# Discovery of a first-in-class gut-restricted RET kinase inhibitor as a clinical candidate for the treatment of IBS

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## Supporting Information

### In Vitro Inhibition of Human RET and KDR Enzyme

The effect of inhibitors on human RET and KDR enzymatic activity was determined in an in vitro biochemical HTRF assay. Inhibitors were diluted from 1 μM for RET or 10 μM for KDR using a 3X dilution over 11 points. 100 nL of diluted inhibitor or DMSO was prepared in 384 well plate. The compound dilution plate was centrifuged at 2,500 rpm for 1 minute, then 1.2 μL of 0.5 M EDTA was manually added to the low control wells. 5 μL of 2X RET Enzyme Mix (1 mM DTT and 0.2 nM RET Kinase in 1X assay buffer (50 mM HEPES pH 7.0, 1 mM CHAPS, 0.1 mg/mL BSA)) or 2X KDR Enzyme Mix (2 mM DTT and 0.0026 ng/μL KDR enzyme in 1X assay buffer (50 mM HEPES pH 7.0, 1 mM CHAPS, 0.1 mg/mL BSA)) was added to each well, the plate was covered to prevent evaporation, centrifuged at 1,900 rpm for 30 seconds and incubated for 30 minutes at 23 ºC. 5 μL of 2X Substrate (RET: 20 μM ATP, 20 mM $MgCl_2$, 1 μM Cisbio TK biotin-peptide substrate in 1X assay buffer or KDR: 6 μM ATP, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.6 μM Cisbio TK biotin peptide substrate and 3.2 nM supplement enzymatic buffer (SEB) in 1X assay buffer) was added to each well, the plate was covered, centrifuged at 1,900 rpm for 30 seconds and the enzyme was incubated with the substrate for 1 hour for RET or 30

min for KDR at 23 ºC. 10 µL of Stop/Detection Mix (RET: 50 mM EDTA, 200X dilution of Ab Europium Cryptate and 62.5 nM Streptavidin-XL665 in 50 mM HEPES pH 7.0 or KDR: 50 mM EDTA, 200X dilution of Ab Europium Cryptate and 37.5 nM Streptavidin-XL665 in 50 mM HEPES pH 7.0) was added to each well, the plate was covered, centrifuged at 1,900 rpm for 30 seconds and incubated for 1 hour at 23 ºC. The fluorescence emission was measured at 590 nm (Cryptate) and 665 nm (XL665) for RET and KDR and the ratio (665/590) was calculated for each well.

Table S1. Assay standards run in each plate.

| | RET $IC_{50}$ nM | KDR $IC_{50}$ nM | TT ELISA $IC_{50}$ nM |
|---|---|---|---|
| Sorafenib | 0.70 ± 0.49 (n = 265) | 6.77 ± 4.18 (n = 237) | 80.67 ± 27.10 (n = 198) |
| AMG-706 | 46.85 ± 25.37 (n = 263) | 4.77 ± 1.86 (n = 236) | 559.94 ± 219.51 (n = 55) |
| Sunitinib | 9.28 ± 3.90 (n = 259) | 121.30 ± 68.96 (n = 233) | 421.59 ± 155.22 (n = 4) |
| Vandetinib | 15.48 ± 7.25 (n = 248) | 50.85 ± 24.20 (n = 231) | 418.76 ± 241.66 (n = 10) |

**Cell-based RET Phosphorylation ELISA Assay**

The effect of inhibitors on RET phosphorylation in TT cells was determined in an in vitro ELISA assay. TT cells were maintained in culture media (F12 Kaighn's medium with 10% FBS, 1X Non-Essential Amino Acid (NEAA), 1X Glutamax and 1X Penicillin/Streptomycin). TT cells were seeded manually into 96 well plate at 105 cells per well in 100 µL culture media and incubated overnight at 37 ºC in 5% $CO_2$ incubator. The plated cells were treated with the addition of 100 µL of diluted compound/well such that the final starting concentration of inhibitor was 10 µM using a 4X dilution over 10 points and incubated for 2 hours. All cells were rinsed once with ice cold phosphate-buffered saline (PBS), lysed with 200 µL/well of ice-cold 1X lysis mix (1X Protease Inhibitor, 1X Phosphatase Inhibitor Cocktail 2, 1X Phosphatase Inhibitor Cocktail 3, 50 mM Na-β-glycerophosphate, 1 mM sodium orthovanadate in 1X lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 2 mM EDTA pH 8.0)), subjected to freezing at -80 °C for 20 minutes, and thawed on ice to ensure complete lysis. RET protein in the cell lysate was captured in an anti-RET capture antibody (Cell Signaling cat.# 3223) coated plate that had been blocked with 1% bovine serum albumin (BSA) and incubated overnight at 4 °C. Phosphorylated RET was detected by the addition of anti-phosphotyrosine (Cell Signaling cat.#5465), anti mouse

IgG-horseradish peroxidase (HRP) secondary antibody (Cell Signaling cat.#7034). HRP activity was detected by incubation with 3,3′ ,5,5′ -tetramethylbenzidine (TMB) substrate and absorbance measured at 450 nm on Spectra Max (Molecular Devices, Sunnyvale, CA).

**Homology Model Description**

The input amino acid sequence of RET was obtained from the Universal Protein Resource (http://www.uniprot.org). BLAST then was used to find the homologous protein structures in PDB database. Among them, FGFR1, FGFR2, KDR had the highest sequence identity to RET kinase domain (52%, 49%, 41% respectively). Moreover, only KDR had the crystal structures in the DFG-out conformation. The DFG-out structures bound with urea/amide linker analogues have been selected as a template to build homology models for RET.

The crystal structures of KDR was downloaded from Protein Data Bank database (http://www.rcsb.org/, PDB code: 3EWH, 2OH4). A pairwise sequence alignment of KDR and RET kinase domain had been performed, then the sequence of RET was subjected to model building using knowledge based methods. Firstly, the atom coordinates of backbone and conserved residues were assigned to the models. Then the coordinates of the side chains were generated and optimized. Finally, insertions in the alignment were built, and deletions were closed. Loop refinements for the generated models were performed, followed by all-atom minimizations for the intact structures. All the calculations were performed in Prime module of Schrodinger suite 2012.


- Jacobson, M. P.; Pincus, D. L.; Rapp, C. S.; Day, T. J. F.; Honig, B.; Shaw, D. E.; Friesner, R. A., "A Hierarchical Approach to All-Atom Protein Loop Prediction," *Proteins: Structure, Function and Bioinformatics*, **2004**, *55*, 351-367

- Jacobson, M. P.; Friesner, R.A.; Xiang, Z.; Honig, B., "On the Role of Crystal Packing Forces in Determining Protein Sidechain Conformations," *J. Mol. Biol*., **2002**, *320*, 597-608

- Schrödinger Release 2017-4: Prime, Schrödinger, LLC, New York, NY, 2017.


