Supporting Information

Discovery and Preclinical Evaluation of BMS-986242, a Potent, Selective Inhibitor of Indoleamine-2,3-dioxygenase 1

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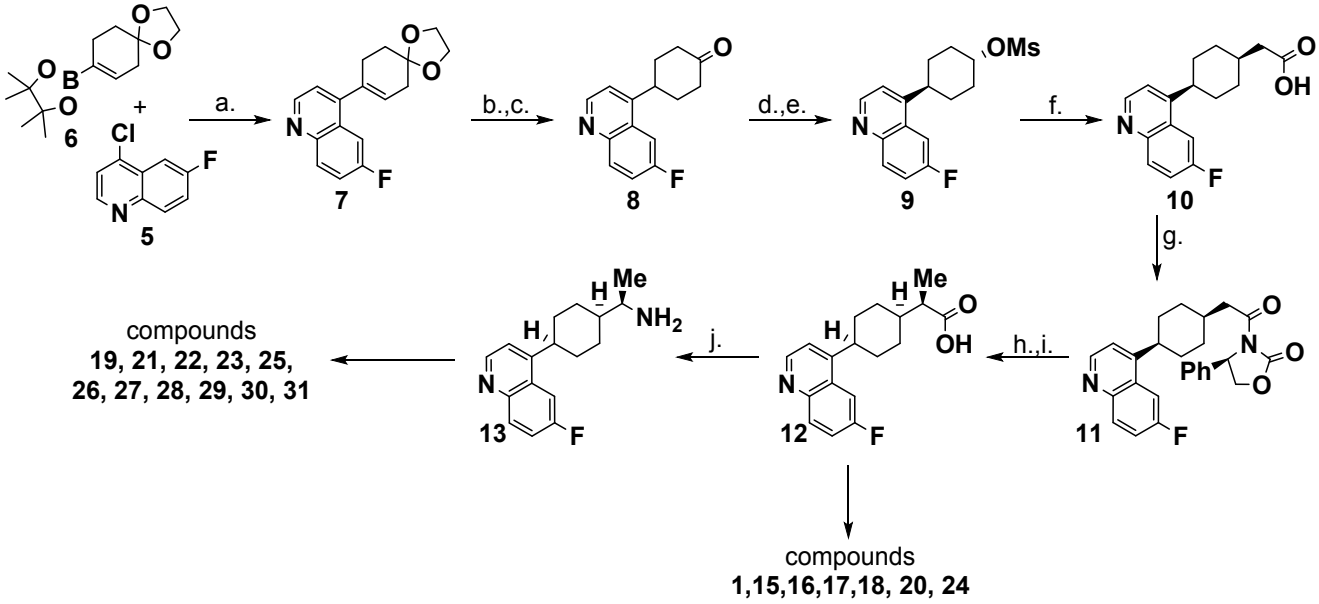
Part 1: Synthetic Procedures and Compound Characterization

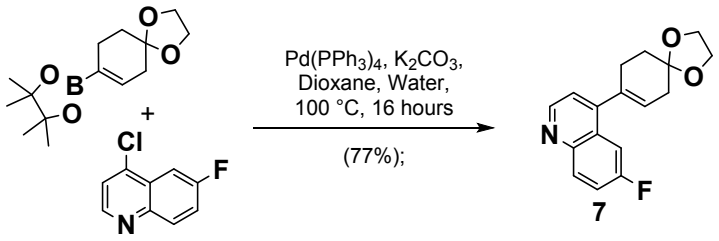
General Methods: Reagents and solvents were used as obtained form commercial suppliers without further purification unless otherwise stated. All reactions were carried out under a nitrogen atmosphere in a roundbottom flask or in a chemglass vial fitted with a pressure relief cap unless otherwise stated. NMR spectra were recorded on a Bruker 400 MHz or Bruker 500 MHz spectrometer. Splitting patterns were designated as "s, d, t, q, m, and br" indicated "singlet, doublet, triplet, quartet, multiplet, and broad," respectively. Chemical shifts (δ) are reported in reference to CD_3OD (δ = 3.31 ppm), D_6-DMSO (δ = 2.50 ppm), or $CDCl_3$ (δ = 7.26 ppm) as an internal standard. All *J* values are given in Hz. Reaction progress was monitored by TLC or LC-MS on a Waters ZQ 2000 single quadropole mass spectrometer (milford, MA) interfaced to a Shimadzu Discovery VP LC (Columbia, MD). Chromatographic separations for reaction monitoring were achieved employing analytical HPLC/MS using **Method A:** Waters Acquity SDS using the following method: Linear Gradient of 2% to 98% solvent B over 1.7 min; UV visualization at 220 nm; Column: BEH C18 2.1 mm x 50 mm; 1.7 um particle (Heated to Temp. 50 °C); Flow rate: 0.8 ml/min; Mobile phase A: 100% Water, 0.05% TFA; Mobile phase B: 100% Acetonitrile, 0.05% TFA. Electrospray ionization (ESI) high resolution mass spectrometry (HRMS) was obtained on a thermo Fisher Finnigan LTQ-FT instrument. Column chromatography was carried out using an ISCO system, and performed using pre-packed silica gel (SiO_2) columns. Preparative HPLC was usually performed using a Waters system with the following conditions: Column: Waters Xbridge C18, 19 x 250 mm, 5-µm particles; Guard Column: Waters Xbridge C18, 19 x 10 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10-mM ammonium acetate; Gradient 15-50% B over 30 minutes, then a 15 minutes hold at 100% B; Flow: 20 mL/min. The purity of all new compounds was determined by reverse phase HPLC according to Methods B and C: **Method B**: Column: Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0-100% B over 3 minutes, then a 0.75-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 220 nm. **Method C**: Column: Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile:water with 0.1% trifluoroacetic acid; Temperature: 50 °C; Gradient: 0-100% B over 3 minutes, then a 0.75-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 220 nm.

General Scheme[a]



[a]Reagents and Conditions: a. $Pd(PPh_3)_4$, K_2CO_3, Dioxane, Water, 100 °C, 16 hours (77%); b. Pd/C (Degussa), NH_4CO_2H; C. 4 M HCl, Acetone (75% over 2 steps); d. $NaBH_4$ (5:1 trans:cis dr); e. MsCl, Pyr. (76% over 2 steps); f. i. NaH, di-^{t}Bu-Malonate, ii. AcOH, 130 °C (76%); g. PivCl, TEA; lithium-(*R*)-2-oxo-4-phenyloxazolidin-3-ide (85%), h. NaHMDS, −40 °C; MeI (>20:1 dr) (68%); i. LiOH, H_2O_2 (82%); j. DPPA, TEA; LiOH, H_2O (90%)

Compound 7:



Experimental: A mixture of 4,4,5,5-tetramethyl-2-(1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (368g, 1.38 mol, 1.3eq) (for preparation, see: US2010/56576 A1), 4-Chloro-6-fluoroquinoline (195 g, 1.07 mol, 1eq), K_2CO_3 (445 g, 3.22 mol, 3eq) and Pd(PPh3)4 (25 g, 22 mmol, 0.02eq) in dioxane-water (3L, 4:1) was heated to reflux overnight. The solution was concentrated and extracted with EtOAc. Purification by flash column chromatography on silica gel (ethyl acetate/pet ether) to give 6-fluoro-4-(1,4-dioxaspiro[4.5]dec-7-en-8-yl)quinoline (236 g, 77%) as a yellow oil.

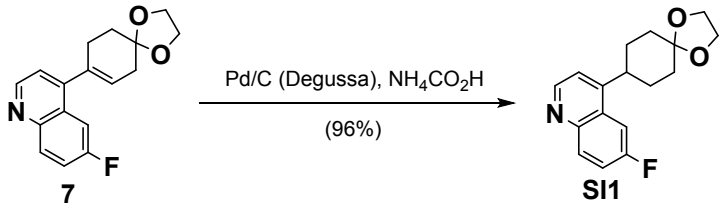
TLC: R_f = 0.3 (pet ether:ethyl acetate = 2:1, UV 254 nm).

LCMS: MS: Anal. Calc'd for $C_{17}H_{16}FNO_2$ 285.117, found [M+H] 286.1 LC: tr = 0.62 min (**Method A**)

^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.81 (d, *J*=4.4 Hz, 1H), 8.11 (dd, *J*=9.2, 5.6 Hz, 1H), 7.64 (dd, *J*=10.1, 2.7 Hz, 1H), 7.47 (br ddd, *J*=9.2, 8.1, 2.9 Hz, 1H), 7.24 (d, *J*=4.3 Hz, 1H), 5.75 (tt, *J*=3.7, 1.8 Hz, 1H), 4.10 - 4.06 (m, 4H), 2.63 (tq, *J*=6.4, 2.1 Hz, 2H), 2.57 - 2.53 (m, 2H), 2.00 (t, *J*=6.4 Hz, 2H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 160.4 (d, *J*=247.0 Hz), 149.7 (d, *J*=5.4 Hz), 149.3 (d, *J*=1.8 Hz), 145.7, 134.6, 132.2 (d, *J*=9.1 Hz), 127.4 (d, *J*=9.1 Hz), 126.5, 120.1, 119.4 (d, *J*=26.3 Hz), 108.9 (d, *J*=22.7 Hz), 107.3, 64.6, 36.0, 31.4, 29.6

Compound SI1:



Experimental: 6-fluoro-4-(1,4-dioxaspiro[4.5]dec-7-en-8-yl)quinoline (12.0 g, 42.1 mmol) was dissolved in MeOH (210 ml) and ammonium formate (13.26 g, 210 mmol) was added. The vessel was equipt with a reflux condenser and vacated and flushed with nitrogen gas three times. Then, Pd/C (1.209 g, 11.36 mmol) was added and the reaction was heated to reflux for 1 hour. Reaction was then cooled, concentrated in vacuo, and diluted with DCM. Solids were filtered off and the filtrate was concentrated and dried on high vac ON to give crude 6-fluoro-4-(1,4-

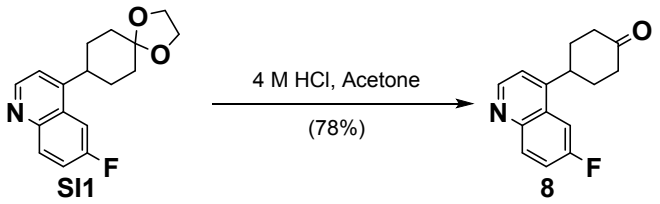
dioxaspiro[4.5]decan-8-yl)quinoline (11.55 g, 40.2 mmol, 96 % yield) as an off white solid. (Used directly without further purification)

LCMS: MS: Anal. Calc'd for $C_{17}H_{18}FNO_2$ 287.132, found [M+H] 288.1 LC: tr = 0.62 min (**Method A**)

^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.81 (d, *J*=4.6 Hz, 1H), 8.12 (dd, *J*=9.2, 5.7 Hz, 1H), 7.68 (dd, *J*=10.5, 2.8 Hz, 1H), 7.47 (ddd, *J*=9.3, 7.9, 2.7 Hz, 1H), 7.34 (d, *J*=4.6 Hz, 1H), 4.04 - 3.99 (m, 4H), 3.26 - 3.17 (m, 1H), 2.04 - 1.79 (m, 8H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 160.5 (d, *J*=247.0 Hz), 151.4 (d, *J*=6.4 Hz), 149.6 (d, *J*=2.7 Hz), 145.5, 132.9 (d, *J*=9.1 Hz), 127.7 (d, *J*=9.1 Hz), 119.0 (d, *J*=25.4 Hz), 118.1, 108.2, 106.5 (d, *J*=22.7 Hz), 64.4, 64.4, 37.8, 35.2, 30.4

Compound 8:



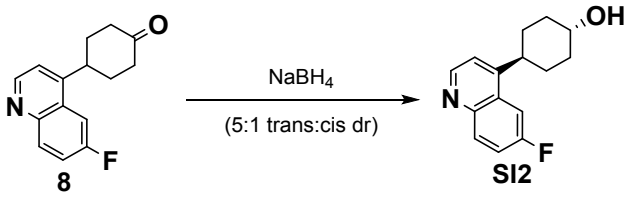
Experimental: 6-fluoro-4-(1,4-dioxaspiro[4.5]decan-8-yl)quinoline (11.55 g, 40.2 mmol) taken up in Acetone (129 ml) and 4M HCl (aq) (32.2 ml) was added. The reaction was stirred at 45 °C for 16 hours. Note: LCMS after ON looks similar to reaction on shows some ketal forming with MeOH on LCMS, but starting material mostly consumed. Reaction was concentrated *in vacuo* to remove acetone and the residue was treated with 1N NaOH to adjust to pH 9. The mixture was then extracted with EtOAc, dried with sodium sulfate, filtered, and concentrated *in vacuo*. The residue was treated with ether and rotovapped down three times to give a crystalline solid. The solid was triturated with ether. Triturated matieral is clean by NMR. The mother liquor was then purified *via* ISCO (40 g column, 0-70% EtOAc in hexanes) to give a combined yield of 4-(6-fluoroquinolin-4-yl)cyclohexanone (7.61 g, 31.3 mmol, 78 % yield) as an off white solid.

LCMS: MS: Anal. Calc'd for $C_{15}H_{14}FNO$ 243.106, found [M+H] 244.1 LC: tr = 0.53 min (**Method A**)

^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.84 (d, *J*=4.6 Hz, 1H), 8.16 (dd, *J*=9.2, 5.7 Hz, 1H), 7.72 (dd, *J*=10.4, 2.7 Hz, 1H), 7.52 (ddd, *J*=9.3, 7.9, 2.7 Hz, 1H), 7.30 (d, *J*=4.6 Hz, 1H), 3.69 (tt, *J*=12.1, 3.1 Hz, 1H), 2.72 - 2.58 (m, 4H), 2.37 (ddt, *J*=10.6, 5.8, 3.0 Hz, 2H), 2.05 (qd, *J*=12.8, 5.2 Hz, 2H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 209.6, 160.7 (d, *J*=248.0 Hz), 149.7 (d, *J*=5.4 Hz), 149.6 (d, *J*=2.7 Hz), 145.6, 133.2 (d, *J*=9.1 Hz), 127.4 (d, *J*=9.1 Hz), 119.3 (d, *J*=25.4 Hz), 117.8, 106.3 (d, *J*=22.7 Hz), 41.2, 37.3, 32.7

Compound SI2:



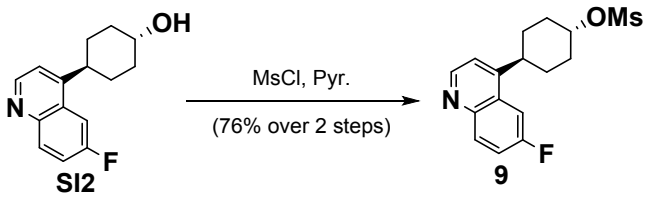
Experimental: Ketone (57.8 g, 237.8 mmol) was dissolved in EtOH (240 mL) and cooled to 0 °C. $NaBH_4$ (9.94g, 261.6 mmol) was added portionwise maintaining the temperature with 0-10 °C range (exothermic reaction). The resulting suspension was stirred for 20 minutes. The reaction was quenched at 0 °C by the slow addition of acetone (58 mL) over 15 minutes(exotherm). The reaction was poured slowly onto 500 mL of saturated aqueous ammonium chloride and 500 g of ice. The resulting aqueous solution was extracted with EtOAc (3 x 300 mL) and the combined organic fractions were washed with saturated aqueous ammonium chloride (250 mL) and saturated aqueous sodium chloride (250 mL). The organic portion was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Sufficient silica to adsorb the oil was added and diluted with 10 % MeOH in CH_2Cl_2. A similar quantity of silica was used as a silica plug to purify the material. The silica plug was washed with 10 % MeOH in CH_2Cl_2 until UV-active material no longer could be detected by TLC (7:3 EtOAc/Hexanes, Rf =0.4). The filtrate was concentrated then suspended in 500 mL of toluene and concentrated again. The yellow solid (58.2 g) was used in the subsequent step without further purification.

