Supporting Information

Discovery of MK-8318, a Potent and Selective CRTh2 Receptor Antagonist for the Treatment of Asthma†

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†Dedicated to Professor E. J. Corey on the occasion of his 90th birthday

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1. Biological assay protocols

1.1 Radioligand binding assay:

Radioligand Binding Assay was performed at room temperature in 50 mM Tris- HCl pH 7.4, 1 mM EDTA containing 2 mM $MnCl_2$ and 3.0 nM [^{3}H]PGD2 (New England Nuclear,

Boston, MA) (171 Ci $mmol^{-1}$, in a final volume of 0.2 mL. Competing ligands were diluted in dimethylsulfoxide (Me_2SO) that was kept constant at 1% (v/v) of the final incubation volume. The reaction was initiated by the addition of 8-20 μg of membrane protein prepared from a human embryonic kidney (HEK)-hCRTH2 cell line. Total and non-specific binding were determined in the absence and the presence of 10 μM PGD_2, respectively. Under these conditions, specific binding (total minus non-specific) of the radioligand to the receptor reached equilibrium within 50 min and was stable up to 180 min. The reaction was routinely conducted for 60 min at room temperature and terminated by rapid filtration through prewetted (0.3% polyethyleneimine) 96-well printed filtermate™ (Wallac) using a Tomtec® harvester (Harnden, CT). After washing with cold buffer, the filter was dried for 2 minutes in microwave, and Meltilex Scintillator sheet (Wallac) was melted on for 2 min. The radioactivity was measured with Betaplate model 1205 (Wallac).

1.2 i[cAMP] measurements:

The ability of the compounds to antagonize the formation of cAMP can be assayed using the ELISA-based assay described in this example. HEK-hCRTH2 cells are grown to 80-90% confluency. On the day of the assay, the cells are washed with phosphate buffered saline (PBS), incubated for 2 min in cell dissociation buffer, harvested by centrifugation at 300 g for 7 min at room temperature and resuspended at $1.25el0^6$ cells mL^{-1} in Hanks' balanced salt solution containing 20 mM HEPES pH 7.4 and 0.75 mM IBMX (HBSS/HEPES/IBMX). The assay is performed in 384-plate format with 0.01 mL HBSS/HEPES/IBMX per well containing 12500 cells and 70 to 75 nl of the test compound and DK-PGD2 at various concentrations. Following a 0 to 10 min pre-incubation of the cells with the test compound at 37°C, 0.005 mL of 30 μM Forskolin dilute in HBSS 20 mM HEPES, is added at a final concentration of 10 uM to initiate the reaction. After 10 to 60 min incubation at room temperature or 37 °C, the cAMP content was quantified using the cAMP XS+ HitHunter chemiluminescence assay (GE Healthcare 90-0075). Percent inhibition is calculated using the Forskolin and EC85 DK-PGD2 controls.

1.3 beta-Arrestin assay:

CHO-Kl cells obtained from DiscoverX are stably transfected with human CRTH2 (propagation medium: F-12, 10% FBS, 300 ug/mL hygB and 800 ug/mL G418). Cells are

grown in Tl 75 cm^2 flask. While in log phase, cells are collected via 0.05% trypsin treatment. Triturated cells are filtered and 40 uL (lOK cells) are plated per well in a 384-well white clear bottom plate and incubated O/N. Cell plate is emptied via inversion and blotted dry. Each well is filled with 35uL of HBSS (with Ca^{++} and Mg^{++}) and incubated for 5 min. Compounds are added in volumes of 1 µL and the plate is gently shaken for 2 min., followed by incubation at °C for 20 min. All compounds and controls are diluted in HBSS assay buffer (with Ca^{++} and Mg^{++}) with a final concentration range of 10^{-5} M to 3 x 10^{-11} M, 11 point Dose response curves at appropriate half-log increments. Final DMSO % is <= 0.3%. Agonist Assay: 1 µl/well of compounds are added into cell plate and left to incubate at 37 °C for 90 min. Antagonist Assay: 1 µl/well of compounds are added into a cell plate. Incubate 30 minutes at 37 °C. Stimulate cells with 1 ul/well of PGD2 [100 nM] final. Incubate plate for 60 minutes at 37 °C. Resulting luminescent signal is detected via Discoverx PathHunter Detection Kit per manufacturer's instructions. A total of 12 µI/well is added to each well. The plate is covered and incubated for 60 min. with gentle shaking. Chemiluminescent detection is done by a SpectraMax plate reader.

1.4 Eosinophil shape change assay in human whole blood:

Blood is collected in vacutainers containing EDTA. The antagonist is added to blood and incubated for 10 min at room temperature. DK-PGD2 (13,14-dihydro-15-keto prostaglandin D2) are then added to blood for 4 min at 37 °C in a running water bath. Blood cells are then fixed in presence of cold 0.25%(v/v) paraformaldehyde prepared in 75%(v/v) DPBS without Ca^{++} and Mg^{++} for 1 min on ice. 175 µL of fixed blood is transferred into 870 µL of cold 155 mM NH_4Cl lysis solution and incubated at 4 °C for at least 40 min. The solution is then centrifuged at 430 g for 5 min and the supernatant is discarded. Centrifuged cells are resuspended in residual supernatant and sodium azide is added (1% final concentration). Samples are analyzed with a FACs Calibur flow cytometer (Becton Dickinson). Flow cytometry raw data is analyzed with Diva software by isolating the eosinophils from the neutrophils based on their high autofluorescence and determining the percent of total eosinophils with increased forward light scatter. Maximum (100%) and minimum (0%) shape change is determined in the presence of 10 µM DK-PGD2 and DPBS, respectively. A dose response curve with DK-PGD2 is performed with every assay to determine the EC_{50} for each blood donor. Compounds are tested in 11-dose titration curves in the presence of 50 nM DK-PGD2 to determine an antagonist IC_{50}.

1.5 Effect of compound 15c with oral dosing on recoverable BAL cells from ovalbumin sensitized and challenged brown norway rats.[1]

All animal studies were approved by the Institutional Animal Care and Use Committee and conducted in compliance with all national and local guidelines and regulations.

Study Protocol: 40 Brown Norway rats were divided into 5 groups of 8 (8 Alum and 32 OVA). Each group of animals will receive 1 PO dose of either vehicle or compound daily over a period of 3 days. On day 3 (2 hours after PO dose), animals will be exposed to aerosolized saline or 1% ovalbumin (ova) for a period of 30 minutes. BAL collected 24 hours post challenge and processed for total counts and differentials. Blood was collected from drug treated animals for PK.

1 Negative control (Alum) - (MC Vehicle + saline challenge)

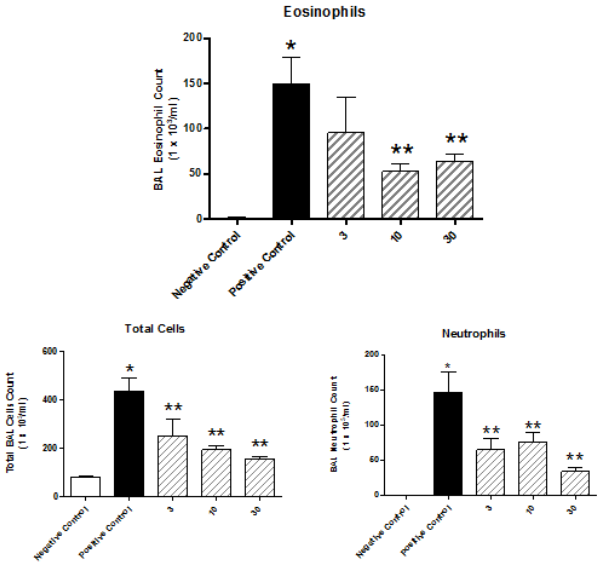
2 Positive control (OVA) - (MC Vehicle + ova challenge)

3 15c (3mpk)* - (drug PO + ova challenge)

4 15c (10mpk)* – (drug PO + ova challenge)

5 15c (30mpk)* – (drug PO + ova challenge)

*Compound was made fresh each dosing day and formulated with 0.4% methyl cellulose (MC). Compound was vortexed, homogenized and sonicated prior to dosing.

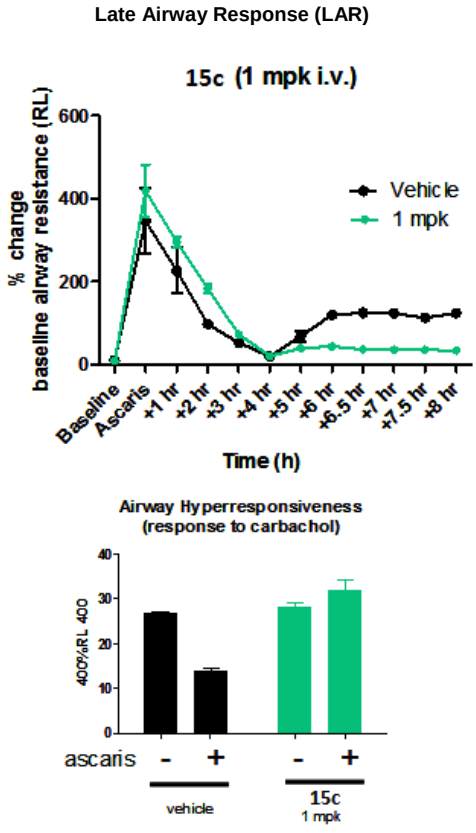


Effect of oral **15c** on recoverable BAL cells from ovalbumin sensitized and challenged Brown Norway rats. Figure displays the effect of **15c** (3 - 30 m/kg, p.o.; QD for 3 days) or vehicle control on differential BAL recoverable inflammatory cells measured 24 hr post antigen (ovalbumin 1%; 30 min) challenge. On day 3 drug or vehicle was given 2 hrs before antigen provocation. Each bar represents mean ± SEM (n=8/treatment group). *p<0.05 compared to negative control; #p<0.05 compared to positive control.

1.6 Evaluation of 15c in a sheep lung allergen challenge model.[1]

All animal studies were approved by the Institutional Animal Care and Use Committee and conducted in compliance with all national and local guidelines and regulations.

Study Protocol: Three sheep were initially dosed intravenously with a vehicle control (5% dextrose solution) and then challenged with an antigen (aerosols of Ascaris suum extract) to establish their control lung function response: the antigen challenge in vehicle-treated animals triggered a rapid increase in airway resistance (early phase bronchoconstriction between 0 and 4 h after challenge) as well as a delayed increase in airway resistance (late phase bronchoconstriction between 4 and 8 h after challenge). This also caused an increase in airway responsiveness measured 24 h after challenge as reflected by a decrease in the amount of carbachol (muscarinic agonist) which indicates the development of airway hyper-responsiveness. The same three sheep were infused for 4 h with 1 mg/kg **15c** which was formulated with 5% Tween 80/10 mM Na phoshate (pH 8). The infusion was initiated 1 h before an inhaled antigen challenge with an Ascaris extract. Effect on early phase (0–4 h) and late phase (4–8 h) bronchoconstriction were measured as well as airway hyper-responsiveness to carbachol 24 h after the antigen challenge. Compound **15c** showed 76% LAR efficacy and 114% AHR efficacy.



2. In vitro activity profile and ancillary profiles of compound 15c (**MK-8318**).

In vitro activity profile of 15c:

In vitro assay	**15c**
CRTh2 binding (Ki, nM)	5.0 ± 1.8 (n = 4)
β-arrestin (IC_{50}, nM)	3.5 ± 2.1 (n = 6)
cAMP (IC_{50}, nM)	8.0 ± 6.8 (n = 12)
Human whole blood eosinophil shape change (IC_{50}, nM)	7.9 ± 5.5 (n = 5)
Human whole blood eosinophil CD11b (IC_{50}, nM)	4.7 ± 0.96 (n = 6)
EP1, EP2, EP3, EP4, FP, and IP IC_{50} (μM)	All >100
DP and TP IC_{50} (μM)	All >10

Ancillary profiles of 15c:[a]

CYP 3A4/2C9/2D6 IC_{50} (μM)[2]	50/50/50
TDI IC_{50} shift ratio[3]	1.7
PXR EC_{50} (μM)[4]	19
hERG IC_{50} (μM)[5]	60
PPB (h, r, rh), % unbound[6]	4.5, 5.1, 4.1
Hepatocyte $Cl_{int(app)}$ h / r (uL/min/million cells)[7]	<1 / 21
Papp (10e-6cm/s, human MDR1)[8]	29
FASSIF, PBS solubility (uM)[9]	163, 152

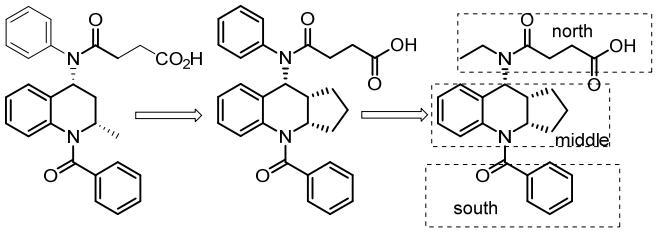
a. Ancillary studies were conducted using industry standard protocols.

3. in vivo metabolism of 15c in Wistar Han rat and Rhesus monkey

- **15c** was extensively metabolized in rat (n = 3) following an oral dose of 10 mg/kg with elimination primarily in the bile (85% of the dose). The major metabolic pathways included glucuronidation (~ 61% of the dose) and oxidation (~ 23% of the dose) with ~ 9 % of the dose excreted as unchanged parent in bile. Acyl-migration and hydrolysis of glucuronidation metabolite was observed in non-acid stabilized bile samples.
- **15c** was extensively metabolized in rhesus monkey (n = 3) following an oral dose of 10 mg/kg with elimination primarily in the bile (80% of the dose). The major metabolic pathway was glucuronidation (75% of the dose) with minor contributions from oxidative metabolism (3% of the dose) and oxidation with subsequent glucuronidation (9% of the dose). Approximately 4.5% of the dose was excreted as unchanged parent in the bile. Acyl-migration and hydrolysis of glucuronide conjugate was observed in non-acid stabilized bile samples.

4. Homology modeling of CRTh2 structure and modeling of ligand binding

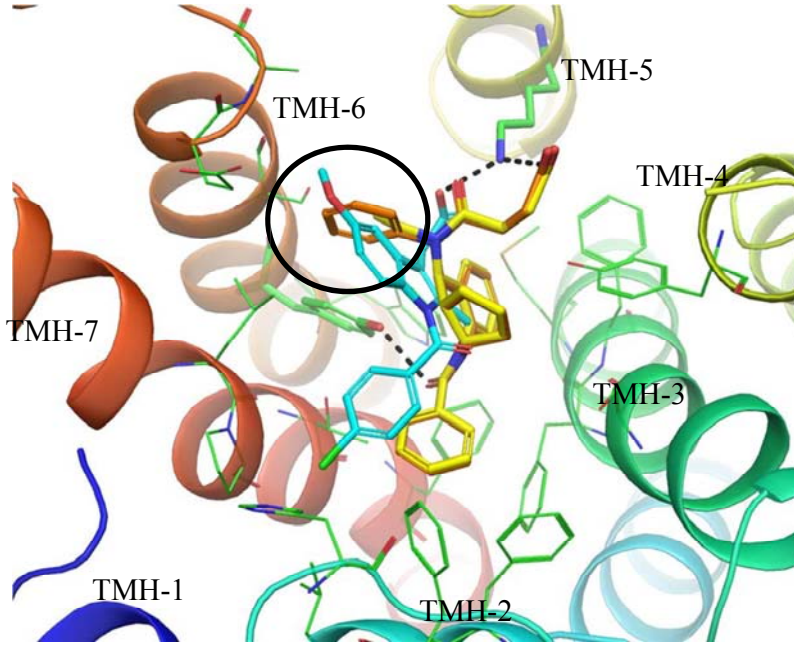
The X-ray structure of angiotensin II type 1 receptor (AT1R) in complex with an inverse agonist olmesartan, PDB id 4ZUD, was selected as the template to build the model of human CRTh2 transmembrane helix (TMH) domain, using MOE of CCG. Both AT1R and CRTh2 belong to Class A GPCR. The sequence identity between the two is 26%, with key residues in each membrane helix aligned. The ligands were modeled into the orthosteric ligand binding site of GPCR using Glide docking program from Schrodinger.



	2	**3**	**4**
$CRTh_2$ Ki	3.9 nM	8.7 nM	11 nM
cAMP IC_{50}	270 nM	18 nM	37 nM
cAMP(Ago.) EC_{50}	10000 nM	3.6 nM	10000 nM
EOS shape IC_{50}	245 nM	930 nM	51 nM

In the CRTh2 model, where ECL-2 was removed, the carboxylic acid of all three ligands forms a salt-bridge with Lys210 side-chain amino group; carbonyl of **3** and **4** makes hydrogen bond with Tyr262 sidechain hydroxy group, as shown in Fig. 1. Interestingly, SAR revealed replacing carbonyl with a methyl significantly reduced binding of **3** to CRTh2. The area occupied by methoxy benzo of indomethacin, defined as agonist selectivity pocket, points directly toward TMH-6. Presumably difference in the effect to TMH-6 from agonist or antagonist binding could travel along the helix to the intracellular end of TMH domain, thus in response, produces distinct structural changes between agonist and antagonist bound CRTh2. In our models we kept CRTh2 structure the same when it binds either to **3** or **4**. In the future, when X-ray structures of CRTh2 in complex with **3** or **4** are made available, it should provide better understanding of the structural changes induced by agonist and antagonist of the receptor. If it follows published GPCR structures, these structural changes would be mainly at the intracellular end of TMH domain, away from the orthosteric ligand binding site.

The mutants of the corresponding residues Lys210 and Tyr262 in mouse CRTh2 were shown to influence binding of indomethacin. In our model, these residues are located in the ligand binding site, display direct VDW contacts with all three ligands.

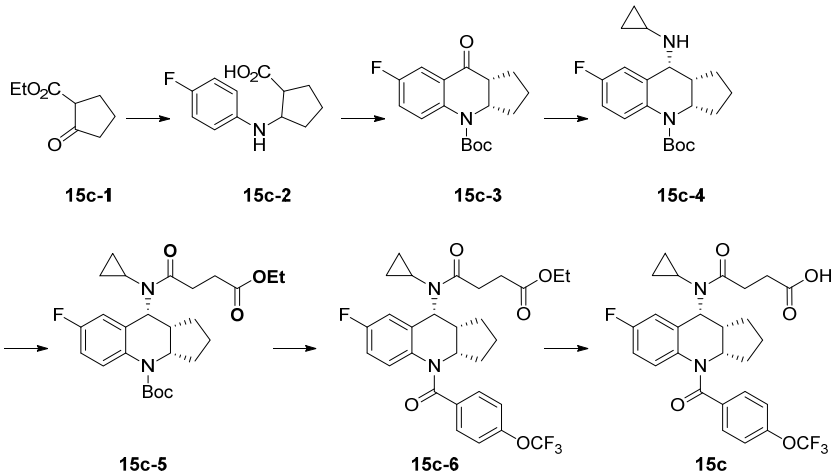


7 helices colored based on their order

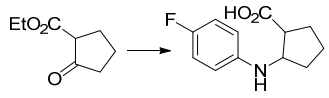
5. Chemistry Experimental Procedures[10]

Analytical methods: All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. 1H NMR spectra were measured on a Varian Oxford 400 or Bruker 500 UltraShield spectrometer. Chemical shifts δ are reported relative to CDCl3 at 7.26 ppm as an internal standard. Normal phase column chromatography was performed on prepacked silica gel columns using ISCO CombiFlash system. Reverse phase column chromatography was performed on Phenomenex Luna 10 μm C18 columns using Alltech 627 HPLC pump or Varian SD-1 HPLC system. Chiral columns were from Chiral Technologies. The purity of final compounds was analyzed on two independent reverse phase HPLC systems with different gradient. LC−electrospray mass spectrometry with a C-18 column using a gradient of 5−95% MeCN in water as the mobile phase was used to determine the molecular mass and retention time. The purity of the samples was assessed using a mass detector and a UV detector at 254 nm. An additional analytical reverse phase HPLC system was used to assess the purity of final compounds using a UV detector monitored at both 219 and 254 nm and an ELSD detector.

5.1 Typical procedure for the preparation of the cyclopentyl fused tetrahedroquiniline analogs (Example 15c):



Step1: 2-((4-fluorophenyl)amino)cyclopentane-1-carboxylic acid

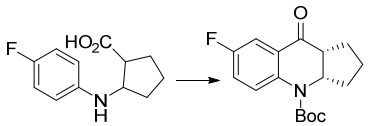


15c-1 **15c-2**

In a 500 mL round bottom flask was added acetic acid (81 mL), water (8.05 mL, 447 mmol), 4-fluoroaniline (10 mL, 97 mmol), ethyl 2-oxocyclopentanecarboxylate **15c-1** (13.13 mL, 97 mmol) followed by zinc (25.4 g, 388 mmol). The reaction was warmed to 80 °C, during which time gas evolution was noted. After 2 h, the bubbling had stopped and the reaction was cooled to rt. The slurry was then diluted with MeOH and the zinc solids were removed by filtering through a fritted funnel. The collected filtrate was then concentrated on the rotovap and then diluted with DCM and wet ice. The solution was neutralized with NH_4OH to pH = 10. The mixture was then poured into a separation funnel containing DCM and water. The mixture was extracted 3x with DCM. The combined fractions were washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The resulting oil was used without further purification.

The above obtained ester (24.0 g, 96 mmol) was dissolved in dioxane (95 mL) and treated with NaOH (96 mL, 5 N, 480 mmol). The reaction mixture was stirred at 100 ^oC for one hour before it was cooled to rt and acidified with 2 N HCl to pH=3. The reaction mixture was then poured into a separation funnel containing EtOAc and water. The mixture was extracted 3x with EtOAc. The combined fractions were washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The resulting oil **15c-2** was used without further purification.

Step 2: tert-butyl (3aS,9aR)-7-fluoro-9-oxo-1,2,3,3a,9,9a-hexahydro-4H-cyclopenta[b]quinoline-4-carboxylate

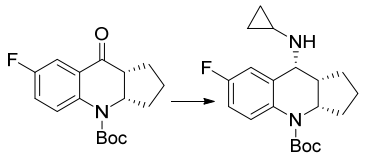


15c-2 **15c-3**

Acid **15c-2** (21.3 g, 95 mmol) was treated with Eaton's reagent (100 mL, 630 mmol) and warmed to 70 °C. The reaction was stirred for one hour. LCMS shows that the starting material was consumed. The reaction was cooled to rt and then quenched by the portionwise addition of ice

water. The mixture was then treated with solid NaOH pellets until the pH was 10. The reaction mixture was then poured into a separation funnel containing EtOAc and water. The mixture was extracted 3x with EtOAc. The combined fractions were washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography (Biotage, 340 g, 0-50% EtOAc/hexane gradient) to give 7-fluoro-2,3,3a,4- tetrahydro-1H-cyclopenta[b]quinolin-9(9aH)-one (10 g). Boc_2O (9.50 g, 41.7 mmol) was added to the solution of above obtained 7-fluoro-2,3,3a,4- tetrahydro-1H-cyclopenta[b]quinolin-9(9aH)-one (13.9 mmol), Et_3N (5.80 mL, 41.7 mmol) and DMAP (1.70 g, 13.9 mmol) in 1,4-dioxane (100 mL). The resultant mixture was kept stirring at rt overnight. The mixture was diluted with EtOAc (100 mL), washed with H_2O (100 mL), and brine (100 mL), the organic was dried over $MgSO_4$ and concentrated. The residue was purified via silica gel column chromatography (EtOAc/Hexane = 1:10) to obtain the racemic, Boc protected ketone amine **15c-3'** as a light yellow syrup (4.0 g). The racemic ketone **15c-3'** was resolved with chiral HPLC to give enantiomerically pure **15c-3** using an OJ-H, 4.6 x 250mm column eluting with an isocratic system of 15% w/ IPA in supercritical CO_2 at a flow rate of 3 mL/min. The fast eluting isomer (first peak) was determined to be the active isomer based on SAR information and was used for the preparation of all active analogs. The configuration of **15c-3** was determined to have the 3aS', 9aR configuration by inference from the configuration of the subsequently prepared product **15c** below with single crystal x-ray structure. Calcd E/Z $[M+H]^+$ for $C_{17}H_{20}FNO_3$: 306.2, Found: 306.0.

Step 3: <u>tert-butyl (3aS,9R,9aS)-9-(cyclopropylamino)-7-fluoro-1,2,3,3a,9,9a-hexahydro-4H-cyclopenta[b]quinoline-4-carboxylate</u>

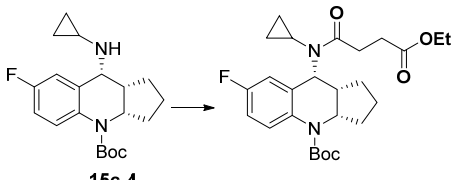


15c-3 **15c-4**

Titanium (IV) ethoxide (1.222 mL, 5.89 mmol) was added to a stirred, room temperature mixture of (3aS,9aR)-tert-butyl 7-fluoro-9-oxo-3,3a,9,9a-tetrahydro-1H-cyclopenta[b]quinoline-4(2H)-carboxylate **15c-3** (720 mg, 2.358 mmol) and cyclopropylamine (0.416 mL, 5.89 mmol) in tetrahydrofuran (12 mL) and the mixture was stirred at 80 °C for overnight. The mixture was cooled, poured into brine, stirred at room temperature for 15 mins, diluted with ethyl acetate,

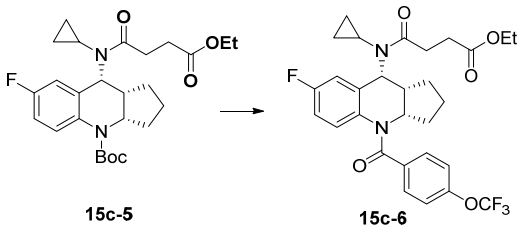
filtered through a short pad of Celite. The biphasic mixture was separated, the aqueous phase was extracted once more with ethyl acetate. The combined organic layers were washed with brine, dried $MgSO_4$, and concentrated to give a yellow gum. The residue was dissolved in dichloromethane and methanol, $NaBH_4$ (178 mg, 4.72 mmol) was added, the resultant mixture was kept stirring at room temperature for 2 h. The mixture was diluted with dichloromethane (20mL), washed with aqueous sodium hydrogen carbonate (saturated, 10mL) and brine (5mL) dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give **15c-4** (735 mg, 2.122 mmol, 90 % yield) as a yellow gum which was used directly without purification. Calcd E/Z $[M+H]^+$ for $C_{20}H_{27}FN_2O_2$: 347.2, Found: 347.0.

Step 4: tert-butyl (3aS,9R,9aR)-9-(N-cyclopropyl-4-ethoxy-4-oxobutanamido)-7-fluoro-1,2,3,3a,9,9a-hexahydro-4H-cyclopenta[b]quinoline-4-carboxylate



Ethyl 4-chloro-4-oxobutanoate (1.201 ml, 8.45 mmol) was added to a stirred, room temperature mixture of **15c-4** (732 mg, 2.113 mmol) and Hunig's base (1.476 ml, 8.45 mmol) in dioxane (20 ml) and the mixture was stirred at room temperature for 90 min. The mixture was diluted with ethyl acetate (15 mL), washed with aqueous sodium hydroxide (20 %, 3 x 10mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel Teledyne ISCO column (80g prepacked), eluting with EtOAc/hexane = 25% to give **15c-5** (900 mg, 1.896 mmol, 90 % yield) as a yellow gum. Calcd E/Z $[M+H]^+$ $C_{26}H_{35}FN_2O_5$: 475.3, Found: 474.9.

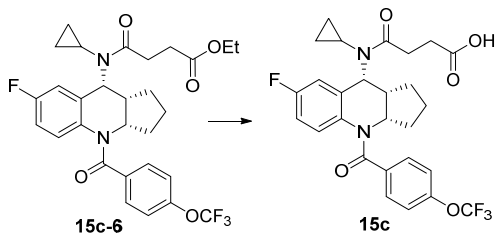
Step 5: ethyl 4-(cyclopropyl((3aS,9R,9aR)-7-fluoro-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoate



TFA (11.69 mL, 152 mmol) was added to a stirred, room temperature mixture of **15c-5** (7.2g, 15.17 mmol) in CH_2Cl_2 (50 mL) and the mixture was stirred at room temperature for overnight. LCMS check, completed, starting material disappeared. The mixture was concentrated, the residue was diluted with DCM (200 ML), basified with aqueous sodium hydrogen carbonate saturated (~150 mL) to pH ~ 9 -10, the aqueous was extracted once more with DCM (200 mL), the combined organic was dried $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel Teledyne ISCO column (220g prepacked) eluting with EtOAc/hexane = 30% to give Boc deprotected amine intermediate **15c-6'** (5.44g, 14.53 mmol, 96 % yield) as a colorless gum. Calcd E/Z $[M+H]^+$ for $C_{21}H_{27}FN_2O_3$: 375.2, Found: 375.0.

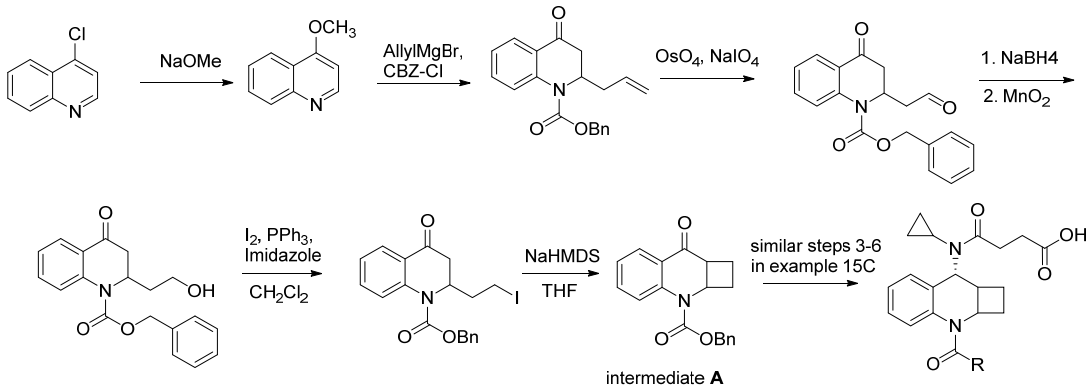
4-Trifluoromethoxy benzoylchloride (0.811 mL, 5.13 mmol) was added to a stirred, room temperature mixture of **15c-6'** (640 mg, 1.709 mmol), DMAP (41.8 mg, 0.342 mmol) and Hunig's Base (0.896 mL, 5.13 mmol) in dichloromethane (10 mL) and the mixture was stirred at room temperature for 1 h. The mixture was diluted with dichloromethane (20 mL), washed with aqueous sodium hydrogen carbonate (saturated, 2x 10mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel Teledyne ISCO column (40g prepacked) eluting with EtOAc/hexane = 1:1 to give **15c-6** (835 mg, 1.484 mmol, 87 % yield) as a white foam. Calcd E/Z $[M+H]^+$ $C_{29}H_{30}F_4N_2O_5$: 563.2, Found: 562.8.

Step 6: <u>4-(cyclopropyl((3aS,9R,9aR)-7-fluoro-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoic acid</u>

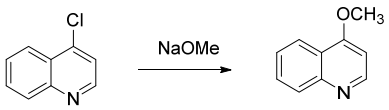


NaOH (1M aq) (1.5 mL, 1.500 mmol) was added to a stirred, room temperature mixture of ethyl 4-(cyclopropyl((3aS,9R,9aR)-7-fluoro-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoate **15c-6** (815mg, 1.449 mmol) in MeOH (1.5 mL) and THF (1.5 mL) and the mixture was stirred at room temperature for 2 h. THF and methanol was removed under reduced pressure, the residue was diluted with water (5mL), acidified with 2N HCl to pH 2~3, extracted with dichloromethane (3 x 10mL), dried $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel ISCO column (40g prepacked) eluting with CH_2Cl_2/MeOH = 12:1 to give 4-(cyclopropyl((3aS,9R,9aR)-7-fluoro-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoic acid **15c** (705mg, 1.319 mmol, 91 % yield) as a white solid. ^{1}H NMR (500 MHz, CDCl3) δ ppm: 7.29 (d, 2H); 7.08 (d, 2H); 6.60 (m, 2H); 6.38 (m, 1 H); 5.168 (m, 1 H); 3.09 (m, 3H); 2.82 (m, 3H); 2.42 (m, l H); 2.03 (m, 1 H); 1.57 (m, 2H); 1.40 (m, 2H); 1.22-1.00 (m, 5H). Calcd E/Z $[M+H]^+$ $C_{27}H_{26}F_4N_2O_5$: 535.2, Found: 534.8 as observed in LCMS. Optical rotation: $[\alpha]_D^{20}$ = +174.1^o (c = 0.1, DMF).

5.2 Typical procedure for the preparation of the cyclobutyl fused tetrahedroquiniline analogs:

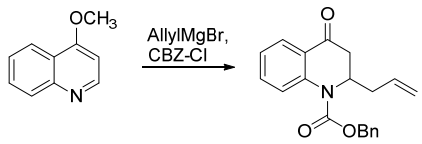


Step 1: <u>4-methoxyquinoline</u>



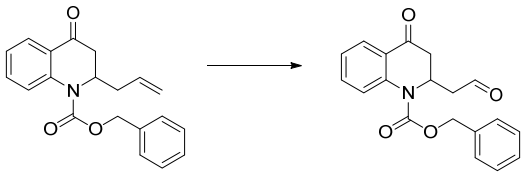
4-Chloroquinoline (3.0 g, 18.34 mmol) was dissolved in MeOH (20 mL) followed with addition of NaOMe (5.0 eq, 25wt% in MeOH) at room temperature. The resulting mixture was heated at 70 oC overnight before it was cooled and the solvent was removed under reduced pressure. The residue was taken up with EA and water. The organic phase was washed with brine, and the washed solution was dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated to give the desired product 4-methoxyquinoline (2.9 g, 99%). Calcd E/Z $[M+H]^+$ $C_{10}H_9NO$: 160.1, Found: 160.1.

Step 2: benzyl 2-allyl-4-oxo-3,4-dihydroquinoline-1(2*H*)-carboxylate



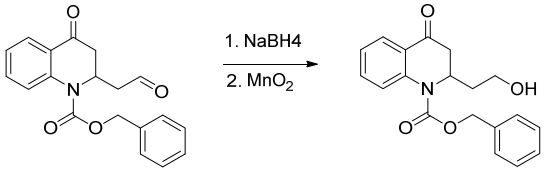
A solution of allylmagnesium bromide (1.00 M in tetrahydrofuran, 101 mL, 101 mmol, 2.00 equiv) was added to a solution of 4-methoxy quinoline (8.00 g, 50.3 mmol, 1 equiv) in tetrahydrofuran (335 mL) at –78 °C. The reaction mixture was stirred at –78 °C for 1 h, then benzyl chloroformate (14.35 mL, 101.0 mmol, 2.00 equiv) was added via syringe over 5 min. The mixture was stirred for an additional 15 minutes at –78 °C, then the cooling bath was removed and the reaction mixture was allowed to warm to 23 °C. After 1 h, methanol (40 mL) was added. After stirring for 5 min, aqueous hydrochloric acid solution (2 N, 20 mL) was added and the mixture was stirred for 10 min. The mixture was then concentrated by rotary evaporation to remove most of the tetrahydrofuran and methanol, and the residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, and the washed solution was dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue was purified by silica gel flash-column chromatography (5% ethyl acetate-hexanes, grading to 20% ethyl acetate-hexanes) to afford benzyl 2-allyl-4-oxo-3,4-dihydroquinoline-1(2*H*)-carboxylate as a colorless oil (13.7 g, 85% yield). Calcd E/Z $[M+H]^+$ $C_{20}H_{19}NO_3$: 322.1, Found: 322.2.

Step 3: benzyl 4-oxo-2-(2-oxoethyl)-3,4-dihydroquinoline-1(2H)-carboxylate



Osmium tetroxide (5% in water, 2.0 mL, 0.33 mmol, 0.025 equiv) was added to a biphasic mixture of benzyl 2-allyl-4-oxo-3,4-dihydroquinoline-1(2*H*)-carboxylate (4.20 g, 13.1 mmol, 1 equiv), sodium periodate (11.2 g, 52.3 mmol, 4.00 equiv) and 2,6-lutidine (3.04 mL, 26.1 mmol, 2.0 equiv) in water (33 mL) and dioxane (98 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 3 h, then was partitioned between water and dichloromethane. The aqueous layer was further extracted with dichloromethane (1x), and the combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue was purified by silica gel flash-column chromatography (15% ethyl acetate-hexanes, grading to 70% ethyl acetate-hexanes) to afford aldehyde in quantitative yield. Calcd E/Z $[M+Na]^+$ $C_{19}H_{17}NO_4$: 346.1, Found: 346.0.

Step 4: benzyl 2-(2-hydroxyethyl)-4-oxo-3,4-dihydroquinoline-1(2H)-carboxylate

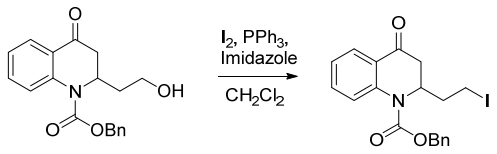


Sodium borohydride (1.27 g, 33.5 mmol, 3.0 equiv) was added to a solution of the aldehyde (11.2 mmol, 1 equiv) in methanol (112 mL) at 0 °C. After stirring at 0 °C for 25 min, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with saturated aqueous sodium chloride solution, and the washed solution was dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated.

The residue was dissolved in 1,2-dichloroethane (50 mL), and manganese dioxide (4.41 g, 50.7 mmol, 5 equiv) was added. The reaction mixture was heated to 65 °C for 2.5 h, and then the heating bath was removed. The cooled reaction mixture was filtered through celite, and the

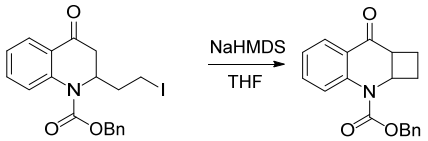
filtrate was concentrated to afford the primary alcohol benzyl 2-(2-hydroxyethyl)-4-oxo-3,4-dihydroquinoline-1(2H)-carboxylate, which was used in subsequent steps without further purification (~70% 3 steps). Calcd E/Z $[M+H]^+$ $C_{19}H_{19}NO_4$: 326.1, Found: 326.2.

Step 5: benzyl 2-(2-iodoethyl)-4-oxo-3,4-dihydroquinoline-1(2H)-carboxylate



Iodine (12.5 g, 49.4 mmol, 1.30 equiv) was added to a solution of the primary alcohol (38.0 mmol, 1 equiv), triphenylphosphine (13.0 g, 49.4 mmol, 1.30 equiv), and imidazole (6.47 g, 95.0 mmol, 1 equiv) in dichloromethane (380 mL) at 23 °C. After stirring for 15 minutes, the reaction mixture was partitioned between dichloromethane and water. The organic phase was dried over sodium sulfate, and the dried solution was filtered. The filtrate was concentrated, and the residue was purified by silica gel flash-column chromatography (hexanes, grading to 30% ethyl acetate-hexanes) to afford the desired product benzyl 2-(2-iodoethyl)-4-oxo-3,4-dihydroquinoline-1(2H)-carboxylate as a colorless oil (15.7 g, 95% yield). Calcd E/Z $[M+H]^+$ $C_{19}H_{18}INO_3$: 436.0, Found: 436.0.

Step 6: benzyl 8-oxo-2,2a,8,8a-tetrahydrocyclobuta[b]quinoline-3(1H)-carboxylate



intermediate **A**

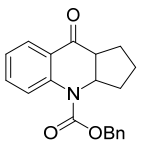
A solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran (1.0 M, 39.4 mL, 39.4 mmol, 1.10 equiv) was added to a solution of benzyl 2-(2-iodoethyl)-4-oxo-3,4-dihydroquinoline-1(2H)-carboxylate (35.8 mmol, 1 equiv) in tetrahydrofuran (716 mL) at –78 °C. The reaction mixture was stirred at –78 °C for 30 minutes, then the cooling bath was removed and the reaction mixture was allowed to warm. After stirring for 3 hours, NH_4Cl solution was added, and the

reaction mixture was concentrated by rotary evaporation to remove most of the tetrahydrofuran. The residue was partitioned between ethyl acetate and water. The organic phase was washed with saturated aqueous sodium chloride solution, and the washed solution was dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue was purified by silica gel flash-column chromatography (hexanes, grading to 40% ethyl acetate-hexanes) to afford the desired product benzyl 8-oxo-2,2a,8,8a-tetrahydrocyclobuta[b]quinoline-3(1H)-carboxylate (intermediate **A**) as a colorless oil (9.4 g, 85%). Calcd E/Z $[M+H]^+$ $C_{19}H_{17}NO_3$: 308.1, Found: 308.2.

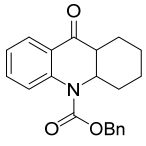
The racemic ketone was resolved with chiral HPLC to give enantiomerically pure isomers using an OJ-H, 4.6 x 250mm column eluting with an isocratic system of 15% w/ IPA in supercritical CO_2 at a flow rate of 3 mL/min. The fast eluting isomer (first peak) was determined to be the active isomer based on SAR information and was used for the preparation of all active analogs.

The ketone intermediate from step 6 can be converted to the final products using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol.

This procedure was also employed for the preparation of key intermediates of cyclopentyl (intermprocediate **B**) and cyclohexyl (intermediate **C**) fused tetrahedroquiniline analogs. The fast eluting isomer (first peak using above mentioned SFC conditions) was determined to be the active isomer based on SAR information and was used for the preparation of all active analogs.

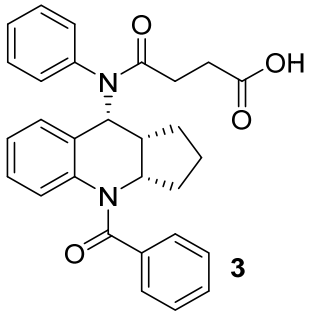


intermediate **B**



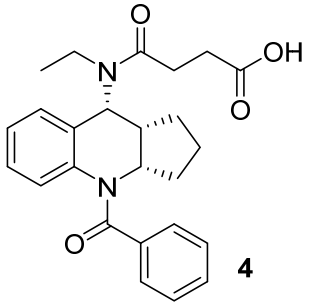
intermediate **C**

5.3 Experimental reagent details and spectra data of key compounds: *These compounds were prepared using the general procedure outlined above for **15c** with experimental descriptions. The purity of all final compounds was determined by LCMS to be >95% before submission for biological testing.*



3

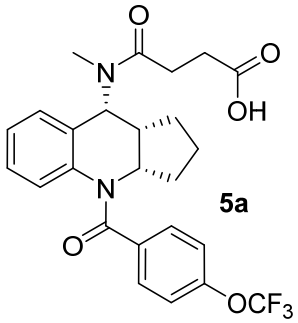
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that aniline was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol and benzyl chloride was used in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.47 (m, 2H), 7.42 (m, 3H), 7.33 (d, J = 12 Hz, 1H), 7.31 (d, J = 12 Hz, 2H), 7.26 (t, J = 12 Hz, 1H), 7.20 (t, J = 7.6 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.84 (t, J = 7.7 Hz, 1H), 6.40 (d, J = 12 Hz, 1H), 5.85 (d, J = 4.0 Hz, 1H), 5.09 (q, J = 7.5 Hz, 1H), 2.82 (m, 1H), 2.74 (m, 1H), 2.63 (m, 1H), 2.47 (m, 1H), 2.37 (m, 1H), 2.17 (m, 1H), 1.31 (m, 1H), 1.06 (m, 1H), 1.00 (m, 1H), 0.25 (m, 1H), -0.04 (m, 1H). Calcd E/Z $[M+H]^{+}$ for C29H28N2O4: 469.2, Found: 469.2 as observed in LCMS.



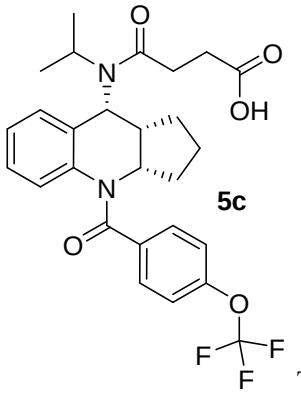
4

The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethylmine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol and benzoyl chloride was used in step 5. (mixture of two rotamers) ^{1}H NMR (600 MHz, Chloroform-d) δ 8.81 (br. s, 1H), 7.32 (t, J = 7.2 Hz), 7.28 (t, J = 7.2 Hz), (1H), 7.25 – 7.15 (m, 4H), 7.13 (t, J = 7.6 Hz), 7.04 (t, J = 7.6 Hz), (1H), 7.05 (d, J = 7.5 Hz), 6.90 (d, J = 7.5 Hz), (1H), 6.94 (t, J = 7.7 Hz), 6.86 (t, J = 7.7 Hz), (1H), 6.50 (br. d), 6.45 (br. d), (1H), 5.24 – 5.09 (m, 2H), 4.01 (dt, J = 13.8, 7.0 Hz), 3.81 (dq, J = 14.4, 7.0 Hz), (1H), 3.56 – 3.41 (m), 3.19 (dt, J = 14.0, 6.8 Hz), (1H), 3.05 (br. m), 2.97 (m), (2H), 2.83 (m, 2H), 2.71 (t, J = 5 Hz, 1H), 2.57 (dt, J = 16.5, 6.5 Hz), 2.49 – 2.35 (m), (2H), 1.84 (m), 1.79 (m), (1H), 1.59 – 1.47 (m,

1H), 1.41 (m, 1H), 1.39 (t, J = 6.9 Hz), 1.24 (t, J = 6.9 Hz), (3H), 0.94 (m, 1H). Calcd E/Z $[M+H]^+$ for C25H28N2O4: 421.2, Found: 421.2 as observed in LCMS.

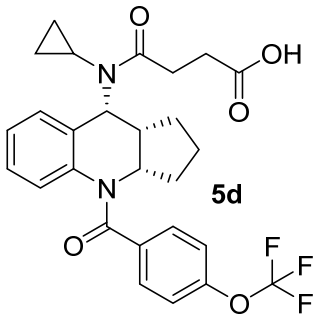


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that methylamine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. (mixture of two rotamers) ^{1}H NMR (600 MHz, Chloroform-d) δ 7.30 (d, J = 8.4 Hz, 2H), 7.15 (t, J = 6.0 Hz), 7.06 (t, J = 6.0 Hz), (1H), 7.02 (d, J = 8.0 Hz, 2H), 6.98 (t, J = 6.0 Hz), 6.88 (t, J = 6.0 Hz), (1H), 6.91 (d, J = 12 Hz), 6.48 (br. d), (1H), 6.40 (d, J = 12 Hz), 5.87 (br. s), (1H), 5.14 (br. m, 1H), 4.03 (dq, J = 13.9, 7.0 Hz), 3.81 (dq, J = 14.4, 7.0 Hz), (1H), 3.48 (dt, J = 15.6, 7.1 Hz), 3.18 (dd, J = 13.9, 7.0 Hz), (1H), 3.05 (br. dt), 2.96 (br. m), (1H), 2.95 (br. m), 2.88 (br. m), (1H), 2.84 (m, 3H), 2.80 (br. t, J = 6.0 Hz), 2.73 (br. t, J = 6.0 Hz), (1H), 2.54 (dt, J = 12, 6.0 Hz), 2.40 (m), (1H), 1.84 (br. m), 1.81 (br. m), (1H), 1.52 (m, 1 H), 1.40 (m, 2 H), 1.38 (t, J = 7.0 Hz), 1.26 (t, J = 7.0 Hz), (3H), 0.95 (m, 1H). Calcd E/Z $[M+H]^+$ for C26H27F3N2O5: 505.2, Found: 505.1 as observed in LCMS.

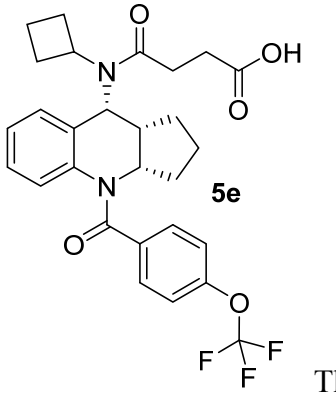


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that isopropyl amine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. (mixture of two rotamers) ^{1}H NMR (500 MHz, Chloroform-d)

δ 7.38-7.17 (m, 3H), 7.14-7.02 (m, 4H), 6.91 (br. t), 6.53 (br. d), (1H), 5.17 (br. m, 1H), 4.54 (br. s), 4.41 (br. s), (1H), 3.62 (m, 1H), 3.18 – 2.67 (m, 3H), 2.63 – 2.31 (m, 2H), 1.89 (m), 1.71 (m), (1H), 1.75 (d, J = 5.0 Hz), 1.45 (d, J = 5.0 Hz), (3H), 1.65-1.45 (m, 2H), 1.53 (d, J = 5.0 Hz), 1.30 (d, J = 5.0 Hz), (3H), 1.28 (m), 0.98 (m), (2H). Calcd E/Z $[M+H]^+$ for C27H29F3N2O5: 519.2, Found: 519.3 as observed in LCMS.

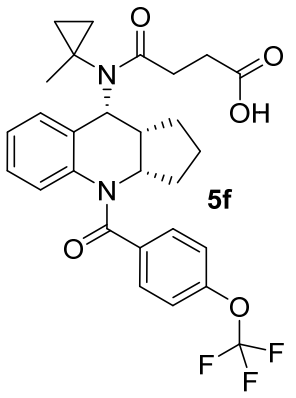


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.31 – 7.19 (m, 2H), 7.01 (m, 3H), 6.83 (m, 2H), 6.39 (d, J = 7.8 Hz, 1H), 5.71 (br. s, 1H), 5.12 (m, 1H), 3.07 (m, 2H), 2.87 – 2.70 (m, 3H), 2.42 (m, 1H), 2.00 (m, 1H), 1.54 (m, 1H), 1.38 (m, 2H), 1.24 (br. s, 1 H), 1.14 (m, 3H), 1.00 (m, 1H), 0.90 (m, 1H). Calcd E/Z $[M+H]^+$ for C27H27F3N2O5: 517.2, Found: 517.2 as observed in LCMS.

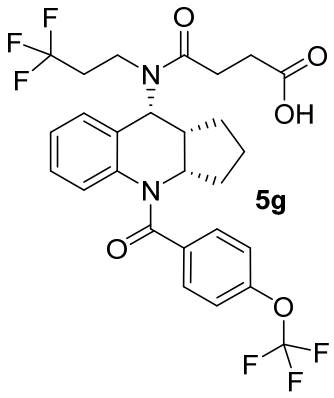


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that cyclobutyl amine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.39 – 6.80 (m, 8H), 5.12 (m, 1H), 5.01 (d, J = 4.2 Hz, 1H), 3.90 – 3.73 (m, 1H), 3.44 (m, 1H), 3.08 (m, 1H), 2.97 – 2.84 (m, 1H), 2.84 – 2.66 (m, 3H), 2.61 (m, 1H), 2.49 (m, 1H), 2.45 – 2.17 (m, 2H), 2.00 (m,

2H), 1.81 (m 1H), 1.68 – 1.51 (m, 2H), 1.37 (m, 1H), 1.30 – 1.18 (m, 1H), 1.16 (m 1H), 1.02 (m 1H). Calcd E/Z $[M+Na]^+$ for C28H29F3N2O5: 552.2, Found: 552.5 as observed in LCMS.

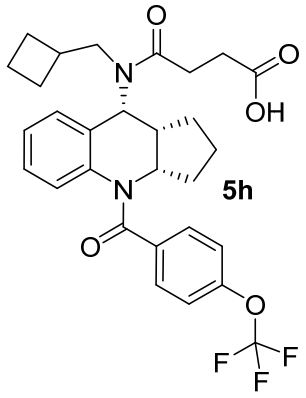


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that 1-methylcyclopropan-1-amine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.22 (d, J = 5.0 Hz, 2H), 7.06 (m, 3H), 6.89 (d, J = 5.0 Hz, 2H), 6.44 (d, J = 7.9 Hz, 1H), 5.14 (m, 1H), 3.18 (m, 2H), 3.06 – 2.73 (m, 3H), 2.46 (m, 1H), 2.12 (m, 1H), 1.73 (m, 1H), 1.62 (s, 3H), 1.37 (m, 3H), 1.24-1.03 (m, 3H), 0.81 (m, 2 H). Calcd E/Z $[M+H]^+$ for C28H29F3N2O5: 531.2, Found: 531.3 as observed in LCMS.

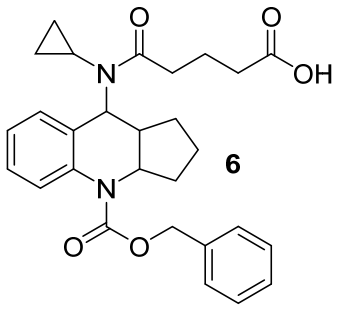


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that 3,3,3-trifluoropropan-1-amine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.34-7.18 (m, 3 H), 7.11 (d, J = 10 Hz, 2 H), 7.05 (m, 2 H), 6.54 (d, J = 10 Hz, 1H), 5.22 (d, J = 5.0 Hz, 1 H), 5.18 (m, 1H), 4.23 (td, J = 12.8, 4.7 Hz, 1H), 3.42 (td, J = 12.8, 4.4 Hz, 1H), 3.05 (m, 1H), 2.98 – 2.83 (m, 2H), 2.77 (dt, J = 12.8, 4.4 Hz, 2H), 2.57 (dt, J = 15, 5.0 Hz, 1H), 2.49

(m, 1H), 2.30 (m, 1H), 1.84 (m, 1H), 1.48 (m, 3H), 1.01 (m, 1H). Calcd E/Z $[M+H]^+$ for C27H26F6N2O5: 573.2, Found: 573.3 as observed in LCMS.

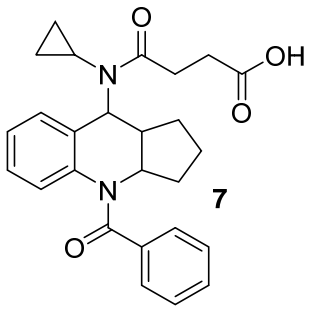


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that and cyclobutylmethyl amine was used in step 3, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. (mixture of two rotamers) ^{1}H NMR (500 MHz, Chloroform-d) δ 7.32 (d, J = 10 Hz, 2H), 7.18 (t, J = 5.0 Hz), 7.11 (t, J = 5.0 Hz) (1H), 7.10 (t, J = 5.0 Hz), 7.02 (t, J = 5.0 Hz), (1H), 7.06 (d, J = 10 Hz, 2H), 6.92 (br. t, J = 5.0 Hz, 1H), 6.51 (br. d, J = 10 Hz), 6.45 (d, J = 10 Hz), (1H), 5.14 (d, J = 11.1 Hz, 1H), 4.06 (dd, J = 14.1, 8.2 Hz), 3.74 (dd, J = 14.1, 8.2 Hz), (1H), 3.51 (dd, J = 16.0, 5.3 Hz), 3.29 (dd, J = 14.1, 5.1 Hz), (1H), 3.09 (m), 3.03 (m), (1H), 2.98 (m, 1H), 2.91 (m, 1H), 2.82 (m, 1H), 2.75 (t, J = 5.0 Hz, 1H), 2.65-2.38 (m, 2H), 2.30 – 1.89 (m, 7H), 1.85 (m, 2H), 1.78 (m), 1.71 (m), (1H), 1.46 (m, 1H), 0.92 (m, 1H). Calcd E/Z $[M+H]^+$ for C29H31F3N2O5: 545.2, Found: 545.3 as observed in LCMS.



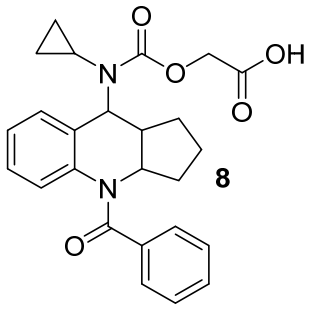
The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was not deprotected, and ethyl 5-chloro-5-oxopentanoate was used in step 4. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.44 – 7.26 (m, 6H), 7.18 – 7.10 (m, 1H), 7.04 – 6.95 (m, 1H), 6.75 –

6.64 (m, 1H), 5.25 (d, J = 12.2 Hz, 2H), 5.18 – 5.09 (m, 1H), 4.87 – 4.80 (m, 1H), 3.09 – 2.75 (m, 3H), 2.76 – 2.63 (m, 1H), 2.56 – 2.28 (m, 2H), 2.18 – 1.80 (m, 4H), 1.61 – 1.29 (m, 3H), 1.27 – 1.11 (m, 1H), 1.08 – 0.98 (m, 2H), 0.93 – 0.75 (m, 2H). Calcd E/Z $[M+H]^+$ for C28H32N2O5: 477.2, Found: 477.2 as observed in LCMS.



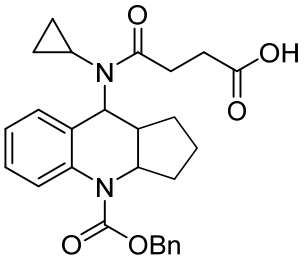
7

The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and benzoyl chloride was used in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.27 (t, *J* = 7.2 Hz, 1H), 7.18 (m, 4H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.82 (t, J = 7.6 Hz, 1H), 6.78 (br. d, 1H), 6.41 (d, *J* = 7.9 Hz, 1H), 5.67 (br. s, 1H), 5.14 (m, 1H), 3.10 (m, 2H), 3.03 (m, 1H), 2.83 (m, 1H), 2.80 (m, 2H), 2.45 – 2.37 (m, 1H), 2.01 – 1.92 (m, 1H), 1.54 (m, 1H), 1.43 – 1.31 (m, 2H), 1.23 – 1.07 (m, 3H), 0.99 (m, 1H), 0.93 (m, 1H). Calcd E/Z $[M+H]^+$ for C26H28N2O4: 433.2, Found: 433.2 as observed in LCMS.



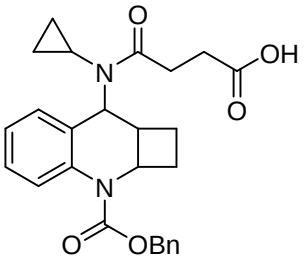
8

The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 2-((chlorocarbonyl)oxy)acetate was used in step 4, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol and benzoyl chloride was used in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.24 (s, 6H), 7.05 (m, 1H), 6.85 (m, 1H), 6.43 (m, 1H), 5.34 (br. s, 1H), 5.14 (br. s, 1H), 4.67 – 4.13 (m, 2H), 3.15 (br. dd, 1H), 2.68 (br. m, 1H), 2.34 (br. dd, 1H), 1.97 (br. m, 1H), 1.44 (br. s, 2H), 1.36 (br. m, 1H), 1.09 (br. m, 2H), 0.99 (m, 2H) 0.87 (br t., 1H). Calcd E/Z $[M+H]^+$ for C25H26N2O5: 435.2, Found: 435.2 as observed in LCMS.



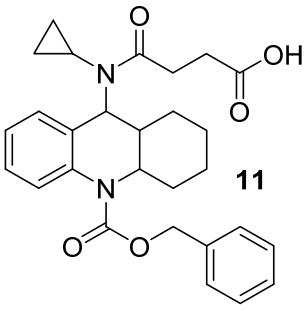
9

The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was not deprotected. Calcd E/Z $[M+H]^+$ for C27H30N2O5: 463.2, Found: 463.3 as observed in LCMS.



10

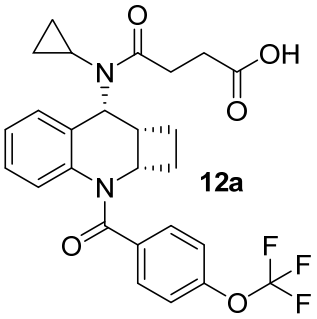
The ketone intermediate **A** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was not deprotected. Calcd E/Z $[M+H]^+$ for C26H28N2O5: 449.2, Found: 449.2 as observed in LCMS.



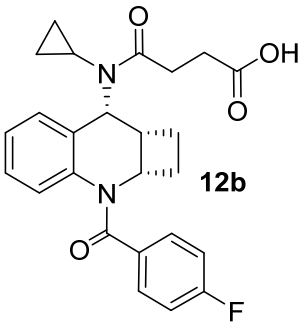
11

The ketone intermediate **C** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was not deprotected. ^{1}H NMR (600 MHz, DMSO-d6) δ 11.99 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.33 (m, 4H), 7.30 – 7.24 (m, 1H), 7.11 (t, J = 7.7 Hz, 1H), 6.95 (t, J = 7.5 Hz, 1H), 6.76 – 6.69 (m, 1H), 5.16 (d, J = 12.6 Hz, 1H), 5.07 (d, J = 12.6 Hz, 1H), 4.21 (s, 1H), 2.87 (m, 2H), 2.74 (s, 1H), 2.63 (s, 1H), 2.46 (m, 2H), 2.29 (m, 1H), 1.92 (bs, 1H), 1.47 (m, 1H), 1.40 (m, 1H), 1.31

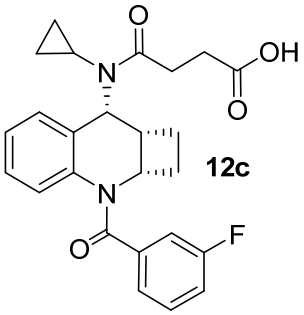
(m, 1H), 1.20 (m, 2H), 1.11 (m, 1H), 0.99 (m, 1H), 0.91 (m, 2H), 0.85 – 0.77 (m, 2H), 0.53 (m, 1H). Calcd E/Z $[M+H]^+$ for C28H32N2O5: 477.2, Found: 477.2 as observed in LCMS.



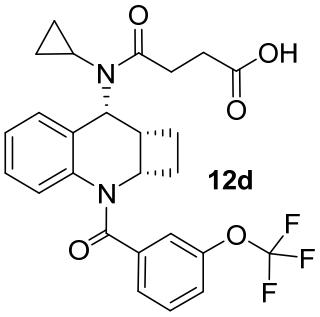
The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.31 (d, J = 8.3 Hz, 2H), 7.12 – 6.99 (m, 3H), 6.93 (s, 1H), 6.80 (d, J = 7.5 Hz, 1H), 6.57 (m, 1H), 5.56 (s, 1H), 5.24 (s, 1H), 3.68 (s, 1H), 3.13 (m, 1H), 3.08 (m, 1H), 2.78 (m, 2H), 2.63 (m, 1H), 2.07 (m, 1H), 1.74 (m, 2H), 1.53 (m, 1 H), 1.26 (m, 2H), 1.07 (m, 1H), 0.70 (m, 1H). Calcd E/Z $[M+H]^+$ for C26H25F3N2O5: 503.2, Found: 503.1 as observed in LCMS.



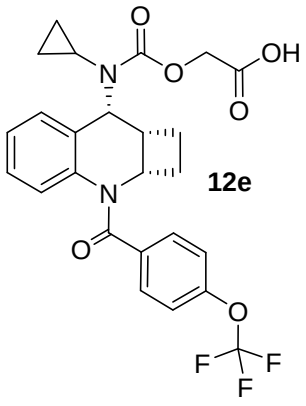
The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and 4-fluorobenzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.36 (dd, J = 8.3, 5.3 Hz, 2H), 7.13 (t, J = 7.5 Hz, 1H), 6.97 (m, 3H), 6.88 (d, J = 7.6 Hz, 1H), 6.63 (d, J = 7.9 Hz, 1H), 5.64 (s, 1H), 5.29 (br. t, 1H), 3.74 (br. m, 1H), 3.23 – 3.01 (br. dd, 2H), 2.85 (t, J = 6.4 Hz, 2H), 2.69 (m, 2H), 2.13 (br. m, 1H), 1.81 (m, 2H), 1.13 (m, 1H), 1.02 (m, 1H), 0.94 (m, 1H), 0.76 (m, 1H). Calcd E/Z $[M+H]^+$ for C25H25FN2O4: 437.2, Found: 437.0 as observed in LCMS.



The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and 3-fluorobenzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.22 (br. d, 1H), 7.18 (br. d, 1H), 7.15 (br. t, 1H), 7.06 (br. t, 1H), 6.98 (m, 2H), 6.89 (br. d, J = 7.5 Hz, 1H), 6.68 (br. s, 1H), 5.65 (br. s, 1H), 5.29 (br. m, 1H), 3.73 (br. m, 1H), 3.12 (br. dd, 2H), 2.85 (t, J = 6.3 Hz, 2H), 2.69 (br. m, 2H), 2.12 (m, 1H), 1.82 (m, 2H), 1.12 (br. s, 1H), 1.02 (br. s, 1H), 0.94 (br. s, 1H), 0.75 (br. s, 1H). Calcd E/Z $[M+H]^{+}$ for C25H25FN2O4: 437.2, Found: 437.0 as observed in LCMS.

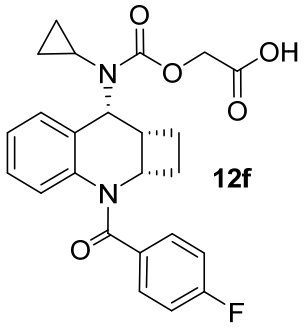


The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and 3-trifluoromethoxybenzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37 (br. s, 1H), 7.22 (br. m, 2H), 7.14 (br. m, 2H), 6.99 (br. t, 1H), 6.90 (br. d, 1H), 6.65 (br. s, 1H), 5.66 (s, 1H), 5.40 – 5.17 (br. m, 1H), 3.74 (br. m, 1H), 3.13 (br. dd, 2H), 2.85 (br. m, 2H), 2.69 (br. m, 2H), 2.12 (br. m, 1H), 1.82 (br. m, 2H), 1.12 (br. m, 1H), 1.01 (br. m, 1H), 0.93 (br. m, 1H), 0.76 (br. m, 1H). Calcd E/Z $[M+H]^{+}$ for C26H25F3N2O5: 503.2, Found: 503.0 as observed in LCMS.



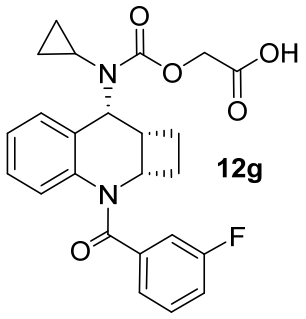
12e

The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 2-((chlorocarbonyl)oxy)acetate was used in step 4, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.35 (d, J = 8.3 Hz, 2H), 7.16 (m, 2H), 7.07 (d, J = 8.2 Hz, 2H), 7.00 (m, 1H), 6.64 (bs, 1H), 5.31 (br. m, 2H), 4.69 (br. m, 2H), 3.77 (m, 1H), 2.60 (br. m, 1H), 2.49 (br. m, 1H), 2.10 (br. m, 1H), 1.74 (br. m, 2H), 0.99 (br. m, 1H), 0.91 (br. m, 2H), 0.72 (br. m, 1H). Calcd E/Z $[M+H]^{+}$ for C25H23F3N2O6: 505.2, Found: 505.2 as observed in LCMS.

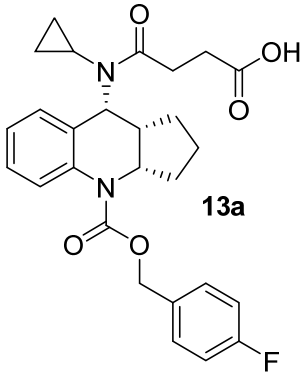


12f

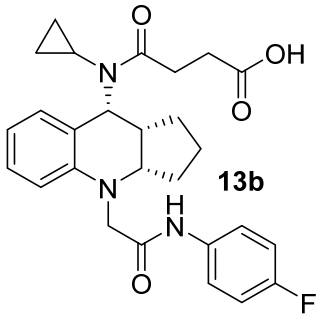
The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 2-((chlorocarbonyl)oxy)acetate was used in step 4, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol and 4-fluorobenzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.31 (dd, J = 8.5, 5.3 Hz, 2H), 7.15 (m, 2H), 6.99 (t, J = 7.7 Hz, 1H), 6.91 (t, J = 8.4 Hz, 2H), 6.63 (s, 1H), 5.29 (br. s, 1H), 5.08 (br. m, 1H), 4.80 (br. m, 1H), 4.69 (br. m, 1H), 3.76 (br. m, 1H), 2.59 (br. m, 1H), 2.49 (br. m, 1H), 2.09 (br. m, 1H), 1.73 (br. m, 2H), 0.98 (br. m, 1H), 0.91 (br. m, 2 H), 0.73 (br. m, 1H). Calcd E/Z $[M+H]^{+}$ for C24H23FN2O5: 439.3, Found: 439.2 as observed in LCMS.



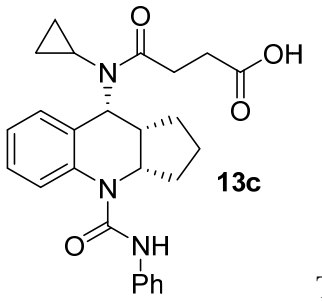
The ketone intermediate **A** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 2-((chlorocarbonyl)oxy)acetate was used in step 4, the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and 3-fluorobenzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.22 – 7.09 (m, 4H), 7.04 - 6.90 (m, 3H), 6.67 (s, 1H), 5.29 (br. s, 2H), 4.71 (br. d, 1H), 4.36 (br. m, 1H), 3.76 (br. m, 1H), 2.60 (br. m, 1H), 2.49 (br. m, 1H), 2.11 (m, 1H), 1.74 (s, 2H), 1.11 – 0.82 (br. m, 3 H), 0.73 (br. m, 1H).Calcd E/Z $[M+H]^{+}$ for C24H23FN2O5: 439.3, Found: 439.2 as observed in LCMS.



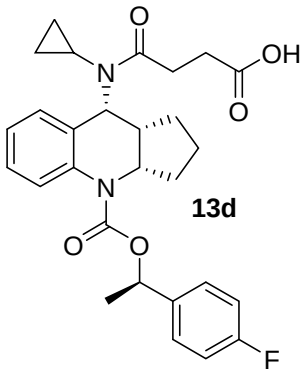
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and triphosgene and 4-fluorobenzyl alcohol were used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.64 (d, J = 7.9 Hz, 2H), 7.48 (d, J = 7.9 Hz, 2H), 7.41 (d, J = 7.8 Hz, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 6.77 (d, J = 7.6 Hz, 1H), 5.35 – 5.28 (m, 1H), 5.24 (d, J = 13.5 Hz, 1H), 4.89 (m, 1H), 3.09 (m, 2H), 2.94 – 2.87 (m, 1H), 2.83 – 2.74 (m, 3H), 2.18 (m, 1H), 1.98 – 1.87 (m, 1H), 1.55 (m, 2H), 1.42 (m, 1H), 1.22 (m, 1H), 1.18 (m, 2H), 0.97 (m, 1H), 0.91 (m, 1H). Calcd E/Z $[M+H]^{+}$ for C27H29FN2O5: 481.2, Found: 480.8 as observed in LCMS.



The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was first deprotected through hydrogenation using Pd on C in ethanol, and then treated with benzyl 2-bromoacetate followed with debenzylation through hydrogenation and amide coupling with 4-fluoroaniline in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 9.59 – 9.17 (br. s, 1H), 8.69 – 7.99 (br. s, 1H), 7.55 (m, 2H), 7.10 (t, J = 10 Hz, 1H), 7.00 (t, J = 8.4 Hz, 2H), 6.83 (br. s, 1 H) 6.75 (t, J = 7.3 Hz, 1H), 6.52 (d, J = 8.2 Hz, 1H), 4.04 – 3.84 (m, 3H), 3.07 (m, 2H), 2.74 (br. m, 1H), 2.65 (m, 1H), 1.98 – 1.70 (m, 8H), 1.10 (m, 2H), 1.06 (m, 2H), 0.96 (m, 1H). Calcd E/Z $[M+H]^+$ for C27H30FN3O4: 480.2, Found: 479.7 as observed in LCMS.

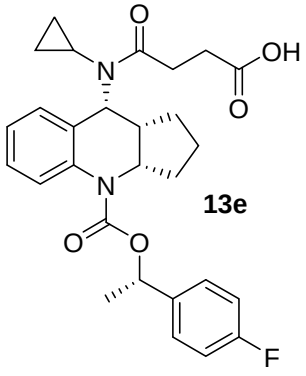


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and followed with triphosgene and aniline to form the urea in step 5. Calcd E/Z $[M+H]^+$ for C26H29N3O4: 448.2, Found: 448.2 as observed in LCMS.



13d

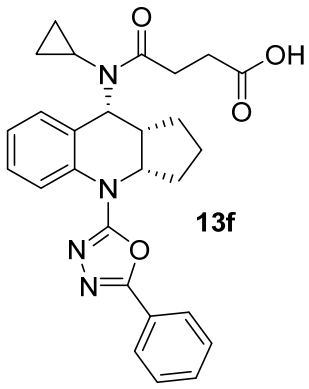
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and followed with triphosgene and 1-(4-fluorophenyl)ethan-1-ol to form the carbamate in step 5, and chiral SFC to give peak 1. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.36 (m, 3H), 7.16 (t, J = 5.0 Hz, 1H), 7.07 (m, 3 H), 6.74 (br. d, J = 5.0 Hz, 1H), 5.90 (t, J = 5.0 Hz, 1H), 4.83 (m, 1H), 3.09 (m, 2H), 2.88 (m, 1H), 2.78 (m, 3H), 2.16 (m, 1H), 1.90 (m, 1H), 1.55 (d, J = 5 Hz, 3H), 1.49 (m, 2H), 1.42 (m, 1H), 1.24-1.08 (m, 4H), 0.97 (m, 1H), 0.89 (m, 1H). Calcd E/Z $[M+Na]^{+}$ for C28H31FN2O5: 517.2, Found: 516.8 as observed in LCMS.



13e

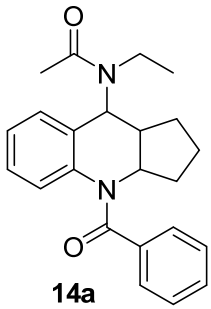
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and followed with triphosgene and 1-(4-fluorophenyl)ethan-1-ol to form the carbamate in step 5, and chiral SFC to give peak 2. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.44 (d, J = 5.0 Hz, 1H), 7.27 (br. d, 2H), 7.22 (t, J = 5.0 Hz, 1H), 7.07 (t, J = 5.0 Hz, 1H), 7.02 (t, J = 10 Hz, 2H), 6.76 (br. d, J = 5.0 Hz, 1H), 5.88 (t, J = 5.0 Hz, 1H), 4.86 (m, 1H), 3.09 (m, 2H), 2.88 (m, 1H), 2.78 (m, 3H), 2.16 (m, 1H), 1.90 (m, 1H), 1.58 (d, J = 5 Hz, 3H), 1.52 (m, 2H), 1.40 (m, 1H), 1.24-1.08 (m, 4H), 0.97 (m,

1H), 0.89 (m, 1H). Calcd E/Z $[M+Na]^+$ for C28H31FN2O5: 517.2, Found: 516.8 as observed in LCMS.



13f

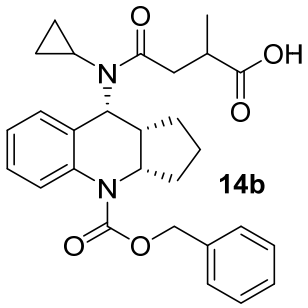
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that the Cbz group was deprotected through hydrogenation using Pd on C in ethanol, and followed with triphosgene and benzohydrazide and Burgess reagent to give the oxadiazole in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.86 (d, J = 7.4 Hz, 2H), 7.46 (m, 4H), 7.29 (t, J = 7.6 Hz, 1H), 7.13 (t, J = 7.5 Hz, 1H), 6.84 (s, 1H), 4.89 (s, 1H), 3.06 (br. m, 3H), 2.80 (br. m, 1H), 2.75 (m, 3H), 2.27 (s, 1H), 1.99 (s, 1H), 1.82 (s, 1H), 1.54 (br. s, 2H), 1.32 (s, 1H), 1.10 (br. d, J = 30 Hz, 2H), 0.94 (br. d, 2H). Calcd E/Z $[M+H]^+$ for C27H28N4O4: 473.2, Found: 473.2 as observed in LCMS.