**Figure S1.** Compound **4** Hinge and Gate Keeper Residues

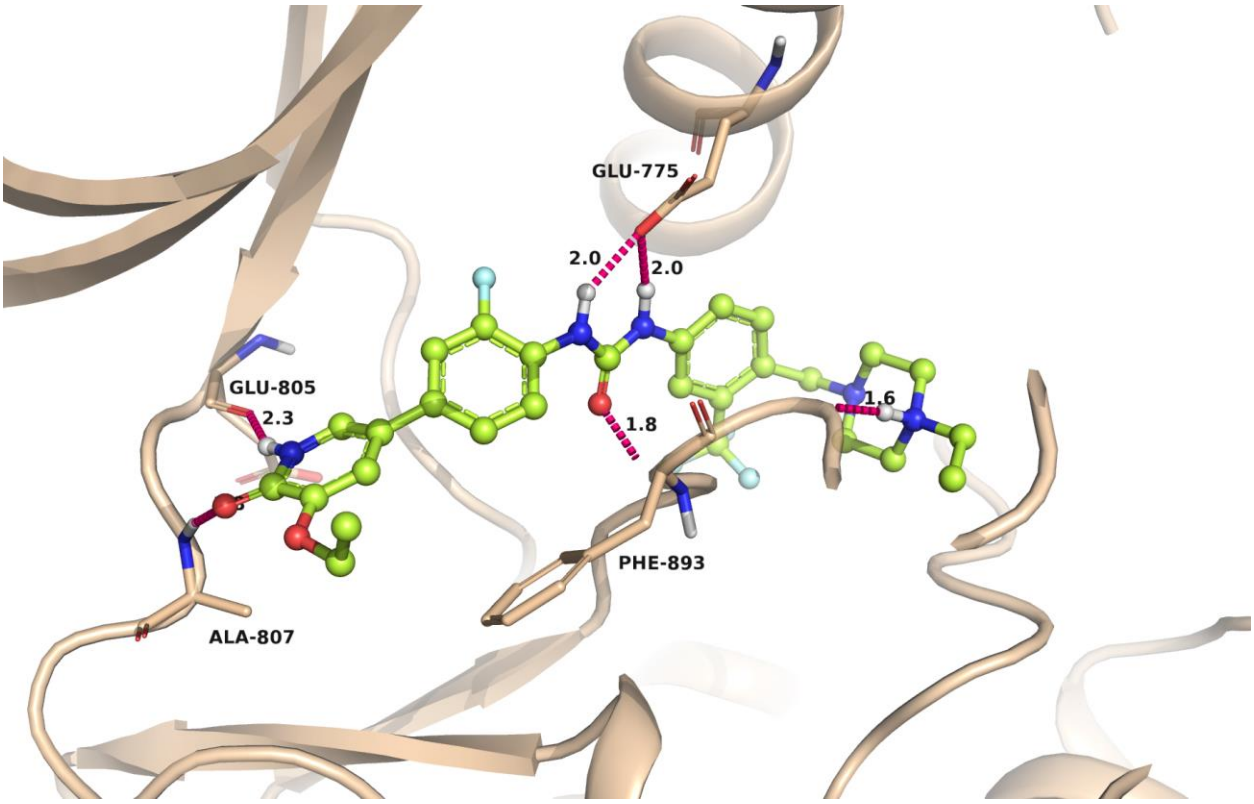



**Figure S2.** Compound **15** Hinge and Gate Keeper Residues

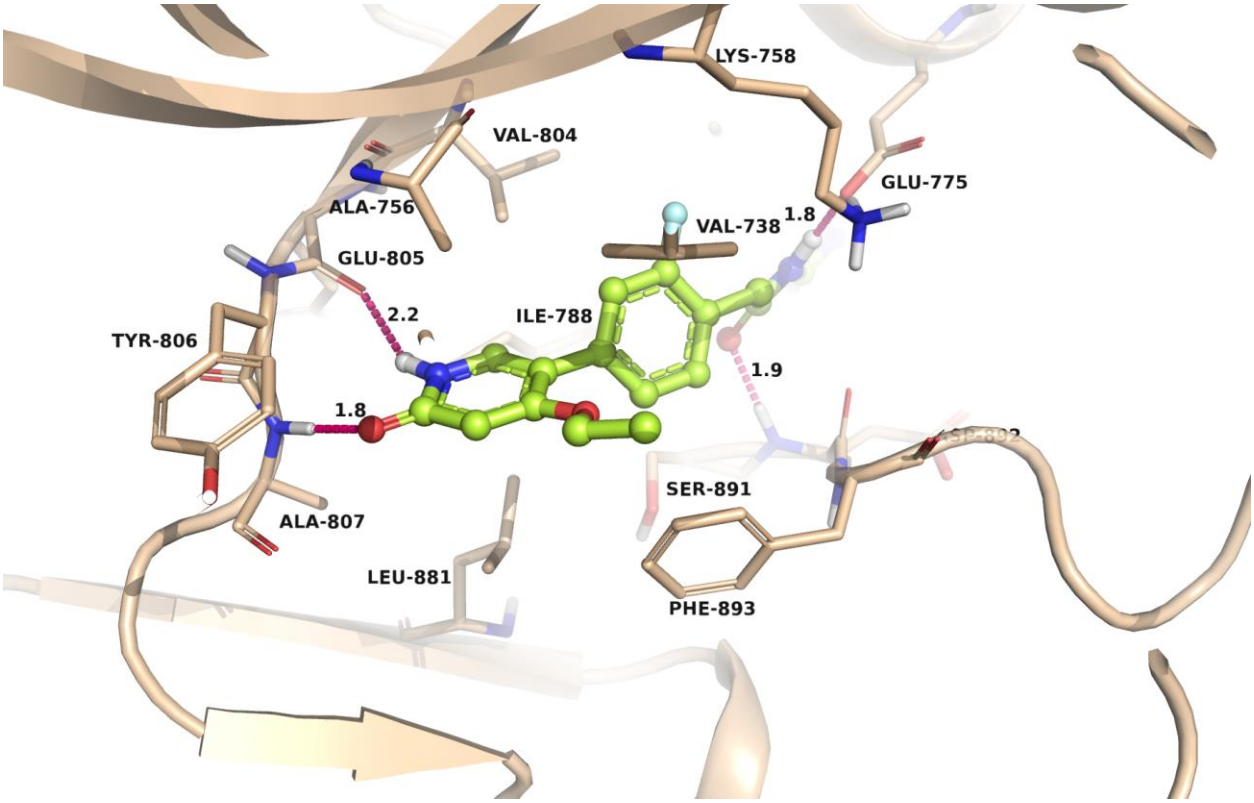


**Figure S3.** Compound **15** isoxazole site

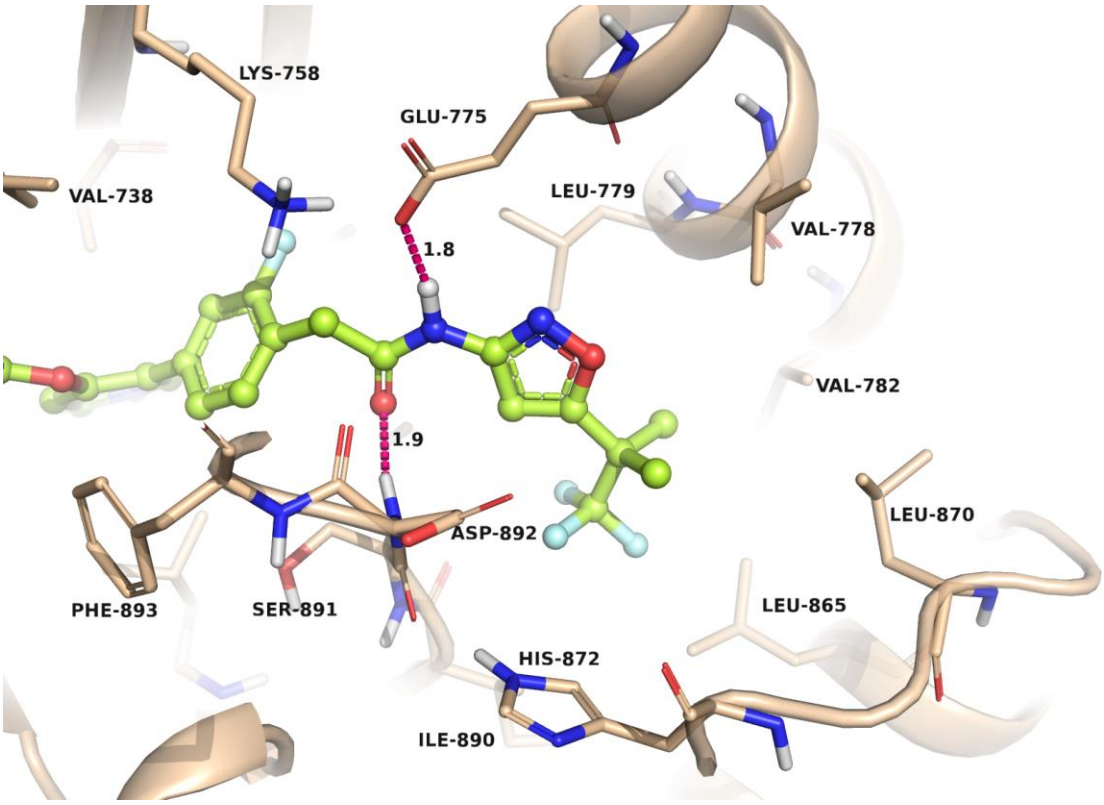



**Figure S4.** Compound **15** isoxazole pocket

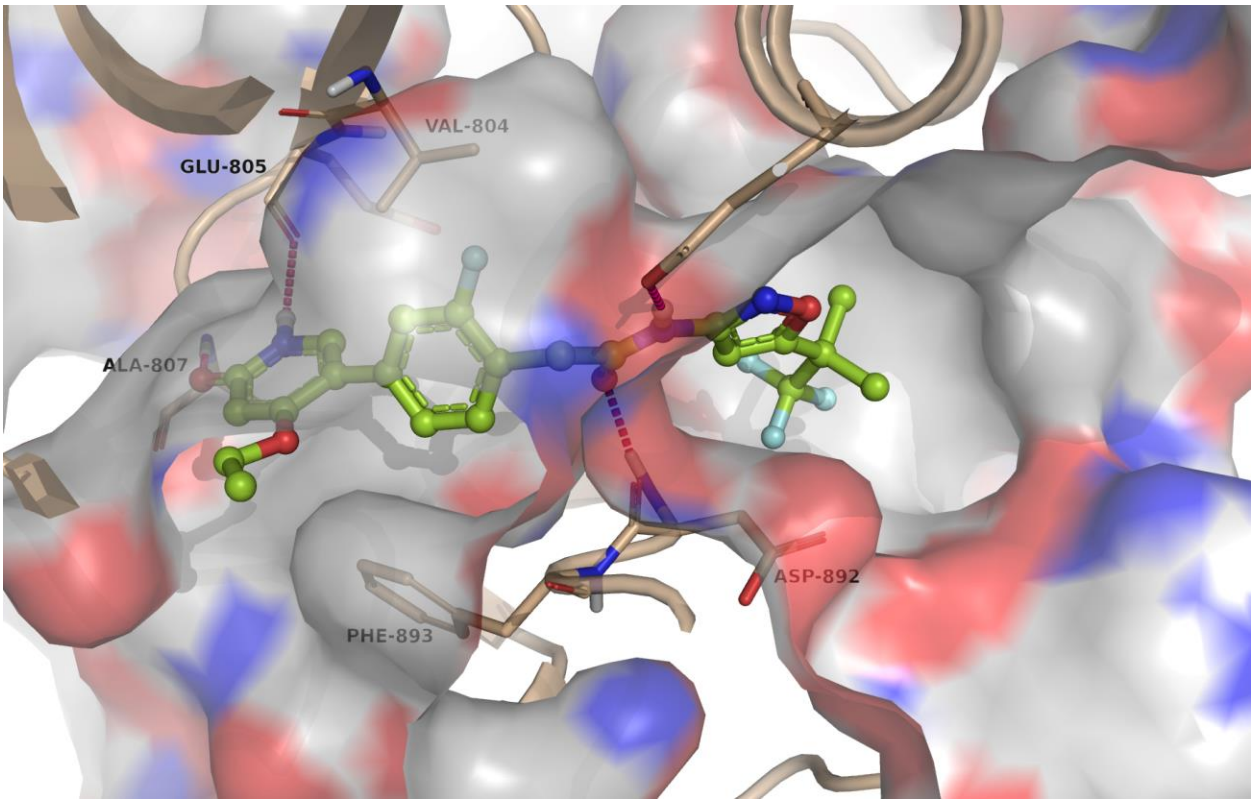



**Figure S5.** Compound **15** A ring ethoxy interactions

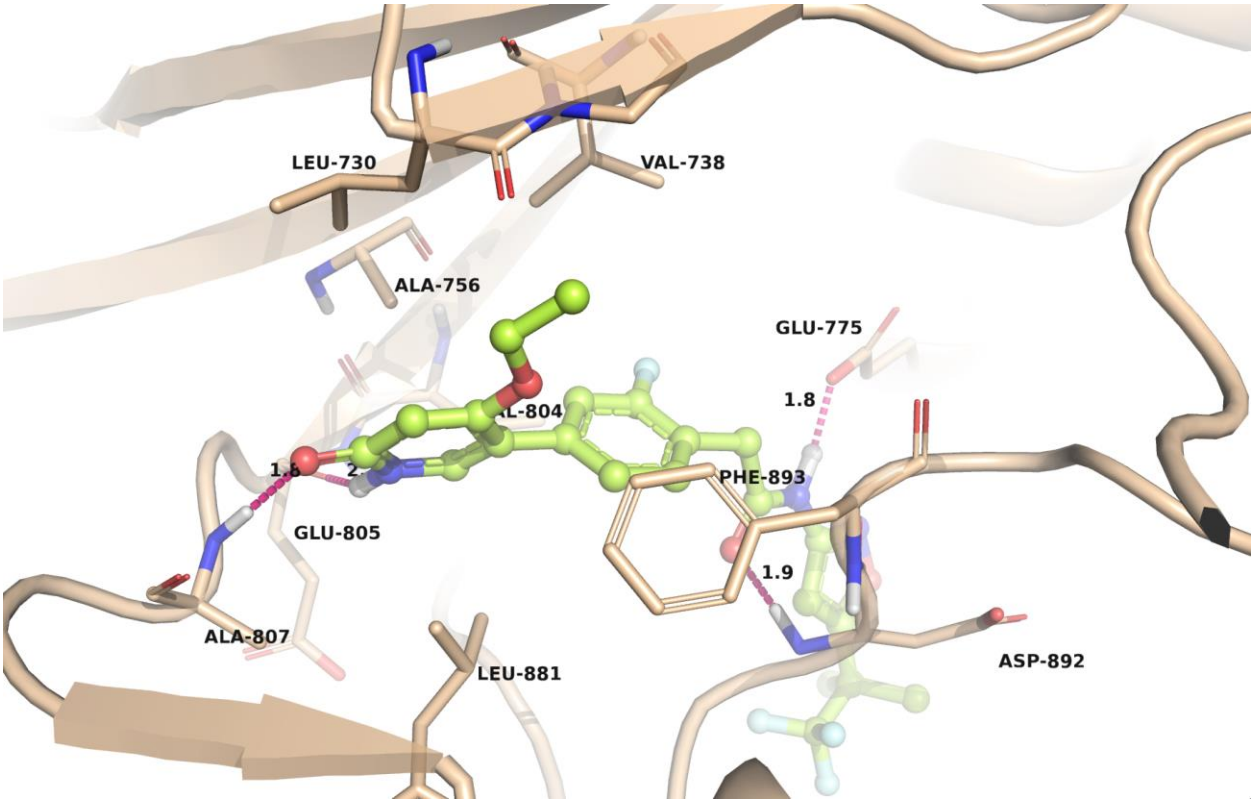


Table S2. AMES predictions and results for the A/B ring anilines

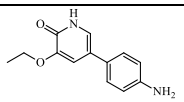
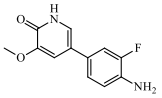
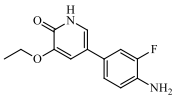
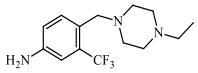
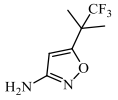
| Aniline | Structure | Nitrenium Ion Formation Energy (kcal/mol) | Ames (TA98) result |
|---|---|---|---|
| A |  | 134.24 | + |
| B |  | 135.26 | + |
| C |  | 134.21 | + |

Table S3. AMES predictions and results for the C ring anilines

| Aniline | Structure | Nitrenium Ion Formation Energy (kcal/mol) | Ames (TA98) result |
|---|---|---|---|
| D |  | 152.12 | + |
| E |  | 190.95 | - |

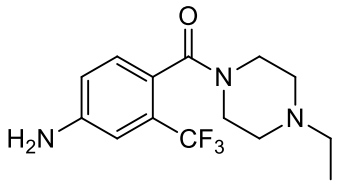
Methods reference for Ames test:

The test methodology was based on established procedures for bacterial mutagenicity testing [Ames, 1973; Ames, 1975; Garner, 1972; Green, 1976 , Green 1984, Maron 1983] and in accordance with the general principles of the current national and international regulatory guidelines [ICH-S2 (R1); OECD (1997)].

- Ames B.N., et al. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proceedings of the National Academy of Science.* 1973;70:2281-2285.
- Ames B.N., et al. Methods for detecting carcinogens and mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test. *Mutation Research.* 1975;31:347-364.
- Garner R.C., et al. Liver microsomal metabolism of Aflatoxin B1 to a reactive derivative toxic to Salmonella typhimurium TA 1530. *Cancer Research.* 1972;32:2058-2066.
- Green M.H.L., et al. Mutagen testing using Trp+ Reversion in Escherichia coli. *Mutation Research.* 1976;38:3-32.
- Green M.H.L., et al. Mutagen testing using Trp+ Reversion in Escherichia coli. *Mutation Research.* 1976;38:3-32.
- Maron D.M., et al. Revised methods for the Salmonella Mutagenicity test. *Mutation Research.* 1983;113:173-215.
- OECD "Bacterial Reverse Mutation Test", in: OECD Guideline for the Testing of Chemicals, Test Guideline 471, 1997.
- ICH S2(R1) (2011) ''Guidance on Genotoxicity and data interpretation for pharmaceuticals intended for human use'', Adopted EMA/CHMP/ICH/126642/2008. December 2011.


Compound 1

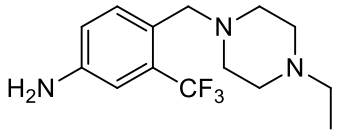
Step 1. (4-amino-2-trifluoromethyl-phenyl)-(4-ethyl-piperazin-1-yl)-methanone




A mixture of 4-amino-2-trifluoromethyl-benzoic acid (15 g, 73.1 mmol), HOBT (14.56 g, 95 mmol), EDC (16.82 g, 88 mmol), $Et_3N$ (20.38 mL, 146 mmol), 1-ethyl-piperazine (8.35 g, 73.1 mmol) in DCM (200 mL) was stirred at 25 °C for 2 h. To the mixture was added DCM (200 mL) and then washed with $H_2O$, 2 mol/L NaOH (2 x 150 mL) and brine. The organic layer was dried over $Na_2SO_4$ and concentrated to give a off white solid of (4-amino-2-trifluoromethyl-phenyl)-(4-ethyl-piperazin-1-yl)-methanone (20 g, 65.2 mmol, 89.0% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 2.4 Hz, 1H), 6.79 (dd, *J* = 2.0, 8.0 Hz, 1H), 3.99 (s, 2H), 3.84-3.76 (m, 2H), 3.25-3.23 (m, 2H), 2.50-2.39 (m, 4H), 2.33-2.31 (m, 2H), 1.08

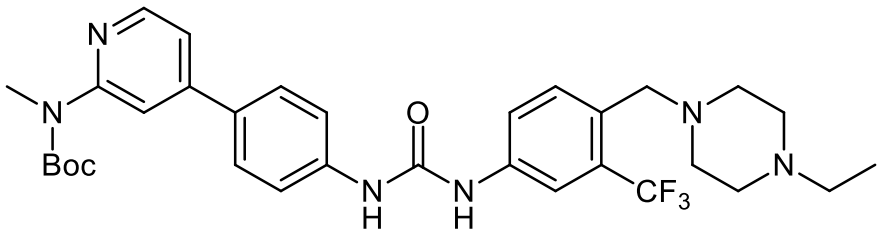
(t, $J$ = 7.2 Hz, 3H); Calculated for $C_{14}H_{18}F_3N_3O$ MW 301: found ES-LCMS $m/z$ 302 (M+H).

Step 2. 4-(4-ethyl-piperazin-1-ylmethyl)-3-trifluoromethyl-phenylamine




To a mixture of (4-amino-2-trifluoromethyl-phenyl)-(4-ethyl-piperazin-1-yl)-methanone (20 g, 66.4 mmol) in THF (500 mL) was added $BH_3$·DMS (19.91 mL, 199 mmol) dropwise. Then the mixture was stirred at 80 °C for 4 h. The mixture was quenched by adding MeOH and then concentrated. The residue was purified by silica column chromatography on silica gel (PE:EA = 2:1, $R_f$ = 0.35) to give a white solid of 4-(4-ethyl-piperazin-1-ylmethyl)-3-trifluoromethyl-phenylamine (14 g, 46.0 mmol, 69.4% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, $J$ = 8.4 Hz, 1H), 6.91 (d, $J$ = 2.8 Hz, 1H), 6.79 (dd, $J$ = 2.4, 8.4 Hz, 1H), 3.76 (s, 2H), 3.53 (s, 2H), 2.45-2.39 (m, 8H), 1.08 (t, $J$ = 7.2 Hz, 3H); Calculated for $C_{14}H_{20}F_3N_3$ MW 287: found ES-LCMS $m/z$ 288 (M+H).

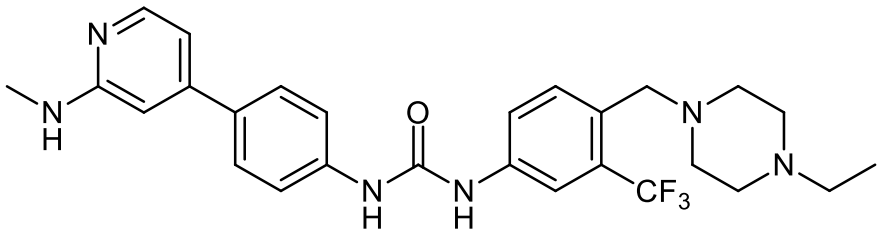
Step 3. tert-butyl (4-(4-(3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)ureido)phenyl)pyridin-2-yl)(methyl)carbamate




To the mixture of 4-(4-Ethyl-piperazin-1-ylmethyl)-3-trifluoromethyl-phenylamine (25 mg, 0.087 mmol) in Toluene (15 mL) was added Triphosgene (9.04 mg, 0.030 mmol), then the mixture was refluxed for 4 hrs. The mixture was concentrated, the residue was added Tetrahydrofuran (THF) (15.00 mL), [4-(4-Amino-phenyl)-pyridin-2-yl]-methyl-carbamic acid tert-butyl ester (22.14 mg, 0.074 mmol), triethylamine (0.036 mL, 0.261 mmol), and the whole mixture was stirred at 25 °C for 10 hr. The mixture was concentrated, purified by prepare HPLC to give the product, tert-butyl (4-(4-(3-(4-((4-ethylpiperazin-1-yl)methyl)-3-

(trifluoromethyl)phenyl)ureido)phenyl)pyridin-2-yl)(methyl)carbamate (10 mg, 0.016 mmol, 18.8% yield). LCMS purity is 100% by area. Calculated for $C_{32}H_{39}F_3N_6O_3$ MW 612: found ES-LCMS *m/z*: 613 (M+H).

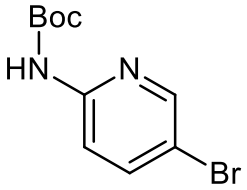
Step 4. 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(2-(methylamino)pyridin-4-yl)phenyl)urea



The mixture of [4-(4-{3-[4-(4-Ethyl-piperazin-1-ylmethyl)-3-trifluoromethyl-phenyl]-ureido}-phenyl)-pyridin-2-yl]-methyl-carbamic acid tert-butyl ester (10 mg, 0.016 mmol), hydrochloride acid (2.033 mg, 0.016 mmol) in Ethyl acetate (20 mL) was stirred at 25 °C for 2 hr. The mixture was concentrated to give 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(2-(methylamino)pyridin-4-yl)phenyl)urea (2.3 mg, 3.65 umol, 22.4% yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.67-7.98 (m, 8H), 7.18 (2H), 4.40 (s, 2H), 3.52-3.64 (m, 2H), 2.88-3.39 (m, 13H), 1.28 (t, 3H); LCMS purity is 99.2% by area. Calculated for $C_{27}H_{31}F_3N_6O$ MW 512: found ES-LCMS *m/z*: 513 (M+H).

Compound 2

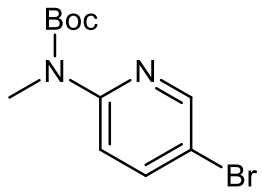
Step 1. tert-butyl (5-bromopyridin-2-yl)carbamate



To a solution of 5-bromopyridin-2-amine (1 g, 5.78 mmol) and $Et_3N$ (2.417 mL, 17.34 mmol) in Dichloromethane (DCM) (15 mL) stirred under nitrogen at 20°C was added $Boc_2O$ (1.610 mL,

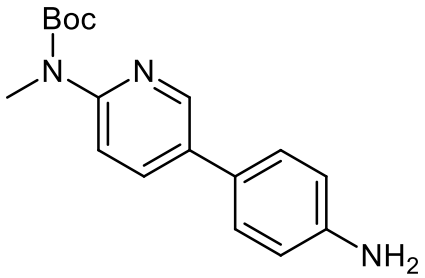
6.94 mmol) in one charge. The reaction mixture was stirred at 20 °C for 12 hr. The solution was washed with water, the organic layer was separated, dried over $Na_2SO_4$, concentrated in vacuo to give the crude product, tert-butyl (5-bromopyridin-2-yl)carbamate (0.8 g, 2.08 mmol, 36% yield). Calculated for $C_{10}H_{13}BrN_2O_2$ MW 273: found ES-LCMS m/z m/z 217 (M-tBu).

Step 2. tert-butyl (5-bromopyridin-2-yl)(methyl)carbamate




To a solution of tert-butyl (5-bromopyridin-2-yl)carbamate (1 g, 3.66 mmol) and NaH (0.088 g, 3.66 mmol) in N,N-Dimethylformamide (DMF) (5 mL) stirred under nitrogen at 0°C was added iodomethane (0.520 g, 3.66 mmol) in one charge . The reaction mixture was stirred at 0 °C for 12 hr. The mixture was poured into water and extracted into ethyl acetate,the organic layer was separated ,dried (Na2SO4) and concentrated under reduced pressure, and gave tert-butyl (5-bromopyridin-2-yl)(methyl)carbamate (300 mg, 1.003 mmol, 27.4 % yield). Calculated for $C_{11}H_{15}BrN_2O_2$ MW 287: found ES-LCMS *m/z*: 230 (M-tBu).

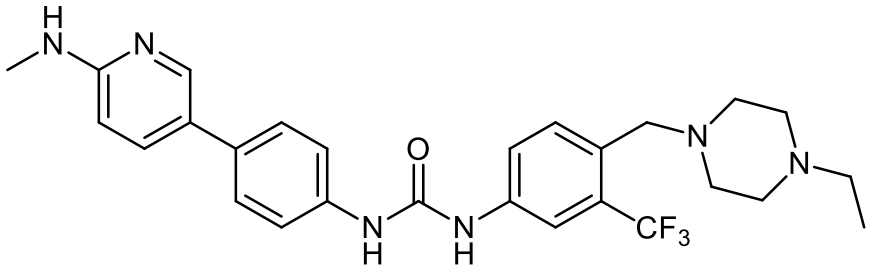
Step 3. tert-butyl (5-(4-aminophenyl)pyridin-2-yl)(methyl)carbamate




To a solution of tert-butyl (5-bromopyridin-2-yl)(methyl)carbamate (200 mg, 0.696 mmol), (4-aminophenyl)boronic acid, 2 Hydrochloride (146 mg, 0.696 mmol) and sodium carbonate (148 mg, 1.393 mmol) in 1,4-Dioxane (3 mL) and Water (1.000 mL) stirred under nitrogen at 20°C was added $PdCl_2$(dppf) (25.5 mg, 0.035 mmol) in one charge. The reaction mixture was stirred at 110 °C for 15 min. The solution was concentrated in vacuo to give the crude product. The crude

product was purified by TLC to give the desired product, tert-butyl (5-(4-aminophenyl)pyridin-2-yl)(methyl)carbamate (150 mg, 0.49 mmol, 70.5% yield). Calculated for $C_{17}H_{21}N_3O_2$ MW 299: found ES-LCMS *m/z*: 300 (M+H).

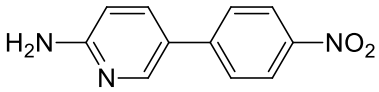
Step 4. 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(6-(methylamino)pyridin-3-yl)phenyl)urea



To a solution of 4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (173 mg, 0.601 mmol) in Tetrahydrofuran (THF) (10 mL) stirred under nitrogen at room temperature was added solid triphosgene (52.0 mg, 0.175 mmol) in one charge . The reaction mixture was stirred at 50 °C for 1 hr. Then the solution was warmed to room temperature,added a solution of tert-butyl (5-(4-aminophenyl)pyridin-2-yl)(methyl)carbamate (150 mg, 0.501 mmol), $Et_3N$ (0.210 mL, 1.503 mmol) and N,N-dimethylpyridin-4-amine (6.12 mg, 0.050 mmol) in Tetrahydrofuran (THF) (5 mL). The mixture solution was stirred at 20 °C for 12 hr. Then the solution was concentrated in vacuo to give the crude product.The crude product was purified by sepecial HPLC separation to give the desired product .1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(6-(methylamino)pyridin-3-yl)phenyl)urea, 3 Hydrochloride (58 mg, 0.093 mmol, 18.6% yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 8.17 (m, 2H), 8.06 (m, 2H), 7.83 (m, 1H), 7.55 (q, 4H), 7.13 (dd, 1H), 4.62 (s, 2H), 3.60-4.07 (m, 9H), 3.30 (m, 2H), 3.00 (3, 3H), 1.42 (t, 3H). LCMS purity is 99.8% by area. Calculated for $C_{27}H_{31}F_3N_6O$ MW 512: found ES-LCMS *m/z*: 513 (M+H).

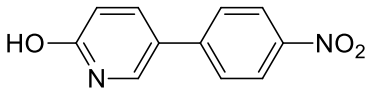
Compound 3.

Step 1. 5-(4-nitrophenyl)pyridin-2-amine



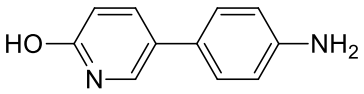
A mixture of 5-bromopyridin-2-amine (2 g, 11.56 mmol), (4-nitrophenyl)boronic acid (1.930 g, 11.56 mmol), $PdCl_2$(dppf) (0.423 g, 0.578 mmol), $Cs_2CO_3$ (7.53 g, 23.12 mmol) in 1,4-dioxane (30 mL) and water (5 mL) was heated to 100 °C for 1 hr at microwave. Then the mixture was concentrated to give the residue which was extracted with DCM (20 mL x 2), dried over $Na_2SO_4$, concentrated to give the residue which was purified by *via* column chromatography to give 5-(4-nitrophenyl)pyridin-2-amine (1 g, 4.65 mmol, 40.2% yield); Calculated for $C_{11}H_9N_3O_2$ MW 215: found ES-LCMS *m/z* 216.1 (M+1).

Step 2. 5-(4-nitrophenyl)pyridin-2(1H)-one



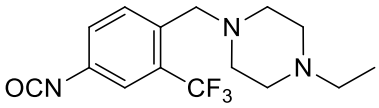

To a mixture of 5-(4-nitrophenyl)pyridin-2-amine (1 g, 4.65 mmol) in $H_2SO_4$ (33.2 ml, 3.5 M, 116 mmol) was added sodium nitrite (20.10 ml, 2 M , 40.2 mmol) at 0 °C. After the mixture was stirred for 2 hrs later, the mixture was pour into ice water and extracted with DCM (200 mL x 2), dried over $Na_2SO_4$, concentrated to give 5-(4-nitrophenyl)pyridin-2(1H)-one (800 mg, 3.70 mmol, 80% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.30-8.27 (dd, *J* = 8.8, 2.8 Hz, 2H), 8.02 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.89 (m, 1H), 7.80-7.77 (m, 2H), 6.68-6.65 (m, 1H); Calculated for $C_{11}H_8N_2O_3$ MW 216: found ES-LCMS *m/z* 217.1 (M+H).

Step 3. 5-(4-aminophenyl)pyridin-2(1H)-one



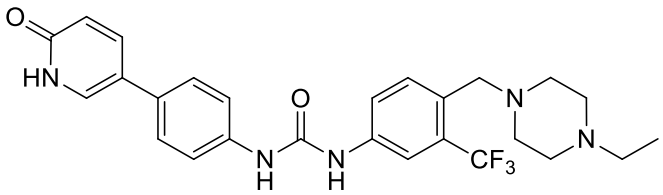

A mixture of 5-(4-nitrophenyl)pyridin-2(1*H*)-one (800 mg, 3.70 mmol), nickel (21.72 mg, 0.370 mmol) in methanol (20 mL) was stirred overnight at 20 psi under $H_2$ atmosphere. Then the mixture was filtered and the filtrate was concentrated to give 5-(4-aminophenyl)pyridin-2(1H)-one (400 mg, 2.148 mmol, 58.1% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.88-7.85 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.58-7.57 (d, *J* = 2.8 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.79-6.77 (d, *J* = 2.0 Hz, 1H), 6.62-6.59 (d, *J* = 10.2 Hz, 1H); Calculated for $C_{11}H_{10}N_2O$ MW 186: found ES-LCMS *m/z* 187.1 (M+H).

Step 4. 1-ethyl-4-(4-isocyanato-2-(trifluoromethyl)benzyl)piperazine




To a mixture of 4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (80 mg, 0.278 mmol) in THF (10 mL) was added triphosgene (27.3 mg, 0.092 mmol), then mixture was heated to 70 °C for 30 min, the mixture was concentrated to give 1-ethyl-4-(4-isocyanato-2-(trifluoromethyl)benzyl)piperazine (84 mg, 0.268 mmol, 96% yield) which was used for the next step without purification or characterization.

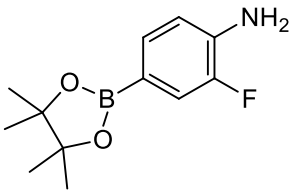
Step 5. 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(6-hydroxypyridin-3-yl)phenyl)urea




A mixture of 5-(4-aminophenyl)pyridin-2-ol (50 mg, 0.269 mmol), 1-ethyl-4-(4-isocyanato-2-(trifluoromethyl)benzyl)piperazine (84 mg, 0.269 mmol), $Et_3N$ (0.075 mL, 0.537 mmol) in THF (10 mL) was stirred for overnight. Then the mixture was concentrated to give the residue which was purified by preparative HPLC to give 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(6-hydroxypyridin-3-yl)phenyl)urea, 2 Hydrochloride (119.55 mg, 0.209 mmol, 78% yield) : $^1$H NMR (400 MHz, $CD_3OD$) δ 8.60 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.41-8.40 (d, *J* = 2.0 Hz, 1H), 8.17-8.16 (d, *J* = 2.0 Hz, 1H), 8.05-8.03 (m, 1H), 7.85-7.83 (m, 1H), 7.66 (s, 4H), 7.32-7.29 (m, 1H), 4.64 (m, 2H), 3.88-3.70 (m, 8H), 3.40-3.34 (m, 2H), 1.43 (t, *J* = 7.20 Hz, 3H). LCMS purity is 100% by area. Calculated for $C_{26}H_{28}F_3N_5O_2$ MW 499: found ES-LCMS *m/z* 500.1 (M+H).

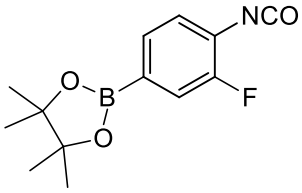
**Compound 4**. 1-(4-(5-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea

Step 1. 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline



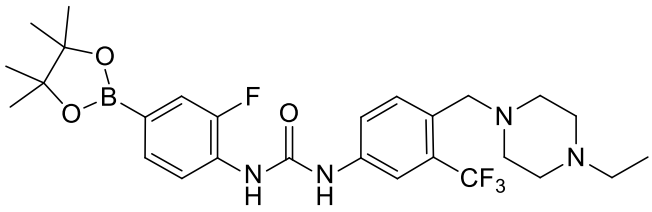

To a solution of 4-bromo-2-fluoroaniline (40 g, 211 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (64.1 g, 253 mmol) and potassium acetate (41.3 g, 421 mmol) in 1,4-Dioxane (500 mL) stirred under nitrogen at 20 °C was added $PdCl_2$(dppf) (7.70 g, 10.53 mmol) in one charge. The reaction mixture was stirred at 100 °C for 3 h. The solution was concentrated in vacuo to give the crude product 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (44 g, 158 mmol, 74.9% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.46-7.40 (m, 2H), 6.75-6.71 (m, 1H), 1.30 (s, *J* = 3.6 Hz, 12H); Calculated for $C_{12}H_{17}BFNO_2$ MW 237: found ES-LCMS *m/z* 238.1 (M+H).

Step 2. 2-(3-fluoro-4-isocyanatophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane



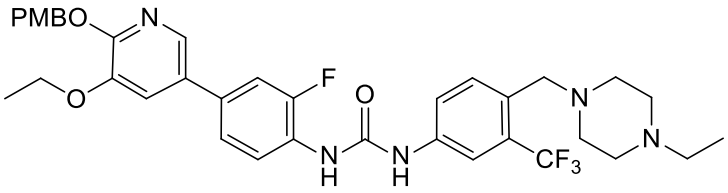

To a mixture of 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (500 mg, 2.109 mmol) in THF (10 mL) was added triphosgene (250 mg, 0.844 mmol). The mixture was stirred at 60 °C for 30 minutes. The residue was evaporated to give 2-(3-fluoro-4-isocyanatophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (500 mg, 1.616 mmol, 77% yield); Calculated for $C_{13}H_{15}BFNO_3$ MW 263: found ES-LCMS *m/z* 296.1 (M+MeOH+H).

Step 3. 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



To a solution of 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (500 mg, 2.109 mmol) in tetrahydrofuran (50 mL) was added triphosgene (219 mg, 0.738 mmol). The resulting miature was stirred at 70 °C. After 30min, LCMS analysis showed the starting material was disappeared. The solvent was removed in *vacuo* to give 2-(3-fluoro-4-isocyanatophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (520 mg, 1.977 mmol, 94 % yield). To a solution of 4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (568 mg, 1.977 mmol), $Et_3N$ (0.827 mL, 5.93 mmol) and DMAP (24.15 mg, 0.198 mmol) in tetrahydrofuran (50 mL) was added a solution of 2-(3-fluoro-4-isocyanatophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (520 mg, 1.977 mmol) at 70 °C. The resulting mixture was stirred at 70 °C. After LCMS analysis showed the starting material was disappeared. The solvent was removed in *vacuo*. The residue was dissolved in DCM (100 mL) and washed with $H_2O$ (30 mL) and brine (30 mL). The organic layer was dried over $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography (DCM/MeOH = 20/1) to yiled 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (0.67 g, 0.851 mmol, 43.0 % yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.17-8.14 (m, 1H), 7.86 (s, 1H), 7.69-7.67 (m, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 11.2 Hz, 1H), 4.59 (s, 2H), 3.60 (s, 2H), 2.52-2.47 (m, 8H), 1.33 (s, 12H), 1.10 (t, *J* = 7.2 Hz, 3H); Calculated for $C_{27}H_{35}BF_4N_4O_3$ MW 550: found ES-LCMS m/z m/z 551.2 (M+H).

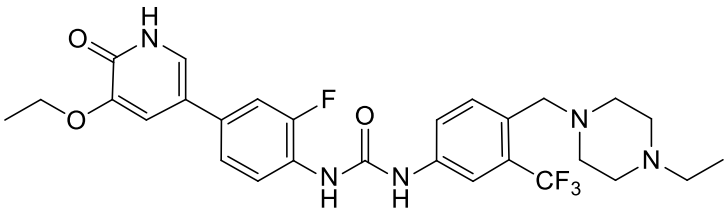
Step 4. 1-(4-(5-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea




A solution of 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (0.67 g, 1.217 mmol), 5-bromo-3-ethoxy-2-((4-methoxybenzyl)oxy)pyridine (0.412 g, 1.217 mmol), $PdCl_2$(dppf)-$CH_2Cl_2$ adduct (0.099 g, 0.122 mmol) and $Cs_2CO_3$ (0.793 g, 2.435 mmol) in 1,4-dioxane (12 mL) and water (4 mL) was stirred at 110 °C overnight under a $N_2$ atmosphere. After LCMS analysis showed the starting material was disappeared. The solvent was removed in *vacuo*. The residue was

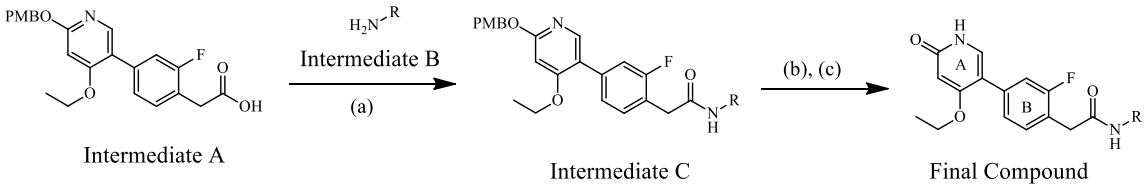
dissolved in EtOAc (120 mL) and washed with $H_2O$ (40 mL) and brine (40 mL). The organic layer was dried over $Na_2SO_4$, filtered and concentrated. The crude material was purified by silica column chromatography (DCM/MeOH = 30/1 to 20/1). All fractions found to contain product by TLC (DCM/MeOH = 10/1) were combined and concentrated to yield a brown solid of 1-(4-(5-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea (0.53g, 0.638 mmol, 52.4 % yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.17 (m, 1H), 7.91-7.89 (m, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.61 (m, 1H), 7.44-7.38 (m, 5H), 6.90 (d, *J* = 8.8 Hz, 1H), 5.35 (s, 2H), 4.17-4.11 (s, 2H), 3.70 (s, 3H), 3.67-3.65 (m, 2H), 2.53-2.44 (m, 8H), 1.41 (t, *J* = 7.0 Hz, 3H), 1.12-1.08 (m, 3H); Calculated for $C_{36}H_{39}F_4N_5O_4$ MW 681: found ES-LCMS m/z 682.2 (M+H).

Step 5. 1-(4-(5-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea



A solution of 1-(4-(5-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea (0.53 g, 0.777 mmol) in hydrogen chloride, methanol (10 mL) was stirred at 25 °C. After LCMS analysis showed the starting material was disappeared. The solvent was removed in *vacuo* to give the crude product, which was purified by preparative HPLC to yield a yellow solid of 1-(4-(5-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea, 2 hydrochloride (293.81 mg, 0.459 mmol, 59.0 % yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.18 (t, *J* = 8.4 Hz, 1H), 8.02 (s, 1H), 7.82 (m, 1H), 7.73 (m, 1H), 7.49-7.41 (m, 4H), 4.24-4.18 (m, 4H), 3.76-3.25 (m, 8H), 3.12 (m, 2H), 1.49 (t, *J* = 7.0 Hz, 3H), 1.37 (t, *J* = 7.2 Hz, 3H); LCMS purity is 99.0% by area. Calculated for $C_{28}H_{31}F_4N_5O_3$ MW 561: found ES-LCMS m/z 562.1 (M+H).

Scheme S1. Compounds 5-9, 11-14.



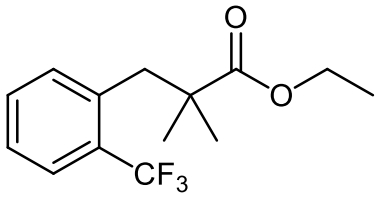

**Compounds 5-14.**

| Compound | Intermediate B | Final Compound $^1$H NMR and LCMS data |
|---|---|---|
| 5 | commerically available | $^1$H NMR (400 MHz, $CD_3OD$): δ 8.03 (br. s., 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.69 (s, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.46 - 7.36 (m, 2H), 7.31 - 7.24 (m, 2H), 6.28 (s, 1H), 4.24-4.19 (m, 2H), 3.84 (s, 2H), 1.41 (t, *J* = 6.8 Hz, 3H); LCMS purity is 100% by area. Calculated for $C_{22}H_{18}F_4N_2O_3$ MW 434: found ES-LCMS: *m/z* 435.1 (M+H) |
| 6 | commercially available | $^1$H NMR (400 MHz, $CD_3OD$) δ 8.21 (d, *J*=1.8 Hz, 1H), 8.04 -7.97 (m, 2H), 7.77 (d, *J*=8.6 Hz, 1H), 7.47 (t, *J*=7.9 Hz, 1H), 7.38 - 7.29 (m, 2H), 6.59 (s, 1H), 4.48 (s, 2H), 4.32-4.30 (m, 2H), 3.88 (s, 2H), 2.92 (s, 6H), 1.44 (t, *J*=6.9 Hz, 3H); LCMS purity is 100% by area. Calculated for $C_{25}H_{25}F_4N_3O_3$ MW 491: found ES-LCMS *m/z* 492 (M+H) |
| 7 | synthesis details below | $^1$H NMR (400 MHz, DMSO-d6) δ 10.49 (br. s., 1H), 8.04 (br. s., 1H), 7.81-7.54 (m,1H), 7.45-7.15 (m, 2H), 5.80 (s, 1H), 4.04 (d, *J* = 7.0 Hz, 2H), 3.74 (br. s., 2H), 2.74 (br. s., 2H), 1.28 (t, *J* = 6.5 Hz, 3H), 0.98 (br. s., 6H); LCMS purity is 100% by area. Calculated for $C_{26}H_{27}F_4N_3O_3$ MW 505: found EC-LCMS *m/z* 506.3 (M+H) |
| 8 | synthesis details below | $^1$H NMR (400 MHz, $CD_3OD$) d = 8.54 (d, *J*=5.0 Hz, 1H), 7.76 (d, *J*=5.5 Hz, 1H), 7.64 (s, 1H), 7.45 (t, *J*=8.0 Hz, 1H), 7.34 - 7.25 (m, 2H), 6.23 (s, 1H), 4.54 (s, 2H), 4.22 (q, *J*=7.0 Hz, 2H), 3.96 (s, 2H), 2.96 (s, 6H), 1.43 (t, *J*=6.8 Hz, 3H); LCMS purity is 100% by area. Calculated for $C_{25}H_{24}F_5N_3O_3$ MW 509: found ES-LCMS *m/z* 510.2 (M+H) |
| 9 | commercially available | $^1$H NMR (400 MHz, $CD_3OD$) 8.08 (d, *J*=2.0 Hz, 1H), 7.89 (d, *J*=8.6 Hz, 1H), 7.75 - 7.67 (m, 2H), 7.41 (t, *J*=7.9 Hz, 1H), 7.29 - 7.23 (m, 2H), 6.27 (s, 1H), 4.31 (d, *J*=7.3 Hz, 1H), 4.21 (q, *J*=7.1 Hz, 2H), 3.82 (s, 2H), 1.62 (d, *J*=7.1 Hz, 3H), 1.40 (t, *J*=6.9 Hz, 3H); LCMS purity is 98.8% by area. Calculated for $C_{25}H_{21}F_4N_3O_3$ MW 487: found ES-LCMS *m/z* 488 (M+H) |
| 10 | synthesis details below | $^1$H NMR (400 MHz, $CD_3OD$) δ 7.95 (s, 1H), 7.67 (m, 2H),7.54 (s,2H), 7.24 (t, *J* = 6.0 Hz, 1H), 7.24 (m, 2H), 6.13 (s, 1H), 4.15 (m, 2H), 3.79 (s, 2H), 2.91 (s, 2H), 1.38 (t, *J* = 6.8 Hz, 3H), 1.145 (s, 6H); LCMS purity is 100% by |

| | | |
|---|---|---|
| | | area. Calculated for $C_{26}H_{26}F_4N_2O_4$ MW 506: found ES-LCMS *m/z* 507.2 (M+H) |
| 11 | synthesis details below | $^1$H NMR (400 MHz, $CD_3OD$) δ 7.95 (d, *J*=2.0 Hz, 1H), 7.74 - 7.65 (m, 2H), 7.47 - 7.37 (m, 2H), 7.31 - 7.23 (m, 2H), 6.29 (s, 1H), 4.21 (q, *J*=7.0 Hz, 2H), 3.81 (s, 2H), 3.33 - 3.30 (m, 2H), 2.77 (s, 2H), 1.40 (t, *J*=6.9 Hz, 3H), 0.82 (s, 6H); LCMS purity is 100% by area. Calculated for $C_{27}H_{28}F_4N_2O_4$ MW 520: found ES-LCMS *m/z* 521.1 (M+H) |
| 12 | synthesis details below | $^1$H NMR (400 MHz, $CD_3OD$) δ: 7.98 (d, *J* = 2.0 Hz, 1H), 7.76 (d, *J* = 6.8 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.42-7.33 (m, 2H), 7.27-7.17 (m, 2H), 5.99 (s, 1H), 4.10 (q, *J* = 6.8 Hz, 2H), 3.79 (s, 2H), 3.20 (s, 2H), 3.05 (s, 2H), 2.97 (s, 3H), 1.36 (t, *J* = 6.8 Hz, 3H), 1.18 (s, 6H); LCMS purity is 99.5% by area.Calculated for $C_{28}H_{30}F_4N_2O_5S$ MW 582: found ES-LCMS *m/z* 583 (M+H) |
| 13 | commercially available | $^1$H NMR (400 MHz, DMSO-d6) δ 11.35 (br. s., 1H), 10.86 (br. s., 1H), 8.51 (br. s., 1H), 8.21 (br. s., 1H), 7.86 (br. s., 1H), 7.35 (dd, *J*=7.6, 15.5 Hz, 2H), 7.28 - 7.19 (m, 2H), 5.78 (br. s., 1H), 4.02 (d, *J*=6.8 Hz, 2H), 3.79 (s, 2H), 2.65 (br. s., 3H), 1.26 (t, *J*=7.1 Hz, 3H); LCMS purity is 99.8% by area.Calculated for $C_{25}H_{20}F_4N_4O_4$ MW 516: found ES-LCMS m/z 517 (M+1) |
| 14 | synthesis details below | $^1$H NMR (400 MHz, $CD_3OD$) δ 7.39-7.32 (m, 1H), 7.26-7.18 (m, 1H), 6.37 (s, 1H), 5.99 (s, 1H), 4.10 (q, *J* = 6.8 Hz, 2H), 3.82 (s, 2H), 1.53 (s, 6H), 1.37 (t, *J* = 7.2 Hz, 3H); LCMS purity is 100% by area. Calculated for $C_{22}H_{21}F_4N_3O_4$ MW 467: found ES-LCMS *m/z* 468 (M+H) |

Intermediate B (Scheme S1) for final compound 7

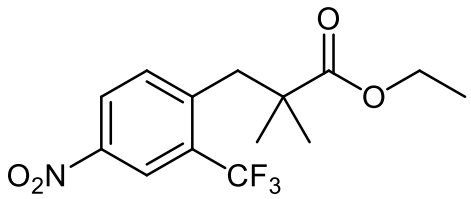
Step 1. ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate




To a mixture of ethyl isobutyrate (6.95 g, 59.8 mmol) in Tetrahydrofuran (THF) (500 mL) cooled to -30 °C was added LDA (34.5 mL, 69.0 mmol) dropwise. The mixture was stirred at -30 °C for 1 hour. To the mixture was added a solution of 1-(bromomethyl)-2-

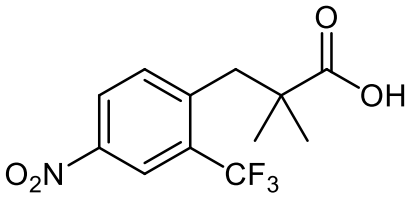
(trifluoromethyl)benzene (11 g, 46.0 mmol) in THF (50 mL) at -30 °C. The whole mixture was stirred at -30 °C for 3 hours and then stirred at 25°C for 2 hours. The mixture was quenched with $NH_4Cl$ (aq, 100 mL), extracted with EtOAc (300 mL x 2). The organic layer was washed with brine (350 mL), dried over $MgSO_4$, filtered and concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 200:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, Rf = 0.6) were combined and concentrated to yield a light yellow solid of ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate (13 g, 30.3 mmol, 65.9 % yield). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 1H), 7.45-7.37 (m, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.14 (s, 2H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.17 (s, 6H).

Step 2. ethyl 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoate



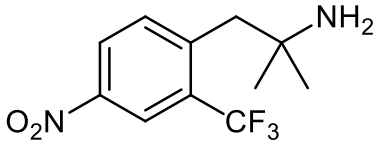

To a solution of ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate (13 g, 47.4 mmol) in $H_2SO_4$ (50 ml, 938 mmol) cooled to 0 °C was added potassium nitroperoxous acid (5.27 g, 52.1 mmol) in portions. The mixture was stirred at 0 °C for 30 mins. The mixture was poured into ice-water (200 mL), extracted with EtOAc (200 mL x 3). The organic layer was washed with *aq.* $Na_2CO_3$ (100 mL x 2), dried over $Na_2SO_4$ and concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 200:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, Rf = 0.65) were combined and concentrated to give a yellow solid of ethyl 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoate (12 g, 33.8 mmol, 71.2 % yield). 1H NMR (400MHz, CDCl3) δ = 8.80-8.10 (m, 2H), 7.64-7.31 (m, 1H), 4.43-4.00 (m, 2H), 3.24 (s, 2H), 1.67-1.56 (m, 3H), 1.35-1.12 (s, 6H). Calculated for $C_{14}H_{16}F_3NO_4$ MW 319: found ES-LCMS m/z: 320 (M+H).

Step 3. 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoic acid




LiOH (1.875 g, 78 mmol) was added to a solution of ethyl 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoate (5g, 15.66 mmol) in Tetrahydrofuran (THF) (40 mL) and water (20 mL). The mixture was at 60 °C for 12 hr. Then the solution was concentrated and distributed between ethyl acetate and saturated $NaHCO_3$ solution. The combined organic extract was washed with Brine, dried over $MgSO_4$, filtered and concentrated. The crude material was purified by silica column chromatography (PE/EtOAc = 1:1). All fractions found to contain product by TLC (PE/EtOAc =:1, Rf 0.4) were combined and concentrated to yield a light yellow solid of 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoic acid (2.93g, 9.05 mmol, 57.8 % yield). $^1$H NMR (400 MHz, CHLOROFORM-d) δ = 8.56 (d, *J*=2.0 Hz, 1H), 8.34 (dd, *J*=2.2, 8.6 Hz, 1H), 7.61 (d, *J*=8.6 Hz, 1H), 3.29 (s, 2H), 1.28 (s, 6H).

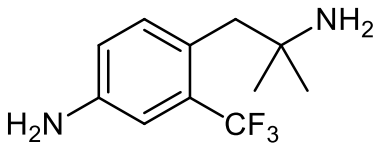
Step 4. 2-methyl-1-(4-nitro-2-(trifluoromethyl)phenyl)propan-2-amine




$Et_3N$ (1.434 mL, 10.29 mmol) was added to a solution of 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoic acid (2.8 g, 9.61 mmol) in toluene (30 mL). diphenylphosphorazidate (2.83 g, 10.29 mmol) was added under 0 °C and the mixture was stirred under 0 °C for 1 hour. The mixture was stirred at 26 °C for an additional 1 hour, and then the mixture was at 100 °C for 3 hr. The solution was cooled and washed with water (3*10 mL), and the toluenn phase was separated, dried over $Na_2SO_4$, and evaporated in vacuum. A mixture of 15% hydrochloric acid (6 mL) and acetic acid (6 mL) was added, and the resulting mixture was stirred at 26 °C overnight. The mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (4*10 mL), and the aqueous was concentrated in vacuum to afford the HCl salt of the title compound 2-methyl-1-(4-nitro-2-(trifluoromethyl)phenyl)propan-2-amine

(770 mg, 2.467 mmol, 25.7 % yield). $^1$H NMR (400MHz, CHLOROFORM-d) δ 8.55 (d, *J*=2.2 Hz, 1H), 8.34 (dd, *J*=2.2, 8.6 Hz, 1H), 8.00 - 7.87 (m, 1H), 2.99 (s, 2H), 1.44 - 1.32 (m, 2H), 1.22 -1.12 (m, 6H). Calculated for $C_{11}H_{13}F_3N_2O_2$ MW 262: found LCMS: 263.1 (M+H).

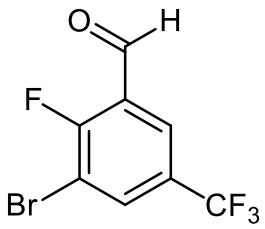
Step 5. 4-(2-amino-2-methylpropyl)-3-(trifluoromethyl)aniline




To a suspension of 2-methyl-1-(4-nitro-2-(trifluoromethyl)phenyl)propan-2-amine (770 mg, 2.94 mmol) in methanol (10 mL) was added palladium on carbon (156 mg, 0.147 mmol)(10%). The mixture was hydrogenated under H2 atmosphere (15 Psi) at 26 °C for 3 hr. Then the solution was filtered and concentrated. The crude material was purified by preparative HPLC (Instrument: DC /Column: ASB C18 150*25mm /Mobile phase A: Water+0.1% HCl/Mobile phaseB: MeCN / Flowrate:25ml/min / Gradient Profile Description: 1-20 (B%)) to yield a white solid of 4-(2-amino-2-methylpropyl)-3-(trifluoromethyl)aniline, 2 Hydrochloride (601.47 mg, 1.962 mmol, 66.8 % yield). TLC (DCM/MeOH = 10:1, Rf 0.4). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.48-7.35 (m, 2H), 7.27 (dd, *J* = 2.1, 8.3 Hz, 1H), 3.18-3.08 (m, 2H), 1.37 (s, 6H). Calculated for $C_{11}H_{15}F_3N_2$ MW 232: found EC-LCMS: 233 (M+H).

Intermediate B (Scheme S1) for final compound 8

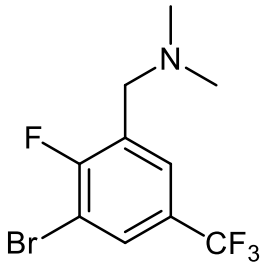
Step 1. 3-bromo-2-fluoro-5-(trifluoromethyl)benzaldehyde




A solution of BuLi (1.975 mL, 4.94 mmol) (2.5 M in hexanes) was added to a solution of diisopropylamine (0.416 g, 4.12 mmol) in tetrahydrofuran (THF) (10 mL) at -30 °C. After 15 min, the mixture was cooled to - 78 °C, then 2-bromo-1-fluoro-4-(trifluoromethyl)benzene (1 g, 4.12 mmol) was added. After 30 min, anhydrous DMF (0.319 mL, 4.12 mmol) was added

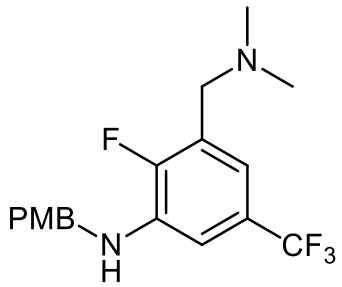
dropwise. After 15 min, acetic acid (0.471 mL, 8.23 mmol) was added, then the mixture was diluted between EA (50 mL) and $H_2O$ (20 mL), extracted with EA (30 mL x 2). The organic layer was washed with brine (20 mL), dried over $Na_2SO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography (PE/EA = 50/1 to 20/1). All fractions found to contain product by TLC (PE/EA = 10/1, Rf = 0.8) were combined and concentrated to yield a yellow oil of 3-bromo-2-fluoro-5-(trifluoromethyl)benzaldehyde (0.8 g, 2.66 mmol, 64.6 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 10.35 (s, 1H), 8.08 (dd, *J*=5.9, 9.0 Hz, 2H).

Step 2. 1-(3- bromo-2-fluoro-5-(trifluoromethyl)phenyl)-N,N-dimethylmethanamine



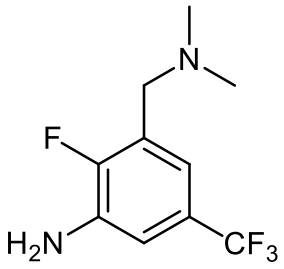

A solution of 3-bromo-2-fluoro-5-(trifluoromethyl)benzaldehyde (0.2 g, 0.738 mmol) and dimethylamine (2M in THF) (0.738 mL, 1.476 mmol) in Dichloromethane (DCM) (10 mL) was stirred at 25 °C for 2 hr. sodium cyanoborohydride (0.139 g, 2.214 mmol) was added. The resulting mixture was stirred at 25 °C for 16 hr. After LCMS analysis showed the starting material was disappeared. The mixture was distributed between DCM (30 mL) and $H_2O$ (20 mL), extracted with DCM (30 mL x 2). The organic layer was washed with brine (20 mL), dried over $Na_2SO_4$, filtered and concentrated to give the crude product, which was purified by preparative TLC (PE/EA = 5/1) to yield a colorless oil of 1-(3- bromo-2-fluoro-5-(trifluoromethyl)phenyl)-N,N-dimethylmethanamine (0.1 g, 0.283 mmol, 38.4 % yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.93 - 7.88 (m, 1H), 7.75 (d, *J*=4.4 Hz, 1H), 3.62 (s, 2H), 2.26 (s, 6H); Calculated for $C_{10}H_{11}BrF_4N$ MW 300: found ES-LCMS m/z 302.0 (M+2H).

Step 3. 3-((dimethylamino)methyl)-2-fluoro-N-(4-methoxybenzyl)-5-(trifluoromethyl)aniline




A solution of 1-(3-bromo-2-fluoro-5-(trifluoromethyl)phenyl)-N,Ndimethylmethanamine (90 mg, 0.300 mmol), (4-methoxyphenyl)methanamine (82 mg, 0.600 mmol), BINAP (18.67 mg, 0.030 mmol), $Pd_2dba_3$ (27.5 mg, 0.030 mmol) and potassium tert-butoxide (67.3 mg, 0.600 mmol) in Toluene (6 mL) was stirred at 120 °C for 2 hr in a microwave. The solvent was removed in vacuo. The residue was purified by preparative TLC (PE/EA = 5/1, Rf = 0.2) to yield a yellow oil of 3-((dimethylamino)methyl)-2-fluoro-N-(4-methoxybenzyl)-5-(trifluoromethyl)aniline (70 mg, 0.169 mmol, 56.3 % yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.31 (d, *J*=8.5 Hz, 2H), 6.90 (d, *J*=8.5 Hz, 3H), 6.82 (d, *J*=7.5 Hz, 1H), 4.37 (s, 2H), 3.79 (s, 3H), 3.71 (br. s., 2H), 2.39 (s, 6H); Calculated for $C_{18}H_{20}F_4N_2O$ MW 356: found ES-LCMS m/z 357.1 (M+H).

Step 4. 3-((dimethylamino)methyl)-2-fluoro-5-(trifluoromethyl)aniline

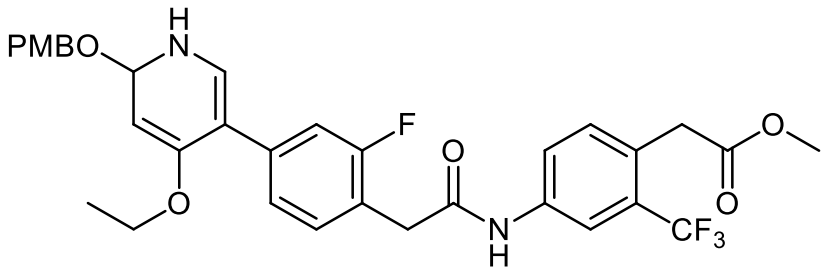



A solution of 3-((dimethylamino)methyl)-2-fluoro-N-(4-methoxybenzyl)-5-(trifluoromethyl)aniline (70 mg, 0.196 mmol) in 2,2,2-trifluoroacetic acid (20 % in Dichloromethane) (2 mL, 2.98 mmol) was stirred at 25 °C for 0.5 hr. After LCMS analysis showed the starting material was disappeared. The solvent was removed in vacuo to give an off-white solid of 3-((dimethylamino)methyl)-2-fluoro-5-(trifluoromethyl)aniline, 2-trifluoroacetic acid salt (90 mg, 0.184 mmol, 94 % yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.78 (br. s., 1H), 7.46 - 7.26 (m, 5H), 7.23 (d, *J*=2.2 Hz, 1H), 7.03 (br. s., 1H), 4.29 (dd, *J*=4.0, 10.1 Hz, 1H), 4.25 - 4.19

(m, 1H), 4.12 (q, *J*=7.1 Hz, 2H), 3.86 (d, *J*=4.4 Hz, 1H), 3.81 (br. s., 2H), 3.66 (br. s., 2H), 1.47 (t, *J*=6.9 Hz, 3H); Calculated for $C_{10}H_{12}F_4N_2$ MW 256: found ES-LCMS m/z 237.1 (M+H).

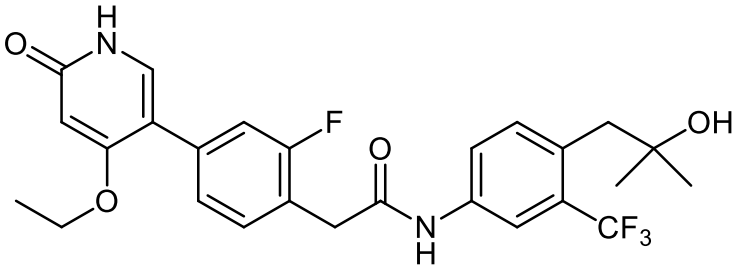
Compound 10 (differentated step after amide formation from Scheme S1)

Step 1. methyl 2-(4-(2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)-1,6-dihydropyridin-3-yl)-2-fluorophenyl)acetamido)-2-(trifluoromethyl)phenyl)acetate




A solution of methyl 2-(4-amino-2-(trifluoromethyl)phenyl)acetate (200 mg, 0.858 mmol), 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)acetic acid (353 mg, 0.858 mmol), HATU (489 mg, 1.287 mmol) and DIEA (0.225 mL, 1.287 mmol) in Dichloromethane (DCM) (20 mL) was stirred at 20 °C for 3 hours. After LCMS analysis showed the starting material was disappeared. DCM (20mL) was added and the resulting mixture was washed with $H_2O$ (20 mL) and brine (20 mL). The separated organic layer was dried over $Na_2SO_4$, filtered and concentrated to give crude material which was purified by preparative TLC (DCM/MeOH=30/1) to give pure product methyl 2-(4-(2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)-1,6-dihydropyridin-3-yl)-2-fluorophenyl)acetamido)-2-(trifluoromethyl)phenyl)acetate (277 mg, 0.313 mmol, 36.5 % yield). $^1$H NMR (400 MHz, $CD_3OD$): δ 1.35 (t, *J* = 7.0 Hz, 3 H) 3.65 (s, 3 H) 3.77 (s, 2 H) 3.79 (s, 3H) 4.07 - 4.13 (m, 2 H) 5.17 - 5.31 (m, 2 H) 5.33 - 5.59 (m, 2 H) 6.43 (s, 1 H) 6.89 (d, *J*=8.6 Hz, 2 H) 7.26 (d, *J*=9.8 Hz, 2 H) 7.31 - 7.42 (m, 4 H) 7.77 (d, *J*=6.2 Hz, 1 H) 7.94 (s, 1 H) 7.98 (s, 1 H). Calculated for $C_{33}H_{32}F_4N_2O_6$ MW 628: found LCMS:628.2.

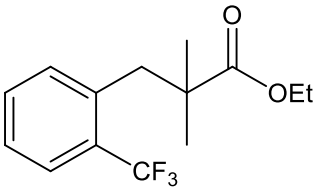
Step 2. 2-(4-(4-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-N-(4-(2-hydroxy-2-methylpropyl)-3 (trifluoromethyl)phenyl)acetamide



To a solution of methyl 2-(4-(2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)-1,6-dihydropyridin-3-yl)-2-fluorophenyl)acetamido)-2-(trifluoromethyl)phenyl)acetate (40 mg, 0.064 mmol) in Tetrahydrofuran (THF) (20 mL) cooled to 0 °C was added methyllithium (13.98 mg, 0.636 mmol) in dropwise. Then the mixture was stirred at 0 °C for 2 hours. After the mixture was warmed to 25 ºC and quenched with $NH_4Cl$ (aq), the solution was extracted with EA (15mLx 2), washed with $H_2O$ and brine . The organic layer was dried over $Na_2SO_4$, filtered and concentrated. The crude product was purified by preparative HPLC to give pure product 2-(4-(4-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-N-(4-(2-hydroxy-2-methylpropyl)-3 (trifluoromethyl)phenyl)acetamide (3.03 mg, 5.95 μmol, 9.34 % yield). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.95 (s, 1H), 7.67 (m, 2H),7.54 (s,2H), 7.24 (t, *J* = 6.0 Hz, 1H), 7.24 (m, 2H), 6.13 (s, 1H), 4.15 (m, 2H), 3.79 (s, 2H), 2.91 (s, 2H), 1.38 (t, *J* = 6.8 Hz, 3H), 1.145 (s, 6H); LCMS purity is 100% by area. Calculated for $C_{26}H_{26}F_4N_2O_4$ MW 506: found ES-LCMS *m/z* 507.2 (M+H).

Intermediate B (Scheme S1) for final compound 11

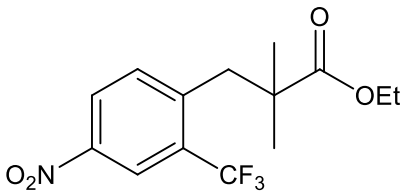
Step 1. ethyl 2,2-dimethyl-3-(2- (trifluoromethyl)phenyl)propanoate



To a mixture of diisopropylamine (8.00 mL, 57.1 mmol) in THF (300 mL) cooled to 0 °C, *n*-BuLi (24.60 mL, 61.5 mmol) was added dropwise. The mixture was stirred at 0 °C for 1 hour. Then the mixture was cooled to -30 °C and a solution of ethyl isobutyrate (6.12 g, 52.7 mmol) in THF (2 mL) was added. The mixture was stirred at -30 °C for 1 hour. To the mixture was

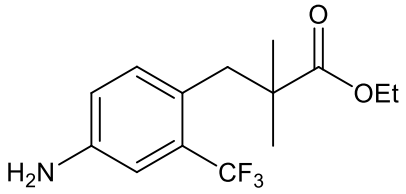
added a solution of 1-(bromomethyl)-2-(trifluoromethyl)benzene (10.5 g, 43.9 mmol) in THF (5 mL) at -30 °C. The whole mixture was stirred at -30 °C for 3 hours and then stirred at 25 °C for 12 hours. The mixture was quenched with $NH_4Cl$ (aq), extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 200:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, $R_f$ = 0.6) were combined and concentrated to yield a light yellow solid of ethyl 2,2-dimethyl-3-(2- (trifluoromethyl)phenyl)propanoate (10 g, 35.3 mmol, 80% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.14 (s, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.18 (s, 6H); Calculated for $C_{14}H_{17}F_3O_2$ MW 274: found ES-LCMS *m/z* 275 (M+H).

Step 2. ethyl 2,2-dimethyl-3-(4-nitro-2- (trifluoromethyl)phenyl)propanoate



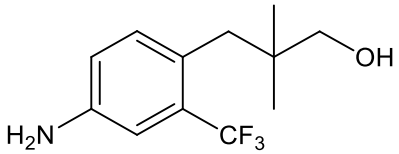

To a solution of ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate (10 g, 36.5 mmol) in $H_2SO_4$ (5 mL, 94 mmol) cooled to 0 °C was added potassium nitroperoxous acid (4.05 g, 40.1 mmol) in portions. The mixture was stirred at 0 °C for 30 min. The mixture was poured into ice-water, extracted with DCM (100 mL x 2). The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated to give a yellow solid of ethyl 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoate (8.5 g, 24.54 mmol, 67% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.59 (d, *J* = 2.4 Hz, 1H), 8.47 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 5.97-5.83 (m, 2H); Calculated for $C_{14}H_{16}F_3NO_4$ MW 319: found ES-LCMS *m/z* 320 (M+H).

Step 3. ethyl 3-(4-amino-2-(trifluoromethyl)phenyl)-2,2-dimethylpropanoate



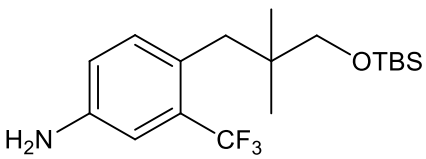
A reaction mixture of ethyl 2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propanoate (8.5 g, 26.6 mmol) and Pd-C (0.283 g, 2.66 mmol) in MeOH (50 mL) was hydrogenated using the H-cube (settings: 50 °C, 50 psi, 24 hours). The mixture was filtered, and the filtrate was concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 10:1). All fractions found to contain product by TLC (PE/EtOAc = 5:1, $R_f$ = 0.4) were combined and concentrated to yield a off white solid of ethyl 3-(4-amino-2-(trifluoromethyl)phenyl)-2,2-dimethylpropanoate (7 g, 22.42 mmol, 84% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.71 (dd, *J* = 2.4, 8.4 Hz, 1H), 4.15 (q, *J* = 6.8 Hz, 2H), 3.00 (s, 2H), 1.25 (t, *J* = 7.2Hz, 3H), 1.14 (s, 6H); Calculated for $C_{14}H_{18}F_3NO_2$ MW 289: found ES-LCMS *m/z* 290 (M+H).

Step 4. 3-(4-amino-2-(trifluoromethyl)phenyl)- 2,2-dimethylpropan-1-ol




To a mixture of ethyl 3-(4-amino-2-(trifluoromethyl)phenyl)-2,2-dimethylpropanoate (2 g, 6.91 mmol) in THF (200 mL) was added $LiAlH_4$ (0.525 g, 13.83 mmol) in portions. The mixture was stirred at 25°C for 10 hours. The mixture was quenched with 15% NaOH solution (aq, 10 mL). The mixture was dried over $Na_2SO_4$, filtered, and the filtrate was concentrated. The residue was purified on silica column chromatography (PE/EtOAc = 8:1). All fractions found to contain product by TLC (PE/EtOAc = 2:1, $R_f$ = 0.35) were combined and concentrated to yield a light yellow oil of 3-(4-amino-2-(trifluoromethyl)phenyl)- 2,2-dimethylpropan-1-ol (1.1 g, 4.45 mmol, 64% yield): $^1$H NMR (400 MHz, $CD_3Cl$) δ: 7.17 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 2.4, 8.0 Hz, 1H), 3.31 (s, 2H), 2.67 (d, *J* = 1.2 Hz, 2H), 0.84 (s, 6H); Calculated for $C_{12}H_{16}F_3NO$ MW 247: found ES-LCMS *m/z* 248 (M+H).

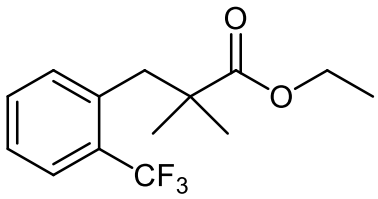
Step 5. 4-(3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-3-(trifluoromethyl)aniline



To a mixture of 3-(4-amino-2-(trifluoromethyl)phenyl)-2,2-dimethylpropan-1-ol (300 mg, 1.213 mmol) in DCM (150 mL) was added imidazole (124 mg, 1.820 mmol) and TBSCl (219 mg, 1.456 mmol). Then the mixture was stirred at 25 °C for 5 hours. The mixture was filtered and the filtrate was concentrated. The crude material was purified by preparative TLC (PE/EtOAc = 2:1, $R_f$ = 0.5) to yield a light yellow solid of 4-(3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-3-(trifluoromethyl)aniline (350 mg, 0.930 mmol, 77% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.14 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 2.8 Hz, 1H), 6.70 (dd, *J* = 2.8, 8.4 Hz, 1H), 3.20 (s, 2H), 2.62 (d, *J* = 1.2 Hz, 2H), 0.87 (s, 9H), 0.73 (s, 6H), 0.00 (s, 6H); Calculated for $C_{18}H_{30}F_3NOSi$ MW 361: found ES-LCMS *m/z* 362 (M+H).

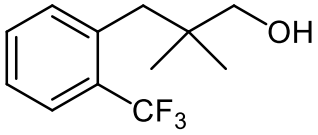
Intermediate B (Scheme S1) for final compound 12

Step 1. ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate



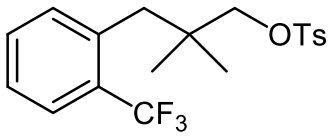

To a mixture of ethyl isobutyrate (6.32 g, 54.4 mmol) in Tetrahydrofuran (THF) (200 mL) cooled to -30 °C was added LDA (0.031 L, 62.0 mmol) dropwise. The mixture was stirred at -30 °C for 1 hour. To the mixture was added a solution of 1-(bromomethyl)-2 (trifluoromethyl)benzene (10g, 41.8 mmol) in THF (150 mL) at -30 °C. The whole mixture was stirred at -30 °C for 1 hours and then stirred at 25 °C for 1 hours. The mixture was quenched with $NH_4Cl$ (aq, 200 mL). The mixture was added $H_2O$ (200 mL), extracted with EtOAc (800 mL x 3). The organic layer was washed with brine (800 mL), dried over $Na_2SO_4$, filtered and concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 200:1). All fractions found to contain product by TLC (PE/EtOAc =10:1, Rf = 0.6) were combined and concentrated to yield a light yellow solid of ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate (10 g, 31.0 mmol, 74.1 % yield). Attached NMR and LCMS are consistent with product. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.68 (d, *J*=7.5 Hz, 1H), 7.56 - 7.49 (m, 1H), 7.44 - 7.37 (m, 1H), 7.32 (d, *J*=7.5 Hz, 1H), 4.18 (q, *J*=7.2 Hz, 2H), 3.16 (s, 2H), 1.26 (t, *J*=7.0 Hz, 3H), 1.19 (s, 6H).

Step 2. 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propan-1-ol



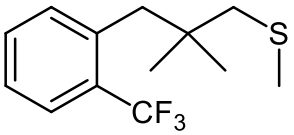

To a mixture of $LiAlH_4$ (2.77 g, 72.9 mmol) in Tetrahydrofuran (THF) (200 mL) cooled to 0 °C was added a solution of ethyl 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propanoate (10 g, 36.5 mmol) in THF (200 mL) dropwise. The mixture was stirred at 25 °C for 5 hours. The mixture was quenched with 15% NaOH (aq, 80 mL). To the mixture was added $Na_2SO_4$ (20 g) and stirred for 0.5 hour. After filtered, the filtrate was concentrated to yield a light yellow oil of 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propan-1-ol (7.5 g, 30.7 mmol, 84 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 7.6 Hz, 1H), 7.48-7.39 (m, 2H), 7.33-7.26 (m, 1H), 3.40 (s, 2H), 2.84 (s, 2H), 0.89 (s, 6H).

Step 3. 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl 4-methylbenzenesulfonate



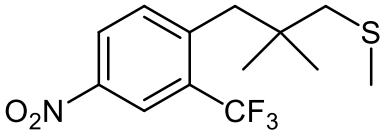

A mixture of 4-methylbenzene-1-sulfonyl chloride (8.00 g, 42.0 mmol) and 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propan-1-ol (7.5 g, 32.3 mmol) in pyridine (30 mL) stirred at 60 °C for 10 hour. LCMS showed the started material was consumed completely, the mixture was concentrated. The residue was purified on silica column chromatography (PE/EtOAc = 30:1~20:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, Rf = 0.5) were combined and concentrated to yield a light yellow oil of 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl 4-methylbenzenesulfonate (10 g, 24.58 mmol, 76 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 3H), 7.32-7.26 (m, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 3.75 (s, 2H), 2.77 (s, 2H), 2.46 (s, 3H), 0.90-0.83 (m, 6H); Calculated for $C_{19}H_{21}F_3O_3Si$ MW 386: found ES-LCMS *m/z*: 404 (M+18).

Step 4. (2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane



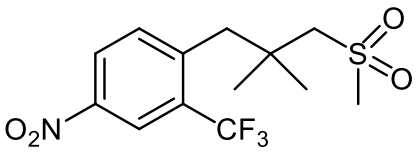

A mixture of 2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl 4-methylbenzenesulfonate (5g, 12.94 mmol) and sodium methanethiolate (3.06 mL, 64.7 mmol) in N-Methyl-2-pyrrolidone (NMP) (20 mL) was stirred at 160 °C for 2 hours. After cooled, the mixture was added $H_2O$ (50 mL) and extracted with EtOAc (50 mL x 3). The organic layer was concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 20:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, Rf = 0.6) were combined and concentrated to yield a light yellow oil of (2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane (2.5 g, 9.34 mmol, 72.2 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 1H), 7.48-7.42 (m, 1H), 7.41-7.37 (m, 1H), 7.33-7.27 (m, 1H), 2.87 (s, 2H), 2.53 (s, 2H), 2.15 (s, 3H), 0.97 (s, 6H).

Step 5. (2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane




To a solution of (2,2-dimethyl-3-(2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane (2 g, 7.62 mmol) in $H_2SO_4$ (10 mL, 188 mmol) cooled to 0 °C was added potassium nitroperoxous acid (0.809 g, 8.01 mmol) in portions. The mixture was stirred at 0 °C for 5 mins. The mixture was poured into ice-water (20 mL), extracted with EtOAc (20 mL x 3). The organic layer was washed with saturated $Na_2CO_3$ (20 mL x 2), dried over $MgSO_4$ and concentrated. The residue was purified on silica column chromatography (PE/EtOAc = 20:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, Rf = 0.55) were combined and concentrated to yield a yellow oil of (2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane (450 mg, 1.391 mmol, 18.25 %yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 2.0 Hz, 1H), 8.31 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 2.98 (s, 2H), 2.53 (s, 2H), 2.17 (s, 3H), 0.99 (s, 6H).

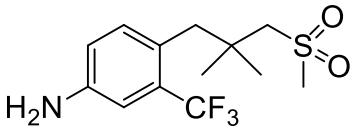
Step 6. 1-(2,2-dimethyl-3-(methylsulfonyl)propyl)-4-nitro 2-(trifluoromethyl)benzene



To a mixture of (2,2-dimethyl-3-(4-nitro-2-(trifluoromethyl)phenyl)propyl)(methyl)sulfane (450 mg, 1.464 mmol) in Dichloromethane (DCM) (50 mL) was added mCPBA (1011 mg, 5.86 mmol).

Then the mixture was stirred at 25 °C for 12 hr. The mixture was added $Na_2S_2O_3$ (3 g) and $Na_2CO_3$ (1 g). The mixture was stirred for 30 min. The mixture was filtered and the filtrate was concentrated. The residue was purified by preparative HPLC (Column: ASB C18 150*25mm; Mobile phase A: Water + 0.1% HCl; Mobile phaseB: Acetonitrile; Flowrate:25mL/min; Gradient Profile Description: 35-75 (B%)) to give a yellow oil of 1-(2,2-dimethyl-3-(methylsulfonyl)propyl)-4-nitro 2-(trifluoromethyl)benzene (400 mg, 1.155 mmol, 79 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 2.2 Hz, 1H), 8.35 (dd, *J* = 2.2, 8.6 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 1H), 3.26 (s, 2H), 3.06 (s, 2H), 2.97 (s, 3H), 1.25 (s, 7H); Calculated for $C_{13}H_{16}F_3NO_4S$ MW 339: found ES-LCMS *m/z* 362 (M+23).

Step 7. 4-(2,2-dimethyl-3-(methylsulfonyl)propyl)-3-(trifluoromethyl)aniline

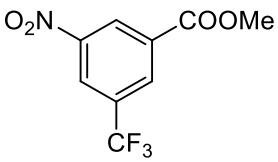


To a mixture of 1-(2,2-dimethyl-3-(methylsulfonyl)propyl)-4-nitro-2-(trifluoromethyl)benzene (70 mg, 0.206 mmol) in methanol (30 mL) was added Pd/C (2.195 mg, 0.021 mmol) under $N_2$. The mixture was stirred under a $H_2$ balloon at 25 °C for 1 hour. The mixture was filtered and concentrated to give a yellow oil of 4-(2,2-dimethyl-3-(methylsulfonyl)propyl)-3-(trifluoromethyl)aniline (50 mg, 0.155

mmol, 75 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.77 (dd, *J* = 2.4, 8.4 Hz, 1H), 3.79 (s, 2H), 2.97 (s, 2H), 2.90 (s, 3H), 2.87 (s, 2H), 1.26 (s, 6H); Calculated for $C_{13}H_{18}F_3NO_2S$ MW 309: found ES-LCMS *m/z*: 317 (M+18).

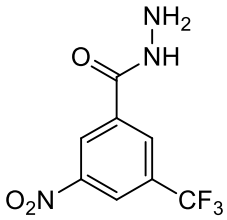
Intermediate B (Scheme S1) for final compound 14

Step 1. methyl 3-nitro-5-(trifluoromethyl)benzoate



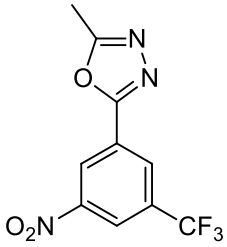

To a mixture of 3-nitro-5-(trifluoromethyl)benzoic acid (20 g, 85 mmol) in MeOH (200 mL) was added $H_2SO_4$ (12 mL, 225 mmol) at 0 °C dropwise, then the mixture was stirred for 16 hours at 25 °C. Then the solvent was concentrated and was adjusted pH = 9 with $NaHCO_3$ solution, the solvent was concentrated to give the residue which was extracted with DCM (200 mL x 2), dried over $Na_2SO_4$, concentrated to yield an oil of methyl 3-nitro-5-(trifluoromethyl)benzoate (20 g, 76 mmol, 90% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.99 (s, 1H), 8.74 (s, 1H), 8.62 (s, 1H), 4.01 (s, 3H); Calculated for $C_9H_6F_3NO_4$ MW 249: found ES-LCMS *m/z* 250 (M+1).

Step 2. 3-nitro-5-(trifluoromethyl)benzohydrazide




A mixture of methyl 3-nitro-5-(trifluoromethyl)benzoate (20 g, 80 mmol), hydrazine hydrate (5.56 mL, 96 mmol) in MeOH (100 mL) was stirred for 16 hours at 25 °C. Then the solvent was concentrated to yield an off white solid of 3-nitro-5-(trifluoromethyl)benzohydrazide (20 g, 72.2 mmol, 90% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 8.89 (s, 1H), 8.65 (s, 1H), 8.50 (s, 1H); Calculated for $C_8H_6F_3N_3O_3$ MW 249: found ES-LCMS *m/z* 250 (M+H).