LCMS: MS: Anal. Calc'd for $C_{15}H_{16}FNO$ 245.122, found [M+H] 246.1 LC: tr = 0.50 min (**Method A**)

^{1}H NMR: (400 MHz, CHLOROFORM-d) δ 8.72 (d, *J*=4.6 Hz, 1H), 8.08 (dd, *J*=9.2, 5.7 Hz, 1H), 7.60 (dd, *J*=10.4, 2.8 Hz, 1H), 7.41 (ddd, *J*=9.2, 7.9, 2.8 Hz, 1H), 7.21 (d, *J*=4.6 Hz, 1H), 3.98 (br s, 1H), 3.81 - 3.63 (m, 1H), 3.17 - 3.03 (m, 1H), 2.30 - 2.11 (m, 2H), 2.11 - 1.89 (m, 2H), 1.63 - 1.46 (m, 4H)

^{13}C NMR: (101 MHz, CHLOROFORM-d) δ 160.3 (d, *J*=247.4 Hz), 151.7 (d, *J*=5.4 Hz), 149.2 (d, *J*=3.1 Hz), 145.1, 132.4 (d, *J*=9.2 Hz), 127.6 (d, *J*=8.5 Hz), 119.0 (d, *J*=26.2 Hz), 117.7, 106.5 (d, *J*=22.3 Hz), 69.8, 37.9, 35.6, 31.2

Compound 9:



Experimental: To the yellow solid (58.2 g, 237.8 mmol) obtained from the previous step was added MeCN (125 mL) and pyridine (38.7 mL, 480 mmol) and the reaction mixture was cooled to 5 °C using ice/water bath. Methanesulfonyl chloride (26.0 mL, 336 mmol) was added dropwise at 5 °C (exothermic reaction), the reaction mixture stirred for 1 hr at 5 °C and then brought up to room temperature and stirred for additional 16 h during which time a white precipitate formed. The heterogeneous mixture was quenched by the addition of saturated aqueous ammonium chloride (200 mL) and extracted with CH_2Cl_2 (3 x 300 mL). The combined organic fractions were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Excess pyridine was removed by azeotroping from toluene (3 x 300 mL). The crude material was recrystallized from H_2O/MeOH as follows: 1 mL/mmol of H_2O was added and the slurry was heated to 120 °C in an oil bath. MeOH was added until the solids went into solution (~0.5 L). After cooling white crystals were collected by filtration. (58.6 g, >20:1 dr, 76 % over two steps).

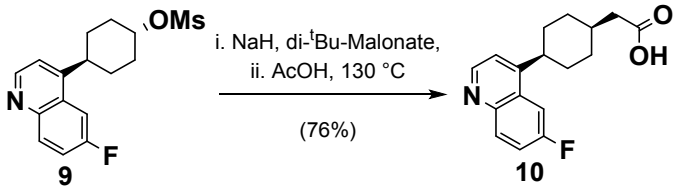
LCMS: MS: Anal. Calc'd for $C_{16}H_{18}FNO_3S$ 323.099, found [M+H] 324.1 LC: tr = 0.63 min (**Method A**)

^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.81 (d, *J*=4.6 Hz, 1H), 8.12 (dd, *J*=9.2, 5.7 Hz, 1H), 7.63 (dd, *J*=10.5, 2.7 Hz, 1H), 7.48 (ddd, *J*=9.2, 8.0, 2.7 Hz, 1H), 7.25 (d, *J*=4.6 Hz, 1H), 4.78 (tt, *J*=11.2, 4.6 Hz, 1H), 3.19 (tt, *J*=12.0, 3.1 Hz, 1H), 3.07 (s, 3H), 2.39 (ddd, *J*=13.3, 2.6, 1.1 Hz, 2H), 2.17 - 2.09 (m, 2H), 1.93 - 1.80 (m, 2H), 1.78 - 1.63 (m, 2H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 160.6 (d, *J*=247.0 Hz), 150.2 (d, *J*=5.5 Hz), 149.6 (d, *J*=2.7 Hz), 145.5, 133.0 (d, *J*=9.1 Hz), 127.5 (d, *J*=9.1 Hz), 119.2 (d, *J*=25.4 Hz), 117.8, 106.3 (d, *J*=22.7 Hz), 80.3, 38.9, 37.3, 33.0, 31.0

2-D NMR: A 2D NOESY data supported *trans*- stereochemistry. The *J*-coupling of the two methine protons is consistent with axial-axial coupling. In addition the two ring methines have noe crosspeaks with axial protons.

Compound 10:



Experimental: Di-*tert*-butyl malonate (33.5 mL, 150 mmol) was added dropwise to a stirred suspension of NaH (6.0 g, 60% suspension in oil, 150 mmol) in 1,2-dimethosyethane (100mL) under Argon, cooled in an ice-water bath. After stirring for 10 minutes, mesylate **9** (16.2 g, 50 mmol) was added and the

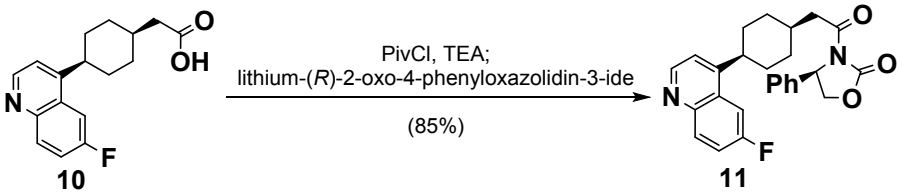
reaction was heated to 85 °C for 20 hours. After this time, acetic acid (100 mL) was added, the reaction flask was fitted with a distillation head, and the temperature was increase to 130 °C. 1,2-dimethosyethane was distilled off under atmospheric pressure until the distillate was acidic (~100 mL). The distillation head was removed, a reflux condenser was attached, water (20 mL) was added, and the reaction was heated to 130 °C for 12 hours. The reaction was then concentrated under reduced pressure and poured into 200 g of ice and 100 mL sat. aq. sodium acetate. The desired product was isolated as a white solid *via* filtration and further dried by refluxing with toluene in a dean stark apparatus to give dry **10** (11.0 g, 76% yield).

LCMS: MS: Anal. Calc'd for $C_{17}H_{18}FNO_2$ 287.132, found [M+H] 288.2 LC: tr = 0.60 min (**Method A**)

^{1}H NMR: (400 MHz, DMSO-d_6) δ 12.07 (br s, 1H), 8.81 (d, *J*=4.5 Hz, 1H), 8.08 (dd, *J*=9.3, 5.9 Hz, 1H), 7.95 (dd, *J*=11.0, 2.8 Hz, 1H), 7.65 (td, *J*=8.7, 2.8 Hz, 1H), 7.51 (d, *J*=4.5 Hz, 1H), 3.41 - 3.27 (m, 1H), 2.44 (d, *J*=7.6 Hz, 2H), 2.27 (br s, 1H), 1.94 - 1.78 (m, 3H), 1.77 - 1.61 (m, 5H).

^{13}C NMR: (101 MHz, DMSO-d_6) δ 174.1, 159.9 (d, *J*=244.3 Hz), 152.3 (d, *J*=5.4 Hz), 149.8 (d, *J*=2.3 Hz), 145.1, 132.6 (d, *J*=9.2 Hz), 127.1 (d, *J*=9.2 Hz), 118.9 (d, *J*=25.4 Hz), 118.7, 107.1 (d, *J*=23.1 Hz), 37.5, 36.2, 29.4, 29.1, 27.3.

Compound 11:



Experimental: To a dried flask (heat gun on high vac and let cool) was added **10** (4 g, 13.92 mmol). Flask was then vacated and back filled with nitrogen a few times before being placed under N2 atmosphere. THF (55.7 ml) was added followed by TEA (3.88 ml, 27.8 mmol). Slurry was cooled to -78 °C and pivaloyl chloride (2.141 ml, 17.40 mmol) was added over ~10 minutes. After addition complete, the reaction stirred at room temperature for 1 hour. Meanwhile, in another dry flask under N2 atmosphere, (*R*)-4-phenyloxazolidin-2-one (2.95 g, 18.10 mmol) was taken up in THF (55.7 ml) and cooled to -78 C. N-butyllithium (7.24 ml, 18.10 mmol) (a 2.5 M solution in hexanes) was added and the reaction stirred 15 minutes at -78 °C before being removed from bath (goes from yellow to red upon warming). The first flask containing the pivayl anhydride was re-cooled to -78 °C and the contents of the deprotonated oxazolidinone in the second flask were added via cannula over the course of ~10 minutes. After addition complete, the bath was removed and the reaction was allowed to stir at rt for 3 hours. After 3 hours, the reaction was quenched with sat. aq NH_4Cl and extracted with EtOAc. Combined organics

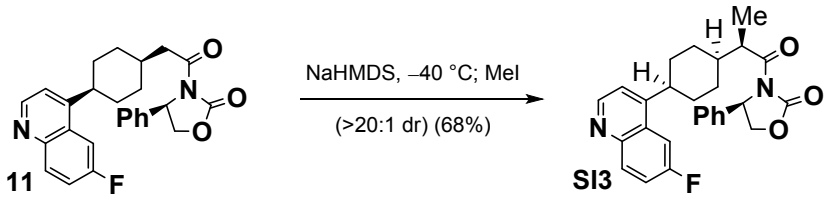
were washed with brine, dried over sodium sulfate, filtered and concentrated. Crude residue purified via ISCO (40 g column, 40 mL/min, 0-100% EtOAc) to give **11** (5.12 g, 11.83 mmol, 85 % yield).

LCMS: MS: Anal. Calc'd for $C_{26}H_{25}FN_2O_3$ 432.185, found [M+H] 433.0 LC: tr = 0.79 min (**Method A**)

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.82 (d, *J*=4.5 Hz, 1H), 8.08 (dd, *J*=9.2, 5.9 Hz, 1H), 7.95 (dd, *J*=11.0, 2.7 Hz, 1H), 7.65 (ddd, *J*=9.2, 8.3, 2.8 Hz, 1H), 7.47 (d, *J*=4.5 Hz, 1H), 7.41 - 7.35 (m, 2H), 7.34 - 7.27 (m, 3H), 5.49 (dd, *J*=8.6, 3.7 Hz, 1H), 4.75 (t, *J*=8.7 Hz, 1H), 4.16 (dd, *J*=8.7, 3.8 Hz, 1H), 3.40 - 3.28 (m, 1H), 3.22 - 2.98 (m, 2H), 2.35 (br s, 1H), 1.95 - 1.48 (m, 8H)

^{13}C NMR: (101 MHz, DMSO-d_6) δ 171.6, 159.9 (d, *J*=244.3 Hz), 153.7, 152.2 (d, *J*=5.4 Hz), 149.8 (d, *J*=2.3 Hz), 145.1, 140.0, 132.6 (d, *J*=9.2 Hz), 128.7, 127.9, 127.1 (d, *J*=9.2 Hz), 125.7, 118.9 (d, *J*=25.4 Hz), 118.5, 107.1 (d, *J*=22.3 Hz), 70.0, 56.9, 37.4, 36.5, 29.6, 29.0, 28.8, 27.4

Compound SI3:



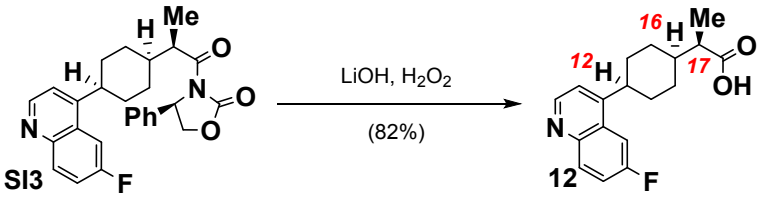
Experimental: A solution of the imide 11 (21.6 g, 50 mmol) in anhydrous THF (200 mL) was cooled to –40 °C (using ACN/ dry ice bath, some precipitation occurs) and 2M NaHMDS solution in THF (30 mL, 60 mmol) was added over 5 minutes. The resulting yellow reaction mixture was stirred for 10 minutes, became homogeneous, and MeI (10.6 g, 75 mmol) was added dropwise over 2 minutes. The reaction mixture was stirred for 1 hour at –40 °C. The reaction was rapidly diluted with saturated aqueous ammonium chloride solution (400 mL) and the biphasic mixture was stirred for 15 minutes. ^{i}PrOAc was added. The layers were separated, and the aqueous layer was again extracted with ^{i}PrOAc. The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated. The resulting residue was recyrstallized from acetone and water (~3:1) to give SI3 as white needles (15.04 g, 68%).

LCMS: MS: Anal. Calc'd for $C_{27}H_{27}FN_2O_3$ 446.201, found [M+H] 447.3 LC: tr = 0.85 min **(Method A)**

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.85 (d, *J*=4.5 Hz, 1H), 8.09 (dd, *J*=9.2, 5.9 Hz, 1H), 7.98 (dd, *J*=11.0, 2.8 Hz, 1H), 7.75 - 7.61 (m, 1H), 7.43 - 7.36 (m, 3H), 7.36 - 7.28 (m, 3H), 5.53 (dd, *J*=8.6, 3.7 Hz, 1H), 4.76 (t, *J*=8.7 Hz, 1H), 4.35 - 4.23 (m, 1H), 4.15 (dd, *J*=8.7, 3.8 Hz, 1H), 3.48 - 3.35 (m, 1H), 2.06 - 1.95 (m, 1H), 1.91 - 1.51 (m, 8H), 1.05 (d, *J*=6.8 Hz, 3H)

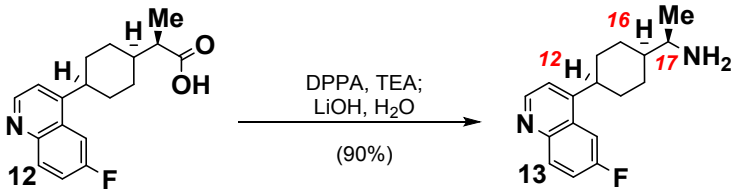
^{13}C NMR: (101 MHz, DMSO-d_6) δ 175.9, 159.9 (d, *J*=244.3 Hz), 153.6, 152.3 (d, *J*=5.4 Hz), 149.8 (d, *J*=2.3 Hz), 145.2, 139.9, 132.6 (d, *J*=10.0 Hz), 128.8, 128.0, 127.1 (d, *J*=9.2 Hz), 125.5, 119.0 (d, *J*=25.4 Hz), 118.4, 107.2 (d, *J*=22.3 Hz), 69.8, 57.0, 37.1, 36.0, 34.7, 28.4, 27.9, 27.5, 26.1, 15.9

Compound 12:



Experimental: To a solution of methyl imide **SI3** (82.0 g, 183.6 mmol) in THF (610 mL) at 0 °C was added aqueous hydrogen peroxide (35 wt%, 82 mL) and LiOH (7.04 g, 293.8 mmol) in water (189 mL). The resulting reaction mixture was allowed to slowly warm to room temperature and stirred overnight. The reaction was cooled to 0 °C and saturated aqueous sodium bisulfite solution (250 mL) was added. After stirring for 30 minutes, the THF was removed under reduced presssure. Acetic acid (34 mL) was added followed by EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc three times. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The brown crude reaction mixture was suspended in ACN and the suspension was brought to reflux with vigorous stirring. After cooling to ambient temperature, the solids were collected by filtration washing with additional ACN. The desired product **12** was obtained as a white solid (45.4 g, 82% yield).
LCMS: MS: Anal. Calc'd for $C_{18}H_{20}FNO_2$ 301.148, found [M+H] 302.2 LC: tr = 0.65 min (**Method A**)
^{1}H NMR: (400 MHz, DMSO-d_6) δ 12.08 (s, 1H), 8.81 (d, *J*=4.5 Hz, 1H), 8.08 (dd, *J*=9.3, 5.9 Hz, 1H), 7.96 (dd, *J*=11.1, 2.8 Hz, 1H), 7.65 (ddd, *J*=9.1, 8.3, 2.8 Hz, 1H), 7.50 (d, *J*=4.5 Hz, 1H), 3.49 - 3.35 (m, 1H), 2.70 (dq, *J*=10.1, 6.8 Hz, 1H), 1.94 - 1.53 (m, 9H), 1.10 (d, *J*=6.8 Hz, 3H).
^{13}C NMR: (101 MHz, DMSO-d_6) δ 177.6, 159.9 (d, *J*=244.3 Hz), 152.2 (d, *J*=5.4 Hz), 149.8 (d, *J*=3.1 Hz), 145.1, 132.6 (d, *J*=9.2 Hz), 127.2 (d, *J*=9.2 Hz), 118.9 (d, *J*=25.4 Hz), 118.7, 107.1 (d, *J*=22.3 Hz), 37.2, 35.6, 28.7, 27.8, 27.2, 26.2, 15.6.
Other NMR Experiments: COSY, NOESY, dept-1H-13C-HSQC, 1H-13C-HMBC, 1H-15N-HMBC, 1H-1H-homonuclear decoupling
Results: Homonuclear decoupling experiments in D_6-benzene of H17 at 2.68 ppm (dq, *J*=10.7 [H17/H16], 6.9 Hz [Me/H17], 1H), reveal H16a at 1.89 ppm (ddt, *J*=10.4, 8.8, 4.1 Hz, 1H) to have ax/eq and eq/eq *J* coupling which is consistent for being in the equatorial position, The *J* coupling of H12 at 2.74 ppm (tt, *J*=10.4, 3.7 Hz, 1H), is consistent with an axial postion, and thus supporting that the ring substituents are *cis*.

Compound 13:



Experimental: Propanoic acid **12** (5 g, 16.59 mmol) was taken up in toluene (55.3 ml) in a sealed tube and diphenyl phosphorazidate (5.02 g, 18.25 mmol) and TEA (2.78 ml, 19.91 mmol) were added. The vessel was sealed and heated to 70 °C. After 2 hours, the reaction was cooled to room temperature and concentrated under reduced pressure. The crude residue was taken up in 40 mL THF and 40 mL of water and LiOH (1.987 g, 83 mmol) was added. Reaction stirred at room temperature for 1 hour. Reaction was carefully acidified with 1N HCl (white precipitate forms) and extracted with EtOAc to remove DPPA related impurities. Then basified with 1N NaOH (precipitate forms again) and extracted with EtOAc (x5). Basic extracts were concentrated *in vacuo* to give amine **13** (4.5 g, 14.87 mmol, 90 % yield) as a white solid.

LCMS: MS: Anal. Calc'd for $C_{17}H_{21}FN_2$ 272.169, found [M+H] 273.2 LC: tr = 0.49 min (**Method A**)

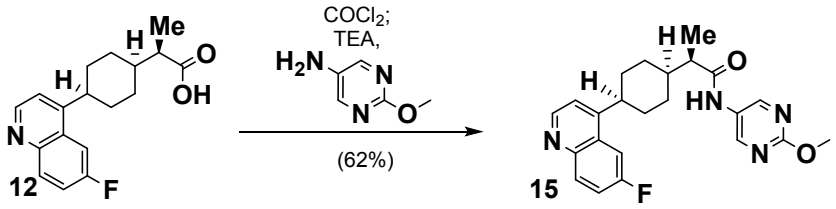
^{1}H NMR: (400 MHz, CHLOROFORM-d) δ 8.77 (d, *J*=4.5 Hz, 1H), 8.08 (dd, *J*=9.2, 5.7 Hz, 1H), 7.64 (dd, *J*=10.6, 2.8 Hz, 1H), 7.43 (ddd, *J*=9.2, 7.9, 2.8 Hz, 1H), 7.29 (d, *J*=4.5 Hz, 1H), 3.28 (tt, *J*=9.1, 4.4 Hz, 1H), 3.10 (dq, *J*=9.4, 6.3 Hz, 1H), 2.07 - 1.97 (m, 1H), 1.91 - 1.64 (m, 9H), 1.52 - 1.38 (m, 1H), 1.13 (d, *J*=6.2 Hz, 3H)

^{13}C NMR: (101 MHz, CHLOROFORM-d) δ 160.4 (d, *J*=246.6 Hz), 152.1 (d, *J*=6.2 Hz), 149.5 (d, *J*=2.3 Hz), 145.5, 132.8 (d, *J*=9.2 Hz), 127.7 (d, *J*=9.2 Hz), 118.8 (d, *J*=25.4 Hz), 118.3, 106.7 (d, *J*=22.3 Hz), 46.0, 41.7, 38.0, 28.3, 28.1, 27.8, 27.0, 22.2

Other NMR Experiments: COSY, NOESY, dept-1H-13C-HSQC, 1H-13C-HMBC, 1H-15N-HMBC, 1H-1H-homonuclear decoupling

Results: Homonuclear decoupling experiments of H17 @ 2.77 (dq, *J*=8.9, 6.3 Hz, 1H), revealed the simplification of H16a @ 1.15 ppm from a ddt, (*J*=10.4, 8.8, 4.1 Hz, 1H) to a quin (*J*=4.1 Hz, 1H), thus having ax/eq and eq/eq *J* coupling no greater than 4.1 Hz., which is consistent for being in the equatorial position. The *J* coupling of H12a @ 2.86 (tt, *J*=9.5, 4.1 Hz, 1H) is consistent with an axial position, and thus supporting that the ring substituents are *–cis*.

Compound 15:



Experimental: To a solution of **12** (200 mg, 0.664 mmol) in CH_2Cl_2 (5 mL) at room temperature was added oxalyl chloride (0.061 mL, 0.697 mmol) dropwise, followed by 1 drop of DMF. The reaction was stirred at room temperature for 1h. The reaction was then concentrated to dryness. The residue was dissolved in DCM and concentrated to dryness (repeated three times). The crude acyl chloride of **12** (212 mg, 0.663 mmol, 100 % yield) was obtained as white solid.

To a solution the crude acyl chloride intermediate of **12** (15 mg, 0.047 mmol) in THF (1 mL) at room temperature was added 2-methoxypyrimidin-5-amine (11.74 mg, 0.094 mmol), followed by triethylamine (0.020 mL, 0.141 mmol). The reaction was stirred at room temperature for 3 hours. The reaction was then concentrated *in vacuo*, diluted with DMF and filtered through 0.45 μM membrane. The filtrate was purified directly *via* HPLC to give **15** (11.9 mg, 62%).

LCMS: MS: Anal. Calc'd for $C_{23}H_{25}FN_4O_2$ 408.196, found [M+H] 409.1 LC: tr = 0.68 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{23}H_{26}FN_4O_2$ $[M+H]^+$ 409.20343, found 409.20370

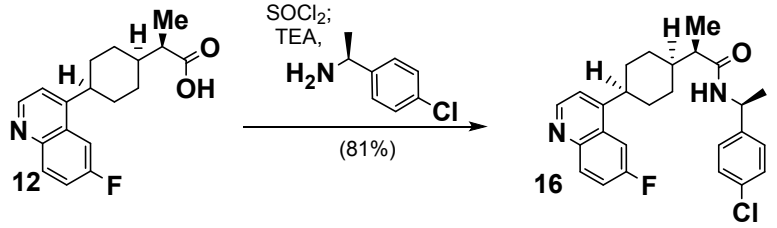
HPLC (purity): >99% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.81 - 8.74 (m, 3H), 8.08 (dd, *J*=9.2, 5.6 Hz, 1H), 7.89 (dd, *J*=10.7, 2.6 Hz, 1H), 7.62 - 7.55 (m, 2H), 3.98 (s, 3H), 3.45 (br t, *J*=10.5 Hz, 1H), 2.95 - 2.87 (m, 1H), 2.15 - 1.71 (m, 9H), 1.27 (d, *J*=6.9 Hz, 3H) (amide NH exchanges-not observed)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 178.5, 163.4, 162.3 (d, *J*=247.0 Hz), 155.4 (d, *J*=5.4 Hz), 152.8, 150.6 (d, *J*=1.8 Hz), 146.3, 132.9 (d, *J*=9.1 Hz), 130.1, 129.4 (d, *J*=9.1 Hz), 120.7 (d, *J*=26.3 Hz), 120.0, 108.4 (d, *J*=22.7 Hz), 55.8, 42.5, 39.8, 37.7, 30.2, 29.4, 29.0, 28.0, 16.7

OR: $[\alpha]_D^{23.2°C}$ = (c 0.5, MeOH) -97.0 °

Compound 16:



Experimental: Intermediate **12** (20 mg, 0.066 mmol) was dissolved in thionyl chloride (48.4 μl, 0.664 mmol) and DMF (2.57 μl, 0.033 mmol) was added. The reaction was stirred at room temperature. After 1

hour, the reaction was concentrated in vacuo, taken up in toluene, concentrated again and placed on high vacuum to remove excess thionyl chloride. After 15 minutes, the crude acyl chloride was taken up in ACN (664 µl) and added to a solution of (*S*)-1-(4-chlorophenyl)ethanamine (20.66 mg, 0.133 mmol) in ACN (664 µl) and TEA (46.3 µl, 0.332 mmol) at 0 °C. After stirring at room temperature overnight, the reaction was concentrated *in vacuo*, taken up in DMF filtered, and purified directly *via* HPLC to give **16** (23.7 mg, 81%).

LCMS: MS: Anal. Calc'd for $C_{26}H_{28}ClFN_2O$ 438.187, found [M+H] 439.3 LC: tr = 0.83 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{26}H_{29}ClFN_2O$ $[M+H]^+$ 439.19470, found 439.19514

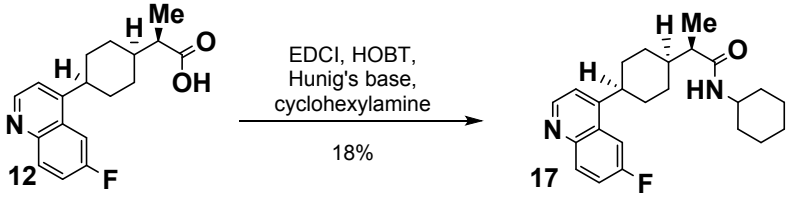
HPLC (purity): 99% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.76 (d, *J*=4.7 Hz, 1H), 8.07 (dd, *J*=9.2, 5.6 Hz, 1H), 7.88 (dd, *J*=10.6, 2.7 Hz, 1H), 7.63 - 7.54 (m, 2H), 7.32 (s, 4H), 5.02 (q, *J*=7.0 Hz, 1H), 3.46 - 3.37 (m, 1H), 2.83 - 2.71 (m, 1H), 2.05 - 1.63 (m, 9H), 1.43 (d, *J*=7.0 Hz, 3H), 1.12 (d, *J*=6.7 Hz, 3H) (amide NH exchanges-not observed)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 178.7, 162.4 (d, *J*=247.0 Hz), 155.7 (d, *J*=5.4 Hz), 150.8 (d, *J*=2.7 Hz), 146.4, 144.5, 134.0, 133.1 (d, *J*=10.0 Hz), 129.9, 129.6 (d, *J*=9.1 Hz), 129.1, 120.9 (d, *J*=25.4 Hz), 120.2, 108.5 (d, *J*=23.6 Hz), 50.2, 41.8, 40.1, 37.5, 30.4, 29.5, 29.2, 28.2, 22.6, 16.8

OR: $[\alpha]_D^{22.1°C}$ = (c 1.0, MeOH) -64.8 °

Compound 17:



Experimental: Intermediate **12** (50 mg, 0.166 mmol) was taken up in DMF (2 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (33.5 mg, 0.216 mmol), 1-hydroxybenzotriazole hydrate (33.0 mg, 0.216 mmol) were added and reaction stirred for 2 minutes prior to the addition of cyclohexanamine (49.4 mg, 0.498 mmol) and Hunig's Base (0.145 mL, 0.830 mmol). The reaction was stirred at 65°C for 16 hours. The mixture was diluted with water and EtOAc. Organic layer was separated and washed with 0.5N NaOH, water, and then brine. The combined organic extracts were dried over magnesium sulfate, filtered and concentrated to dryness. The crude material was purified via HPLC to give compound **17** (11.3 mg, 0.030 mmol, 17.80 % yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{31}FN_2O$ 382.242, found [M+H] 383.2 LC: tr = 0.77 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{32}FN_2O$ $[M+H]^+$ 383.24932, found 383.24878

HPLC (purity): >99% by Methods B and C

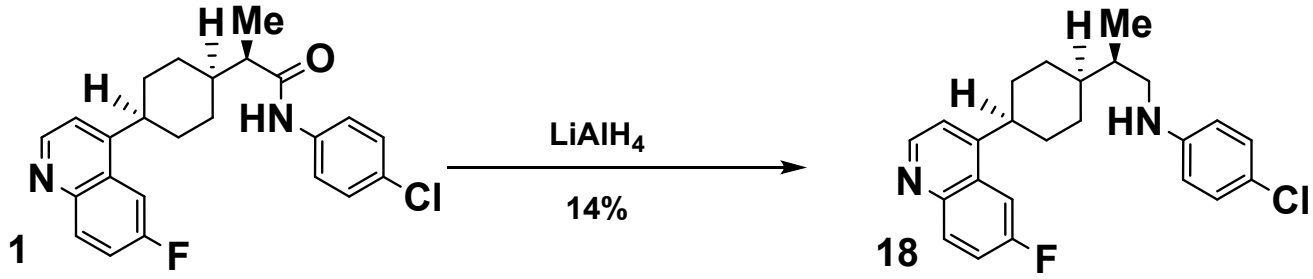
^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.81 (d, *J*=4.4 Hz, 1H), 8.12 (dd, *J*=9.2, 5.7 Hz, 1H), 7.65 (dd, *J*=10.5, 2.7 Hz, 1H), 7.47 (ddd, *J*=9.2, 7.9, 2.7 Hz, 1H), 7.33 (d, *J*=4.6 Hz, 1H), 5.36 (br d, *J*=8.2 Hz, 1H), 3.88 - 3.73 (m, 1H), 3.42 - 3.22 (m, 1H), 2.39 (dq, *J*=10.8, 6.8 Hz, 1H), 2.04 - 1.58 (m, 15H), 1.43 - 1.31 (m, 2H), 1.18 (d, *J*=6.7 Hz, 3H), 1.16 - 1.02 (m, 2H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 175.1, 160.5 (d, *J*=247.0 Hz), 152.1 (d, *J*=5.5 Hz), 149.5 (d, *J*=2.7 Hz), 145.6, 132.9 (d, *J*=10.0 Hz), 127.7 (d, *J*=9.1 Hz), 119.0 (d, *J*=25.4 Hz), 118.2, 106.8 (d, *J*=22.7 Hz), 47.9, 42.0, 38.2, 36.4, 33.4, 33.2, 28.9, 28.7, 27.7, 27.0, 25.5, 24.9, 24.8, 16.3.

OR: $[\alpha]_D^{23.0°C}$ = (c 1.0, MeOH) -74.9 °

MP: 141-144 °C

Compound 18:



Experimental: Compound **1** (50 mg, 0.122 mmol) (For preparation, see: *Org. Process Res. Dev.* **2019**, *23*, 11, 2482-2498) was taken up in THF (1217 µl) and cooled to 0 °C and placed under N_2 atmosphere. $LiAlH_4$ (18.47 mg, 0.487 mmol) was added slowly portionwise. Once the addition was complete the reaction stirred at 50 °C. After 1 hour, more $LiAlH_4$ (18.47 mg, 0.487 mmol) was added and reaction stirred at 50 °C ON. The reaction was quenched with 0.1 mL water, then 0.2 mL 1N NaOH, followed by 0.3 mL water. After stirring 30 minutes, the reaction was diluted with EtOAc, dried with sodium sulfate, filtered over pressed celite, and concentrated *in vacuo*. The crude residue was taken up in DMF, filtered, and purified via HPLC to give compound **18** (6.8 mg, 14%).

LCMS: MS: Anal. Calc'd for $C_{24}H_{26}ClFN_2$ 396.177, found [M+H] 397.2 LC: tr = 0.93 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{27}ClFN_2$ $[M+H]^+$ 397.18413, found 397.18409

HPLC (purity): 97% by Methods B and C

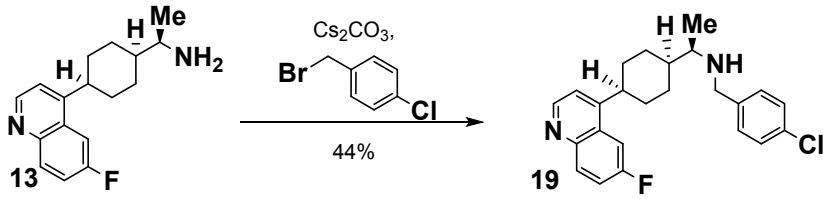
^{1}H NMR: (500 MHz, CHLOROFORM-d) δ 8.81 (d, *J*=4.6 Hz, 1H), 8.13 (dd, *J*=9.2, 5.8 Hz, 1H), 7.67 (dd, *J*=10.5, 2.6 Hz, 1H), 7.52 - 7.43 (m, 1H), 7.33 (d, *J*=4.6 Hz, 1H), 7.12 (d, *J*=8.7 Hz, 2H), 6.54 (d, *J*=8.7 Hz, 2H), 3.76 (br s, 1H), 3.33 (td, *J*=9.1, 4.5 Hz, 1H), 3.28 (dd, *J*=12.2, 3.7 Hz, 1H), 2.88 (dd, *J*=12.2, 7.9 Hz, 1H), 2.04 - 1.69 (m, 9H), 1.65 - 1.58 (m, 1H), 1.05 (d, *J*=6.7 Hz, 3H)

^{13}C NMR: (126 MHz, CHLOROFORM-d) δ 160.5 (d, *J*=247.0 Hz), 152.1 (d, *J*=6.4 Hz), 149.5 (d, *J*=2.7 Hz), 147.1, 145.5, 132.8 (d, *J*=9.1 Hz), 129.0, 127.7 (d, *J*=9.1 Hz), 121.6, 119.0 (d, *J*=26.3 Hz), 118.4, 113.6, 106.8 (d, *J*=22.7 Hz), 48.3, 37.9, 36.7, 32.1, 28.6, 28.2, 28.1, 27.6, 16.1

OR: $[\alpha]_D^{23.7°C}$ = (c 1.0, MeOH) -43.2 °

MP: 135-136 °C

Compound 19:



Experimental: Intermediate **13** (100 mg, 0.367 mmol) was taken up in DMF (1469 µl). 1-(bromomethyl)-4-chlorobenzene (91 mg, 0.441 mmol) was added followed by cesium carbonate (239 mg, 0.734 mmol). The reaction was stirred at room temperature for 16 hours. The reaction was then diluted with water and extracted with EtOAc. The combined organic extracts were dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was taken up in DMF, filtered, and purified via HPLC to give compound compound **19** (63.6 mg, 0.160 mmol, 43.6 % yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{26}ClFN_2$ 396.177, found [M+H] 397.3 LC: tr = 0.73 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{27}ClFN_2$ $[M+H]^+$ 397.18413, found 397.18407

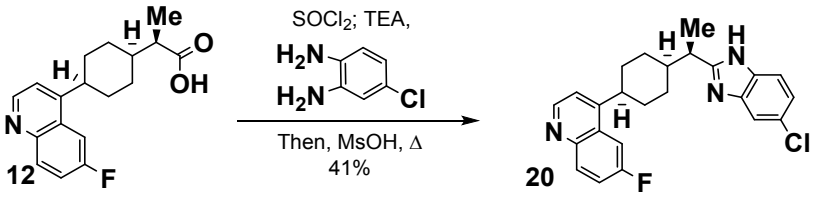
HPLC (purity): 99% by Methods B and C

^{1}H NMR: (400 MHz, METHANOL-d_4) δ 8.73 (d, *J*=4.6 Hz, 1H), 8.05 (dd, *J*=9.2, 5.6 Hz, 1H), 7.82 (dd, *J*=10.6, 2.7 Hz, 1H), 7.62 - 7.53 (m, 1H), 7.45 - 7.29 (m, 4H), 7.21 (d, *J*=4.8 Hz, 1H), 3.96 (d, *J*=13.4 Hz, 1H), 3.75 (d, *J*=13.4 Hz, 1H), 3.44 - 3.33 (m, 1H), 2.91 - 2.82 (m, 1H), 2.07 - 1.97 (m, 1H), 1.94 - 1.83 (m, 1H), 1.83 - 1.60 (m, 3H), 1.60 - 1.49 (m, 2H), 1.45 - 1.33 (m, 2H), 1.17 (d, *J*=6.2 Hz, 3H) (NH not observed)

^{13}C NMR: (101 MHz, METHANOL-d_4) δ 165.0, 162.2 (d, *J*=245.8 Hz), 155.3 (d, *J*=6.2 Hz), 150.6 (d, *J*=2.3 Hz), 146.2, 134.3, 132.9 (d, *J*=9.2 Hz), 131.9, 129.8, 129.3 (d, *J*=9.2 Hz), 120.7 (d, *J*=26.2 Hz), 120.0, 108.3 (d, *J*=23.1 Hz), 51.1, 50.5, 40.1, 39.1, 29.5, 29.1, 28.5, 27.8, 17.0

OR: $[\alpha]_D^{24.1°C}$ = (c 1.0, MeOH) -83.6 °

Compound 20:



Experimental: Intermediate **12** (100 mg, 0.332 mmol) was dissolved in thionyl chloride (242 µl, 3.32 mmol) and DMF (12.85 µl, 0.166 mmol) was added. Reaction stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo*, taken up in toluene and concentrated again (2x) and placed on high vacuum to remove excess thionyl chloride. After 15 minutes, the crude acyl chloride was taken up in ACN (1659 µl) and added to a solution of 4-chlorobenzene-1,2-diamine (95 mg, 0.664 mmol) in ACN (1659 µl) and TEA (231 µl, 1.659 mmol) at 0 °C. Reaction was then allowed to warm to room temperature. After 30 minutes, the reaction was diluted with water and extracted with EtOAc. Organics were combined, dried with sodium sulfate, filtered and concentrated in vacuo.

The resulting crude residue was taken up in $POCl_3$ (309 µl, 3.32 mmol). Reaction heated to 90 °C. After 1 hour, the reaction was quenched into cold 1N NaOH (over ice) and extracted with EtOAc. Organics were dried with sodium sulfate, filtered and concentrated. Crude material was purified *via* HPLC to give compound **20** (56.1 mg, 0.138 mmol, 41.4 % yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{23}ClFN_3$ 407.156, found [M+H] 408.2 LC: tr = 0.76 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{24}ClFN_3$ $[M+H]^+$ 408.16370, found 408.16373

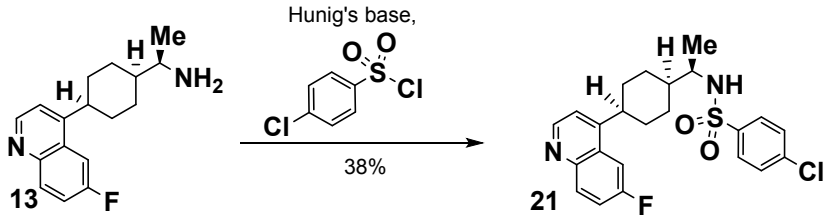
HPLC (purity): 96% by Methods B and C

^{1}H NMR: (400 MHz, METHANOL-d_4) δ 9.09 (br d, *J*=5.6 Hz, 1H), 8.42 - 8.24 (m, 2H), 8.17 (br d, *J*=5.6 Hz, 1H), 8.01 - 7.91 (m, 1H), 7.84 (d, *J*=1.7 Hz, 1H), 7.78 (d, *J*=8.8 Hz, 1H), 7.61 - 7.46 (m, 1H), 4.02 - 3.87 (m, 1H), 3.73 (br d, *J*=3.3 Hz, 1H), 2.40 - 2.21 (m, 2H), 2.16 - 1.75 (m, 7H), 1.59 (br d, *J*=7.0 Hz, 3H), 1.38 - 1.18 (m, 1H)

^{13}C NMR: (101 MHz, METHANOL-d_4) δ 165.8, 165.7, 163.6 (d, *J*=252.0 Hz, 1C), 160.6, 145.6, 137.6, 133.3 (d, *J*=17.7 Hz, 1C), 131.3, 130.3 (d, *J*=10.0 Hz, 1C), 128.0, 126.6 (d, *J*=10.0 Hz, 1C), 125.4 (br d, *J*=27.0 Hz, 1C), 121.1, 116.4, 115.1, 110.2 (br d, *J*=24.7 Hz, 1C), 40.7, 39.7, 34.2, 29.6, 28.4, 28.2, 27.8, 17.6

OR: $[\alpha]_D^{23.9°C}$ = (c 1.0, MeOH) -83.5 °

Compound 21:



Experimental: Intermediate **13** (20 mg, 0.073 mmol) was dissolved in DCM (500 uL) and 4-chlorophenyl sulfonyl chloride (31 mg, 0.147 mmol) was added to the solution followed by DIPEA (64.1 µl, 0.367 mmol). The reaction was then stirred at room temperature overnight. After 16 hours, the reaction was diluted with 1.5 mL of DMF, filtered, and purified directly *via* HPLC to give Compound **21**. (12.6 mg, 38% yield).

LCMS: MS: Anal. Calc'd for $C_{23}H_{24}ClFSN_2O_2$ 446.123, found [M+H] 447, LC: tr = 2.74 min (Method: Injection volume : 10 µL; Waters XBridge C18, 4.6x50mm, 5um; Gradient : 4 min, 0%B to 100%B; A = 5 :95 ACN :H2O with 10mM NH_4OAc, B = 95:5 ACN:H2O with 10mM NH_4OAc; Flow rate: 4 mL/min; UV detection Wavelength: 220 nm; Column temp : 33° C)

HRMS: (ESI) *m/z* calculated for $C_{23}H_{25}ClFSN_2O_2$ $[M+H]^+$ 447.13038, found 447.13043

HPLC (purity): 98% by Methods B and C

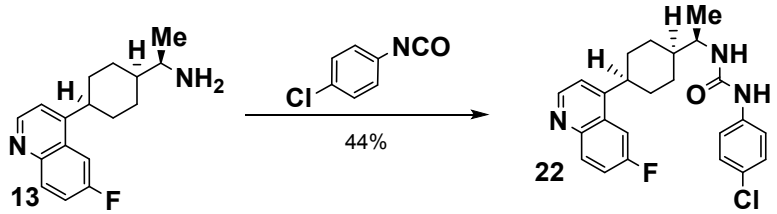
^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.78 (d, *J*=4.6 Hz, 1H), 8.07 (dd, *J*=9.2, 5.6 Hz, 1H), 7.90 (d, *J*=8.7 Hz, 2H), 7.84 (dd, *J*=10.7, 2.6 Hz, 1H), 7.64 - 7.52 (m, 3H), 7.42 (d, *J*=4.6 Hz, 1H), 3.61 - 3.50 (m, 1H), 3.42 - 3.33 (m, 1H), 2.03 (br d, *J*=13.0 Hz, 1H), 1.91 - 1.46 (m, 8H), 1.02 (d, *J*=6.4 Hz, 3H)

2D NMR: (126 MHz, METHANOL-d_4) δ 162.2 (d, *J*=247.0 Hz, 1C), 155.4 (d, *J*=5.4 Hz, 1C), 150.7 (d, *J*=1.8 Hz, 1C), 146.2, 142.5, 139.7, 132.9 (d, *J*=9.1 Hz, 1C), 130.6, 129.9, 129.4 (d, *J*=9.1 Hz, 1C), 120.7 (d, *J*=26.3 Hz, 1C), 120.0, 108.3 (d, *J*=23.6 Hz, 1C), 50.7, 41.1, 39.5, 29.1, 28.9, 28.5, 27.9, 20.2.

OR: $[\alpha]_D^{24.7°C}$ = (c 1.0, DCM) -8.0 °

MP: 217-219 °C

Compound 22:



Experimental: To a solution of Intermediate **13** (18 mg, 0.066 mmol) in THF (1 mL) was added 1-chloro-4-isocyanatobenzene (11.16 mg, 0.073 mmol). The reaction mixture was stirred at room temperature for 0.6 hours. The reaction mixture was concentrated *in vacuo* and the residue was extracted

with ethyl acetate. The organic layer was concentrated *in vacuo* and dissolved in MeOH, filtered, and purified *via* HPLC to give Compound **22** (12.7 mg, 44%).

LCMS: MS: Anal. Calc'd for $C_{24}H_{25}ClFN_3O$ 425.167, found [M+H] 426.3 LC: tr = 0.80 min (**Method A**).

HRMS: (ESI) *m/z* calculated for $C_{24}H_{26}ClFN_3O$ $[M+H]^+$ 426.17429, found 426.17454

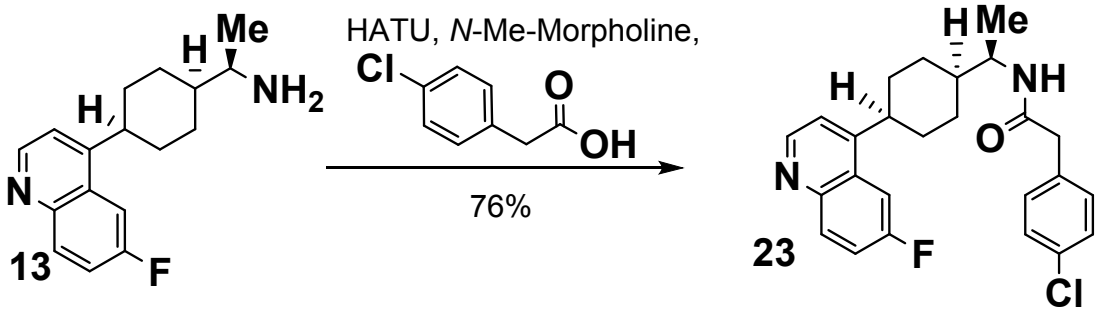
HPLC (purity): 98% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.76 (d, *J*=4.7 Hz, 1H), 8.07 (dd, *J*=9.2, 5.6 Hz, 1H), 7.87 (dd, *J*=10.7, 2.6 Hz, 1H), 7.64 - 7.53 (m, 2H), 7.35 (d, *J*=8.9 Hz, 2H), 7.22 (d, *J*=8.9 Hz, 2H), 4.26 - 4.13 (m, 1H), 3.49 - 3.38 (m, 1H), 2.12 - 1.72 (m, 8H), 1.71 - 1.63 (m, 1H), 1.24 (d, *J*=6.6 Hz, 3H) (2 NH of urea are not observed)

^{13}C NMR: 126 MHz, METHANOL-d_4) δ 162.2 (d, *J*=246.1 Hz, 1C), 157.8, 155.5 (d, *J*=6.4 Hz, 1C), 150.7 (d, *J*=2.7 Hz, 1C), 146.2, 140.2, 132.9 (d, *J*=10.0 Hz, 1C), 129.8, 129.4 (d, *J*=9.1 Hz, 1C), 128.1, 121.4, 120.6 (d, *J*=25.4 Hz, 1C), 120.2, 108.3 (d, *J*=22.7 Hz, 1C), 46.0, 41.2, 39.7, 29.4, 29.3, 28.9, 28.5, 20.4.

OR: $[\alpha]_D^{24.3°C}$ = (c 0.35, MeOH) -52.8 °

Compound 23:



Experimental: To a solution of 4-chlorophenyl acetic acid (12.21 mg, 0.072 mmol) in DMF (1 mL) was added HATU (27.2 mg, 0.072 mmol). The reaction mixture was stirred at room temperature for 10 min, followed by addition of intermediate **13** (15 mg, 0.055 mmol) in THF (0.4 mL) and *N*-methyl morpholine (0.026 mL, 0.220 mmol). The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated in vacuo, taken up in DMF, filtered, and purified *via* HPLC to give Compound **23** (18 mg, 76% yield).

LCMS: MS: Anal. Calc'd for $C_{25}H_{26}ClFN_2O$ 424.172, found [M+H] 425.1 LC: tr = 0.83 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{25}H_{27}ClFN_2O$ $[M+H]^+$ 425.17905, found 425.17887

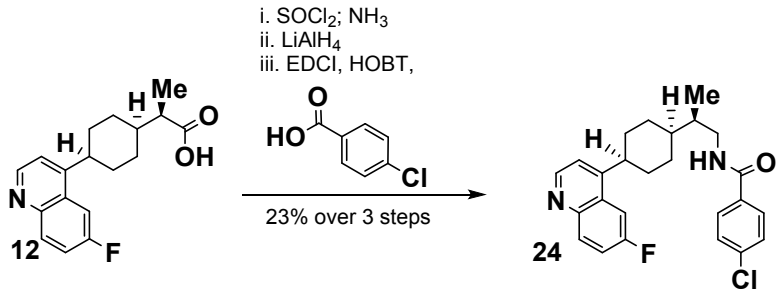
HPLC (purity): >99% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.73 (d, *J*=4.7 Hz, 1H), 8.06 (dd, *J*=9.2, 5.6 Hz, 1H), 7.84 (dd, *J*=10.6, 2.7 Hz, 1H), 7.61 - 7.53 (m, 1H), 7.50 (d, *J*=4.7 Hz, 1H), 7.28 (s, 4H), 4.35 - 4.23 (m, 1H), 3.49 (s, 2H), 3.41 - 3.35 (m, 1H), 1.99 - 1.58 (m, 9H), 1.18 (d, *J*=6.4 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 171.4, 160.7 (d, *J*=246.1 Hz, 1C), 153.9 (d, *J*=5.4 Hz, 1C), 149.2 (d, *J*=1.8 Hz, 1C), 144.7, 134.7, 132.4, 131.4 (d, *J*=9.1 Hz, 1C), 130.2, 128.2, 127.8 (d, *J*=9.1 Hz, 1C), 119.1 (d, *J*=26.3 Hz, 1C), 118.6, 106.7 (d, *J*=23.6 Hz, 1C), 47.6, 44.1, 41.9, 38.9, 38.2, 27.7, 27.2, 27.0, 18.2.

OR: $[\alpha]_D^{20.9°C}$ = (c 1.0, MeOH) -46.6 °

Compound 24:



Experimental: Step 1: Intermediate **12** (75 mg, 0.249 mmol) was dissolved in a minimum amount of thionyl chloride (182 µl, 2.489 mmol) and stirred at room temperature. After 30 minutes, reaction was concentrated in vacuo, diluted with toluene and concentrated again (x2). The crude acyl chloride was taken up in DCM (498 µl) and ammonia (4978 µl, 2.489 mmol) (0.5 M in dioxane). A few drops of ammonium hydroxide (conc. aq solution) was added until pH of reaction is basic. After 10 minutes, the reaction was concentrated *in vacuo*, diluted with sodium bicarbonate solution and extracted with EtOAc. The organics were dried with sodium sulfate, filtered, and concentrated to give intermediate primary amide (76 mg, 0.253 mmol, 102 % yield). (Crude material carried forward as is.)

Step 2: Intermediate primary amide (76 mg, 0.253 mmol) was taken up in THF (2530 µl) and cooled to 0 °C and placed under N_2 atmosphere. $LiAlH_4$ (38.4 mg, 1.012 mmol) was added slowly portionwise. Once the addition was complete the reaction stirred at room temperature. After 3 hours, another portion of $LiAlH_4$ (38.4 mg, 1.012 mmol) added at room temperature. After 1 hour, the temperature was increased to 50 °C. After 2 hours, the reaction was quenched with 0.1 mL water, then 0.2 mL 1N NaOH, followed by 0.3 mL water. After stirring 30 minutes, the reaction was diluted with EtOAc, dried with sodium sulfate, filtered over pressed celite, and concentrate *in vacuo* to give intermediate primary amine (66 mg, 0.230 mmol, 91 % yield)

Step 3: Intermediate primary amine (66 mg, 0.230 mmol) was taken up in DMF (1152 µl) and HOBT (45.9 mg, 0.300 mmol), EDC (57.4 mg, 0.300 mmol), 4-chlorobenzoic acid (72.2 mg, 0.461 mmol) and TEA (161 µl, 1.152 mmol) were added and reaction was stirred at room temperature. After 2 hours, the reaction was diluted with DMF, filtered, and purified *via* HPLC to give Compound **24** (25.5 mg, 26% yield).

LCMS: MS: Anal. Calc'd for $C_{25}H_{26}ClFN_2O$ 424.172, found [M+H] 425.2 LC: tr = 0.83 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{25}H_{27}ClFN_2O$ $[M+H]^+$ 425.17905, found 425.17908

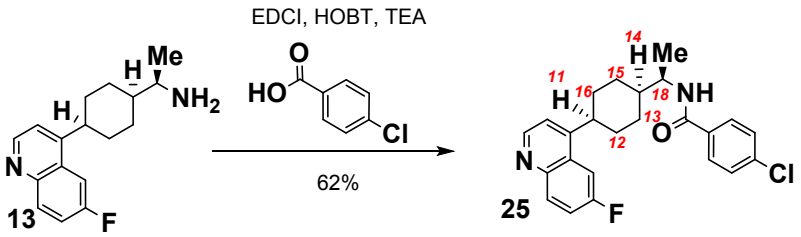
HPLC (purity): 99% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.74 (d, *J*=4.7 Hz, 1H), 8.06 (dd, *J*=9.2, 5.6 Hz, 1H), 7.86 (dd, *J*=10.7, 2.6 Hz, 1H), 7.81 (d, *J*=8.5 Hz, 2H), 7.61 - 7.52 (m, 2H), 7.47 (d, *J*=8.7 Hz, 2H), 3.65 (dd, *J*=13.2, 3.9 Hz, 1H), 3.53 - 3.39 (m, 1H), 3.09 (dd, *J*=13.3, 9.0 Hz, 1H), 2.21 - 2.03 (m, 2H), 2.02 - 1.89 (m, 2H), 1.88 - 1.72 (m, 5H), 1.61 - 1.49 (m, 1H), 1.02 (d, *J*=6.7 Hz, 3H).

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 169.4, 162.2 (d, *J*=246.1 Hz, 1C), 155.5 (d, *J*=5.4 Hz, 1C), 150.6 (d, *J*=2.7 Hz, 1C), 146.2, 138.7, 134.8, 132.9 (d, *J*=9.1 Hz, 1C), 130.1, 129.9, 129.4 (d, *J*=9.1 Hz, 1C), 120.6 (d, *J*=26.3 Hz, 1C), 120.2, 108.4 (d, *J*=23.6 Hz, 1C), 45.7, 39.5, 38.6, 33.8, 29.5, 29.5, 29.2, 28.9, 16.4.

OR: $[\alpha]_D^{24.3°C}$ = (c 1.0, MeOH) +1.1 °

Compound 25:



Experimental: Intermediate **13** (24 g, 88 mmol) was taken up in DMF (441 ml) and HOBT (16.19 g, 106 mmol), EDC (20.27 g, 106 mmol), 4-chlorobenzoic acid (17.94 g, 115 mmol) and TEA (61.4 ml, 441 mmol) were added. The reaction was stirred at room temperature overnight. The reaction was diluted with water/sat $NaHCO_3$ (1:1), extracted with DCM, and the combined organics were washed with 1/2 sat brine. The organics were then dried with sodium sulfate, filtered, and concentrated *in vacuo* to give a white solid. The crude product was purified *via* ISCO in three batches (220 g column, 0-80% EtOAc in hexanes). The material was then taken up in 15% EtOH in water (500 mL total) and heated to 85 °C with

vigorous stirring for 6 hours. After cooling, white solid was filtered to isolate, and dried on high vac overnight to give Compound **25** (22.49 g, 54.4 mmol, 61.7 % yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{24}ClFN_2O$ 410.156, found [M+H] 411.2 LC: tr = 0.80 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{25}ClFN_2O$ $[M+H]^+$ 411.16394, found 411.16325

Elemental Analysis: calculated for $C_{24}H_{24}ClFN_2O$: C, 70.15%; H, 5.89%; N, 6.82%; Found: C, 70.46 %; H, 5.74%; N, 6.81%

HPLC (purity): 99.9% by UPLC/MS with five minor impurities at or below 0.05% (Method: Waters UPLC; Column: Waters BEH CSH C18, 1.7 µm, 150mm (L) x 2.1mm; Temperature: 40˚C**;** Flow rate: 0.30 mL/min; Mobile Phase: A: 0.05% TFA in water B: Acetonitrile; Gradient Profile: Time (min): 0 min=10% B; 15 min = 95% B; 17 min = 95% B; 17.5 min = 10% B; Post Run Time: 3 min (under the initial mobile phase conditions), Injection volume: 1µL of 0.5mg/mL sample in methanol; MS Conditions: Mass Range: m/z 150-1200; Ionization and Mode: ESl+/-)

^{1}H NMR: (500MHz, BENZENE-d_6) δ 8.73 (d, J=4.4 Hz, 1H), 8.22 (dd, J=9.3, 5.8 Hz, 1H), 7.58 (dd, J=10.4, 2.8 Hz, 1H), 7.39 (d, J=8.5 Hz, 2H), 7.14 - 7.09 (m, 1H), 7.07 - 6.99 (m, 2H), 6.93 (d, J=4.7 Hz, 1H), 5.18 (br d, J=9.5 Hz, 1H), 4.62 (tq, J=9.9, 6.5 Hz, 1H), 2.91 - 2.75 (m, 1H), 2.03 - 1.84 (m, 1H), 1.74 - 1.61 (m, 1H), 1.61 - 1.51 (m, 1H), 1.51 - 1.43 (m, 2H), 1.42 - 1.32 (m, 2H), 1.32 - 1.15 (m, 2H), 0.99 (d, J=6.6 Hz, 3H)

^{13}C NMR: (126MHz, BENZENE-d_6) δ 165.4, 161.2 (d, J=245.2 Hz, 1C), 151.7 (d, J=5.5 Hz, 1C), 150.7 (d, J=2.7 Hz, 1C), 146.9, 137.8, 134.4 (d, J=10.0 Hz, 1C), 134.2, 129.2 (s, 2C), 129.0 (s, 2C), 128.3, 119.2, 119.1 (d, J=25.4 Hz, 1C), 107.2 (d, J=21.8 Hz, 1C), 44.9, 40.4, 38.8, 28.6, 28.3 (s, 2C), 28.1, 20.0

19F NMR: (471MHz, CHLOROFORM-d) δ -113.6 (1F)

OR: $[\alpha]_D^{20°C}$ = (c 0.32, MeOH) -117.6 °

MP: 175 °C

Other NMR Experiments Performed:

Spectrometer: LVL L2 Bruker 500 (nmrl2b500)

Probe: Prodigy N2 cooled probe/5mm

Experiments: 1H-1D, 13C-1D (1H dec), 19F-1D (1H coupled), COSY, NOESY, dept-1H-13C-HSQC, 1H-13C-HMBC, 1H-15N-HMBC, 1H-1H-homedec

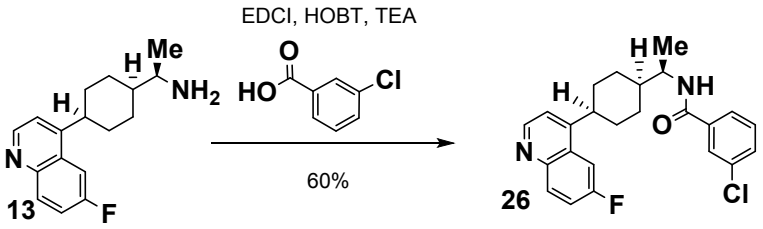
Solvent: d6-benzene,

Temperature: 27°C, 60°C (some peak sharpening)

Sample: ~ 5 mg in ~ 650 mL in a 5 mm NMR tube, prepared by analyst

Results: The 1H, 13C, 15N and 19F are consistent with the proposed structure. The cyclohexyl ring methylene protons and carbons are diastereotopic. The ring methine protons were confirmed to be −*cis* by the observation of an noe from H-18 to H-16/12 (ax) and H11 to H13/15 (ax).

Compound 26:



Experimental: Intermediate **13** (20 mg, 0.073 mmol) was dissolved in DMF (500 uL). HOBT (16.87 mg, 0.110 mmol) was added followed by EDC (21.12 mg, 0.110 mmol). Next, 3-chlorobenzoic acid (23 mg, 0.147 mmol) was added followed by TEA (51.2 µl, 0.367 mmol). The reaction was stirred at room temperature over night. After 16 hours, the reaction was diluted with DMF, filtered, and purified *via* HPLC to give Compound **26** (18.2 mg, 60% yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{24}ClFN_2O$ 410.156, found [M+H] 411.2 LC: tr = 2.11 min (Method: Injection volume: 10 µL; Waters XBridge C18, 4.6x50mm, 5um; Gradient : 4 min, 0% B to 100% B; A = 5 :95 ACN:H_2O with 10 mM NH_4OAc, B = 95:5 ACN :H_2O with 10 mM NH_4OAc; Flow rate: 4 mL/min; UV detection Wavelength: 220 nm; Column temp: 33° C)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{25}ClFN_2O$ $[M+H]^+$ 411.16340, found 411.16334

HPLC (purity): >99% by Methods B and C

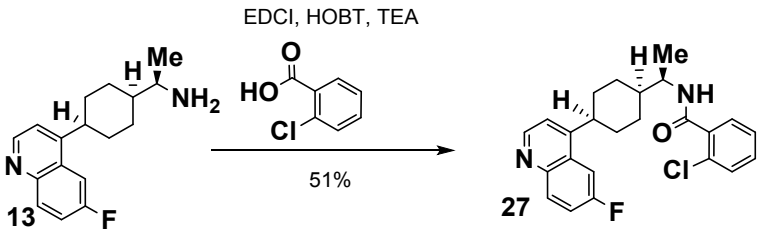
^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.77 (d, *J*=4.7 Hz, 1H), 8.08 (dd, *J*=9.2, 5.6 Hz, 1H), 7.88 (dd, *J*=10.7, 2.6 Hz, 1H), 7.85 (t, *J*=1.7 Hz, 1H), 7.77 (d, *J*=7.8 Hz, 1H), 7.62 - 7.51 (m, 3H), 7.50 - 7.42 (m, 1H), 4.62 - 4.51 (m, 1H), 3.48 - 3.35 (m, 1H), 2.09 - 1.73 (m, 9H), 1.31 (d, *J*=6.6 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 168.2, 162.2 (d, *J*=246.1 Hz, 1C), 155.5 (d, *J*=5.4 Hz, 1C), 150.7 (d, *J*=2.7 Hz, 1C), 146.2, 138.2, 135.7, 132.9 (d, *J*=9.1 Hz, 1C), 132.6, 131.3, 129.4 (d, *J*=10.0 Hz, 1C), 128.6, 126.8, 120.6 (d, *J*=26.3 Hz, 1C), 120.2, 108.3 (d, *J*=22.7 Hz, 1C), 46.2, 40.2, 39.9, 29.4, 29.2, 28.8, 28.7, 19.6

OR: $[\alpha]_D^{20.8°C}$ = (c 1.0, MeOH) -79.2°

MP: 96-97 °C

Compound 27:



Experimental: Intermediate **13** (20 mg, 0.073 mmol) was dissolved in DMF (500 uL). HOBT (16.87 mg, 0.110 mmol) was added followed by EDC (21.12 mg, 0.110 mmol) in 6.5mL of DMF. Next, 2-chlorobenzoic acid (23 mg, 0.147 mmol) was added followed by TEA (51.2 µl, 0.367 mmol). The reaction was stirred at room temperature over night. After 16 hours, the reaction was diluted with DMF, filtered, and purified *via* HPLC to give Compound **27** (15.4 mg, 51% yield).

LCMS: MS: Anal. Calc'd for $C_XH_XFNO_X$ $C_{24}H_{24}ClFN_2O$ 410.156, found [M+H] 411.2 LC: tr = 1.99 min (Method: Injection volume : 10 µL; Waters XBridge C18, 4.6x50mm, 5um; Gradient : 4 min, 0%B to 100%B; A = 5:95 ACN:H_2O with 10 mM NH_4OAc, B = 95:5 ACN:H_2O with 10 mM NH_4OAc; Flow rate: 4 mL/min; UV detection Wavelength: 220 nm; Column temp : 33° C)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{25}ClFN_2O$ $[M+H]^+$ 411.16340, found 411.16447

HPLC (purity): >99% by Methods B and C

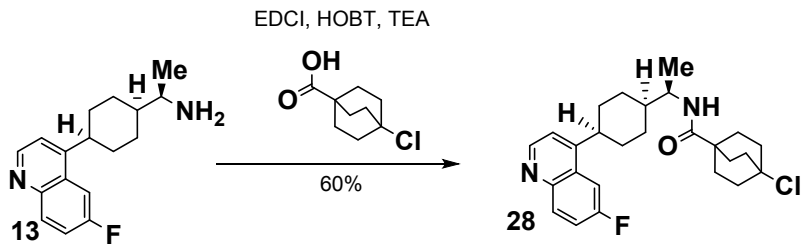
^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.77 (d, *J*=4.7 Hz, 1H), 8.08 (dd, *J*=9.2, 5.6 Hz, 1H), 7.89 (dd, *J*=10.7, 2.6 Hz, 1H), 7.64 - 7.56 (m, 2H), 7.49 - 7.34 (m, 4H), 4.60 - 4.48 (m, 1H), 3.48 - 3.38 (m, 1H), 2.18 - 1.75 (m, 10H), 1.31 (d, *J*=6.6 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 169.7, 162.3 (d, *J*=246.1 Hz, 1C), 155.5 (d, *J*=5.4 Hz, 1C), 150.7 (d, *J*=2.7 Hz, 1C), 146.2, 138.4, 132.9 (d, *J*=9.1 Hz, 1C), 132.1, 131.9, 131.1, 129.8, 129.4 (d, *J*=9.1 Hz, 1C), 128.3, 120.7 (d, *J*=26.3 Hz, 1C), 120.2, 108.3 (d, *J*=22.7 Hz, 1C), 46.2, 40.3, 39.8, 29.4, 29.2, 28.8, 28.5, 19.6.

OR: $[\alpha]_D^{21.3°C}$ = (c 1.0, MeOH) -40.7°

MP: 130-132 °C

Compound 28:



Experimental: Intermediate **13** (30 mg, 0.110 mmol) was taken up in DMF (1101 µl). Then, HOBT (21.93 mg, 0.143 mmol), EDC (27.5 mg, 0.143 mmol), 4-chlorobicyclo[2.2.2]octane-1-carboxylic acid (41.6 mg, 0.220 mmol) and TEA (77 µl, 0.551 mmol) were added and the reaction was stirred at room temperature. After 2 hours, the reaction was diluted wtih another 1 mL of DMF, filtered and purified *via* HPLC to give Compound **28** (29.9 mg, 60% yield).

LCMS: MS: Anal. Calc'd for $C_{26}H_{32}ClFN_2O$ 442.219, found [M+H] 443.3 LC: tr = 0.84 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{26}H_{33}ClFN_2O$ $[M+H]^+$ 443.22600, found 443.22593

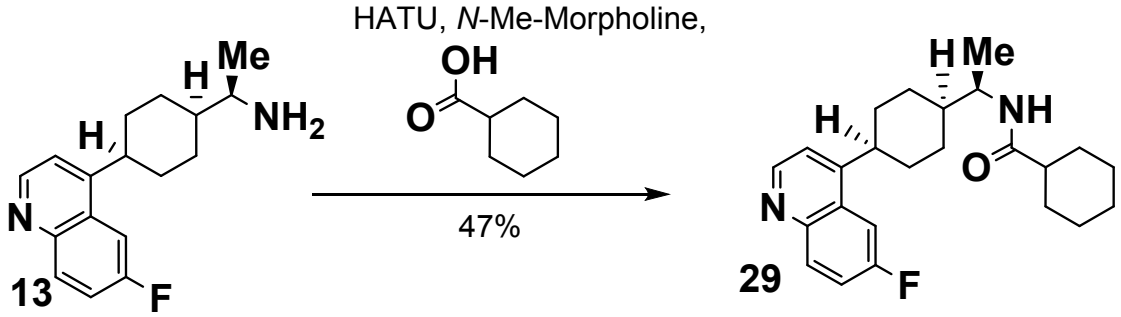
HPLC (purity): 98% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.75 (d, *J*=4.7 Hz, 1H), 8.07 (dd, *J*=9.2, 5.6 Hz, 1H), 7.86 (dd, *J*=10.7, 2.6 Hz, 1H), 7.61 - 7.55 (m, 1H), 7.54 (d, *J*=4.6 Hz, 1H), 7.22 (br d, *J*=9.2 Hz, 1H), 4.43 - 4.28 (m, 1H), 3.43 - 3.35 (m, 1H), 2.11 - 2.02 (m, 6H), 2.00 - 1.91 (m, 7H), 1.91 - 1.65 (m, 8H), 1.15 (d, *J*=6.6 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 178.7, 162.2 (d, *J*=247.0 Hz, 1C), 155.5 (d, *J*=6.4 Hz, 1C), 150.7 (d, *J*=1.8 Hz, 1C), 146.2, 132.9 (d, *J*=9.1 Hz, 1C), 129.4 (d, *J*=9.1 Hz, 1C), 120.7 (d, *J*=25.4 Hz, 1C), 120.2, 108.3 (d, *J*=22.7 Hz, 1C), 67.3, 45.3, 40.0, 39.8, 39.0, 37.1, 31.8, 29.3, 29.2, 28.8, 28.6, 19.6

OR: $[\alpha]_D^{21.6°C}$ = (c 1.0, MeOH) -52.6°

Compound 29:



Experimental: To a solution of cyclohexane carboxylic acid (10.69 µl, 0.086 mmol) in DMF (1 mL) was added HATU (30.2 mg, 0.079 mmol). The reaction mixture was stirred at rt for 10 min, followed by addition of Intermediate **13** (18 mg, 0.066 mmol) in THF (1 mL) and N-methyl morpholine (0.032 mL, 0.264 mmol). The resulting mixture was stirred at room temperature for 20 hours. The reaction mixture was diluted with ethyl acetate and saturated $NaHCO_3$ solution. The organic layer was separated and was concentrated *in vacuo*. The residue was taken up in DMF, filtered, and purified *via* HPLC to give Compound **29** (12 mg, 47% yield).

