), 6.56 (s, 1H), 5.65 (s, 2H), 5.18 (q, *J* = 9.1 Hz, 2H), 4.75 (d, *J* = 1.6 Hz, 2H), 3.17 (s, 3H), 2.90 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.78, 161.79, 152.27, 150.59, 131.40, 124.12 (q, *J* = 279.7 Hz), 123.57, 121.86, 110.56, 109.44, 62.58, 52.10 (q, *J* = 33.5 Hz), 48.79, 43.80, 14.46; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.2; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{15}H_{15}F_3N_4O_4SH]^+$: 405.0839, found: 405.0835.

5-(3-Furyl)-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**18**). Yield: (44 mg, 35%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.85 (d, *J* = 0.8 Hz, 1H), 7.80 (dd, *J* = 2.1, 1.0 Hz, 1H), 6.69 (dd, *J* = 3.3, 2.1 Hz, 1H), 6.61 (dd, *J* = 3.3, 1.0 Hz, 1H), 6.52 (q, *J* = 1.3 Hz, 1H), 5.18 (q, *J* = 9.1 Hz, 2H), 4.77 (d, *J* = 1.5 Hz, 2H), 2.43 – 2.39 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.6, 162.5, 152.7, 150.8, 143.0, 142.2, 131.3, 124.1 (q, *J* = 279.8 Hz), 123.6, 121.8, 112.2, 110.2, 110.1, 108.2, 52.1 (q, *J* = 33.5 Hz), 49.0, 14.3; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.24; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{17}H_{13}F_3N_4O_3H]^+$: 379.1013, found: 379.1012

4-Methyl-5-(spiro[3.3]heptan-2-yl)-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**19**). Yield: (39 mg, 80%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 – 8.24 (m, 1H), 7.81 (d, *J* = 0.7 Hz, 1H), 6.29 (d, *J* = 1.5 Hz, 1H), 5.16 (q, *J* = 9.1 Hz, 2H), 4.73 (p, *J* = 8.7 Hz, 1H), 4.62 (d, *J* = 1.4 Hz, 2H), 2.98 (td, *J* = 9.0, 2.8 Hz, 2H), 2.80 (s, 3H), 2.36 (td, *J* = 8.4, 2.7 Hz, 2H), 2.11 – 2.04 (m, 2H), 1.99 (dd, *J* = 8.4, 6.1 Hz, 2H), 1.86 – 1.75 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.4, 163.5, 150.5, 150.3, 131.2, 124.1 (q, *J* = 279.8 Hz), 123.9, 121.5, 110.6, 109.3, 52.1 (q, *J* = 33.3 Hz), 48.2, 37.2, 35.3, 35.0, 17.0, 14.5. ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{20}H_{21}F_3N_4O_2H]^+$: 407.1689, found: 407.1691.

4-Methyl-5-(1-methyl-1H-pyrazol-4-yl)-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridine-3,6(5H)-dione (**20**). Yield: (44 mg, 64%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.92 (s, 1H), 7.84 (d, *J* = 0.8 Hz, 1H), 7.51 (q, *J* = 2.1, 1.5 Hz, 1H), 6.49 – 6.45 (m, 1H), 5.19 (q, *J* = 9.1 Hz, 2H), 4.74 (d, *J* = 1.5 Hz, 2H), 3.91 (d, *J* = 0.8 Hz, 3H), 2.47 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1, 162.6, 151.6, 151.4, 137.2, 131.3, 129.6, 124.1 (q, *J* = 279.8 Hz), 123.8, 121.6, 118.4, 109.5, 109.4, 52.1 (q, *J* = 33.3 Hz), 48.8, 15.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.23; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{17}H_{15}F_3N_6O_2H]^+$: 393.1281, found: 393.1283.

5-(4-Fluorophenyl)-4-methyl-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**21**). Yield: (48 mg, 27 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.85 (s, 1H), 7.44 – 7.36 (m, 4H), 6.51 (s, 1H), 5.18 (q, *J* = 9.1 Hz, 2H), 4.77 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.16, 163.54, 162.68, 161.10, 151.82, 150.36, 134.28 (d, *J* = 3.0 Hz), 131.33, 130.96 (d, *J* = 8.8 Hz), 124.13 (q, *J* = 279.6 Hz), 123.73, 121.68, 116.97 (d, *J* = 22.9 Hz), 109.92, 109.39, 52.09 (q, *J* = 33.4 Hz), 48.86, 15.81; ^{19}F NMR (376 MHz,

DMSO-d_6) δ -70.22, -113.09; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{19}H_{14}F_4N_4O_2H]^+$: 407.1126, found: 407.1127.

4-Methyl-5-[(2R)-2-phenylcyclopropyl]-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-3,6-dione (**22**). Yield: (56 mg, 12 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, *J* = 0.7 Hz, 1H), 7.82 (d, *J* = 0.7 Hz, 1H), 7.38 – 7.27 (m, 4H), 7.27 – 7.21 (m, 1H), 6.38 (q, *J* = 1.2 Hz, 1H), 5.17 (q, *J* = 9.1 Hz, 2H), 4.67 (d, *J* = 1.4 Hz, 2H), 3.12 (ddd, *J* = 7.6, 4.9, 3.8 Hz, 1H), 2.82 (s, 3H), 2.36 (ddd, *J* = 10.4, 7.0, 3.9 Hz, 1H), 1.73 (dt, *J* = 7.4, 6.5 Hz, 1H), 1.48 (ddd, *J* = 9.9, 6.4, 4.9 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 163.26, 152.97, 150.48, 140.62, 131.24, 128.72, 126.77, 126.62, 124.12 (q, *J* = 279.7 Hz), 123.81, 121.56, 109.83, 109.27, 52.08 (q, *J* = 33.5 Hz), 48.52, 37.45, 27.10, 19.43, 15.27; ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.23; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{22}H_{19}F_3N_4O_2H]^+$: 429.1533, found: 429.1531

6-Chloro-4-methyl-2-(1-(2,2,2-trifluoroethyl)-1H-pyrazol-4-yl)-1,2-dihydro-3H-pyrrolo[3,4-c]pyridin-3-one (9).

To a solution of ethyl 4-methyl-3,6-dioxo-2-[1-(2,2,2-trifluoroethyl)pyrazol-4-yl]-1H-pyrrolo[3,4-c]pyridine-5-carboxylate (614 mg, 1.58 mmol) in THF (10 mL) was added HCl (6M, 10 mL, 60 mmol) at rt. The mixture was heated at 95 °C overnight and then concentrated under reduced pressure. To this residue was added $POCl_3$ (15 mL) and the mixture heated at 95 °C under nitrogen for 3 h. The reaction mixture was concentrated under reduced pressure. Ice and EtOAc and ethyl acetate were added. On reaction room temperature the layers were separated and the combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified via silica gel chromatography (1:1 Petroleum ether: ethyl acetate, R_f= 0.20) to afford (**2)** (356 mg, 68%, white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.88 (s, 1H), 7.66 (s, 1H), 5.18 (q, *J* = 9.1 Hz, 2H), 4.85 (s, 2H), 2.74 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.8, 157.4, 154.6, 151.2, 131.5, 125.5 (q, *J* = 279.1, 277.5 Hz), 125.1, 123.4, 122.0, 117.5, 52.1 (q, *J* = 33.5 Hz); ^{19}F NMR (376 MHz, DMSO-d_6) δ -70.3; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $[C_{13}H_{10}ClF_3N_4OH]^+$: 331.0568, found: 331.0569.

4. Abbreviations:

CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
DTT	DL-Dithiothreitol
EGTA	Ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid
FBS	Fetal Bovine Serum
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid