

Discovery of Tropifexor (LJN452), a Highly Potent Non-Bile Acid FXR Agonist for the Treatment Cholestatic Liver Diseases and Nonalcoholic Steatohepatitis (NASH)

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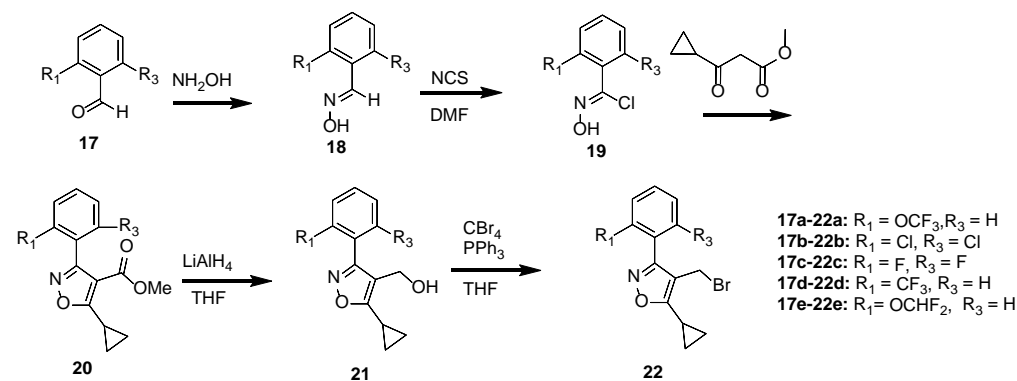
Material and Methods. Commercially available starting materials were used as supplied without further purification. Reactions were carried out using dry organic solvents (DCM, ACN, DMF, etc.) unless otherwise noted. Abbreviations for solvents and protecting groups are in accordance with *Protective Groups in Organic Synthesis* (Theodora Green and Peter G. Wuts, edition 5, Wiley and Sons), and the *Journal of Medicinal Chemistry*. Reactions were monitored using thin layer chromatography and an Agilent Technologies 1200 series 6140 Quadrupole LC-MS with UV detection at 254 nm in electrospray ionization (ESI) mode. For LC-MS, all retention times reported are at 254 nm UV channel unless otherwise noted. Three LC-MS methods were employed. Method A: An instrument configured with the following components: Waters 2525 Binary Gradient Pump, Waters 2488 UV Detector, Leap Technologies HTS Pal Autosampler, Sedex 75 Evaporative Light Scattering Detectors (4), Waters ZQ2000 Mass Spectrometer with 4 channel MUX ion source, HPLC Column: Waters Atlantis dC18 5µm 50x2.1mm (4), Mobile Phase: (A) H₂O + 0.05% TFA and (B) Acetonitrile + 0.05% TFA, Gradient: 4mL/minute (split 4 ways), initial 5% B for 0.1 minutes, ramp to 90% B over 2.9 minutes, then to 100% B at 3.0 and hold until 3.5 minutes, return to 5% B to at 3.51 minutes until end of run at 4.00, MS Scan: 180 to 800 amu in 0.2 seconds per channel, Diode Array Detector: 220 nm and 254 nm. Method B; An instrument configured with the following components: Agilent G1379A Degasser, Agilent G1312A Binary Pump or Shimadzu LC10, Agilent G1315B Diode Array Detector, Leap Technologies HTS Pal Autosampler, Sedex 75 Evaporative Light Scattering Detector, Waters ZQ2000 Mass Spectrometer, HPLC Column: Waters Atlantis dC18 3µm 30x2.1mm, Mobile Phase: (A) H₂O + 0.05% TFA and (B) Acetonitrile + 0.05% TFA. Gradient: 1 mL/minute, initial 5% B for 0.1 minutes, ramp to 90% B over 2.6 minutes, then to 100%B at 2.71 and hold until 2.98 minutes, return to 5% B to at 2.99 minutes until end of run at 3.00. The column is re-equilibrated in the ~30 seconds between injections, MS Scan: 180 to 800amu in 0.4 seconds, Diode Array Detector: 214nm – 400nm. Method C: Column Sunfire C18 3.5 µm 3.0 x 30mm 40 °C. Mobile Phase: (A) H₂O + 0.05% TFA and (B) Acetonitrile + 0.05% TFA. Gradient: 10% to 90% 2 mL/minute (2.1 min total). Diode Array Detector: 214nm – 400nm. Normal phase chromatography was performed on a CombiFlash Companion (Teledyne Isco) with RediSep normal phase silica gel columns and UV detection at 254 nm unless otherwise noted. Preparative reversed-phase HPLC/MS was performed on an HPLC coupled to a single quad-

rupole mass spectrometer. The HPLC/MS consisted of a Waters Acquity uPLC system (Waters Corp., Milford, MA) and a Waters 3100 mass spectrometer (Waters Corp., Milford, MA). The diode array detector was configured to collect data between 214 nm and 400 nm at 20 Hz. The HPLC column used was a Acquity UPLC™ HSS T3 C18, 50 x 2.1 mm ID, 1.8 μ m, part number 186003538 (Waters Corp). Instrument Configuration for HPLC/MS Waters Acquity uPLC/HPLC systems, three conditions were used. Condition A: Column: Acquity HSS T3 1.8 μ m 2.1 x 50 mm, 60 °C, Flow rate: 0.9 mL/min, Mobile phase: A) H₂O + 0.05% TFA, B) acetonitrile +0.05% TFA, Gradient: 10% to 100% in 3.35 min (3.7 min total) or Gradient: 10% to 90 % in 3.35 min (3.7 min total), DAD-UV chromatogram 210-400 nm. Condition B: Column: ACQUITY UPLC® BEH C18 1.7 μ m, 40 °C, Flow rate: 1 mL/min, Mobile phase: A) H₂O +0.05% TFA, B) acetonitrile +0.05% TFA, Gradient: 10% to 100% in 2.0 min (2.1 min total), DAD-UV chromatogram 210-400 nm. Condition C: Column: Sunfire C18 3.5 μ m 3.0 x 30 mm, 40 °C, Flow rate: 2 mL/min, Mobile phase: A) H₂O +0.05% TFA, B) acetonitrile +0.05% TFA, Gradient: 10% to 90% in 2.0 min (2.1 min total), DAD-UV chromatogram 210-400 nm. All NMR spectra were recorded on a Bruker AVANCE-400 spectrometer operating at a frequency of 400.13 MHz for ¹H and 100.61 MHz for ¹³C equipped with a 5mm QNP cryoprobe with Z-gradient. Chemical shifts for ¹H and ¹³C spectra were referenced to residual solvent. MS were obtained on an Agilent Technologies 1200 series 6140 Quadrupole LC-MS in electrospray ionization (ESI) mode. HRMS-ESI data were recorded using an Agilent 6520 Accurate-Mass Q-TOF LC-MS system with HPLC-Chip Cube interface and an Agilent 1200 HPLC. All final compounds were isolated analytically pure, >99% purity by HPLC unless otherwise indicated. Elemental combustion analysis was performed by Midwest Microlab, LLC, Indianapolis, IN. The X-ray crystallography for LJN452 intensity data were collected at 100 K at the Advanced Light Source (ALS), Berkeley CA. using synchrotron radiation (λ =0.97648 Å) one axis oscillation mode. Data were collected on as ADSC Q315R CCD array with beam width of 50 μ m, two sets of 360° wedges were collected with the two-theta angle set to 0.0 and 14.5 degrees respectively, each set was collected with and without attenuation to optimize data completeness and resolution for the equipment which are set up to collect macromolecular data. Data processing and global cell refinement were performed with HKL2000. The structure was solved by direct methods and subsequent DF syntheses and refined based on full-matrix least-squares on F² using the SHELXL program suite. Anisotropic displacement parameters were used for all non-hydrogen atoms. Hydrogen atoms were located in DF maps and refined in idealized positions using a riding model. The atomic coordinates of **1** will deposited to the Cambridge Crystallographic Data Center (CCDC) with accession number to be determined at time of publication. The FT IR spectrum was recorded on a Nicolet Avatar 360 E. S. P. FT-IR, a mid-IR instrument with DTGS detector and KBr splitter. The resolution is 0.05 cm⁻¹. 32 scans were averaged for both the background and sample collection. Optical rotations were measured using an Atego # 5223 POLAX-2L polarimeter with a 100 mm cell and standardization against the literature values of (+)/(-) menthol.