LCMS: MS: Anal. Calc'd for $C_{24}H_{31}FN_2O$ 382.242, found [M+H] 383.2 LC: tr = 0.80 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{24}H_{32}FN_2O$ $[M+H]^+$ 383.24932, found 383.24890

HPLC (purity): 99% by Methods B and C

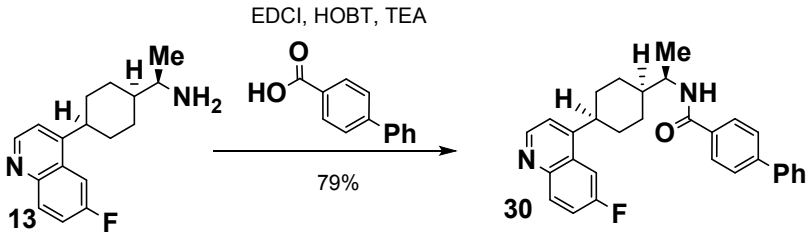
^{1}H NMR: (500 MHz, METHANOL-d_4) δ 8.75 (d, *J*=4.6 Hz, 1H), 8.07 (dd, *J*=9.2, 5.6 Hz, 1H), 7.87 (dd, *J*=10.5, 2.6 Hz, 1H), 7.62 - 7.53 (m, 2H), 4.37 - 4.26 (m, 1H), 3.40 (br t, *J*=10.6 Hz, 1H), 2.19 (tt, *J*=11.8, 3.1 Hz, 1H), 1.99 - 1.64 (m, 15H), 1.58 - 1.40 (m, 2H), 1.39 - 1.21 (m, 3H), 1.17 (d, *J*=6.6 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 178.6, 162.2 (d, *J*=246.1 Hz, 1C), 155.5 (d, *J*=5.4 Hz, 1C), 150.7 (d, *J*=2.7 Hz, 1C), 146.2, 132.9 (d, *J*=9.1 Hz, 1C), 129.4 (d, *J*=9.1 Hz, 1C), 120.6 (d, *J*=25.4 Hz, 1C), 120.2, 108.3 (d, *J*=22.7 Hz, 1C), 46.9, 44.9, 40.4, 39.8, 31.2, 30.7, 29.3, 29.2, 28.8, 28.5, 27.1, 27.0, 19.8

OR: $[\alpha]_D^{24.5°C}$ = (c 0.28, MeOH) -83.8°

MP: 188-190 °C

Compound 30:



Experimental: Compound **13** (13.21 g, 48.5 mmol) was taken up in DMF. Then, (243 ml) and HOBT (9.66 g, 63.1 mmol), EDC (12.09 g, 63.1 mmol), [1,1'-biphenyl]-4-carboxylic acid (14.42 g, 72.8 mmol) and TEA (33.8 ml, 243 mmol) were added and reaction was stirred at room temperature. After 4 hours, the reaction was diluted with water/sat $NaHCO_3$ (1:1), extracted with DCM, and the combined organics were washed with 1/2 saturated brine. The combined organics were subsequently dried with sodium sulfate, filtered, and concentrated *in vacuo* to give a white solid. The crude material was then recrystallized from EtOAc. Entire batch recrystallized from EtOAc. Recrystallized material was then further purified *via* HPLC to give Compound **30** (17.7 g, 79% yield).

LCMS: MS: Anal. Calc'd for $C_{30}H_{29}FN_2O$ 452.226, found [M+H] 453.3 LC: tr = 0.86 min (Method A)

HRMS: (ESI) *m/z* calculated for $C_{30}H_{30}FN_2O$ $[M+H]^+$ 453.23422, found 453.23331

HPLC (purity): 99.96% by UPLC/MS (Method: Waters UPLC; Column: Waters BEH CSH C18, 1.7 µm, 150mm (L) x 2.1mm; Temperature: 40˚C**;** Flow rate: 0.30 mL/min; Mobile Phase: A: 0.05% TFA in water B: Acetonitrile; Gradient Profile: Time (min): 0 min=10% B; 15 min = 95% B; 17 min = 95% B; 17.5 min = 10% B; Post Run Time: 3 min (under the initial mobile phase conditions), Injection

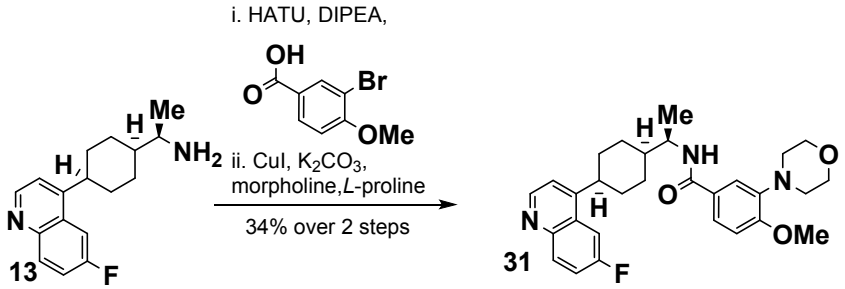
volume: 1μL of 0.5mg/mL sample in methanol; **MS Conditions:** Mass Range: m/z 150-1200; Ionization and Mode: ESI+/-)

^{1}H NMR: (500MHz, BENZENE-d_6) δ 8.72 (d, J=4.6 Hz, 1H), 8.19 (dd, J=9.2, 5.9 Hz, 1H), 7.77 (d, J=8.5 Hz, 2H), 7.57 (dd, J=10.5, 2.7 Hz, 1H), 7.43 (d, J=8.1 Hz, 4H), 7.27 - 7.20 (m, 2H), 7.18 - 7.16 (m, 1H), 7.15 - 7.12 (m, 1H), 7.03 (d, J=4.4 Hz, 1H), 5.36 (br d, J=9.2 Hz, 1H), 4.66 (tq, J=9.6, 6.5 Hz, 1H), 2.97 - 2.87 (m, 1H), 2.08 - 1.94 (m, 1H), 1.85 - 1.73 (m, 1H), 1.69 - 1.59 (m, 1H), 1.57 - 1.49 (m, 3H), 1.48 - 1.42 (m, 1H), 1.41 - 1.34 (m, 1H), 1.34 - 1.29 (m, 1H), 1.07 (d, J=6.6 Hz, 3H)

^{13}C NMR: (126MHz, BENZENE-d_6) δ 165.6, 160.6 (d, *J*=245.2 Hz, 1C), 151.0, 149.9, 146.3, 144.1, 140.3, 134.2, 133.7 (d, *J*=9.1 Hz, 1C), 128.7 (s, 2C), 127.6, 127.5, 127.4 (s, 2C), 127.1 (s, 2C), 127.1 (s, 2C), 118.5, 118.2 (d, *J*=25.4 Hz, 1C), 106.4 (d, *J*=21.8 Hz, 1C), 44.6, 40.0, 38.0, 28.1, 27.8, 27.6, 27.3, 19.3.

OR: $[\alpha]_D^{20°C}$ = (c 0.28, MeOH) -133.6°

Compound 31:



Experimental: Step 1: To a solution of 3-bromo-4-methoxybenzoic acid (106 mg, 0.459 mmol) in DMF (4 mL) was added HATU (181 mg, 0.477 mmol). The reaction mixture was stirred at room temperature for 3 minutes, followed by addition a solution compound **13** (100 mg, 0.367 mmol) in THF (1 mL) and DIPEA (0.128 mL, 0.734 mmol). The reaction mixture was stirred at rt for 1 hour. The reaction mixture was then diluted with ethyl acetate and saturated $NaHCO_3$ solution. The organic layer was separated and washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was dissolved in DCM, purified by flash column chromatography on silica gel to give 3-bromo-*N*-((*R*)-1-((*cis*)-4-(6-fluoroquinolin-4-yl)cyclohexyl)ethyl)-4-methoxybenzamide (125 mg, 69%).

Step 2: A reaction mixture of the intermediate amide (35 mg, 0.072 mmol), morpholine (0.159 mL, 1.803 mmol), K_2CO_3 (19.93 mg, 0.144 mmol) and L-proline (9.96 mg, 0.087 mmol) (s) in DMSO (1.5 mL) was purged with nitrogen for 1 min, followed by addition of copper (I) iodide (6.87 mg, 0.036 mmol). The resulting mixture was heated at 100 °C overnight. The reaction mixture was then diluted with ethyl acetate and sat. $NaHCO_3$ solution. The organic layer was separated and washed with brine

solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH, filtered, and purified *via* HPLC to give Compound **31** (17 mg, 48%) .

LCMS: MS: Anal. Calc'd for $C_{29}H_{34}FN_3O_3$ 491.258, found [M+H] 492.2 LC: tr = 0.70 min (**Method A**)

HRMS: (ESI) *m/z* calculated for $C_{29}H_{35}FN_3O_3$ $[M+H]^+$ 492.26570, found 492.26570

HPLC (purity): >99% by Methods B and C

^{1}H NMR: (500 MHz, METHANOL-d_4) δ 9.01 (d, *J*=5.5 Hz, 1H), 8.27 - 8.16 (m, 3H), 7.98 (d, *J*=5.5 Hz, 1H), 7.92 - 7.84 (m, 1H), 7.60 (dd, *J*=8.4, 2.0 Hz, 1H), 7.51 (d, *J*=1.8 Hz, 1H), 7.05 (d, *J*=8.5 Hz, 1H), 4.67 - 4.54 (m, 1H), 3.93 (s, 3H), 3.90 - 3.82 (m, 4H), 3.68 - 3.59 (m, 1H), 3.19 - 3.05 (m, 4H), 2.15 - 1.70 (m, 8H), 1.32 (d, *J*=6.6 Hz, 3H)

^{13}C NMR: (126 MHz, METHANOL-d_4) δ 169.5, 163.2 (d, *J*=250.7 Hz, 1C), 156.7, 147.0, 141.8, 139.7, 130.2 (d, *J*=10.0 Hz, 1C), 128.9, 128.5, 128.1 (d, *J*=10.0 Hz, 1C), 124.5, 124.2 (d, *J*=27.2 Hz, 1C), 120.8, 118.9, 112.4, 109.8 (d, *J*=23.6 Hz, 1C), 68.1, 56.4, 52.6, 45.9, 40.9, 40.0, 29.3, 29.0, 28.7, 28.6, 19.7.

OR: $[\alpha]_D^{24.4°C}$ = (c 0.29, MeOH) -108.4°

Part 2: Cellular *In Vitro* Biological Assay Protocols

2.1 Human HeLa and Mouse M109 Cell-based IDO1 Assays

Hela or M109 cells were seeded at 20,000 cells per 30 μL per well with RPMI/phenol red free media containing 10% fetal bovine serum (FBS) in a 384-well black wall clear bottom tissue culture plate. Fifty nL of certain concentration of compound was then added to the cells, and the plate was incubated at 37 ˚C for two hours. Then 20 μL of the recombinant human Interferon-gamma (IFNγ) (R&D System, cat# 285-IF, at final concentration of 10 ng/ml) or recombinant murine IFNγ (PEPRO TECH INC, cat#315-05, at final concentration of 5 ng/ml) was added to induce IDO activities. The cells were incubated for 18 hours in 37 ^{o}C incubator with 5% CO_2.

The compound treatments were then stopped by adding TCA to a final concentration at 3%. The cell plate was further incubated at 50 ˚C for 30 minutes. The equal volume supernatant (20 μL) and 2% (w/v) Ehrlich reagent (4-dimethylaminobenzaldehyde) in glacial acetic acid were mixed in a new clear bottom 384-well plate. This plate was then incubated at room temperature for 30 minute. The absorbance at 490nm was measured on an Envision plate reader.

Compound IC_{50} values were calculated using no INFγ induction counts as one hundred percent inhibition, and INF-gamma induction without compound treatment as zero percent inhibition.

2.2 Human Whole Blood IDO1 Assay

2.2.1 Human Whole Blood Incubation and Stimulation

Forty five μL per well human venous whole blood which was obtained from healthy donors was pre-incubated with compounds for four hours at 37 ^{o}C in a humidified 95% air/5% CO_2 incubator. The blood was stimulated with 5 μL per well human IFNγ and LPS at final concentration of 50 ng/ml IFNγ, 5 μg/mL LPS diluted in RPMI-1640 (Thermo Fisher Scientific, Grand Island, NY) for 18 hours at 37 °C in a humidified 95% air/5% CO_2 incubator. The plasma samples were liberated by centrifugation at 2300 rpm for 5 minutes. The tryptophan and kynurenine concentrations in the plasma samples were analyzed using RapidFire mass spectrometry as described below.

2.2.2 Analysis of Plasma Tryptophan and Kynurenine Levels Using RapidFire Mass Spectrometry

2.2.2.1 Preparations of Plasma Samples and Standards

Ten μL of plasma samples from the whole blood assay were diluted with 45 μL of DPBS buffer containing bovine serum albumin (BSA) and an internal standard L-kynurenine sulfate (RING-D4,3,3-D2, Sigma-Aldrich, St. Louis, MO) in a 384-well REMP® plate and 5 μL of a trichloro-acetic acid (TCA) stock solution (40% w/v) was then added. The diluted plasma sample contained a final concentration of 0.92% (w/v) BSA, 1 μM of L-kynurenine sulfate (RING-D4,3,3-D2) and 3.3% (w/v) of TCA. The plate was shaken in an

orbital shaker for 1 min and then centrifuged at 2000 RPM for 20 minutes in a Thermo Scientific Heraues Multifuge X3 bench top centrifuge. The supernatant was transferred to a new 384-well REMP plate for the analysis using RapidFire mass spectrometry.

Standard curves for L-tryptophan and L-kynurenine calibrations were constructed separately by preparing standards at 16 different concentrations from 200 μM stocks by a 2-fold serial dilution in DPBS buffer containing BSA and L-kynurenine sulfate (RING-D4,3,3-D2, internal standard) in a 384-well REMP plate. TCA stock solution (40% w/v) was then added. Each standard sample contained a final concentration of 0.92% (w/v) BSA, 1 μM of L-kynurenine sulfate (RING-D4,3,3-D2) and 3.3% (w/v) of TCA. The plate was shaken in an orbital shaker for 1 min and then centrifuged at 2000 RPM for 20 minutes in a Thermo Scientific Heraues Multifuge X3 bench top centrifuge. The supernatant was transferred to a new 384-well REMP® plate (Brooks Automation, Chelmsford, MA) for the analysis using RapidFire mass spectrometry.

2.2.2.2 RapidFire Mass Spectrometry

The plates were placed on the Rapidfire 300 MS system (Agilent technologies, Santa Clara, CA) connected to a Sciex API400 Qtrap mass spectrometer (Applied Biosystems) for analysis. Samples were first sipped for 1000 ms using an organic solvent of 99.9% acetonitrile: 0.1% formic acid. The samples were then directly loaded to a C18 separations cartridge using a mixture of acetonitrile containing 0.01% formic acid for 3000 ms as a desalting step. The flow rate of the mobile phase was 1.5 mL/min. Once the samples were eluted, the mobile phase of acetonitrile with 0.01% formic acid was used to move the samples to the mass spectrometer equipped with an electrospray ionization (ESI) source to analyze the samples at a flow rate of 1.25 mL/min. MS/MS separation was utilized for L-tryptophan, L-kynurenine and internal standards monitoring the MS/MS of tryptophan 205.1m/z → 188.1, the MS/MS of kynurenine 209.2 m/z → 192.3 and the MS/MS of L-kynurenine sulfate (RING-D4,3,3-D2) 215 m/z → 198 (internal standard). The mass spectrometer dwell time for each sample was 200 ms. The ESI source voltage was 5000 and was run at a temperature of 400 °C. All valves were washed for 3000 ms in methanol prior to the next injection to reduce carry over, along with the introduction of blank methanol injections between blood samples. Peak areas for both kynurenine and tryptophan were normalized using the internal standard.