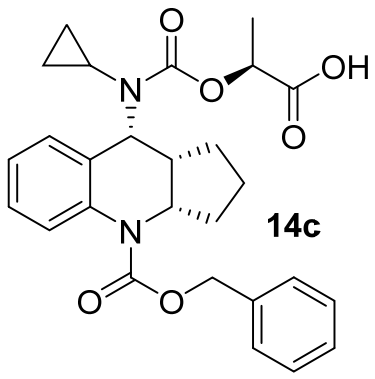
14a

The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that ethylamine was used in step 3, acetyl chloride was used in step 4, and the Cbz group was deprotected through hydrogenation using Pd on C in ethanol and benzoyl chloride was used in step 5. (mixture of two rotamers) ^{1}H NMR (600 MHz, Chloroform-d) δ 7.32 – 7.22 (m, 2H), 7.17 (dq, J = 15.0, 7.6 Hz, 3H), 7.09 (t, J = 7.4 Hz), 7.01 (t, J = 7.5 Hz), (1H), 7.05 (d, J = 7.4 Hz), 6.92 (d, J = 7.4 Hz),

(1H), 6.91 (dd, J = 11.6, 7.6 Hz), 6.83 (t, J = 7.7 Hz), (1H), 6.46 (d, J = 7.8 Hz), 6.42 (d, J = 7.9 Hz) (1H), 5.99 (d, J = 3.7 Hz), 5.07 (d, J = 3.7 Hz), (1H), 5.15 (dq, J = 13.5, 5.6 Hz, 1H), 4.04 (dq, J = 14.0, 7.0 Hz), 3.72 (dq, J = 14.2, 7.0 Hz), (1H), 3.42 (dq, J = 14.4, 7.0 Hz), 3.12 (dq, J = 13.8, 6.9 Hz), (1H), 3.04 (tdd, J = 10.6, 7.2, 3.6 Hz), 2.92 (tdd, J = 10.6, 7.2, 3.6 Hz), (1H), 2.42 (dt, J = 22.3, 8.4 Hz, 1H), 2.32 (s), 2.15 (s), (3H), 1.84 (m), 1.81 (m), (1H), 1.64 – 1.46 (m, 1H), 1.44 – 1.35 (m, 2H), 1.33 (t, J = 7.1 Hz), 1.24 (q, J = 6.9 Hz), (3H), 0.99 – 0.89 (m, 1H). Calcd E/Z $[M+H]^+$ for C23H26N2O2: 363.2, Found: 363.2 as observed in LCMS.

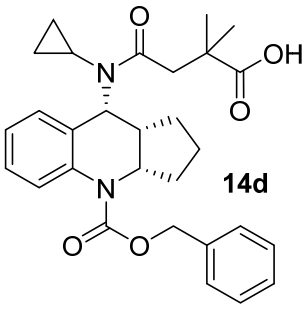


The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 4-chloro-2-methyl-4-oxobutanoate was used in step 4, and the Cbz group was not deprotected in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.40 – 7.27 (m, 6H), 7.16 (t, J = 7.6 Hz, 1H), 7.02 (m, 1H), 6.68 (m, 1H), 5.25 (d, J = 12.6 Hz, 1H), 5.14 (m, 1H), 4.84 (s, 1H), 3.10 (m, 2H), 2.94 – 2.78 (m, 4H), 2.74 (m, 1H), 2.13 (m, 1H), 1.85 (m, 1H), 1.49 (m, 2H), 1.34 (m, 1H), 1.31 (d, J = 10 Hz, 3H), 1.18 (m, 1H), 1.07 (m, 2H), 0.91 (m, 1H). Calcd E/Z $[M+H]^+$ for C28H32N2O5: 477.2, Found: 477.2 as observed in LCMS.



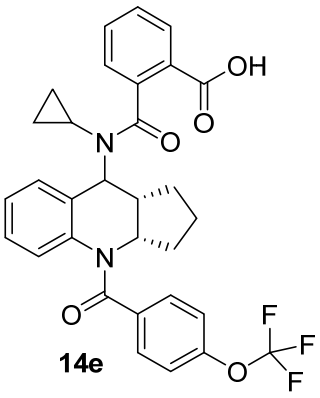
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that triphosgen and methyl (S)-2-hydroxypropanoate was used in step 4, and the Cbz group was not deprotected in step 5. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 7.42 (m, 6H); 7.25 (m, 1H); 7.24

(m 1H); 7.17 (m, 1H); 7.04 (m, 1H); 5.32 (d, *J* = 13Hz, 1H); 5.22 (d, *J* = 13Hz, 1H); 5.11 (q, *J* = 7 Hz, 1H); 3.01 (m, 1H); 2.71 (m 1H); 2.15 (m, 1H); 1.94 (m, 1H); 1.59 (m, 3H); 1.45 (m, 2H); 1.32 (m, 1H); 1.22~1.14 (m, 2H); 1.11~ 0.80 (m, 4H). Calcd E/Z $[M+Na]^+$ for C27H30N2O6: 501.2, Found: 500.7 as observed in LCMS.



14d

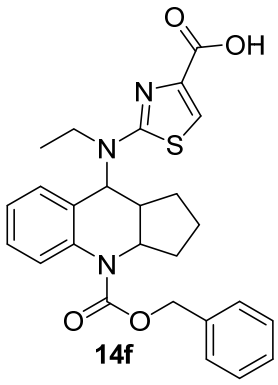
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that ethyl 4-chloro-2,2-dimethyl-4-oxobutanoate was used in step 4, and the Cbz group was not deprotected in step 5. ^{1}H NMR (600 MHz, Chloroform-d) δ 7.40 – 7.26 (m, 6H), 7.14 (t, J = 7.8 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.68 (s, 1H), 5.24 (d, J = 12.0 Hz, 1H), 5.12 (d, J = 12.0 Hz, 1H), 4.91 (br. m, 2H), 4.83 (m, 1H), 3.03 (m, 2H), 2.83 (m, 1H), 2.71 (m, 1H), 2.11 (m, 1H), 1.84 (m, 1H), 1.49 (m, 1H), 1.33 (s, 6H), 1.17 (m, 2H), 1.07 (m, 2H), 0.89 (m, 2H). Calcd E/Z $[M+H]^+$ for C29H34N2O5: 491.2, Found: 491.3 as observed in LCMS.



14e

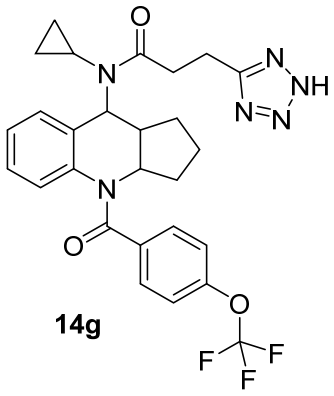
The ketone intermediate **B** (peak 1 from SFC separation) was converted to the final product using a similar procedure in Example **15c** from steps 3 to 6 except that methyl 2-(chlorocarbonyl)benzoate was used in step 4. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.17 (d, J = 7.8 Hz, 1H), 7.72 (s, 1H), 7.57 (q, J = 7.6 Hz, 2H), 7.41 (m, 3H), 7.18 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 8.3 Hz, 2H), 6.96 (t, J = 7.7 Hz, 1H), 6.50 (d, J = 7.9 Hz, 1H), 6.01 (br. m, 1H), 5.34 (d, J = 13.3 Hz, 1H), 3.35 (s, 2H), 2.73 (br. m, 1H), 2.49 (br. m, 1H), 2.10 (s, 2H), 1.63 (br.

m, 1H), 1.51 (br. m, 1H), 1.23 (br. m, 1H), 1.03 (br. m, 1H), 0.62 (br. m, 1H), 0.51 (br. m, 1H). Calcd E/Z $[M+H]^+$ for C31H27F3N2O5: 565.2, Found: 564.6 as observed in LCMS.



14f

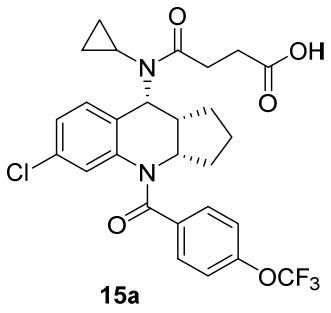
The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that ammonia was used in step 3 to give primary amine which was reacted with ethyl 2-bromo-1,3-thiazole-4-carboxylate using tris(dibenzylideneacetone)dipalladium followed with LHMDS and iodoethan in step 4, and the Cbz group was not deprotected in step 5. (mixture of two rotamers) ^{1}H NMR (600 MHz, Chloroform-d) δ 8.83 (br. s, 1H), 7.32 (t, J = 7.2 Hz), (1H), 7.26-7.15 (m, 4H), 7.13 (t, J = 7.6 Hz, 1H), 7.05 (t, J = 7.6 Hz), (1H), 7.03 (d, J = 7.5 Hz), 6.90 (d, J = 7.5 Hz), (1H), 6.94 (t, J = 7.7 Hz), 6.86 (t, J = 7.7 Hz), (1H), 6.50 (br. m), 6.45 (br. d, J = 6.0 Hz), (1H), 5.88 (br. s), 5.18 (br. s), (1H), 5.16 (br. m, 1H), 4.01 (dt, J = 13.8, 7.0 Hz), 3.81 (dq, J = 14.4, 7.0 Hz), (1H), 3.47 (m), 3.18 (m), (1H), 3.11 – 2.77 (m, 2H), 2.72 (t, J = 6.5 Hz, 1H), 2.88 (m), 2.57 (dt, J = 16.5, 6.5 Hz), (1H), 2.49 – 2.35 (m, 1H), 1.84 (br. m), 1.79 (br. m), (1H), 1.52 (br. m, 1 H), 1.40 (m, 1H), 1.39 (t, J = 6.9 Hz), 1.24 (t, J = 6.9 Hz), (3H), 0.94 (br. m, 1H). Calcd E/Z $[M+H]^+$ for C26H27N3O4S: 478.2, Found: 478.1 as observed in LCMS.



14g

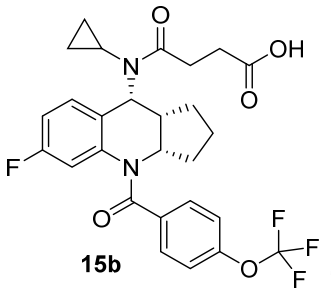
The ketone intermediate **B** was converted to the final product (all cis racemic) using a similar procedure in Example **15c** from steps 3 to 6 except that 3-

cyanopropanoyl chloride was used in step 4, and trimethylsilylazide and dibutyltinoxide were used instead of hydrolysis in step 6. Calcd E/Z $[M+H]^+$ for C27H27F3N6O3: 541.2, Found: 541.3 as observed in LCMS.



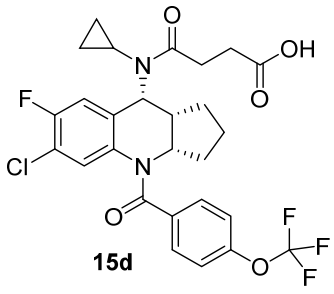
15a

The final product was prepared using a similar procedure in Example **15c** except that 3-chloroaniline was used in step 1. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.32 (m, 2H), 7.08 (d, J = 10 Hz, 2H), 7.03 (d, J = 10 Hz, 1H), 6.97 (s, 1H), 6.75 (br. d, J = 8.2 Hz, 1H), 5.03 (m, 1H), 3.16 – 3.07 (m, 2H), 2.94 (m, 1H), 2.77 (m, 2H), 2.45 (m, 1H), 2.29 (m, 1H), 1.99 (m, 2H), 1.97 (m, 2H), 1.53 (m, 1H), 1.37 (m, 1H), 1.17 (m, 2H), 1.07 (m, 1H), 0.97 (m, 1H). Calcd E/Z $[M+H]^+$ for C27H26ClF3N2O5: 552.2, Found: 552.0 as observed in LCMS.

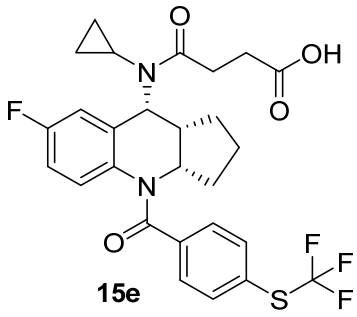


15b

The final product was prepared using a similar procedure in Example **15c** except that 3-fluoroaniline was used in step 1. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37 – 7.24 (m, 2H), 7.09 (d, J = 8.3 Hz, 2H), 6.90 – 6.68 (m, 2H), 6.18 (dd, J = 9.2, 2.3 Hz, 1H), 5.74 – 5.49 (m, 1H), 5.11 (ddd, J = 9.6, 7.3, 5.5 Hz, 1H), 3.07 (m, 3H), 2.89 – 2.74 (m, 3H), 2.44 (m, 1H), 2.08 – 1.96 (m, 1H), 1.57 (m, 1H), 1.41 (m, 2H), 1.20 (m, 2H), 1.12 (m, 1H), 0.98 (m 2H). Calcd E/Z $[M+H]^+$ for C27H26F4N2O5: 535.2, Found: 534.8 as observed in LCMS.

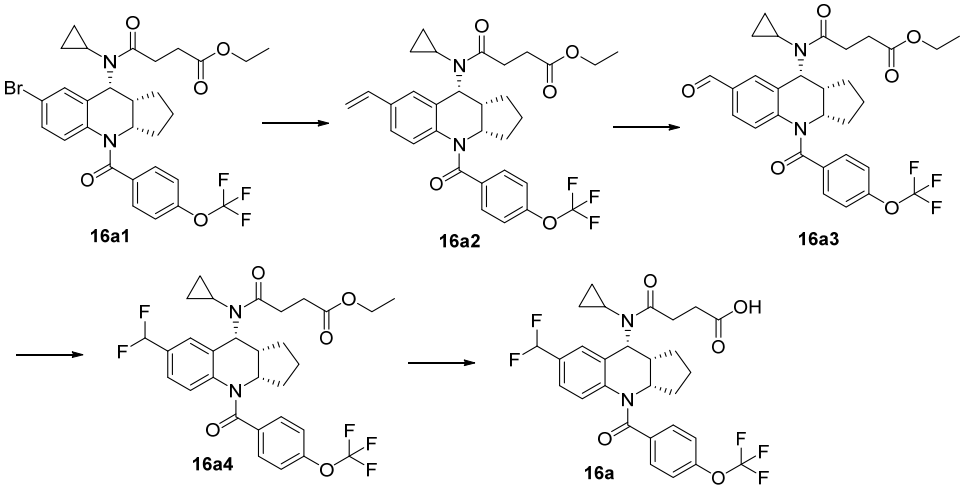


The final product was prepared using a similar procedure in Example **15c** except that 3-chloro-4-fluoroaniline was used in step 1. Calcd E/Z $[M+H]^+$ for C27H25ClF4N2O5: 569.1, Found: 568.8 as observed in LCMS.