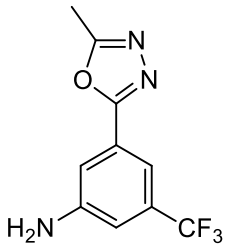
Step 3. 2-methyl-5-(3-nitro-5 -(trifluoromethyl)phenyl)-1,3,4-oxadiazole




A mixture of 3-nitro-5-(trifluoromethyl)benzohydrazide (20 g, 80 mmol) in 1,1,1-triethoxyethane (156 g, 963 mmol) was heated to reflux and stirred for 12 hours . Then the solvent was

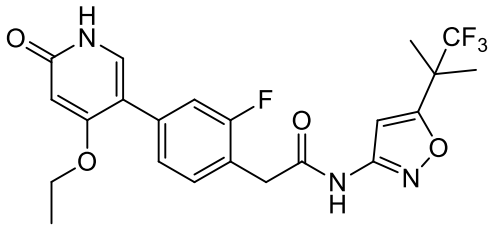
concentrated to yield a black solid of 2-methyl-5-(3-nitro-5 -(trifluoromethyl)phenyl)-1,3,4-oxadiazole (20 g, 65.9 mmol, 82% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 9.04 (s, 1H), 8.71 (s, 1H), 8.66 (s, 1H), 2.67 (s, 1H); Calculated for $C_{10}H_6F_3N_3O_3$ MW 273: found ES-LCMS *m/z* 274 (M+H).

Step 4. 3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(trifluoromethyl)aniline

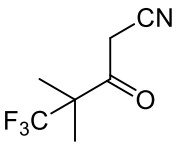



A mixture of 2-methyl-5-(3-nitro-5-(trifluoromethyl)phenyl)-1,3,4-oxadiazole (20 g, 73.2 mmol), Pd/C (0.779 g, 7.32 mmol) in MeOH (25 mL) was stirred for 12 hours at 35 psi under $H_2$ atmosphere at 25 °C. Then the mixture was filtered and the filtrate was concentrated to give the residue which was crystallized with methanol (15 mL) to yield a gray solid of 3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(trifluoromethyl)aniline (17 g, 66.4 mmol, 91% yield): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.46 (s, 1H), 7.42 (s, 1H), 7.07 (s, 1H), 2.61 (s, 3H); Calculated for $C_{10}H_8F_3N_3O$ MW 243: found ES-LCMS *m/z* 244 (M+H).

**Compound 15.** 2-(4-(4-Ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-*N*-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide

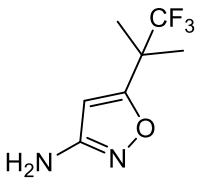



Step 1: 5,5,5-Trifluoro-4,4-dimethyl-3-oxopentanenitrile



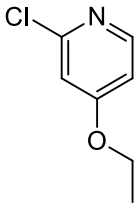
To a mixture of acetonitrile (13.9 mL, 264 mmol) in THF (500 mL) cooled to -78 °C was added *n*-BuLi (106 mL, 264 mmol). The mixture was stirred at -30 °C for 0.5 hour. Then to the mixture was added methyl 3,3,3-trifluoro-2,2-dimethylpropanoate (30 g, 176 mmol) dropwise. The mixture was quenched with $NH_4Cl$ (*aq*, 50 mL), extracted with EtOAc (300 mL x 3). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to yield a crude product of a yellow oil of 5,5,5-trifluoro-4,4-dimethyl-3-oxopentanenitrile (22 g, 122.9 mmol, 70% yield): $^1$H NMR (400MHz, $CDCl_3$) δ 3.75 (s, 2H), 1.41 (s, 6H).

Step 2: 5-(1,1,1-Trifluoro-2-methylpropan-2-yl)isoxazol-3-amine



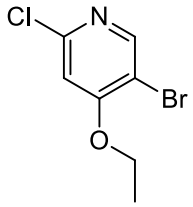

To a mixture of hydroxylamine, hydrochloride (23.2 g, 336 mmol) in water (300 mL) cooled to 0 °C was added $NaHCO_3$ (30 g, 351 mmol) and pH = 7.5 adjusted. Then to the mixture was added a solution of 5,5,5-trifluoro-4,4-dimethyl-3-oxopentanenitrile (30 g, 167.4 mmol) in MeOH (40 mL). The mixture was stirred at 65 °C for 15 hours. After cooled, the mixture was acidified with *conc*. HCl to pH = 1 and then refluxed for 2 hours. After cooling to room temperature, the mixture was neutralized by 4 mol/L NaOH to pH = 8. The mixture was extracted with EtOAc (300 mL x 2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated. The crude material was purified on silica column chromatography (PE/EtOAc = 8:1~3:1). All fractions found to contain product by TLC (PE/EtOAc = 2:1, $R_f$ = 0.6) were combined and concentrated to yield a red solid of 5-(1,1,1-trifluoro-2-methylpropan-2-yl) isoxazol-3-amine (19.5 g, 100.5 mmol, 60% yield): $^1$H NMR (400MHz, $CDCl_3$) δ 5.79 (s, 1H), 3.96 (s., 2H), 1.53 (s, 6H); Calculated for $C_7H_9F_3N_2O$ MW 194: found ES-LCMS *m/z*: 195 (M+H).

Step 3: 2-Chloro-4-ethoxypyridine



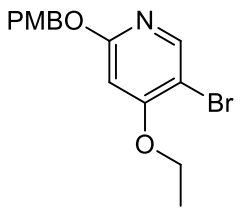
A mixture of 2-chloro-4-nitropyridine (170 g, 1070 mmol) in THF (2 L) was added sodium ethanolate (109.45 g, 1610 mmol) slowly at 0 °C. The mixture was stirred at 25 °C for 12 hours. LCMS and TLC (PE/EtOAc = 5:1, $R_f$ = 0.6) showed the reaction was finished. The mixture was filtrated, and most solvent of the filtrate was removed *in vacuo*. The residue was extracted with EtOAc (800 mL x 3), and the organic layer was washed with saturated NaCl solution (1 L), dried over $Na_2SO_4$ and concentrated to give crude 2-chloro-4-ethoxypyridine (157 g, 1.0 mol, 92% yield) as a solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 8.15 (d, *J* = 6.0 Hz, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.91-6.89 (m, 1H), 4.16-4.14 (m, 2H), 1.41-1.38 (m, 3H); Calculated for $C_7H_8ClNO$ MW 157: found ES-LCMS *m/z* 158 (M+H).

Step 4: 5-Bromo-2-chloro-4-ethoxypyridine



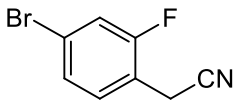

2-Chloro-4-ethoxypyridine (100 g, 0.63 mol) was added to $H_2SO_4$ (500 mL) slowly. Then 1-bromopyrrolidine-2,5-dione (124.2 g, 0.70 mol) was added into above mixture at room temperature. The mixture was stirred at 80 °C for 3 hours. TLC (PE/EtOAc = 10:1, $R_f$ = 0.5) showed the reaction was finished. The reaction mixture was poured into ice-water (2 L), and extracted with EtOAc (1 L x 3). The organic layer was washed with saturated $Na_2CO_3$ solution (1 L x 2), dried over $Na_2SO_4$ and concentrated. The residue was purified on silica column chromatography (PE/EtOAc = 60:1-30:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, $R_f$ = 0.5) were combined and concentrated to yield 5-bromo-2-chloro-4-ethoxypyridine (60.9 g, 0.26 mol, 40% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 8.31 (s, 1H), 7.14 (s, 1H), 4.32-4.10 (m, 2H), 1.58-1.35 (m, 3H); Calculated for $C_7H_7BrClNO$ MW 234: found ES-LCMS *m/z* 236 (M+2).

Step 5: 5-Bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine



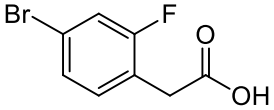

A mixture of 5-bromo-2-chloro-4-ethoxypyridine (75 g, 317.1 mmol) in toluene (500 mL) was added (4-methoxyphenyl)methanol (52.6 g, 380.6 mmol), KOH (35.6 g, 634.3 mmol) and 18-crown-6 (8.4 g, 31.2 mmol) at room temperature. The reaction mixture was stirred at 120 °C for 2 hours. The mixture was portioned between 2-methoxy-2-methylpropane (500 mL) and brine (800 mL). The organic layer was concentrated. The residue was purified by column (PE/EtOAc = 10:1, $R_f$ = 0.5) to give 5-bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine (72.2 g, 221 mmol, 70% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 8.05 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 6.90-6.84 (m, 2H), 6.38 (s, 1H), 5.20 (s, 2H), 4.16-4.05 (m, 2H), 3.77 (s, 3H), 1.43 (q, *J* = 6.8 Hz, 3H); Calculated for $C_{15}H_{16}BrNO_3$ MW 337: found ES-LCMS *m/z* 338 (M+H).

Step 6: 2-(4-Bromo-2-fluorophenyl)acetonitrile



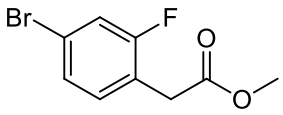

A solution of 4-bromo-1-(bromomethyl)-2-fluorobenzene (500 g, 1.87 mol) in ethanol (2.2 L) stirred under nitrogen at 20 °C was added sodium cyanide (93 g, 1.90 mmol) in one charge. The reaction mixture was stirred at 60 °C for 12 hours. Then the solution was concentrated and distributed between dichloromethane (2000 mL) and saturated $NaHCO_3$ solution (1800 mL). Another batch was repeated using the same procedure. Then the two batches were combined. The combined organic extract was washed with brine, dried over $MgSO_4$, filtered and concentrated to resulting 2-(4-bromo-2-fluorophenyl)acetonitrile (794 g, 99% yield), which was used to the next step without further purification: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38-7.27 (m, 3H), 3.72 (s, 2H).

Step 7: 2-(4-Bromo-2-fluorophenyl)acetic acid



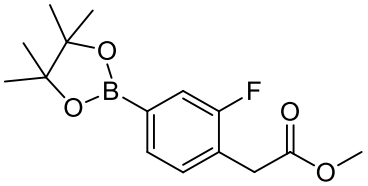
A solution of 2-(4-bromo-2-fluorophenyl)acetonitrile (397 g, 1.82 mol) in methanol (500 mL) stirred under nitrogen at 20 °C was added NaOH (2.22 L, 2.5M, 5.56 mol) solution in one charge. The reaction mixture was stirred at 80 °C for 5 hours. Then the solution was concentrated and neutralized with *conc.* HCl to pH = 5 with stirring. Then the solution was extracted with ethyl acetate (1.5 L x 2). Another two batches were repeated using the same procedure. Then the three batches were combined. The combined organic extract was washed with brine, dried over $Na_2SO_4$, filtered and concentrated in *vacuo* to give the pure 2-(4-bromo-2-fluorophenyl)acetic acid (1200 g, 92% yield): TLC (PE/EtOAc = 5:1, $R_f$ = 0.2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.24 (br. s., 1H), 7.12 (t, *J* = 7.9 Hz, 1H), 3.65 (s, 2H).

Step 8: Methyl 2-(4-bromo-2-fluorophenyl)acetate




To a solution of 2-(4-bromo-2-fluorophenyl)acetic acid (260 g ,1.13 mol ) in methanol (2 L) was added $H_2SO_4$ (30 mL) at room temperature. The solution was heated to reflux overnight. Then the solvent was concentrated and distributed between ethyl acetate and saturated $NaHCO_3$ solution. The combined organic extract was washed with brine, dried over $Na_2SO_4$, filtered and concentrated. Another batch was repeated using the same procedure. Then the two batches were combined. The resulting methyl 2-(4-bromo-2-fluorophenyl)acetate (520 g, 94%) was used to next step without further purification. TLC (PE/EtOAc = 10:1, $R_f$ = 0.7). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.25-7.20 (m, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 3.70 (s, 3H), 3.62 (s, 2H).

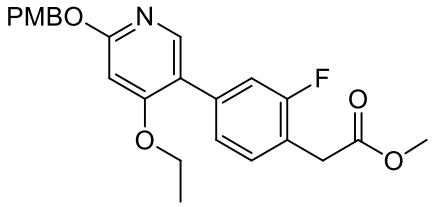
Step 9: Methyl 2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate




To a solution of methyl 2-(4-bromo-2-fluorophenyl)acetate (260 g ,1.05 mol ) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (320 g, 1.26 mol) in dioxane (2 L) was added KOAc (206 g, 2.10 mol) and $Pd(dppf)Cl_2$ (23 g, 0.03 mol) at room temperature. The solution was heated to reflux for 4 hours under $N_2$. Then the solution was filtered and the filtrate was concentrated in *vacuo* to give the crude product. Another batch was repeated using the same

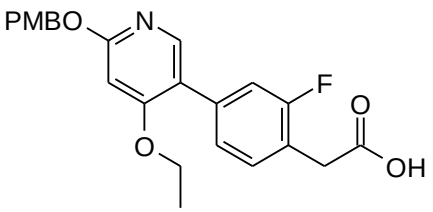
procedure. Then the two batches were combined and purified by silica column chromatography (PE/EtOAc = 30:1 to 10:1). All fractions found to contain product by TLC (PE/EtOAc = 10:1, $R_f$ = 0.5) were combined and concentrated to yield methyl 2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (560 g, 90%) as light yellow oil: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 10.0 Hz, 1H), 7.31-7.26 (m, 1H), 3.73 (s, 2H), 1.34 (s, 12H), 1.27 (s, 3H); Calculated for $C_{15}H_{20}BFO_4$ MW 294: found ES-LCMS *m/z* 295.2 (M+H).

Step 10: Methyl 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl) acetate



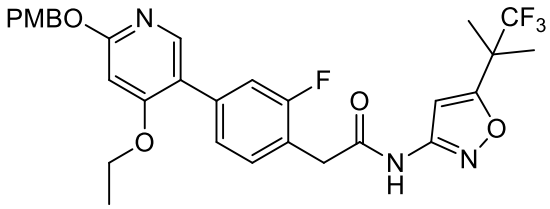

A solution of 5-bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine (175 g, 519 mmol) in dixoane (1.2 L) and $H_2O$ (300 mL) was added methyl 2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (167 g, 569 mmol), $Pd(dppf)Cl_2$ (25 g, 5.19 mmol) and $Cs_2CO_3$ (337 g, 1038 mmol) under $N_2$. The mixture was refluxed for 2 hours. TLC (PE/EtOAc = 5:1, $R_f$ = 0.3) showed the reaction was finished. The mixture was portioned between EtOAc (1 L) and $H_2O$ (800 mL). The organic layer was dried over $Na_2SO_4$ and concentrated. The residue was purified by column (PE/EtOAc = 5:1, $R_f$ = 0.3) to give 5-bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine (210 g, 0.49 mol, 90% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 7.94 (s, 1H), 7.36 (d, *J* = 8.8 Hz, 2H), 7.32-7.22 (m, 3H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.43 (s, 1H), 5.26 (s, 2H), 4.11 (d, *J* = 6.8 Hz, 2H), 3.78 (s, 3H), 3.72 (s, 2H), 3.70 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H); Calculated for $C_{24}H_{24}FNO_5$ MW 425: found ES-LCMS *m/z* 426 (M+H).

Step 11: 2-(4-(4-Ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)acetic acid



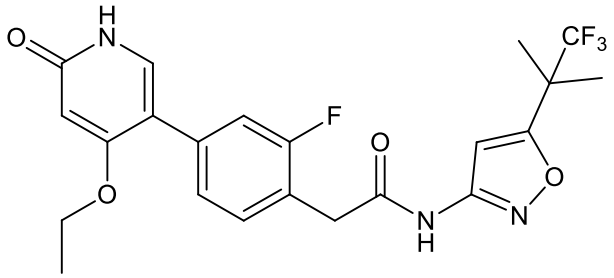
To a solution of methyl 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy) pyridin-3-yl)-2-fluorophenyl) acetate (210 g, 519 mmol) in THF (500 mL) was added a solution of $LiOH \cdot H_2O$ (52 g, 1.23 mol) in $H_2O$ (700 mL). The mixture was stirred at 60 °C for 10 hours. TLC (PE/EtOAc = 5:1, $R_f$ = 0.3) showed the reaction was finished. The mixture was concentrated and neutralized with 1.0 mol/L HCl to pH = 7.0. Then the mixture was filtrated, and the solid was washed with water and dry *in vacuo* to give 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy) pyridin-3-yl)-2-fluorophenyl)acetic acid (183.3 g, 0.45 mol, 93% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 7.94 (s, 1H), 7.41-7.28 (m, 3H), 7.24 (d, *J* = 9.6 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.44 (s, 1H), 5.26 (s, 2H), 4.11 (q, *J* = 6.8 Hz, 2H), 3.78 (s, 3H), 3.67 (s, 2H), 1.36 (t, *J* = 7.2 Hz, 3H); Calculated for $C_{23}H_{22}FNO_5$ MW 411: found ES-LCMS *m/z* 412 (M+H).

Step 12: 2-(4-(4-Ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)-*N*-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide




To a mixture of 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)acetic acid (55.1 g, 134 mmol) and 5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-amine (26 g, 134 mmol) in pyridine (500 mL) was added $T_3P$ (137.5 mL, 134 mmol) dropwise and stirred at 25 °C for 1 hour. After TLC analysis showed the starting material was consumed completely, the mixture was poured into stirring cold water (1 L). The mixture was stirred for 0.5 hour and then let stand for 10 hours. The solid was filtered, washed with $H_2O$ (200 mL x 3) and TBME (200 mL x 2) and dried *in vacuo* to give an off-white solid of 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl)-*N*-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide (65 g, 100 mmol, 74% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 7.94 (s, 1H), 7.40-7.32 (m, 3H), 7.26 (d, *J* = 9.6 Hz, 2H), 6.90 (d, *J* = 8.8 Hz, 3H), 6.43 (s, 1H), 5.26 (s, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 2H), 3.78 (s, 3H), 1.56 (s, 6H), 1.35 (t, *J* = 7.2 Hz, 3H); Calculated for $C_{30}H_{29}F_4N_3O_5$ MW 587: found ES-LCMS *m/z*: 588 (M+H).