SYNTHETIC PROCEDURES

Synthesis of 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylic acid, **1 (LJN452)**

Scheme 1. Synthesis of intermediate **22**



(E)-2-(Trifluoromethoxy)benzaldehyde oxime (18a**).** To a solution of sodium hydroxide (7 g, 175 mmol, 1.19 equiv) in water (120 mL) was added a stirred solution of NH₂OH•HCl (11.8 g, 170 mmol, 1.15 equiv) in water (120 mL) at 0 °C. The resulting solution was stirred for 10 min at 0 °C. A solution of 2-(trifluoromethoxy)benzaldehyde (28 g, 147.29 mmol, 1.00 equiv) in ethanol (120 mL) was added, and the resulting solution was allowed to stir for an additional 1 h at room temperature. The reaction was diluted with water (500 mL) and extracted with ethyl acetate (2 x 700 mL). The organic layers were combined, washed with brine (2 x 300 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum to give

(E)-2-(trifluoromethoxy)benzaldehyde oxime, which was carried directly into the next step, 30.0 g (83% yield). LC-MS m/z 206.0 (M+H); method A; RT = 1.43 min.

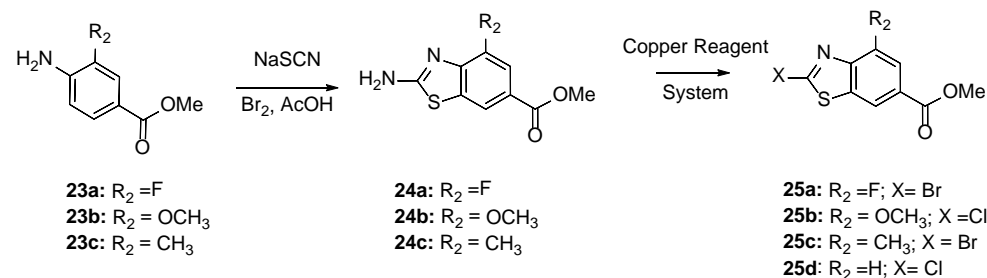
N-hydroxy-2-(trifluoromethoxy)benzimidoyl chloride (19a). NCS (22 g, 166 mmol, 1.12 equiv) was slowly added to a stirred solution of (E)-2-(trifluoromethoxy)benzaldehyde oxime **18a** (30 g, 146.27 mmol, 1.00 equiv) in DMF (300 mL) while keeping the internal temperature below 25 °C. The reaction mixture was stirred for 1 h at room temperature. The resulting solution was diluted with water (300 mL) and extracted with ethyl acetate (2×500 mL). The organic layers were combined, washed with brine (5×300 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum to give 35g of N-hydroxy-2-(trifluoromethoxy)benzimidoyl chloride as a light yellow residue. This material was used directly without further purification (99% yield). ¹H NMR (d_4 -MeOD, 400 MHz) δ 7.95–7.90 (m, 1H), 7.83 (d, J = 7.6, 1H), 7.62–7.43 (m, 2H). LC-MS m/z 240.0 (M+H); method A; RT = 2.03 min.

Methyl 5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazole-4-carboxylate (20a). Potassium carbonate (11.0 g, 79.7 mmol, 1.09 equiv) was suspended in THF (100 mL), and the mixture was cooled to –10 °C and treated with a solution of methyl 3-cyclopropyl-3-oxopropanoate (11 g, 77.5 mmol, 1.06 equiv) in 50 ml THF dropwise over 5 minutes. A solution of N-hydroxy-2-(trifluoromethoxy)benzimidoyl chloride **19a** (17.6 g, 73.6 mmol, 1.00 equiv) in THF (50 mL) was added to the reaction carefully to ensure that the internal temperature did not exceed –5 °C during the time of the addition. Upon complete addition, the reaction was warmed to 35 °C. When the reaction was completed, the mixture was diluted with water (200 mL) and extracted with ethyl acetate (2 × 300 mL). The organic layer was washed with brine (2 × 200 mL), dried over anhydrous sodium sulfate, concentrated under vacuum, and then purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:100–1:20) eluent to afford methyl 5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazole-4-carboxylate as a white solid, 12.5 g (52% yield), M.P. 145–150 °C (broad). ¹H NMR (d_4 -MeOD, 400 MHz) δ 7.65–7.60 (m, 1H), 7.53 (dd, J = 7.6, 2Hz, 1H), 7.48–7.43 (m, 2H), 3.68 (s, 3H), 2.89 (pentet, J = 6.8Hz, 1H), 1.30 (app br d, J = 6.8 Hz, 4H). LC-MS m/z 328.1 (M+H); method A; RT = 2.91 min.

(5-Cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)-methanol (21a). A 250-mL round-bottom flask was purged and maintained with an inert atmosphere of nitrogen. A suspension of LiAlH₄ (2.50 g, 65.8 mmol, 2.87 equiv) in THF (50 mL) was added, followed by the addition of a solution of methyl 5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl) isoxazole-4-carboxylate **20a** (7.5 g, 23 mmol, 1.0 equiv) in tetrahydrofuran (50 mL) dropwise at –10 °C. The resulting solution was stirred for 30 min at –10 °C. The reaction was then quenched by the addition of ethyl acetate (3 mL), followed by water (3 mL), and then finally 15% aqueous NaOH (10 mL). The resulting mixture was filtered through celite, and the filter cake was washed with ethyl acetate (200 mL). The filtrate was washed with brine (2×100 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 7 g of (5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methanol as yellow oil, (93% yield). ¹H NMR (CDCl₃, 400 MHz) δ 7.57–7.55 (m, 2H), 7.41–7.40 (m, 2H), 4.50 (s, 2H), 2.22–2.20 (m, 1H), 1.72 (s, 1H) 1.11–1.28 (m, 4H). LC-MS m/z 300.1 (M+H); method B; RT = 1.69 min.

4-(Bromomethyl)-5-cyclopropyl-3-(2-(trifluoromethoxy)-phenyl)isoxazole (22a). Into a 100 mL round bottom flask was placed (5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)-methanol **21a** (4.0 g, 13.3 mmol) followed by triphenylphosphine (5.6 g, 20 mmol, 1.5 equiv) and dichloromethane (40 mL). The mixture was stirred until it was completely dissolved, and then added dropwise, slowly, to a solution of carbon tetrabromide (6.6 g, 20 mmol, 1.5 equiv) in dichloromethane (20 mL). The mixture was stirred for 1 h, and the solvent was then evaporated *in vacuo*. The residue was purified by silica gel chromatography with a 0–50% gradient of ethyl acetate/hexane, and the product was obtained as a clear oil, 4.18 g (87% yield), and used immediately (owing to stability) in the next step. ¹H NMR (CDCl₃, 400 MHz) δ 7.55–7.50 (m, 1H), 7.49–7.45 (m, 1H), 7.40–7.32 (m, 2H), 4.27 (s, 2H), 2.11–1.99 (m, 1H), 1.24–1.17 (m, 2H), 1.17–1.09 (m, 2H). LC-MS m/z 362.0/364.0 (M + H); (Br isotope pattern); method B; RT = 1.62 min.

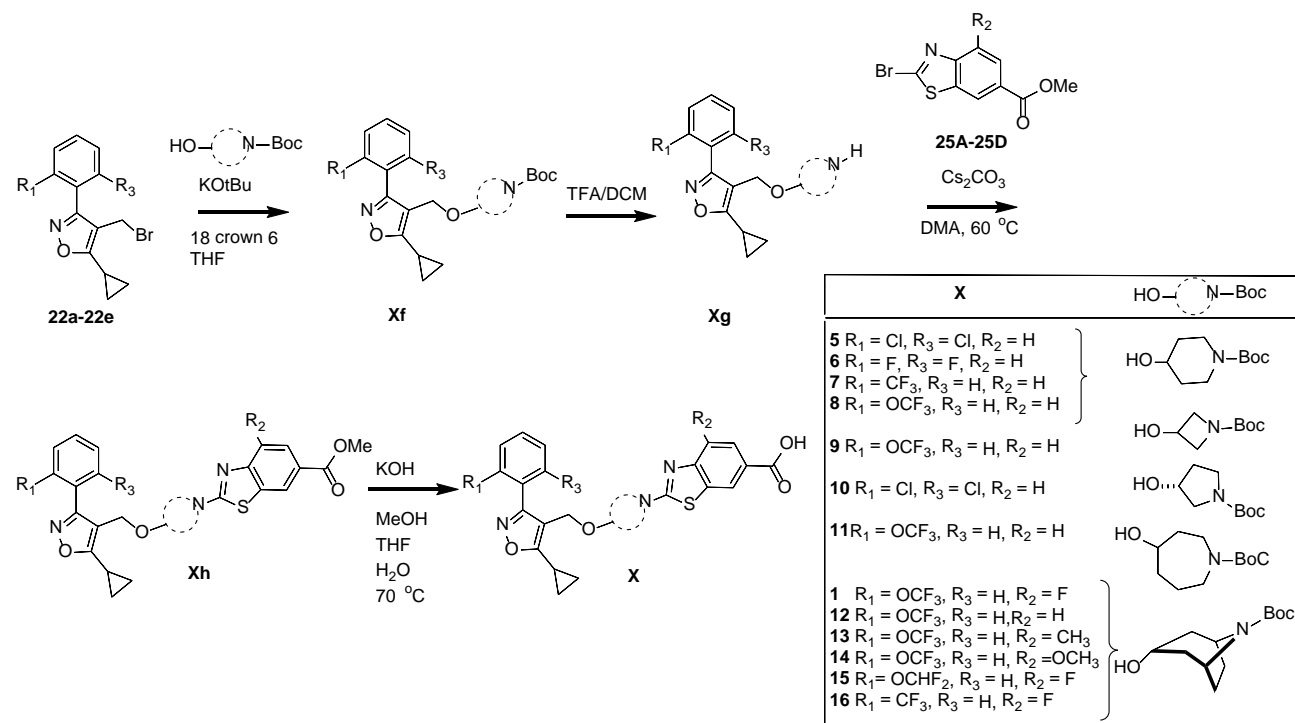
Scheme 2. Synthesis of intermediate 25



Methyl 2-amino-4-fluorobenzo[d]thiazole-6-carboxylate (24a). Into a 1000-mL round-bottom flask was placed a solution of methyl 4-amino-3-fluorobenzoate **23a** (commercial product, Matrix Scientific, 45 g, 266 mmol, 1.0 equiv) and NaSCN (86 g, 266 mmol, 1.06 mol, 4.0 equiv) in AcOH (350 mL). This was followed by the addition of a solution of Br₂ (42 g, 262.5 mmol, 0.99 equiv) in AcOH (150 mL) dropwise at 0 °C over 1 h. The resulting solution was stirred for 48 h at 30 °C. The solids were filtered, and the remaining solution was diluted with H₂O. The pH of the solution was adjusted to pH=8–9 with ammonia. The solids were collected by filtration, which resulted in 40.1 g (66% yield) of methyl 2-amino-4-fluorobenzo[d]thiazole-6-carboxylate as a yellow solid. ¹H NMR: (CDCl₃, 400 MHz) δ 8.50 (br s, 2H), 8.02 (br s, 1H), 7.86 (d, 1H, *J* = 9.0 Hz), 3.75 (s, 3H). LC-MS *m/z* 227.1 (M+H); method A; RT = 1.86.

Methyl 2-bromo-4-fluorobenzo[d]thiazole-6-carboxylate (25a). Into a 2000-mL 3-necked round-bottom flask was placed a suspension of CuBr₂ (61 g, 272 mmol, 1.54 equiv) in acetonitrile (800 mL). This was followed by the addition of *t*-BuONO (48 mL) at 0 °C over a period of 10 min. Methyl 2-amino-4-fluorobenzo[d]thiazole-6-carboxylate **24a** (40 g, 176.99 mmol, 1.00 equiv) was added and the reaction mixture was stirred for 48 h at 30 °C. The reaction was diluted with EtOAc (1000 mL), and the organic layer was washed with water (3×400 mL), and brine (3×400 mL), and then dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column eluting with ethyl acetate/hexanes (1–100% EA gradient, 20 minutes) to afford 12.2 g of crystalline solid (24% yield) of methyl 2-bromo-4-fluorobenzo[d]thiazole-6-carboxylate M.P. = 183–188 °C (uncorrected). ¹H NMR: (CDCl₃, 400 MHz) δ 8.22 (d, 1H, *J* = 0.9 Hz), 7.86 (dd, 1H, *J* = 9.6, 1.2 Hz), 3.99 (s, 3H). LC-MS *m/z* 290.0/292.0 (M+H); (Br isotope pattern); method A; RT = 2.26 min.

Scheme 3. Synthesis of compounds 1 and 5 through 16



***Tert*-Butyl 3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octane-8-carboxylate (1f).** Into a dry 250-mL flask was placed N-Boc nortropine (2.9 g, 12.8 mmol) and 18-crown-6 (3.4 g, 12.8 mmol) followed by anhydrous THF (80 mL). Potassium *tert*-butoxide (2.9 g, 25.6 mmol) was added in portionwise, and the mixture stirred vigorously under nitrogen for 1 h. 4-(Bromomethyl)-5-cyclopropyl-3-(2-(trifluoromethoxy)-phenyl)isoxazole **22a** (4.18 g, 11.6mmol) was dissolved in anhydrous THF (20 mL) and added dropwise. The reaction mixture was stirred overnight under a positive nitrogen pressure. The solvent was removed *in vacuo*, and the mixture was diluted with water (100 mL) and ethyl acetate (100 mL). The organic layer was separated, dried (MgSO₄), evaporated *in vacuo*, and then purified by silica gel chromatography with a gradient of 0–100% ethyl acetate/hexanes to yield a yellow oil, 4.07 g (69% yield). ¹H NMR (DMSO-d₆, 400 MHz) δ 7.98–7.64 (m, 3H), 7.64 (app d, *J* = 7.6, 1H), 4.38 (s, 2H), 3.82 (bs, 2H), 3.56 (t, *J* =

4.5Hz, 1H), 2.36–1.98 (m, 3H), 1.82–1.70 (m, 6H), 1.15 (s, 9H), 1.14–1.07 (m, 4H). LC-MS m/z 509.2 (M+H); method A; RT = 2.42 min.

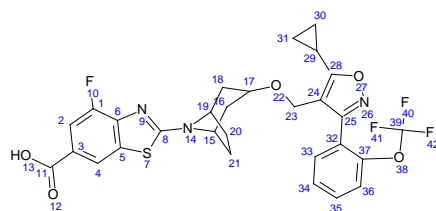
4-((8-Azabicyclo[3.2.1]octan-3-yloxy)methyl)-5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazole (1g). *tert*-Butyl-3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octane-8-carboxylate **1f** (3.98 g) was dissolved in a solution of 20% TFA in dichloromethane (30 mL). The solution was stirred at room temperature for 1 h, and the solvent was evaporated. The residue was dissolved in ethyl acetate (125 mL) and washed with a saturated solution of sodium bicarbonate (100 mL). The organic layer was dried with anhydrous sodium sulfate and evaporated *in vacuo*. The crude residue was purified by silica gel chromatography using a gradient of 0–20% ethanol/dichloromethane to afford the desired product as a colorless oil, 3.01 g (94% yield). ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.51 (br s, 1H, NH), 7.72–7.68 (m, 1H), 7.64 (dd, J = 7.6, 1.8 Hz, 1H), 7.58–7.52 (m, 2H), 4.33 (s, 2H), 3.81 (bs, 2H), 3.55 (t, J = 4.5 Hz, 1H), 2.36–2.33 (m, 1H), 1.98 (app dt, J = 14.8, 4.0 Hz, 2H), 1.91–1.76 (m, 6H), 1.14–1.07 (m, 4H). LC-MS m/z 409.2 (M+H); method A; RT = 1.72 min.