The final product was prepared using a similar procedure in Example **15c** except that 4-((trifluoromethyl)thio)benzoyl chloride was used in step 5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.53 (d, J = 8.0 Hz, 2H), 7.33 – 7.22 (d, J = 8.0 Hz, 2H), 6.68 – 6.47 (m, 2H), 6.38 (br. s, 1H), 5.62 (br. s, 1H), 5.18 (br. dd, 1H), 3.10 (m, 3H), 2.88 (br. m, 1H), 2.83 (m, 2H), 2.41 (m, 1H), 2.12 – 1.95 (m, 1H), 1.58 (m, 1H), 1.40 (br. m, 2H), 1.23 (br. m, 1H), 1.15 (br. m, 2H), 1.02 (s, 2H). Calcd E/Z $[M+H]^+$ for C27H26F4N2O4S: 551.2, Found: 551.0 as observed in LCMS.

*Preparation of compound **16a.***



Step 1: ethyl 4-(cyclopropyl((3aS,9R,9aR)-4-(4-(trifluoromethoxy)benzoyl)-7-vinyl-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoate

Enantiopure compound **16a1** (180 mg, 0.289 mmol, prepared using a similar procedure of compound **15c** from steps 1-5 except that 4-bromoaniline was used in step 1.), 2,6-di-tert-butyl-4-methylphenol (6.36 mg, 0.029 mmol) and palladium tetrakis (16.7 mg, 0.014 mmol) , tributyl(vinyl)stannane (110 mg, 0.346 mmol) and lithium chloride (36.7 mg, 0.866 mmol) were mixed in a microwave vial, degassed and refilled with nitrogen (3 times). The vial was placed in an oil bath at 100 °C overnight. The mixture was diluted with ethyl acetate (15 mL) and water (6 mL). The mixture was filtered through Celite®, and the filtrate was separated. The organic layer was washed with brine (1 x 5 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel silica gel (Si; 40 g prepacked), eluting with EtOAc/isohexane = 1:3 to give **16a2** (120 mg, 72% yield,) as a white foam. Calcd E/Z $[M+H]^+$ for C31H33F3N2O5: 571.2, Found: 571.0.

Step 2: ethyl 4-(cyclopropyl((3aS,9R,9aR)-7-formyl-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoate

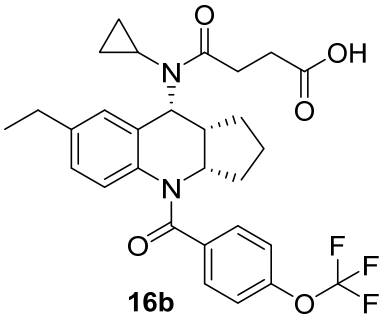
Ozone was bubbled into a stirred, cooled -78 °C mixture of **16a2** (235 mg, 0.412 mmol) in DCM (2 mL) and MeOH (1.000 ml). The mixture was stirred at -78 °C for 15 min. until the mixture turned blue. Oxygen was bubbled in to remove the excess ozone, until the blue color disappeared. Dimethyl sulfide (0.2 ml, 2.70 mmol) was added, and the mixture was kept stirring at room temperature for 1 h. The mixture was diluted with DCM (10 mL), washed with water (5 mL), dried $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to obtain a white foam. The residue was purified by column chromatography on silica gel (Si; 40 g prepacked), eluting with EtOAc/isohexane = 1:1 to give **16a3** (184 mg, 0.321 mmol, 78 % yield,) as a white foam. Calcd E/Z $[M+H]^+$ for C30H31F3N2O6: 573.2, Found: 573.0.

Step 3: ethyl 4-(cyclopropyl((3aS,9R,9aR)-7-(difluoromethyl)-4-(4-(trifluoromethoxy)benzoyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]quinolin-9-yl)amino)-4-oxobutanoate

Deoxo-fluor (bis(2-methoxyethyl)aminosulfur trifluoride) (0.077 mL, 0.419 mmol) was added to a stirred, room temperature mixture of compound **16a3** (60 mg, 0.105 mmol) inTHF (1 mL) and the mixture was stirred at 80 °C overnight. LCMS check revealed that both starting material and

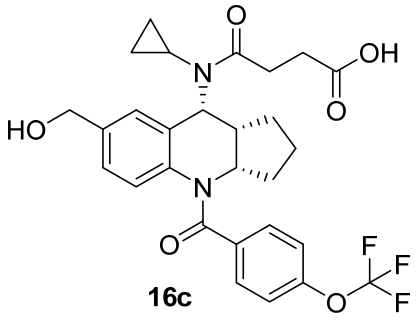
product were present in the mixture. More deoxo-fluor (0.077 mL, 0.419 mmol) was added. The reaction mixture was heated at 80 °C overnight. The mixture was cooled, poured into icy water (3 mL), diluted with ethyl acetate (10 mL), basified with saturated $NaHCO_3$ (5 mL), the organic was separated, washed with brine (1x 5 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to obtain a light brown foam. The residue was purified by preparative HPLC (Reverse phase C-18), eluting with acetonitrile/water + 0.1% TFA, to give **16a4** (25 mg, 0.032 mmol, 30.1 % yield) as a yellow gum. Calcd E/Z $[M+H]^+$ for C30H31F5N2O5: 595.2, Found: 595.0.

Step 4: Compound **16a4** was hydrolyzed with NaOH in MeOH in a similar manner in Example **15c** (step 6) to give **16a.** ^{1}H NMR (500 MHz, Chloroform-d) δ 7.29 (d, J = 10 Hz, 2H), 7.06 (d, J = 10 Hz, 2H), 7.04 (d, J = 10 Hz, 1H), 6.98 (bs, 1H), 6.51 (d, J = 10 Hz, 1H), 5.69 (s, 1H), 5.13 (m, 1H), 3.11 (m, 3H), 2.86 (m, 1H), 2.82 (m, 2H), 2.43 (m, 1H), 2.02 (m, 1H), 1.55 (m, 1H), 1.41 (m, 2H), 1.22 (m, 1H), 1.12 (m, 3H), 1.05 (m, 1H), 0.97 (m, 1H). Calcd E/Z $[M+H]^+$ for C28H27F5N2O5: 567.2, Found: 567.0 as observed in LCMS.



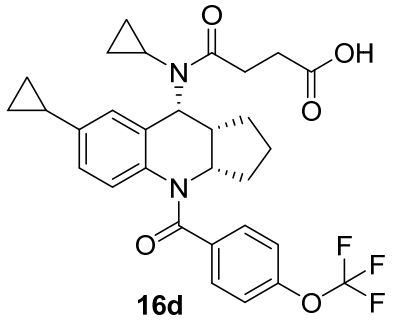
16b

Intermediate **16a2** was hydrogenated with H_2/Pd/C followed with hydrolysis with NaOH in MeOH to give **16b**. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.62 (br. s, 1H), 7.28 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.61 (s, 1H), 6.33 (d, J = 8.0 Hz, 1H), 5.76 (bs, 1H), 5.15 (dd, J = 10, 5.0 Hz, 1H), 3.22 – 2.98 (m, 3H), 2.91 – 2.74 (m, 3H), 2.63 – 2.50 (m, 2H), 2.43 (m, 1H), 2.04 (m, 1H), 1.65 – 1.55 (m, 1H), 1.41 (m, 2H), 1.32 – 1.09 (m, 3H), 1.17 (t, J = 10 Hz, 3 H), 1.05 (m, 1H), 0.93 (m, 1H). Calcd E/Z $[M+H]^+$ for C29H31F3N2O5: 545.2, Found: 544.8 as observed in LCMS.



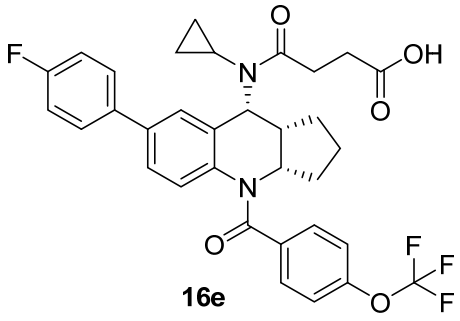
16c

Intermediate **16a3** was reduced with $NaBH_4$ in MeOH/DCM followed with hydrolysis with NaOH in MeOH to give **16c**. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.32 (d, J = 7.1 Hz, 2H), 7.06 (m, 3H), 6.76 (d, J = 8.0 Hz, 1H), 6.38 (d, J = 8.0 Hz, 1H), 5.12 (m, 1H), 4.66 – 4.46 (br dd, J = 45, 10 Hz, 2H), 3.09 (br. m, 2H), 2.91 (br. m, 3H), 2.81 (br. m, 1H), 2.72 (br. m, 1H), 2.36 (br. m, 1H), 1.98 (m, 1H), 1.43 (br. m, 3H), 1.13 (br. m, 5H), 1.05 (br. m, 1H). Calcd E/Z $[M+Na]^+$ for C28H29F3N2O6: 568.2, Found: 568.8 as observed in LCMS.

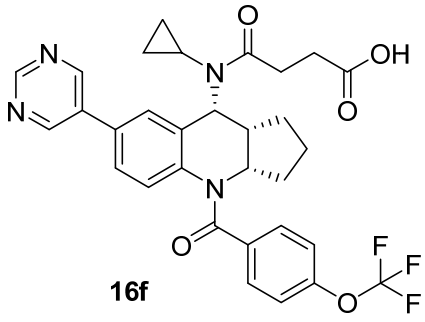


16d

Intermediate **16a1** was coupled with cyclopropyl boronic ester through Suzuki reaction followed with hydrolysis with NaOH in MeOH to give **16d**. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.28 (d, J = 7.5 Hz, 2H), 7.05 (d, J = 8.2 Hz, 2H), 6.78 (br. s, 1H), 6.54 (br. d, 1H), 6.50 (br. s, 1H), 6.30 (d, J = 8.1 Hz, 1H), 5.74 (bs, 1H), 5.14 (br. dd, 1H), 3.12 (br. dd, 2H), 3.04 (br. m, 1H), 2.82 (br. m, 3H), 2.42 (br. m, 1H), 2.03 (br. m, 1H), 1.78 (br. m, 1H), 1.58 (br. m, 1H), 1.39 (br. m, 2H), 1.30 – 1.08 (br. m, 3H), 1.04 (br. m, 1H), 0.93 (br. m, 3H), 0.63 (m, 1H), 0.51 (m, 1H). Calcd E/Z $[M+H]^+$ for C30H31F3N2O5: 557.2, Found: 557.0 as observed in LCMS.



16e Intermediate **16a1** was coupled with 4-fluorophenyl boronic ester through Suzuki reaction followed with hydrolysis with NaOH in MeOH to give **16e**. ^{1}H NMR (500 MHz, Chloroform-d) δ 10.04 (bs, 1H), 7.42 (dd, J = 8.3, 5.1 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 7.11 (t, J = 10 Hz, 2 H), 7.07 (m, 3H), 6.98 (s, 1H), 6.49 (d, J = 8.1 Hz, 1H), 5.79 (bs, 1H), 5.16 (m, 1H), 3.14 (br. m, 2H), 3.09 (br. m, 1H), 2.89 (br. m, 1H), 2.83 (br. m, 2H), 2.48 (m, 1H), 2.07 (m, 1H), 1.63 (m, 1H), 1.45 (m, 2H), 1.26 (m, 3H), 1.05 (br. m, 1H), 0.99 (br. m, 1H). Calcd E/Z $[M+H]^+$ for C33H30F4N2O5: 611.2, Found: 610.6 as observed in LCMS.



16f Intermediate **16a1** was coupled with pyrimidin-5-ylboronic acid through Suzuki reaction followed with hydrolysis with NaOH in MeOH to give **16f**. ^{1}H NMR (500 MHz, Chloroform-d) δ 10.42 (s, 1H), 9.24 (s, 1H), 9.19 (s, 2H), 7.38 (bs, 1H), 7.34 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 8.2 Hz, 1H), 7.10 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 8.2 Hz, 1H), 5.88 (br. s, 1H), 5.11 (m, 1H), 3.22-2.61 (br. m, 7H), 2.48 (br. m, 1H), 2.05 (br. m, 1H), 1.55 (br. m, 1H), 1.42 (br. m, 2H), 1.27 (br. m, 1 H), 1.16 (br. m, 2H), 1.07 (br. m, 1H). Calcd E/Z $[M+H]^+$ for C31H29F3N4O5: 595.2, Found: 595.0 as observed in LCMS.

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