2.2.2.3 Generations of Standard Curves and Calculations of Compound IC50 values

Peak area ratios were calculated from peak of analyte/peak of internal standard. The peak area ratios of the standards were used to generate the standard curves for kynurenine and tryptophan calibrations using a second order polynomial (quadratic) equation in the GraphPad Prism software:

Equation 1:

$$Y = B0 + B1*X + B2*X^2$$

where Y = the measured peak area ratio and X= concentration of the standard.

Standard calibration curves for kynurenine and tryptophan were used separately to calculate the concentration of either kynurenine or tryptophan based on the peak area ratio measured for each plasma sample. The actual concentration of kynurenine or tryptophan was calculated by multiplying the dilution factor of 6. The actual concentration of kynurenine was used to calculate the percent inhibition occurring

at each concentration of inhibitor, and the IC50 value for each compound was calculated using the following equation:

Equation 2:

$$Y = A + \frac{B - A}{1 + (C/x)^D}$$

where Y = percent inhibition at each inhibitor concentration, A = minimal Y value B = maximal Y value, C = IC50, D = Hill Slope, x = concentration of inhibitor.

2.3 Human Primary T-Cell Functional Assays

2.3.1 T-cell Proliferation in Mixed Lymphocyte Reaction

Primary $CD14^+$ monocytes and allogeneic $CD3^+$ T cells from healthy human donors were procured from Biological Specialty Corporation (Colmar, PA). The monocytes were cultured in RPMI1640 medium containing 10% FBS, GM-CSF (100 ng/mL) and IL-4 (200 ng/mL) at 1×10^6 cells/mL for 7 days to differentiate them into dendritic cells. They were further matured in RPMI1640 medium containing 10% FBS, GM-CSF (100 ng/mL), IL-4 (200 ng/mL), TNFα (20 ng/mL), IL-1β (10 ng/mL), IL-6 (25 ng/mL) and PGE2 (5 μM) for 48 hours before being applied to the mixed lymphocyte reaction (MLR).

The mixed lymphocyte reactions, which contained equal number of matured dendritic cells and allogeneic T cells (2×10^5 cells each) in 200 μL of RPMI1640 medium with 10% FBS, were carried out in a U-bottom 96-well plate. The matured dendritic cells (2×10^5) were first pre-incubated with a desired concentration of compound for 2 hours at 37 °C before mixing with allogeneic T cells (2×10^5). After culturing the mixed lymphocytes in tissue culture incubator for 4 days, an aliquot of [3H]-thymidine (4 μCi/mL) was added into each well and cultured for additional 18 hours. The proliferation of T cells in the MLR was monitored by determining the incorporation of radioactivity into the cells after removing excess [3H]-thymidine from the culture by a plate harvester.

2.3.2 T-cell Proliferation in co-culture of SKOV3 Cells and T cells

SKOV3, a human ovarian cancer cell line, was obtained from American Type Culture Collection (ATCC, Manassas, VA). The procedure to monitor T-cell proliferation in co-culture of SKOV3 cells and T cells was similar to that described above for the mixed lymphocyte reaction except that the co-culture experiment was carried out in a flat-bottom 96-well plate, and the matured dendritic cells were replaced with SKOV3 cells.

Part 3: *In Vivo* Pharmacodynamic and Pharmacokinetic Protocols

3.1 Animals and Murine Cell Lines

Seven- to 12-week old female nu/nu mice were used in the studies, and they were purchased from Envigo (Indianapolis, IN). The mice received food and water ad libitum and were maintained in a controlled environment according to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International regulations.

Human ovarian cancer cell line SKOV3 were purchased from American Type Culture Collection (Manassas, VA). These cell lines were maintained in vitro with the growth medium RPMI1640 containing 10% fetal bovine serum. They were harvested while in the log growth phase, and re-suspended in Hank's balanced salt solution at 1 to 6 × 10^7 cells/ml before mixing with Matrigel® (BD Biosciences, San Jose, CA) and implanting in mice.

3.2 The Drug Substance BMS-986242 and Vehicle

Compound **25** was formulated as a solution in the vehicle ethanol (EtOH)/polyethylene glycol 400 (PEG 400)/propylene glycol (PG)/tocopheryl polyethylene glycol 1000 succinate (TPGS) at a volume ratio of 5:55:20:20.

3.3 Human SKOV3 Xenograft Tumor Models

Human SKOV3 cells (6 × 10^7 cells/ml in Hank's balanced salt solution) were mixed with Matrigel® at 1:1 ratio and implanted subcutaneously into the flank of nu/nu mice at 0.1 ml per mouse (3 × 10^6 cells/mouse). Fifteen days after implantation, the tumor volumes were measured and the tumor-bearing mice were randomized to 5 animals per group. Each group was administered orally once a day (QD) or twice a day (BID) with either vehicle or tested compound at the dosage indicated in the figures or tables for 5 days. Tumors were snap-frozen and sera were harvested at designated times on day 5 after dosing for quantifications of the kynurenine and compound levels as the PD and PK endpoints, respectively. The tumor volumes were also measured before harvesting. The tumor and serum samples were stored at –80 ˚C until processed for analyses (within two weeks).

3.4 Analytical Methods

3.4.1 Tumor and Serum Preparations

The frozen tumor samples were weighed and homogenized in 4 volumes of ice-cold phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 1 minute using TissueLyser II (QIAGEN, Valencia, CA) at a frequency of 30 Hertz per second. The tumor homogenates were kept on ice and processed immediately for PD and PK analyses. The tumor and serum processing procedure outlined below was modified from a previously published protocol.[i]

For PD (kynurenine) analysis, 60 µl of internal standards were added to an aliquot (30 µL) of tumor homogenate or serum, and mixed for 1 minute. The PD internal standards were stable isotopes D6-kynurenine (250 nM) and D5-tryptophan (1500 nM) prepared in PBS containing 1% BSA. They were purchased from Cambridge Isotope Laboratories (Tewksbury, MA). The sample containing internal standards was then mixed with 40 µL of 10% trichloroacetic acid for 5 minutes. The supernatant was

separated from the precipitated proteins after a 5-minute centrifugation at 3500×g and transferred to auto-sampler vials or a 96-well plate. An aliquot (3 µl) was injected onto the high-performance liquid chromatography (HPLC) column for LC-MS/MS-based analysis. To establish standard curves, kynurenine (50 to 20000 nM) and tryptophan (100 to 200000 nM) prepared in PBS containing 1% BSA were processed in the same fashion as described above for tumor homogenate and serum.

For PK (compound) analysis, an aliquot (30 µL) of tumor homogenate or serum was mixed with acetonitrile (60 µL) containing internal standard compound **25** (0.2 µM) for 5 minutes. If dilutions were required, an aliquot of the sample was diluted into blank serum before mixing with acetonitrile. The supernatant was then separated from the precipitated proteins after an 8-minute centrifugation at 2000×g and transferred to auto-sampler vials or a 96-well plate. An aliquot (10 µL) was injected onto the ultra high-performance liquid chromatography (uHPLC) column for LC-MS/MS-based analysis. To establish standard curves, compound **25** prepared in serum was processed in the same fashion as described above for tumor homogenate and serum.

3.4.2 Instrumentation for Kynurenine and Tryptophan Measurements

The HPLC system consisted of a Waters® Acquity Ultra Performance LC (UPLC) System (Waters Corporation, Milford, MA) that contained 2 LC pumps, an auto-sampler and a cooling stack that maintained samples at 4°C during analysis. The analytical column utilized was a Waters® UPLC HSS T3 C18, 2.1mm x 50mm, 1.8 µm particle size (Waters Corporation, Milford, MA) maintained at 60°C. The mobile phase, which consisted of 0.1% formic acid with 10 mM ammonium formate in water (A) and acetonitrile with 0.1% formic acid (B), was delivered at a total flow rate of 0.7 mL/min. The gradient is summarized in Table 3.4.2-1.

Table S1: Solvent Gradient for LC-MS/MS Analysis of Kynurenine and Tryptophan

Time (min)	% A	% B	Flow Rate (mL/min)	Curve
0 (Initial)	95	5	0.7	isocratic
0.70	95	5	0.7	isocratic
1.0	2	98	0.7	linear
1.2	2	98	0.7	isocratic
1.21	95	5	0.7	step
1.5	95	5	0.7	isocratic

A: 0.1% formic acid with 10 mM ammonium formate in water; B: 0.1% formic acid in acetonitrile

The retention times for kynurenine and tryptophan were 0.45 and 0.7 minutes, respectively. The total analysis time was 1.5 minutes.

The Acquity UPLC was interfaced to a PE-Sciex API 4000 QTRAP LC-MS/MS tandem mass spectrometer equipped with a turbo-electrospray interface operating in the positive ionization mode. Detection of each analyte was achieved through selected reaction monitoring (SRM). Ions representing the precursor $(M+H)^+$ species for tryptophan, kynurenine and the labeled compounds (internal standards), were selected in quadrupole 1 and collisionally dissociated with UHP nitrogen to generate specific product ions, which were subsequently monitored by quadrupole 2. The transitions monitored and the mass spectrometer settings are summarized in Table 3.4.2-2.

Table S2: Transitions Monitored and Settings for LC-MS/MS Analysis of Kynurenine and Tryptophan

Compound	Precursor Ion	Product Ion	DP Voltage	Collision Energy
Tryptophan	205	146	100	20
Kynurenine	209	94	100	20
D5-Tryptophan	210	150	100	27
D6-Kynurenine	215	98	100	20

Source: ELN 96446-116

3.4.3 Instrumentation for Compound 25 Measurement

The ultra-high performance liquid chromatography (uHPLC) system consisted of a Thermo Vaquish LC System (Thermo Fisher Scientific, San Jose, CA) that contained 4 LC pumps, an auto-sampler and a cooling stack that maintained samples at 5°C during analysis. The analytical column used was a Waters HSS T3, 2.1 mm x 150 mm, (Thermo Fisher Scientific, San Jose, CA) at 60°C. The mobile phase, which consisted of 0.1% formic acid in 95% water/5% acetonitrile (A) and 95% acetonitrile/5% water (B), was delivered at a flow rate of 0.6 mL/min. The gradient is summarized in Table 3.4.3-1.

Table S3: Solvent Gradient for LC-MS/MS Analysis of BMS-986242

Time (min)	% A	% B	Flow Rate (mL/min)
0 (Initial)	96	4	0.600
0.30	96	4	0.600
0.60	75	25	0.600
1.50	50	50	0.600
2.50	5	95	0.600
3.00	5	95	0.600
3.10	96	4	0.600

A: 0.1% formic acid in 95% water/5% acetonitrile; B: 0.1% formic acid in 95% acetonitrile/5% water

The retention times for BMS-986242 and internal standard BMS-986205-05-002 were 2.0 and 2.3 minutes, respectively. The total analysis time was 3.4 minutes.

The uHPLC was interfaced to a Thermo Quantiva LC-MS/MS tandem mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an electrospray interface operating in the positive ionization mode. Ultra-high-purity (UHP) nitrogen was used as the sheath and aux gases at flow rates of 50 L/h for sheath and 35 L/h for aux. The vapor temperature was 450 °C and the capillary temperature was 350□C. Detection of each analyte was achieved through selected reaction monitoring. Ions representing the precursor mass-to-charge ratio $(M+H)^+$ species for BMS-986242 and BMS-986205-05-002 were selected in quadrupole 1 and collisionally dissociated with UHP argon at a pressure of 1.3×10^{-5} torr to generate specific product ions, which were subsequently monitored by quadrupole 2. The transitions monitored and the mass spectrometer settings are summarized in Table 3.4.3-2.

Table S4: Transitions Monitored and Settings for LC-MS/MS Analysis of BMS-986242

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Tube Lens	Collision Energy
BMS-986242	411.3	148.2	120	42
Linrodostat	417.2	147.8	100	40

3.4.4 Standard Curve Ranges

Unless otherwise specified, the analyses of kynurenine, tryptophan, and BMS-986242 were conducted against each standard curve ranging from 50-20000 nM for kynurenine, from 500 to 200000 nM for tryptophan, and from 0.975 to 8000 nM for BMS-986242. The standard curve was fitted with a quadratic regression weighted by reciprocal concentration (1/x). Standards were analyzed in duplicate within each analytical set.

3.5 Calculations of PK and PD Parameters

The PK and PD parameters of BMS-986242 were obtained by non-compartmental analysis of serum or tumor concentration versus time data (Phoenix software, Version 6.3.0.395, Pharsight, A Certara™ Company, Princeton, NJ). The area under the concentration-time curve from time zero to the 24-hour sampling time for serum or tumor concentrations of BMS-986242 [AUC(0-24h)] was calculated using a combination of linear and log trapezoidal summations. The area under the concentration-time curve from time zero to the 24-hour sampling time for serum or tumor concentrations of kynurenine [AUEC(0-24h)] was calculated using a combination of linear and log trapezoidal summations. The PD effect AUEC kynurenine reduction (%) was calculated as the area under kynurenine concentration-time curve from 0 to 24-hour and compared with that of vehicle control.

Equation 3:

$$\text{PD effect AUEC kynurenine reduction \%} = \left(1 - \frac{\text{AUEC}(0-24\text{h}) \text{ in treatment group}}{\text{AUEC}(0-24\text{h}) \text{ in vehicle control group}}\right) \times 100\%$$

3.6 Statistical Analysis Method

A second order polynomial statistical model was fitted to tumor and serum kynurenine data, respectively. The statistical model consisted of main effect terms for treatment and time, a second order term for time, and the interaction effect of treatment with the first order and the second order term for time. For the tumor kynurenine endpoint, the model also consisted of a covariate adjustment term for post dosing tumor weights. Planned comparisons, or tests, were set up using the fitted model parameter estimates to test for the difference in kynurenine levels between doses of tested compound and vehicle control at each time point. Use of the fitted statistical model for the comparisons maximized the effective use of every datum in the study to better estimate the variance used to calculate the t-statistic for testing group differences. The Bonferroni's method was used to protect against false positive results by fixing the experiment-wise error rate, or the accumulated risk associated with a family of five comparisons at each time point to $\alpha = 0.05$.

For the Bonferroni correction, the comparisons tested at each time point were treated as independent sets across time, with dependence assumed among the comparisons only within a time point. Therefore, for individual comparisons, the false positive rate was calculated by dividing $\alpha = 0.05$ by the number of hypotheses being tested at each time point. The SAS 9.2 procedure GLM[ii] was used for the analysis.

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[i] Huang Y, Louie A, Yang Q, et al. A simple LC-MS/MS method for determination of kynurenine and tryptophan concentrations in human plasma from HIV-infected patients. Bioanalysis 2013; 5:1397-407.

[ii] SAS Institute Inc. 2009. Base SAS 9.2 Procedures Guide. Cary, NC: SAS institute Inc.