Step 13: 2-(4-(4-Ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-*N*-(5-(1,1,1- trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide



To a suspension of 2-(4-(4-ethoxy-6-((4-methoxybenzyl)oxy)pyridin-3-yl)-2-fluorophenyl) -*N*-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide (100 g, 170 mmol) in DCM (1 L) was added TFA (80 mL, 1077 mmol) dropwise. The mixture was stirred at 25 °C for 2 hours. The mixture was then concentrated. To the residue was added $H_2O$ (500 mL) dropwise and then neutralized with saturated $Na_2CO_3$ solution to adjust pH to 7.5. The precipitate was filtered, washed with $H_2O$ (350 mL x 3) and dried *in vacuo*. The solid was added PE/EtOAc (3:1, *v*/*v*, 300 mL) and stirred for 0.5 hour. The solid was filtered, washed with PE/EtOAc (3:1, *v*/*v*, 100 mL x 2). The solid was redissolved in DCM/MeOH (20:1, *v*/*v*, 1.5 L) and then concentrated *in vacuo* to a minimal amount of solvent (about 150 mL). The solid was filtered, washed with $CH_3CN$ (50 mL x 2) and dried *in vacuo*. The residual solid was added to EtOH (2.5 L) and heated to 80 °C. After the solid was dissolved completely, the mixture was concentrated *in vacuo* to give a white solid of 2-(4-(4-ethoxy-6-oxo-1,6-dihydropyridin-3-yl)-2-fluorophenyl)-*N*-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)acetamide (61.4 g, 131 mmol, 77% yield): $^1$H NMR (400MHz, $CD_3OD$) δ 7.40-7.30 (m, 2H), 7.25-7.18 (m, 2H), 6.88 (s, 1H), 5.98 (s, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 2H), 1.56 (s, 6H), 1.37 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (300 MHz, $CH_3OD$) δ 169.56, 166.83, 166.80, 165.60, 160.70 (d), 158.07, 135.08, 133.92, 131.18, 126.70 (q), 124.58, 120.70, 115.73, 115.51, 97.31, 96.39, 64.49, 42.35, 35.41, 19.40, 12.92; Calculated for $C_{22}H_{21}F_4N_3O_4$ MW 467: found ES-LCMS *m/z*: 468 (M+H). UPLC purity is 99.9 area% by PDA (210 to 350 nm), rt 5.40 min; CHN analytical results: theoretical (monohydrate of **15**) C: 54.43 H: 4.78 N: 8.66, average (%): C: 54.48 H: 4.69 N: 8.63 (monohydrate).

**Recombinant Kinase Profiling**

The inhibitory effect of 1 μM GSK3179106 on enzyme activity described as percent inhibition on over 300 recombinant kinases was determined in an in vitro screen (Carna Biosciences, Kobe, Japan). Optimal reaction conditions for each kinase were optimized for time and substrate, ATP and metal concentration. The readout value of reaction control (complete reaction mixture) was set as 0% inhibition, and the readout value of background (reaction mixture without enzyme) was set as 100% inhibition and percent inhibition of each test solution was calculated.

**Table S4.** GSK3179106 (compound **15**) at 1 μM inhibits >80% enzyme activity of 26 kinases. Inhibited kinases are highlighted in yellow and include 7 mutants of wildtype kinases.

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 μM (%) |
|---|---|
| ABL | 11.7 |
| ABL(E255K) | 0.5 |
| ABL(T315I) | -3.9 |
| ACK | 3.9 |
| ALK | -8.9 |
| ALK(F1174L) | -8.6 |
| ALK(L1196M) | -6.7 |
| ALK(R1275Q) | -5.7 |
| EML4-ALK | -4.3 |
| NPM1-ALK | -8.5 |
| ARG | 2.8 |
| AXL | 18.0 |
| BLK | 3.7 |
| BMX | 1.2 |
| BRK | 14.7 |
| BTK | -10.2 |
| CSK | 24.7 |
| DDR1 | 102.9 |
| DDR2 | 102.7 |
| EGFR | -6.2 |
| EGFR(d746-750) | -1.5 |
| EGFR(d746-750/T790M) | -12.3 |
| EPHB1 | 66.7 |
| EPHB2 | 73.7 |
| EPHB3 | 39.3 |
| EPHB4 | 28.0 |
| FAK | -3.8 |
| FER | -6.9 |
| FES | -7.9 |
| FGFR1 | 55.3 |
| FGFR1(V561M) | 13.1 |
| FGFR2 | 83.8 |
| FGFR3 | 50.0 |
| FGFR3(K650E) | 54.4 |
| FGFR3(K650M) | 51.1 |
| FGFR4 | 46.1 |
| FGFR4(N535K) | 72.8 |
| FGFR4(V550E) | -11.0 |
| FGFR4(V550L) | 3.8 |
| FGR | 2.5 |
| FLT1 | 97.2 |
| FLT3 | 97.5 |
| FLT4 | 103.0 |
| FMS | 30.1 |

| | | | |
|---|---|---|---|
| EGFR(L858R) | -0.7 | FRK | 45.1 |
| EGFR(L861Q) | -5.0 | FYN | -3.1 |
| EGFR(T790M) | -7.6 | HCK | 21.2 |
| EGFR(T790M/L858R) | -8.9 | HER2 | -4.8 |
| EPHA1 | 5.6 | HER4 | -0.3 |
| EPHA2 | 88.2 | IGF1R | -3.5 |
| EPHA3 | 12.6 | INSR | -3.9 |
| EPHA4 | 66.1 | IRR | -2.2 |
| EPHA5 | 86.4 | ITK | -3.3 |
| EPHA6 | 46.7 | JAK1 | -0.8 |
| EPHA7 | 29.5 | JAK2 | -6.9 |
| EPHA8 | 84.8 | JAK3 | -8.6 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 μM (%) |
|---|---|
| KDR | 95.3 |
| KIT | 101.2 |
| KIT(D816V) | 29.0 |
| KIT(T670I) | 72.9 |
| KIT(V560G) | 100.2 |
| KIT(V654A) | 52.0 |
| LCK | 28.0 |
| LTK | -11.3 |
| LYNa | 61.8 |
| LYNb | 56.5 |
| MER | 20.9 |
| MET | -1.4 |
| MET(Y1235D) | 5.4 |
| MUSK | 79.8 |
| PDGFRα | 102.9 |
| PDGFRα(T674I) | 96.5 |
| PDGFRα(V561D) | 102.6 |
| PDGFRβ | 102.1 |
| PYK2 | -7.3 |
| RET | 101.4 |
| RET(G691S) | 102.7 |
| RET(M918T) | 102.7 |
| RET(S891A) | 103.1 |
| RET(Y791F) | 102.2 |
| RON | 6.7 |
| ROS | -5.0 |
| SRC | -6.2 |
| SRM | -4.5 |
| SYK | 1.7 |
| TEC | -11.1 |
| TIE2 | 23.3 |
| TNK1 | 3.6 |
| TRKA | 99.6 |
| TRKB | 97.9 |
| TRKC | 100.6 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 μM (%) |
|---|---|
| YES | 12.1 |
| YES(T348I) | -4.7 |
| ZAP70 | -13.6 |
| AKT1 | 9.9 |
| AKT2 | -7.9 |
| AKT3 | -12.9 |
| AMPKα1/β1/γ1 | -5.8 |
| AMPKα2/β1/γ1 | -0.3 |
| AurA | 2.1 |
| AurA/TPX2 | -1.0 |
| AurB | -4.7 |
| AurC | 2.5 |
| BRAF_Cascade | -1.2 |
| BRAF(V600E)_Cascade | 17.7 |
| BRSK1 | -6.1 |
| BRSK2 | -1.1 |
| CaMK1α | -4.5 |
| CaMK1δ | -0.6 |
| CaMK2α | -1.8 |
| CaMK2β | -1.7 |
| CaMK2γ | -2.5 |
| CaMK2δ | -2.9 |
| CaMK4 | -0.5 |
| CDC2/CycB1 | 1.0 |
| CDC7/ASK | -3.0 |
| CDK2/CycA2 | -2.3 |
| CDK2/CycE1 | -1.6 |
| CDK3/CycE1 | -13.9 |
| CDK4/CycD3 | -5.1 |
| CDK5/p25 | -11.9 |
| CDK6/CycD3 | -2.8 |
| CDK7/CycH/MAT1 | -2.2 |
| CDK9/CycT1 | -2.7 |
| CGK2 | -2.9 |
| CHK1 | 2.1 |

| | | | |
|---|---|---|---|
| TXK | 8.8 | CHK2 | -7.4 |
| TYK2 | -10.6 | CK1α | -1.9 |
| TYRO3 | -4.1 | CK1γ1 | -0.4 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 µM (%) |
|---|---|
| CK1γ2 | 0.8 |
| CK1γ3 | -1.6 |
| CK1δ | -3.7 |
| CK1ε | -18.8 |
| CK2α1/β | -7.7 |
| CK2α2/β | -7.0 |
| CLK1 | 19.0 |
| CLK2 | 5.9 |
| CLK3 | 12.9 |
| COT_Cascade | 1.6 |
| CRIK | -17.0 |
| DAPK1 | -1.9 |
| DCAMKL2 | -8.4 |
| DLK_Cascade | -2.7 |
| DYRK1A | 1.6 |
| DYRK1B | 1.6 |
| DYRK2 | 42.6 |
| DYRK3 | 68.8 |
| EEF2K | -2.9 |
| Erk1 | -5.1 |
| Erk2 | -7.2 |
| Erk5 | -3.9 |
| GSK3α | -11.3 |
| GSK3β | -7.7 |
| Haspin | -4.4 |
| HGK | 9.9 |
| HIPK1 | -0.6 |
| HIPK2 | 13.2 |
| HIPK3 | 45.1 |
| HIPK4 | 97.7 |
| IKKα | -1.7 |
| IKKβ | -2.3 |
| IKKε | -1.1 |
| IRAK1 | 28.5 |
| IRAK4 | 7.0 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 µM (%) |
|---|---|
| LATS2 | 1.4 |
| LOK | 21.3 |
| MAP2K1_Cascade | 4.2 |
| MAP2K2_Cascade | -6.0 |
| MAP2K3_Cascade | 83.1 |
| MAP2K4_Cascade | -4.2 |
| MAP2K5_Cascade | -6.5 |
| MAP2K6_Cascade | 92.1 |
| MAP2K7_Cascade | -13.4 |
| MAP3K1_Cascade | 18.6 |
| MAP3K2_Cascade | -5.3 |
| MAP3K3_Cascade | 76.1 |
| MAP3K4_Cascade | 84.6 |
| MAP3K5_Cascade | 86.9 |
| MAP4K2 | 2.3 |
| MAPKAPK2 | -16.8 |
| MAPKAPK3 | -8.0 |
| MAPKAPK5 | 11.3 |
| MARK1 | -9.2 |
| MARK2 | -9.9 |
| MARK3 | -7.0 |
| MARK4 | -16.2 |
| MELK | 34.4 |
| MGC42105 | -6.2 |
| MINK | -0.2 |
| MLK1_Cascade | -5.2 |
| MLK2_Cascade | -9.4 |
| MLK3_Cascade | -3.8 |
| MNK1 | 89.1 |
| MNK2 | 100.6 |
| MOS_Cascade | 2.9 |
| MRCKα | -0.3 |
| MRCKβ | -3.5 |
| MSK1 | -0.6 |
| MSK2 | 0.5 |

| | | | |
|---|---|---|---|
| JNK1 | -12.2 | MSSK1 | 14.4 |
| JNK2 | -5.6 | MST1 | 1.4 |
| JNK3 | -11.5 | MST2 | -7.8 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 µM (%) |
|---|---|
| MST3 | -7.9 |
| MST4 | -8.7 |
| NDR1 | -2.0 |
| NDR2 | 5.1 |
| NEK1 | -2.1 |
| NEK2 | 0.8 |
| NEK4 | -1.1 |
| NEK6 | -4.3 |
| NEK7 | -7.4 |
| NEK9 | -5.3 |
| NuaK1 | -7.6 |
| NuaK2 | -3.8 |
| p38α | 70.4 |
| p38β | 81.6 |
| p38γ | 22.4 |
| p38δ | -8.0 |
| p70S6K | 48.2 |
| p70S6Kβ | 9.8 |
| PAK1 | -4.9 |
| PAK2 | 5.4 |
| PAK4 | -9.1 |
| PAK5 | 2.0 |
| PAK6 | -4.6 |
| PASK | -2.9 |
| PBK | -11.0 |
| PDHK2 | -2.8 |
| PDHK4 | 1.5 |
| PDK1 | -1.3 |
| PEK | -4.4 |
| PGK | -4.1 |
| PHKG1 | 30.0 |
| PHKG2 | 1.1 |
| PIM1 | -1.4 |
| PIM2 | 2.1 |
| PIM3 | -1.1 |
| PKACα | -13.5 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 µM (%) |
|---|---|
| PKCα | -1.5 |
| PKCβ1 | -3.5 |
| PKCβ2 | -7.0 |
| PKCγ | -0.9 |
| PKCδ | -0.2 |
| PKCε | -4.3 |
| PKCζ | 4.9 |
| PKCη | 0.7 |
| PKCθ | -2.1 |
| PKCι | 5.9 |
| PKD1 | -5.1 |
| PKD2 | -7.8 |
| PKD3 | 5.5 |
| PKN1 | 4.2 |
| PKR | -4.2 |
| PLK1 | -9.9 |
| PLK2 | -1.5 |
| PLK3 | -10.3 |
| PRKX | 1.0 |
| QIK | -6.9 |
| RAF1_Cascade | 22.2 |
| ROCK1 | -4.9 |
| ROCK2 | 22.1 |
| RSK1 | 5.3 |
| RSK2 | 1.7 |
| RSK3 | 30.7 |
| RSK4 | 0.0 |
| SGK | -2.4 |
| SGK2 | -3.3 |
| SGK3 | -6.8 |
| SIK | 2.3 |
| skMLCK | -7.5 |
| SLK | -3.7 |
| SRPK1 | 27.6 |
| SRPK2 | 3.4 |
| TAK1- | -12.0 |

| | | | |
|---|---|---|---|
| | | TAB1_Cascade | |
| PKACβ | -2.7 | TAOK2 | 1.3 |
| PKACγ | -8.0 | TBK1 | -0.2 |

| Kinase | Inhibition of recombinant kinase by GSK3179106 at 1 μM (%) |
|---|---|
| TNIK | 22.8 |
| TSSK1 | -0.6 |
| TSSK2 | -8.2 |
| TSSK3 | -9.3 |
| WNK1 | 2.3 |
| WNK2 | 4.9 |
| WNK3 | 6.3 |
| PIK3CA/PIK3R1 | 17.6 |
| SPHK1 | -21.9 |
| SPHK2 | -8.5 |

**Chemoproteomic Profiling of GSK3179106**

Male Sprague Dawley rats were purchased from Charles River Laboratories (Margate Kent, UK). Rat colon tissue was cut into pieces, frozen in liquid nitrogen and homogenized in DP buffer (50 mM TRIS/HCl pH 7.4, 150 mM NaCl, 1.5 mM $MgCl_2$, 5% Glycerol, 50 mM NaF, 1 mM $Na_3VO_4$, 1 mM DTT and complete, EDTA-free protease inhibitors) using a Polytron. Igepal CA-630 detergent was then added to 0.8% final concentration and the sample was further homogenized using a douncer. Lysate was incubated for 30 min at 4 $^{o}$C and centrifuged for 10 min at 20,000 g. Supernatant was centrifuged again at 100,000 g for 1 h. Resulting supernatant was aliquoted and stored at -80 $^{o}$C.

Kinobeads are unselective kinase inhibitors immobilized on Sepharose beads that are used as an affinity matrix to capture a large set of endogenous kinases present in any cell/tissue extract. For the affinity purifications, compound stock solutions were prepared in DMSO. Final DMSO concentration in the assay was 0.5% for all samples; 700 μL of a 5% beads slurry was used per data point (35 μL of solid beads). The mixture (5 μL of compound solution (or DMSO) and 1 mL (5 mg) of DP-diluted cell lysate) was pre-incubated for 45 min at 4 °C. After a short centrifugation step at 250 g for 1 min, lysate-compound mixtures were transferred to microlute filter plates containing 35 μL of beads and incubation was performed for 1 h at 4 °C. The filter plate was then centrifuged at 250 g for 1 min and wells washed with 10 mL DP buffer containing

0.4 % Igepal CA-630 and 5 mL DP buffer containing 0.2 % Igepal CA630 by gravity flow. Remaining buffer was discarded by centrifugation at 250 g for 2 min. Proteins were eluted from the bead matrix with 50 µL elution buffer (2x LDS sample buffer with 25 mM DTT) at 50 °C with shaking. The eluates were collected by centrifugation at 250 g for 2 min, and stored at 20 °C. Chemoproteomic samples in elution buffer were alkylated for 30 min at 25 °C in the dark with 20 mg/mL iodoacetamide. After a brief centrifugation at 250 g, 35 µL of supernatant was loaded on a 4-12% Bis-Tris gel and proteins were separated by electrophoresis. Gels were cut under keratin shields into three slices across the entire separation range and subjected to tryptic in-gel digestion. Briefly, gels were destained two times for 1 h at 55 °C with 100 µL 60% 5 mM triethylammonium bicarbonate (TEAB), 40% Ethanol followed by dehydration with absolute Ethanol (3 times) at room temperature (RT). Tryptic digestion was carried out using 25 µL of 10 ng µL-1 trypsin in 5 mM TEAB for 4 h at 30 °C, digestion was quenched by addition of 5 µL 5% formic acid. Trypsinized peptides were extracted consecutively with 20 µL 1% formic acid 2 times, 20 µL 60% acetonitrile in water with 0.1% formic acid, 20 µL acetonitrile and extracts were pooled. After tryptic digestion peptides were labeled with tandem mass tags (TMT). Briefly, peptides were lyophilized in vacuum and dissolved in 10 µL 90% 200 mM TEAB, 10% acetonitrile and 10 µL TMT reagent (5 mg dissolved in 581 µL acetonitrile) were added. Reaction took place for 1 h at 20 °C with shaking at 400 rpm. The reaction was stopped with 5 µL 100 mM TEAB/100 mM glycine. Labeled peptides were pooled into Eppendorf cups and lyophilized in vacuum. Pooled samples were dissolved in 20 µL 60% 200 mM TEAB 40% acetonitrile and 2 µL of 2.5% hydroxylamine were added. Samples were transferred into 96 well plates and lyophilized under vacuum.

Samples were separated into 9 fractions using an off-line HPLC system (Ultimate 3000, Thermo Scientific) at pH 12. Lyophilized samples were re-suspended in 25 µL 1.25% ammonia in water. The whole sample was injected onto a pre-column (XBridgeTM, C18, 5 µm) at a flow rate of 30 µL min-1. Separation was done at 40 µL min-1 on a 150 mm reverse-phase-column with 1 mm internal diameter (I.D.) (XBridgeTM, C18, 3.5 µm) with a gradient of 115 min length ranging from 97% buffer A (1.25% ammonia in water) to 60% B (1.25% ammonia in 70% acetonitrile in water). Fractions were collected for 120 sec with pooling (*1*). Lyophilized samples were re-suspended in 0.05% trifluoroacetic acid in water and 50% were injected onto an

Ultimate 3000 nano-LC system coupled to a Q-Exactive mass spectrometer (Thermo-Finnigan). The nanoLC systems were equipped with an Acclaim PepMap100 pre-column (ThermoScientific) of 100 μm I.D. and 2 cm length with 5 μm C18 100 Å pore size and in house prepared separation columns with 100 μm I.D. and 50 cm length filled with 3 μm C18 120 Å pore size. Peptides were trapped on the pre-column at a flow rate of 5 μL $min^{-1}$ with 0.05% trifluoroacetic acid in water for 5 minutes. Gradient elution was performed at 0.35 μL $min^{-1}$ from 3% formic acid in water to 30% formic acid in acetonitrile at 55 °C.