Methyl-2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylate (1h). Into a 25-mL round-bottom flask equipped with a stir bar, the following were added sequentially: 4-((8-azabicyclo[3.2.1]octan-3-yloxy)methyl)-5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazole **1g** (0.525 g, 1.29 mmol), 3.6 mL of DMA, cesium carbonate (1.08 g, 3.31 mmol) and methyl 2-chloro-4-methoxybenzo[d]thiazole-6-carboxylate **25a** (1.12 g, 3.87 mmol). After stirring the resulting slurry at room temperature for 10 minutes, the mixture was warmed to 60 °C for 1 h. The reaction slurry was allowed to cool to room temperature, and it was then diluted with ethyl acetate (200 mL) and washed with water (3×30 mL). The organic solvent extracts were concentrated under vacuum and purified by silica gel chromatography (40 g silica column) using a 15 min gradient of 10–60% ethyl acetate/hexanes. Desired fractions were vacuum concentrated and the resulting residue crystallized upon standing to furnish 0.60 g of desired intermediate methyl ester **1h** (75% yield). ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.13 (d, J = 1.6 Hz, 1H), 7.67–7.59 (m, 3H), 7.54–7.50 (m, 2H), 4.41 (s, 2H), 4.31 (bs, 2H), 3.90 (s, 3H), 3.60 (t, J = 4.8 Hz, 1H), 2.31–2.25 (m, 1H), 2.10 (app dt, J = 14.8, 4.0 Hz, 2H), 2.02–1.91 (m, 4H), 1.83 (app d, J = 14.8 Hz, 2H), 1.19–1.15 (m, 4H). LC-MS m/z 618.2 (M+H); method A; RT = 3.26 min.

2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylic acid (1). Into a 25-mL round-bottom flask equipped with a stir bar, the following were added sequentially: methyl ester **1h** (0.55 g, 0.89 mmol), THF (4.0 mL), MeOH (2.0 mL), and 3 N aqueous KOH solution (1 mL, 3 mmol). The resulting homogenous solution was stirred for 1 h at 70 °C and then allowed to cool to room temperature. The reaction was quenched with AcOH (0.2 mL of glacial acetic acid, 3 mmol) until pH=6 was achieved as monitored by pH-strip paper (Whatman class type CF Cat. No 2613991). The resulting solution was diluted with ethyl acetate (40 mL) and washed with water (3×5 mL). The ethyl acetate layer was concentrated down to an oily residue via vacuum in a 40 mL Wheaton glass vial, and this oil was then dissolved into MeOH (6 mL). The resulting homogenous solution soon began to crystallize and allowed to stand for 2.5 h. The mother liquor was withdrawn, and the crystals were washed with ice cold MeOH (3×2 mL). The crystals were dried under vacuum (10 mm Hg pressure at 45 °C overnight). A first crop of crystals (516 mg, 91% yield) were harvested and shown to be the methanol solvate by elemental analysis. Elemental Analysis as the methanol solvate: (C₃₀H₂₉F₄N₃O₅S): C 56.69, H 4.60, N 6.61; Found: C 56.79, H 4.61, N 6.65. ^1H NMR (CDCl₃, 400 MHz, for the methanol solvate) δ (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 9.0, 1.0 Hz, 1H), 7.57–7.50 (m, 2H), 7.43–7.36 (m, 2H), 4.37 (s, 2H), 4.35 (bs, 2H), 3.60 (t, J = 4.8 Hz, 1H), 3.50 (br s, 3H, peak corresponding to the presence of the methanol solvate), 2.19–2.10 (m, 3H), 2.03–1.90 (m, 4H), 1.83 (app d, J = 12.8 Hz, 2H), 1.22–1.19 (m, 2H), 1.15–1.09 (m, 2H). LC-MS m/z 604.2 (M + 1).

The methanol solvate form of **1** was converted into a solvate free crystalline form using the following procedure: The methanol solvate of **1** (500 mg) was fully dissolved in dry acetonitrile (50 mL) with gentle heating for 8 minutes (60 °C). This solution was allowed to stand for 4 days to crystallize. Upon drying, 484 mg of crystals were obtained with two collection crops. High Resolution Mass Spectroscopic analysis, 604.1514 for the [M+H]⁺ ion is consistent with the molecular formula C₂₉H₂₅F₄N₃O₅S, with a deviation of 1.6 ppm from the calculated [M+H]⁺ ion mass of 604.1530. Purity was found to be >99% by LC-MS analysis, two separate protocols. First protocol: [M+H]⁺ = 604.2 using LC-MS method A, RT = 3.22 min. Second LC-MS method B, RT = 2.21 min, MS m/z 604.1 (M+H). IR (cm⁻¹): major stretches at 1679 (C=O stretch), 1242 (C-O-C stretch), 1213 (C-N stretch), 1167 (C-F stretch). Elemental Analysis solvate free form (C₂₉H₂₅F₄N₃O₅S): C 57.71, H 4.18, N 6.96; Found: C: 57.66, H: 4.20, N: 6.94. ^1H NMR (DMSO- d_6 , 400 MHz) δ 12.96 (br s, 1H), 8.20 (d, J = 1.5 Hz, 1H), 7.69–7.66 (m, 1H), 7.64 (dd, J = 7.6, 1.7 Hz, 1H), 7.59–7.57 (m, 1H), 7.56–7.55 (m, 1H), 7.54 (d, J = 1.1 Hz, 1H), 4.34 (s, 2H), 4.22 (app d, J = 5.7 Hz, 2H), 3.55 (t, J = 4.7 Hz, 1H), 2.34 (tt, J = 8.39, 5.18, 1H), 2.01–1.95 (m, 2H), 1.86–1.82 (m, 4H), 1.75 (app d, J = 14.5 Hz, 2H), 1.16–1.13 (m, 2H), 1.10–1.07 (m, 2H). ^{19}F (DMSO- d_6 , 376 MHz) –126.4, –56.3 ppm. ^{13}C (DMSO- d_6 , 100 MHz) 171.79, 166.77, 166.37, 159.41, 151.70 (d, J = 249 Hz), 146.35, 145.10, 133.65 (d, J = 5.2 Hz),

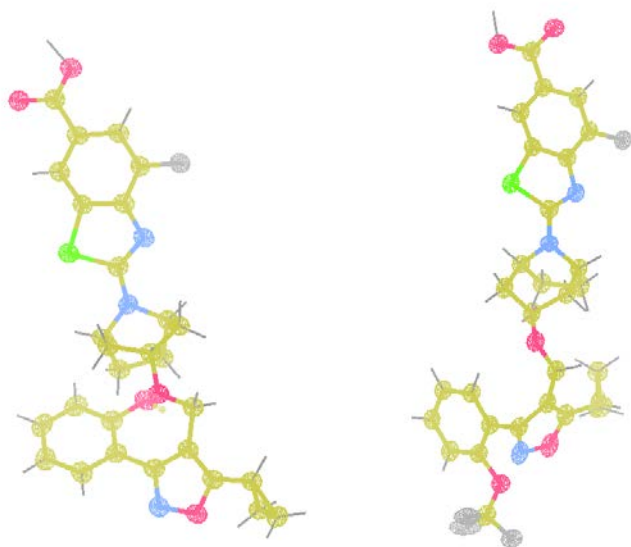
132.26, 132.11, 128.25, 124.09, 123.22, 121.88, 120.40 (q, $J = 258.3$ Hz), 119.78, 113.39 (dd, $J = 19.2, 2.4$ Hz), 112.11, 72.35, 59.68, 57.28, 33.68, 27.91, 8.43, 7.50. M.P. = 221 °C sharp, corrected. See assignment table below for proton and carbon spectra.



No.	Shift1 (ppm)	H's	Type	J (Hz)	Atom1
1	1.08	2	m	-	31<'>, 30<'>
2	1.14	2	m	-	31<'>, 30<'>
3	1.75	2	d	14.49	18<'>, 16<'>
4	1.83	4	m	-	21, 20
5	1.99	2	m	-	18<'>, 16<'>
6	2.34	1	tt	8.39, 5.18	29
7	3.55	1	t	4.68	17
8	4.22	2	d	5.69	19, 15
9	4.34	2	s	-	23
10	7.54	1	d	1.1	36
11	7.56	1	m	-	34
12	7.58	1	m	-	2
13	7.64	1	dd	7.61, 1.74	33
14	7.68	1	m	-	35
15	8.2	1	d	1.47	4
16	12.96	1	br. s.	-	13

No.	Shift1 (ppm)	C's	Type	J (Hz)	Atom1
1	7.5	1	s	-	29
2	8.43	2	s	-	31, 30
3	27.91	2	s	-	21, 20
4	33.68	2	s	-	18, 16
5	57.28	2	s	-	19, 15
6	59.68	1	s	-	23
7	72.35	1	s	-	17
8	112.11	1	s	-	24
9	113.39	1	dd	19.21, 2.38	2
10	119.78	1	br. s.	-	4
11	120.4	1	q	257.61	39
12	121.88	1	s	-	34
13	123.22	1	s	-	32
14	124.09	1	d	6.22	3
15	128.25	1	s	-	36
16	132.11	1	s	-	33
17	132.26	1	s	-	35
18	133.65	1	d	5.12	5
19	145.1	1	d	13.17	6
20	146.4	1	s	-	37
21	151.7	1	d	248.82	1
22	159.41	1	s	-	25
23	166.37	1	s	-	8
24	166.78	1	d	-	11
25	171.79	1	s	-	28

The chemical structure of **1** was further confirmed by x-ray analysis, with two independent molecules in the asymmetric unit of the triclinic space group *P*-1 with unit cell dimensions *a* = 12.250 Å, *b* = 14.827 Å, *c* = 16.282 Å α = 69.879°, β = 76.644°, γ = 83.718°.



Synthesis of 2-(4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid, **5**

Methyl 5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazole-4-carboxylate (**20b**). The compound was prepared following the same sequence used to synthesize **21a** (Scheme 1) starting with 2,6-dichlorobenzaldehyde. The crude residue was purified by silica gel chromatography eluting with a 10–90 ethyl acetate: petroleum ether gradient to provide **20b** (39% yield) as a brown oil. MS *m/z* 312.0/314.0 (*M* + *H*), (*Cl* isotope pattern); method A; RT = 2.40 min.

(5-Cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methanol (**21b**). The compound was prepared according to the same procedure as was used to synthesize **21a** (Scheme 1) starting compound **20b**. The reaction crude was purified by silica gel chromatography eluting with a 10–90 ethyl acetate: petroleum ether gradient to provide **21b** (60% yield). ¹H NMR (CDCl₃, 400 MHz) δ 7.45–7.28 (m, 3H), 4.41 (s, 2H), 2.23–2.14 (m, 1H), 1.30–1.26 (m, 2H), 1.20–1.10 (m, 2H). LC-MS *m/z* 284.0/286.0 (*M* + *H*), (*Cl* isotope pattern); method A; RT = 1.64 min.

4-(Bromomethyl)-5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazole (**22b**). The compound was synthesized according to the same procedure used to synthesize **22a** (Scheme 1). The crude material was purified by silica gel chromatography eluting with 0–100% gradient of ethyl acetate/hexanes to yield **22b** (91% yield). ¹H NMR: (CDCl₃, 400 MHz) δ 7.50–7.46 (m, 2H), 7.45–7.39 (m, 1H), 4.25 (s, 2H), 2.14 (tt, *J* = 8.3, 5.2 Hz, 1H), 1.29–1.17 (m, 4H). LC-MS *m/z* 345.8/347.8/349.8 (*M* + *H*), method B; RT = 1.57 min.

5-Cyclopropyl-3-(2,6-dichlorophenyl)-4-((piperidin-4-yloxy)methyl)isoxazole (**5g**). The compound was prepared following the same sequence as previously described for the synthesis of **1g** (Scheme 3) using the appropriate reagents (**22b** and N-Boc 4-hydroxypiperidine) via intermediate **5f**. It was used as TFA salt direction in the next step (36% yield). LC-MS *m/z* 367.0/369.0 (*M* + *H*), (*Cl* isotope pattern); method A; RT = 2.68 min.

Ethyl-2-(4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylate (**5h**). To a solution of 5-cyclopropyl-3-(2,6-dichlorophenyl)-4-((piperidin-4-yloxy)methyl)isoxazole **5g** (0.28 g, 0.76 mmol) in DMA (2 mL) was added commercially available ethyl 2-chlorobenzo[d]thiazole-6-carboxylate (Key Organics LTD/ Bio-net Research Intermediates, CA-0925) **25d** (185 mg, 0.76 mmol), diisopropylethylamine (0.4 mL, 2.3 mmol) and heated to 60 °C for 20 h. Saturated sodium bicarbonate solution was added to the reaction mixture, which was then extracted with ethyl acetate (3 x 30 mL). The combined organic layer was washed with water, brine, dried over anhydrous sodium sulfate and concentrated. The oily residue was purified by silica gel chromatography eluting with a gradient 0–100% ethyl acetate/hexane to afford 0.14 g of the ester **5h** (31% yield) as a clear oil. LC-MS *m/z* 572.1/574.1 (*M* + *H*), (*Cl* isotope pattern); method A; RT = 3.29 min.

2-(4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid (5). Compound **5h** (0.14 g, 0.24 mmol) was dissolved in dioxane (4 mL) and water (4 mL). LiOH (15 mg, 2.6 mmol) was added, and the resulting suspension was stirred at room temperature for 48 h. The solvent was removed under reduced pressure, and the residue was diluted with citric acid (15 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by mass-directed HPLC (10–90% ACN-water, TFA modifier) using a linear gradient to afford 77 mg of **5** (59% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.70 (s, 1H), 8.34 (d, *J* = 1.6 Hz, 1H), 7.84 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.64–7.62 (m, 2H), 7.56–7.51 (m, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 4.34 (s, 2H), 3.60–3.36 (m, 5H-region obscured by apparent water resonance), 2.40–2.33 (m, 1H), 1.78–1.72 (m, 2H), 1.46–1.38 (m, 2H), 1.19–1.09 (m, 4H). LC-MS *m/z* 544.0/546.0 (M + H), (Cl isotope pattern); method B; RT = 2.03 min. LC-MS purity >99 %.

Synthesis of 2-(4-((5-cyclopropyl-3-(2,6-difluorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid, 6

(5-Cyclopropyl-3-(2,6-difluorophenyl)isoxazol-4-yl)methanol (21c). The compound was prepared following the same sequence used to synthesize **21a** (Scheme 1) starting with 2,6-difluorobenzaldehyde. The crude residue was purified by silica gel chromatography eluting with a gradient of ethyl acetate:petroleum ether (10:90) to provide 8.0 g of **21c** (89% yield). ¹H NMR (CDCl₃, 400 MHz) δ 7.49–7.39 (m, 1H), 7.06–7.01 (m, 2H), 4.48 (s, 2H), 2.23–2.14 (m, 1H), 1.28–1.23 (m, 2H), 1.21–1.10 (m, 2H). LC-MS *m/z* 252.0 (M + H); method A; RT = 1.74 min.

4-(Bromomethyl)-5-cyclopropyl-3-(2,6-difluorophenyl)isoxazole (22c). The compound was prepared following the same procedure used to synthesize **22a** (Scheme 1). The crude bromide was directly carried directly into the next step without further purification. LC-MS *m/z* 314.0/316.0 (M + H), (Br isotope pattern); method A; RT = 2.45 min.

5-Cyclopropyl-3-(2,6-difluorophenyl)-4-((piperidin-4-yloxy)methyl)isoxazole (6g). The compound was synthesized following the same sequence as described for **1g** (Scheme 3) with the appropriate reagents (**22c** and N-Boc 4-hydroxypiperidine) via intermediate **6f**. It was used as TFA salt in the next reaction (76%). LC-MS *m/z* 335.2 (M + H); method B; RT = 1.51 min.

2-(4-((5-Cyclopropyl-3-(2,6-difluorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid (6). Intermediate **6h** was made following the same procedure as was described for **5h**, however it was used as crude in the next step. Compound **6** was prepared from **6h** (100 mg; 0.3 mmol) following the same protocol described for **5**. The crude residue was purified by mass-directed HPLC (10–90% ACN-water, TFA modifier, linear gradient) to afford 136 mg of **6** (90% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.34 (d, *J* = 1.6 Hz, 1H), 7.84 (dd, *J* = 8.4, 2 Hz, 1H), 7.67–7.60 (m, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.33–7.27 (m, 2H), 4.40 (s, 2H), 3.59–3.53 (m, 3H -region obscured by apparent water resonance), 3.42–3.36 (m, 2H), 2.39–2.33 (m, 1H), 1.80–1.74 (m, 2H), 1.46–1.37 (m, 2H), 1.18–1.08 (m, 4H). LC-MS *m/z* 512.1 (M + H); method B; RT = 1.86 min. LC-MS purity >99 %.

Synthesis of 2-(4-((5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid, 7

5-Cyclopropyl-4-((piperidin-4-yloxy)methyl)-3-(2-(trifluoromethyl)phenyl)isoxazole (7g). Intermediate **22d** was prepared according to the same procedure used to synthesize **22a** (Scheme 1) starting from 2-(trifluoromethyl)benzaldehyde. Compound **7g** was prepared following the same sequence as described for **1g** (Scheme 3) with the appropriate reagents (**22d** and N-Boc 4-hydroxypiperidine) via intermediate **7f**. It was used as TFA salt in the next reaction (25% yield). ¹H NMR (CDCl₃, 400 MHz) δ 9.12 (m, 1H), 7.82 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.74–7.61 (m, 2H), 7.50–7.36 (m, 1H), 4.25 (s, 2H), 3.63–3.50 (m, 1H), 3.04–2.87 (m, 4H), 2.07 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.97–1.82 (m, 2H), 1.80–1.67 (m, 2H), 1.32–1.21 (m, 2H), 1.19–1.10 (m, 2H). LC-MS *m/z* 366.7 (M + H), method B; RT = 1.53 min.

2-(4-((5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid (7). Intermediate **7h** was made following the same procedure as for **5h**, however, it was used as crude in the next step. Compound **7** was prepared from **7h** following the same protocol described for **5**. The crude residue was purified by mass-directed HPLC (10–90% ACN-water, TFA modifier, linear gradient) to afford **7** (55% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.69 (s, 1H), 8.35 (d, *J* = 1.7 Hz, 1H), 7.95–7.88 (m, 1H), 7.84 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.83–7.77 (m, 1H), 7.76–7.71 (m, 1H), 7.64–7.57 (m, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 4.30 (s, 2H), 3.61–3.49 (m, 3H), 3.42–3.37 (m, 2H), 2.34 (tt, *J* = 8.3, 5.2 Hz, 1H), 1.83–1.73 (m, 2H), 1.51–1.39 (m, 2H), 1.18–1.07 (m, 4H). LC-MS *m/z* 544.2 (M + H); method B; RT = 2.21 min. LC-MS purity >99 %.

Synthesis of 2-(4-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid, **8**

5-Cyclopropyl-4-((piperidin-4-yloxy)methyl)-3-(2-(trifluoromethoxy)phenyl)isoxazole (**8g**). The compound was prepared following the same procedure as described for **1g** starting from **22a** and N-Boc 4-hydroxypiperidine via intermediate **8f** (Scheme 3). The compound was used as TFA salt without purification in the next step (57% yield). ¹H NMR (CDCl₃, 400 MHz) δ 9.22 (br.s, 2H), 7.61–7.49 (m, 2H), 7.47–7.34 (m, 2H), 3.66–3.50 (m, 1H), 3.03–2.92 (m, 4H), 2.08 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.99–1.87 (m, 2H), 1.81–1.70 (m, 2H), 1.29–1.21 (m, 2H), 1.19–1.10 (m, 2H). LC-MS *m/z* 383.1 (M + H), method A; RT = 2.92 min.

2-(4-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)benzo[d]thiazole-6-carboxylic acid (**8**). The compound was synthesized starting from 5-cyclopropyl-4-((piperidin-4-yloxy)methyl)-3-(2-(trifluoromethoxy)phenyl)isoxazole (**8g**) (450 mg; 1.17 mmol) following the same sequence as was described for **5** via intermediate **8h** (Scheme 3). The crude residue was purified by mass-directed HPLC (10–90% ACN-water, TFA modifier) linear gradient to afford 0.3 g of **8** (46% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.23 (d, *J* = 1.8 Hz, 1H), 7.75 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.60–7.56 (m, 2H), 7.50–7.45 (m, 2H), 7.34 (d, *J* = 8.5 Hz, 1H), 4.32 (s, 2H), 3.60–3.28 (m, 5H, partially obscured by water resonance), 2.34–2.28 (m, 1H), 1.80–1.70 (m, 2H), 1.46–1.32 (m, 2H), 1.14–1.05 (m, 4H). LC-MS *m/z* 560.2 (M + H); method A; RT = 2.46 min. LC-MS purity >99 %.

Synthesis of 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azetidin-1-yl)benzo[d]thiazole-6-carboxylic acid, **9**

Tert-butyl 3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azetidine-1-carboxylate (**9f**). The compound was prepared following the same procedure described for **1f** by reacting intermediate **22a** with N-Boc 3-hydroxyazetidine (Scheme 3). The crude residue was purified by silica gel chromatography with a gradient 0–50 ethyl acetate/heptane to yield **9f** (58% yield). ¹H NMR (CD₂Cl₂, 400 MHz) δ 7.62–7.50 (m, 2H), 7.50–7.36 (m, 2H), 4.25 (s, 2H), 4.15 (tt, *J* = 6.4, 4.2 Hz, 1H), 3.99–3.81 (m, 2H), 3.75–3.56 (m, 2H), 2.12 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.39 (s, 9H), 1.25–1.18 (m, 2H), 1.17–1.09 (m, 2H). LC-MS *m/z* 455.0 (M + H); method C; RT = 1.64 min.

4-((Azetidin-3-yloxy)methyl)-5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazole (**9g**). The compound was prepared in 43.3% yield following the identical procedure described for **1g** (Scheme 3). ¹H NMR (CD₂Cl₂, 400 MHz) δ 7.59–7.52 (m, 2H), 7.45 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.43–7.39 (m, 1H), 4.27–4.17 (m, 3H), 3.56–3.48 (m, 2H), 3.47–3.37 (m, 2H), 2.14 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.23–1.17 (m, 2H), 1.17–1.08 (m, 2H). LC-MS *m/z* 355.2 (M + H); method C; RT = 1.06 min.

Ethyl 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azetidin-1-yl)benzo[d]thiazole-6-carboxylate (**9h**). The compound was prepared by following the same procedure as described for **5h** (Scheme 3) by reacting **9g** with **25d**. The crude residue was purified by silica gel chromatography with a gradient of ethyl acetate–heptane (0–50) to yield **9h** as foamy residue (71% yield). ¹H NMR (CD₂Cl₂, 400 MHz) δ 8.33–8.28 (m, 1H), 7.96 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.58–7.52 (m, 2H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.45–7.39 (m, 2H), 4.45 (tt, *J* = 6.4, 4.2 Hz, 1H), 4.35 (s, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 4.27 (ddd, *J* = 9.4, 6.4, 1.2 Hz, 2H), 3.95 (ddd, *J* = 9.1, 4.2, 1.2 Hz, 2H), 2.14 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.25–1.19 (m, 2H), 1.19–1.10 (m, 2H). LC-MS *m/z* 560.2 (M + H); method C; RT = 1.66 min.

2-(3-((5-Cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azetidin-1-yl)benzo[d]thiazole-6-carboxylic acid (**9**). The compound was prepared from **9h** in 63% yield following the same conditions described for **1** (Scheme 3). ¹H NMR (CD₂Cl₂, 400 MHz) δ 8.36 (d, *J* = 1.7 Hz, 1H), 8.02 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.59–7.51 (m, 3H), 7.46–7.37 (m, 2H), 4.51–4.41 (m, 1H), 4.36 (s, 2H), 4.32–4.25 (m, 2H), 4.01–3.91 (m, 2H), 2.14 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.29–1.10 (m, 4H). ¹³C NMR (CD₂Cl₂, 100 MHz) δ 172.98, 171.19, 170.55, 159.85, 157.52, 147.15, 132.22, 132.03, 131.79, 128.73, 127.63, 123.92, 123.21, 122.04, 121.48, 119.47, 118.64, 110.90, 68.94, 60.46, 60.23, 8.48, 7.84. LC-MS *m/z* 532.0 (M + H); method C; RT = 1.46 min. LC-MS purity >99 %.

Synthesis of (R)-2-(3-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)pyrrolidin-1-yl)benzo[d]thiazole-6-carboxylic acid, **10**

Tert-butyl (R)-3-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)pyrrolidine-1-carboxylate (**10f**). The compound was prepared following the same procedure described for **1f** by reacting **22b** and N-Boc (R)-3-hydroxypyrrolidine (Scheme 3). The crude residue was purified by silica gel chromatography with a gradient 0–50 ethyl acetate/heptane to yield **10f** (90% yield). ¹H NMR (CD₂Cl₂, 400 MHz) δ 7.47–7.42 (m, 2H), 7.41–7.35 (m, 1H), 4.31–4.19 (m, 2H), 3.95–3.88 (m, 1H), 3.35–3.03 (m, 4H), 2.13 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.81–1.69 (m, 2H), 1.42 (s, 9H), 1.26–1.17 (m, 2H), 1.18–1.07 (m, 2H). LC-MS *m/z* 453.0/455.0 (M + H); method C; RT = 1.63 min.

(R)-5-Cyclopropyl-3-(2,6-dichlorophenyl)-4-((pyrrolidin-3-yloxy)methyl)isoxazole (10g). The compound was prepared in 85% yield following the same procedure described for **1g** starting from **10f**. ¹H NMR (CD₂Cl₂, 400 MHz) δ 7.52–7.33 (m, 3H), 4.38–4.21 (m, 2H), 4.14–4.05 (m, 1H), 3.29–3.10 (m, 4H), 2.13 (tt, *J* = 8.3, 5.1 Hz, 1H), 1.97–1.85 (m, 2H), 1.29–1.19 (m, 2H), 1.19–1.09 (m, 2H). LC-MS *m/z* 353.0/355.0 (M + H); method C; RT = 1.01 min.

Ethyl (R)-2-(3-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)pyrrolidin-1-yl)benzo[d]thiazole-6-carboxylate (10h). The compound was prepared by following the same procedure as described for **5h** (Scheme 3) by reacting **10g** with **25d**. The crude residue was purified by silica gel chromatography with a gradient of ethyl acetate–heptane (0–50) to yield **10h** as foamy residue (49% yield). ¹H NMR (CD₂Cl₂, 400 MHz) δ 8.32 (d, *J* = 1.7 Hz, 1H), 7.98 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.57–7.48 (m, 1H), 7.33 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.28 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.12 (t, *J* = 8.1 Hz, 1H), 4.41–4.25 (m, 4H), 4.19–4.12 (m, 1H), 3.59–3.39 (m, 4H), 2.12 (tt, *J* = 8.4, 5.1 Hz, 1H), 2.08–2.00 (m, 2H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.26–1.17 (m, 2H), 1.16–1.08 (m, 2H). LC-MS *m/z* 558.0/560.0 (M + H); method C; RT = 1.62 min.

(R)-2-(3-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)pyrrolidin-1-yl)benzo[d]thiazole-6-carboxylic acid (10). The compound was prepared from **10h** in 43% yield following the same conditions described for **1** (Scheme 3). ¹H NMR (CD₂Cl₂, 400 MHz) δ 8.38 (s, 1H), 8.07–7.98 (m, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.33 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 8.1 Hz, 1H), 4.38–4.25 (m, 2H), 4.19–4.12 (m, 1H), 3.60–3.37 (m, 4H), 2.12 (tt, *J* = 8.4, 5.1 Hz, 1H), 2.08–2.01 (m, 2H), 1.28–1.17 (m, 2H), 1.17–1.08 (m, 2H). ¹³C NMR (CD₂Cl₂, 100 MHz) δ 172.22, 171.47, 167.86, 159.98, 157.56, 135.73, 135.56, 131.65, 131.20, 128.72, 128.41, 128.26, 123.68, 123.17, 118.10, 111.62, 77.54, 59.75, 48.10, 31.72, 8.51, 8.46, 7.90. LC-MS *m/z* 530.0/532.0 (M + H); method C; RT = 1.40 min. LC-MS purity >99 %. Optical rotation: $[\alpha]_D^{25} = +45^\circ$ (c = 1.0, chloroform)

Synthesis of 2-(4-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azepan-1-yl)benzo[d]thiazole-6-carboxylic acid, **11**

4-((Azepan-4-yloxy)methyl)-5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazole (11g). The compound was prepared following the same sequence as described for **1g** (Scheme 3) with the appropriate reagents (**22a** and N-Boc methylazepan-4-ol) via intermediate **11f**. It was used as TFA salt in the next reaction (59% yield). LC-MS *m/z* 381.1 (M + H); method A; RT = 1.92 min.

2-(4-((5-Cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)azepan-1-yl)benzo[d]thiazole-6-carboxylic acid (11). The compound was prepared starting from **11g** following the same procedure described for **5** via intermediate **11h** (Scheme 3). The crude residue was purified by mass-directed HPLC (10–90% ACN-water, TFA modifier, linear gradient) to afford 0.08 g of **11** (74% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.32 (d, *J* = 1.6 Hz, 1H), 7.82 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.67–7.59 (m, 2H), 7.55–7.49 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 4.31 (s, 2H), 3.48–3.46 (m, 3H), 3.41–3.33 (m, 2H), 2.32–2.25 (m, 1H), 1.87–1.80 (m, 1H), 1.75–1.68 (m, 2H), 1.60–1.52 (m, 3H), 1.10–1.01 (m, 4H). LC-MS *m/z* 574.1 (M + H); method B; RT = 1.79 min. LC-MS purity >99 %.

Synthesis of 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)benzo[d]thiazole-6-carboxylic acid, **12**

Methyl 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)benzo[d]thiazole-6-carboxylate (12h). Compound **12h** was made with the same procedure to **1h** and in the same scale by condensation of **1g** and **25d** (Scheme 3). ¹H NMR (CDCl₃, 400 MHz) δ 8.33 (d, *J* = 2.0 Hz, 1H), 7.90–7.55 (m, 3H), 7.53–7.45 (m, 2H), 7.40 (d, *J* = 8.4 Hz, 1H), 4.32 (s, 2H), 4.24 (s, 2H), 3.87 (s, 3H), 3.54 (t, *J* = 4.9 Hz, 1H), 2.27 (t, *J* = 8.5 Hz, 1H), 2.12–1.98 (m, 2H), 1.89–1.70 (m, 4H), 1.84–1.69 (m, 2H), 1.18–0.98 (m, 4H). LC-MS *m/z* 600.2 (M+H); method A; RT = 2.92 min.