**Peptide and Protein Identification and Quantification**

Q-Exactives were operated with XCalibur software (Thermo-Finnigan). Tandem mass spectra were generated for up to 10 precursors using Higher-energy collisional dissociation (HCD) in data dependent mode (*2*). Profile tandem mass spectra were computed by the XCalibur software operating the instrument and data were extracted using customized scripts. Mascot 2.4.1 (Matrix Science) was used for protein identification using 10 ppm mass tolerance for peptide precursors and 0.002 Da for fragment ions. Carbamidomethylation of cysteine residues and TMT modification of lysine residues were set as fixed modifications. Methionine oxidation, N-terminal acetylation of proteins and TMT modification of peptide N-termini were set as variable modifications. The search database consisted of a customized version of the International Protein Index (IPI) database combined with a decoy version of this database created using a script supplied by Matrix Science. Protein identifications were accepted as follows: (i) for single spectrum to sequence assignment, this assignment was required to be the best match with a minimum Mascot score of 33 and a difference in score of 10 of this assignment over the next best assignment. Based on these criteria, the decoy search results indicated <1% false-discovery rate (FDR); (ii) for multiple spectrum to sequence assignments and using the same parameters, the decoy search results indicate <0.1% FDR. For protein quantification, a minimum of two sequence assignments matching to unique peptides were required. FDR for quantified proteins was <0.1%. Furthermore, only peptides unique for identified proteins were used for relative protein quantification denominated quantified unique peptide matches (qupm). Further, quantified unique spectrum matches (qusm) were filtered according to the following criteria: mascot ion score ≥15, signal-to-background ratio of the precursor ion ≥4, signal-to-interference measure ≥0.5. Reporter ion intensities were multiplied with the ion accumulation time yielding

an area value proportional to the number of reporter ions present in the mass analyzer. Protein quantification was derived from individual spectra matching to distinct peptides by using a sum-based bootstrap algorithm; 95% confidence intervals were calculated for all protein fold-changes that were quantified with more than three spectra. Fold-changes were corrected for isotope purity and adjusted for interference caused by co-eluting nearly isobaric peaks as estimated by the signal-to-interference measure (*3*).

Dose-response curves were fitted using R (http://www.r-project.org/) and the drc package (http://www.bioassay.dk) (*4*). $IC_{50}$ values were converted into $K_d^{app}$ using $IC_{50}$ to $K_d^{app}$ correction factors determined in depletion experiments. Briefly, lysate was incubated in independent duplicates for 1 h with a blocked bead matrix or kinobeads matrix, the non-bound lysate was removed from the bead matrix by centrifugation and incubated again for 1 h with kinobeads matrix. Proteins bound to the respective bead matrices in the second incubation step were eluted and subjected to SDS-PAGE as described. The $IC_{50}/K_d^{app}$ correction factor of specifically captured proteins was determined in a quantitative LC-MS analysis as the ratio of the relative fold changes observed in the consecutive incubation with blocked beads and kinobeads matrix and the incubations with 2-times kinobeads matrix as outlined below (*5*).


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**Table S5.** Kinases affected by GSK3179106 with an $IC_{50}$ <30 μM

| Kinase name | Protein accession | $IC_{50}$ (μM) | $pIC_{50}$ | $IC_{50}/K_d^{app}$ | $pK_d^{app}$ |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| RET | IPI00188936 | 0.02 | 7.62 | 2.1 | 7.94 |
| DDR1 | IPI00209121 | 0.04 | 7.37 | 1.7 | 7.61 |
| DDR2 | IPI00895587 | 0.09 | 7.07 | 1.6 | 7.27 |
| FLT3 | IPI00363507 | 0.5 | 6.28 | 1.8 | 6.54 |
| FLT4 | IPI00210269 | 1.3 | 5.89 | 2.5 | 6.28 |
| RIPK2 | IPI00207880 | 1.8 | 5.74 | 3.2 | 6.24 |
| IRAK4 | IPI00372984 | 1.1 | 5.96 | 1.6 | 6.17 |
| PDGFRA | IPI00202498 | 2.1 | 5.68 | 1.1 | 5.73 |
| MUSK | IPI00780249 | 3.9 | 5.41 | 1.4 | 5.57 |
| EPHA2 | IPI00369122 | 4.8 | 5.32 | 1.8 | 5.57 |
| MAPK14 | IPI00829435 | 7.6 | 5.12 | 2.0 | 5.42 |
| TEK | IPI00389063 | 10.0 | 5 | 1.9 | 5.28 |
| PDGFRB | IPI00199968 | 7.2 | 5.14 | 1.2 | 5.22 |
| EPHB4 | IPI00767957 | 15.8 | 4.8 | 1.9 | 5.09 |
| KIT | IPI00205775 | 24.0 | 4.62 | 1.1 | 4.65 |

**Compound 15 Rat IV (bolus)**

0.06 mg/kg IV bolus
Formulation: 0.0400 mg/mL in DMSO:6% HP-beta-CD=5:95, pH = 7, clear solution
Species: Male Sprague-Dawley rat, n = 3
All blood samples are transferred into plastic microcentrifuge tubes containing heparin [Blood: Heparin（1000 unit）=20:1] as an anti-coagulant and placed on wet ice until centrifugation. Harvested blood samples are centrifuged within 30 min of collection.

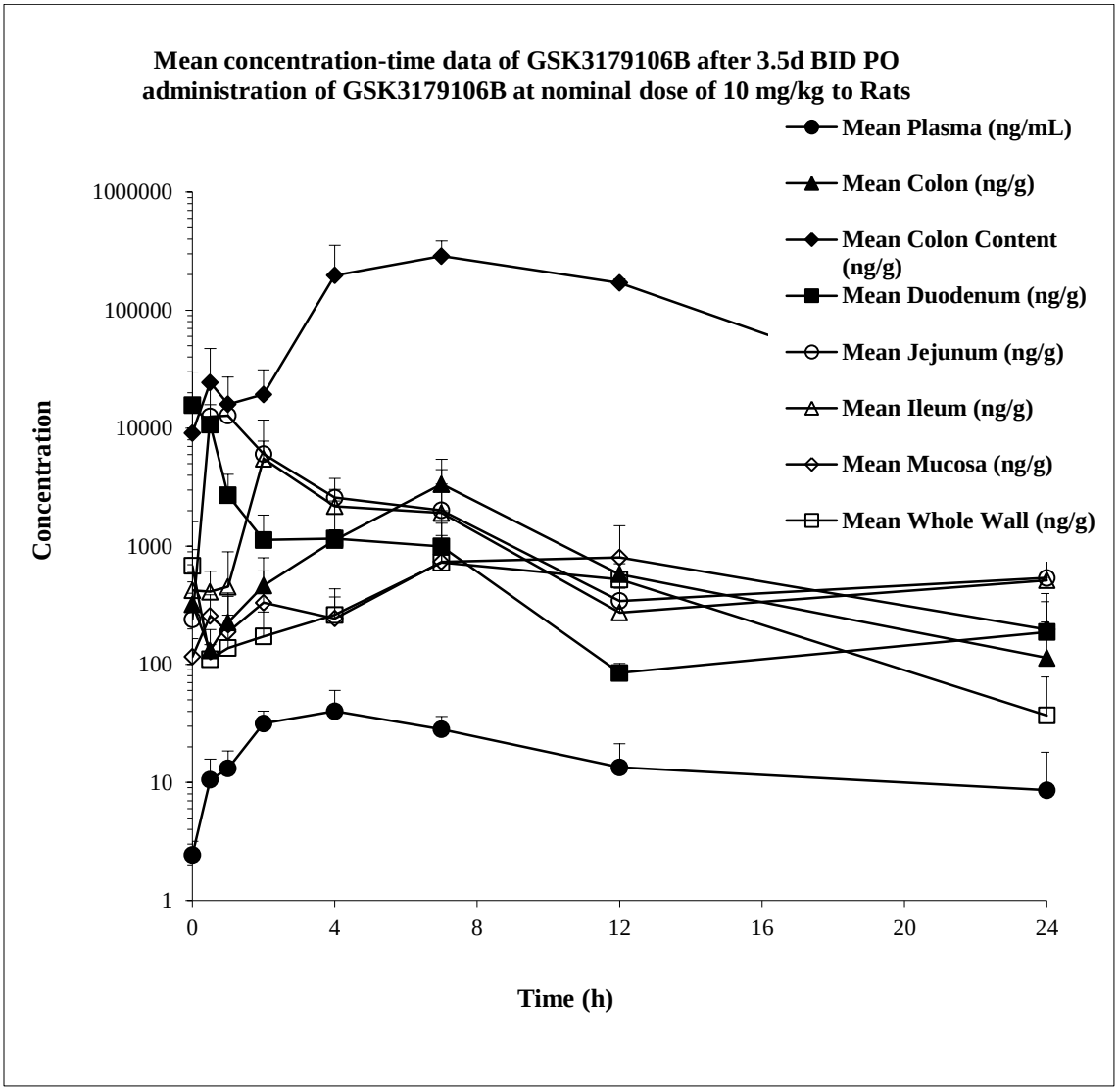
| | **Mean** |
|---|---|
| $C_0$ (ng/mL) | **35.6** |
| $t_{1/2}$ (h) | **6.48** |
| $Vd_{ss}$ (L/kg) | **4.00** |
| Cl (mL/min/kg) | **9.88** |
| $AUC_{0\text{-last}}$ (ng·h/mL) | **102** |
| $AUC_{0\text{-inf}}$ (ng·h/mL) | **116** |
| $AUC_{Extra}$ (%) | **12.5** |
| $MRT_{0\text{-last}}$ (h) | **4.53** |
| $MRT_{0\text{-inf}}$ (h) | **7.20** |
| $AUMC_{Extra}$ (%) | **45.6** |
| $DNAUC_{0\text{-last}}$ (h·kg·ng/mL/mg) | **1639** |

| | |
|---|---|
| $DNAUC_{0\text{-}inf}$ (h·kg·ng/mL/mg) | **1860** |

**Full-gut PK compound 15**

10 mg/kg PO 3.5 BID rat p.o. dosing of compound **15**
Formulation: 1.00 mg/mL in 0.1% Tween80+0.5%HPMC, opaque homogenous suspension
Species: Male Sprague-Dawley rat, fed for PO
All blood samples are transferred into plastic microcentrifuge tubes containing heparin [Blood: Heparin（1000 unit）=20:1] as an anti-coagulant and placed on wet ice until centrifugation. Harvested blood samples are centrifuged within 30 min of collection.
Tissue collection: Weigh each tissue, homogenize with 4-fold 70% ACN on the wet ice and record the weight and volume of tissue homogenate.

**Figure S5.** Satellite full gut PK for 10 mg/kg 3.5 BID rat p.o. dosing of compound **15**.



| Individual concentration-time data of GSK3179106B after 3.5d BID PO administration of GSK3179106B at nominal dose of 10 mg/kg to Rats | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time Point (h) | Animal ID | Body Weight (g) | Concentration | | | | | | | |
| | | | Plasma ng/mL | Colon ng/g | Colon Content ng/g | Duodenum ng/g | Jejunum ng/g | Ileum ng/g | Mucosa ng/g | Whole Wall ng/g |
| 0 | R1 | 241 | 2.12 | 28.8 | 5300 | 540 | 202 | 52.0 | 85.5 | 110 |
| | R2 | 245 | 1.87 | 535 | 13900 | 17800 | 240 | 211 | 173 | 183 |
| | R3 | 236 | 3.28 | 404 | 8100 | 28800 | 278 | 1010 | 90.0 | 1760 |
| 0.500 | R4 | 233 | 7.61 | 96.5 | 5650 | 1855 | 16100 | 231 | 151 | 71.0 |
| | R5 | 229 | 7.36 | 90.5 | 17550 | 4240 | 9600 | 630 | 217 | 119 |
| | R6 | 236 | 16.6 | 207 | 49950 | 26150 | 11950 | 375 | 405 | 142 |
| 1.00 | R7 | 242 | 7.68 | 93.5 | 13500 | 4190 | 14350 | 268 | 63.0 | 97.0 |
| | R8 | 223 | 18.3 | 389 | 28100 | 1545 | 10050 | 139 | 437 | 276 |
| | R9 | 228 | 13.4 | 198 | 6300 | 2395 | 14000 | 960 | 64.0 | 39.8 |
| 2.00 | R10 | 230 | 27.5 | 200 | 7850 | 545 | 6300 | 1870 | 107 | 67.0 |
| | R11 | 227 | 25.8 | 845 | 31500 | 1915 | 4180 | 2040 | 655 | 276 |
| | R12 | 226 | 41.4 | 347 | 18700 | 940 | 7650 | 12650 | 239 | 175 |
| 4.00 | R13 | 225 | 31.1 | 424 | 49600 | 1400 | 2545 | 3945 | 165 | 68.5 |
| | R14 | 225 | 26.1 | 2620 | 360500 | 1095 | 3055 | 875 | 391 | 319 |
| | R15 | 223 | 63.0 | 339 | 181000 | 990 | 2160 | 1730 | 177 | 401 |
| 7.00 | R16 | 230 | 34.0 | 4625 | 259000 | 710 | 970 | 1265 | 1690 | 655 |
| | R17 | 210 | 31.6 | 930 | 207500 | 2110 | 4815 | 910 | 286 | 263 |
| | R18 | 230 | 18.9 | 4520 | 396000 | 173 | 275 | 3575 | 243 | 1265 |
| 12.0 | R19 | 225 | 22.3 | 600 | 183500 | 105 | 590 | 296 | 505 | 411 |
| | R20 | 222 | 10.7 | 540 | 170000 | 76.5 | 364 | 431 | 314 | 419 |
| | R21 | 228 | 7.28 | 600 | 156500 | 72.5 | 79.5 | 97.0 | 1590 | 740 |
| 24.0 | R22 | 233 | 1.04 | 22.9 | 448 | 23.4 | 15.8 | 16.1 | 22.8 | 6.85 |
| | R23 | 217 | 19.1 | 242 | 22700 | 215 | 535 | 1400 | 417 | 84.0 |
| | R24 | 231 | 5.61 | 75.0 | 4455 | 325 | 1070 | 130 | 153 | 20.0 |

**Acetic Acid Model of Acute Colonic Hypersensitivity**

Male Sprague Dawley rats (225-250 g, ~7-8 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed two-per-cage under standard conditions of temperature and humidity with a 12:12 hour light-dark cycle within the Department of Comparative Medicine's animal facility. Food and water were available *ad libitum*. All animals were treated as follows for the induction of acute visceral afferent neuron sensitization. Five minutes after the last dose of vehicle or drug and one hour prior to colonic sensitivity assessment, a volume of 1.5 mL dilute (0.6%) acetic acid was infused into the rat colon via a catheter through the anus to the level of the mid-colon. Sixty-minutes following infusion, visceral afferents have sensitized resulting in acute colonic hypersensitivity. Colonic sensitivity

was assessed by measuring the visceromotor response (VMR) to colorectal distension (CRD) by visual counting of the number of abdominal contractions in response to increasing levels of CRD (0-60 mmHg). On the day of colonic sensitivity assessment, a balloon (5 cm) catheter was inserted 10 cm into the colon. The catheter was taped to the base of the tail to hold it in place. The catheter was then connected to a barostat (Distender Series IIR barostat; G & J Electronics Inc., Toronto, Canada) for controlled, isobaric inflation of the balloon and CRD, using constant pressure (isobaric) tonic distension, was conducted at 0, 20, 40 and 60 mmHg. Each CRD pressure was maintained for a period 10 minutes during which time the number of abdominal muscle contractions were counted visually and a 10-minute recovery period was allowed between each distension. The CRD pressures were ordered in a random fashion and the observer was blinded to the treatment. For oral dosing, GSK3179106 was prepared as a suspension in 0.5% HPMC and 0.1% TWEEN 80 at 2.5 mL/kg and administered BID at 8:00 and 16:00 for 3.5 days. Tegaserod and motesanib were prepared for oral dosing, as a suspension in 0.5% HPMC and 2% TWEEN 80 at 2.5 mL/kg. Tegaserod was administered as single dose 15 minutes prior to the acetic acid induction of acute visceral afferent neuron sensitization and the assessment of colonic sensitivity while motesanib was administered BID at 8:00 and 16:00 for 3.5 days prior to assessment. For all treatment groups (n=7 rats/group), rats were fed a standard diet *ad libitum* during the dosing period but were fasted for 18 hours prior to the acetic acid induction of acute visceral afferent neuron sensitization and the assessment of colonic sensitivity. Dosing solutions were prepared daily and stored in amber bottles at 4 °C.


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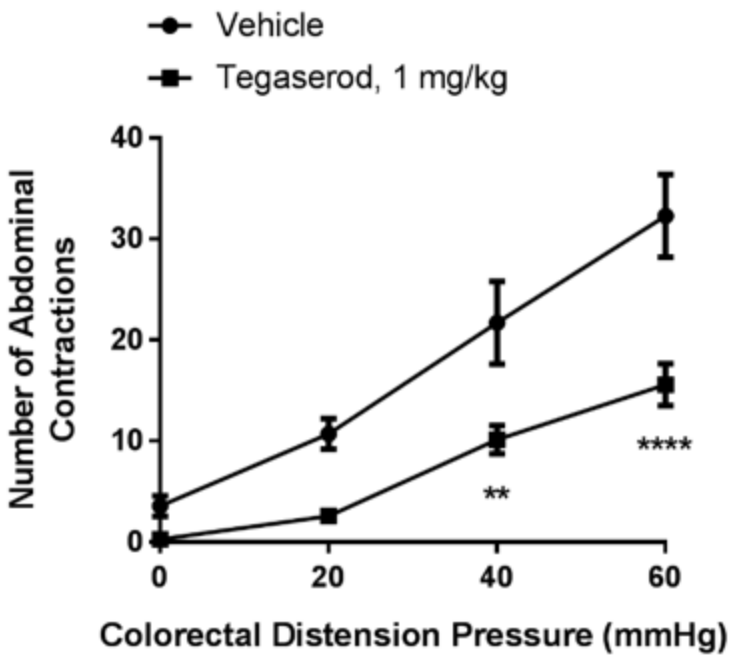
**Figure S6.** Colonic hypersensitivity induced by acute irritation stress is ameliorated by tegaserod. Tegaserod dosed orally at 1 mg/kg (n=7, all groups) for 3.5 days BID reduced the visceromotor response to colorectal distension in comparison to rats given an acetic acid enema and dosed with vehicle (**P<0.01, ****P<0.0001 vehicle compared to either tegaserod treatment, repeated-measure two-way ANOVA, Bonferroni post-test).


Vehicle
Tegaserod, 1 mg/kg
Number of Abdominal Contractions
40
30
20
10
0
0
20
40
60
**
****
Colorectal Distension Pressure (mmHg)