2-(3-((5-Cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)benzo[d]thiazole-6-carboxylic acid (12). Compound **12** was made from **12h** with the same procedure than compound **1** (Scheme 3). The purification utilized silica purification MeOH-DCM (1–5 %, 20 minute gradient on 40 g size column). Crystalline samples of final material were obtained from isopropanol-water (3:1, 2 mL, 100 mg scale). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.65 (s, 1H), 8.27 (d, *J* = 1.8 Hz, 1H), 7.77 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.67–7.55 (m, 2H), 7.55–7.46 (m, 2H), 7.39 (d, *J* = 8.4 Hz, 1H), 4.27 (s, 2H), 4.14 (s, 2H), 3.48 (t, *J* = 4.8 Hz, 1H), 2.27 (tt, *J* = 8.3, 5.2 Hz, 1H), 2.02–1.88 (m, 2H), 1.86–1.72 (m, 4H), 1.74–1.59 (m, 2H), 1.12–1.05 (m, 2H), 1.04–0.98 (m, 2H). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 171.28, 167.07, 165.79, 158.91, 156.46, 145.90, 131.75, 131.61, 130.71, 127.74, 127.51, 123.02, 122.90, 122.73, 121.37, 119.78 (q, *J* = 259 Hz), 117.60, 111.62, 71.90, 59.17, 56.55, 33.06, 27.44, 7.92, 7.00. ¹⁹F (DMSO-*d*₆, 376 MHz) – 56.4 ppm. LC-MS *m/z* 586.1 (M + 1); method B; RT = 1.81 min. LC-MS purity >99%. M.P. = 201 °C, sharp (corrected).

Synthesis of 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methylbenzo[d]thiazole-6-carboxylic acid, 13

Methyl 2-amino-4-methyl-benzothiazole-6-carboxylate (24c). The compound was synthesized by the identical procedures as were used for **24a** and the yellow solid obtained (62% yield) was used without further purification. ¹H NMR (*d*₄-MeOD, 400 MHz) δ 8.17 (*app* d, *J* = 1.0 Hz, 1H), 8.03 (br s, 2H, NH₂), 7.68 (s, 1H), 3.82 (s, 3H), 2.42 (s, 3H). LC-MS *m/z* 223.0 (M+H); method B.

Methyl 2-bromo-4-methyl-benzo[d]thiazole-6-carboxylate (25c). Into a 40 mL reaction vessel equipped with a septum, stir bar, and nitrogen line, was placed solid copper (I) bromide-DMS complex (824 mgs, 4.00 mmol). This was diluted with dry acetonitrile (8 mL), and the resulting dark suspension was cooled to 0 °C with an ice bath. A commercial stock solution of *t*-BuONO (1.0 mL, 90 % weight by weight, furnishes a calculated material delivery of 7.6 mmol) was added in a dropwise fashion over 5 min. Next, a slurry of methyl 2-amino-4-methyl-benzothiazole-6-carboxylate (627 mg, 2.81 mmol) in acetonitrile (3 mL) was added slowly via pipet over 2 min to prevent any possible exothermic event. The bath was removed, and the reaction was allowed to warm to room temperature. After 3 h, the reaction was heated to 45 °C for 45 min. No starting material could be observed after this time as monitored by LC-MS analysis against a standard solution. The reaction was cooled to room temperature, filtered to remove undesired solids, and the resulting mother liquor was added to water (20 mL) as a rapidly stirred solution. The resulting solid was collected, 637 mg (80% yield). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.62 (*app* d, *J* = 1.0 Hz, 1H), 7.93 (*app* dd, *J* = 1.5, 1.0 Hz, 1H), 3.94 (s, 3H), 2.80 (s, 3H). LC-MS *m/z* 285.9/287.9 (M+H) (Br isotope pattern); method A; RT = 2.20 min.

Methyl 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methylbenzo[d]thiazole-6-carboxylate (13h). Compound **13h** was made using the same procedure as **1h** and on the same scale by condensation of **1g** and **25c**. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.21 (br s, 1H), 7.78–7.70 (m, 3H), 7.66–7.56 (m, 2H), 4.38 (s, 2H), 4.28 (s, 2H), 3.83 (s, 3H), 3.52 (t, *J* = 5.0 Hz, 1H), 2.42 (s, 3H), 2.38–2.36 (m, 1H), 2.00 (dt, *J* = 14.0, 4.0 Hz, 2H), 1.89–1.72 (m, 6H), 1.18–1.08 (m, 4H). LC-MS *m/z* 614.2 (M+H), method A; RT = 3.00 min.

2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methylbenzo[d]thiazole-6-carboxylic acid (13). Compound **13** was made from **13h** using the same procedure as compound **1**. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.59 (s, 1H), 8.16 (d, *J* = 1.7 Hz, 1H), 7.80 – 7.60 (m, 3H), 7.60 – 7.46 (m, 2H), 4.34 (s, 2H), 4.21 (s, 2H), 3.56 (t, *J* = 4.8 Hz, 1H), 2.46 (s, 3H), 2.35 (tt, *J* = 8.3, 5.2 Hz, 1H), 2.01 (dt, *J* = 14.9, 4.3 Hz, 2H), 1.90 – 1.80 (m, 4H), 1.75 (d, *J* = 14.8 Hz, 2H), 1.18 – 1.04 (m, 4H). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 171.28, 167.22, 165.16, 158.92, 155.39, 145.89, 131.76, 131.61, 130.14, 127.83, 127.75, 127.03, 122.83, 122.72, 121.37, 120.59, 120.21 (q, *J* = 259.1 Hz), 111.62, 71.90, 59.16, 56.53, 33.00, 27.43, 18.05, 7.93, 7.01. ¹⁹F (DMSO-*d*₆, 376 MHz) – 56.3. LC-MS *m/z* 600.1 (M + 1); method B; RT = 2.46 min. LC-MS purity >99%. M.p. = 221 °C (sharp, uncorrected).

Synthesis 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methoxybenzo[d]thiazole-6-carboxylic acid, 14

Methyl 2-amino-4-methoxybenzo[d]thiazole-6-carboxylate (24b). The compound was synthesized according to the identical procedures and scale as **24a**, and the yellow solid obtained (78% yield) was used without further purification. LC-MS *m/z* 238.1 (M+H).

Methyl 2-chloro-4-methoxybenzo[d]thiazole-6-carboxylate (25b). A 1000-mL 3-necked round-bottom flask was charged with a solution of methyl 2-amino-4-methoxybenzo[d]thiazole-6-carboxylate (5.0 g, 21.01 mmol, 1.00 equiv) and H₃PO₄ (40 mL). To this was added of a solution of NaNO₂ (4.5 g, 65.22 mmol, 3.00 equiv) in water (10 mL) dropwise at 0 °C. The resulting solution was stirred at 0 °C for 1 h. A solution of CuSO₄ (10 g, 62.50 mmol, 5.00 equiv) in water (10 mL) was then added dropwise at 0 °C, followed by a solution of NaCl (18.5 g, 318.97 mmol, 15.00 equiv) in water (10 mL) dropwise at 0 °C. The resulting solution was stirred at room temperature for 1 h, and then diluted with water (100 mL). The aqueous layer was extracted with dichloromethane (2 × 50 mL), and the combined organic layer was concentrated under vacuum. The residue was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (3:1) to give methyl 2-chloro-4-methoxybenzo[d]thiazole-6-carboxylate as a white solid, 4.8 g (89% yield). ¹H NMR (*d*₄-MeOD, 400 MHz) δ 8.59 (*app* d, *J* = 1.6 Hz, 1H), 7.80 (*app* d, *J* = 1.5 Hz, 1H), 3.94 (s, 3H), 3.80 (s, 3H). LC-MS *m/z* 258.0/260.0 (M+H) (Cl isotope pattern); method A; RT = 2.36 min.

Methyl 2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methoxybenzo[d]thiazole-6-carboxylic acid (14h). Compound **14h** was synthesized using the same procedure as **1h** and on the same scale by condensation of **1g** and **25b**. ¹H NMR (CDCl₃, 400 MHz,) δ 8.02 (s, 1H), 7.70 (*app* d, *J* = 8.2 Hz, 2H),

7.65–7.29 (m, 5H), 4.27 (s, 2H), 3.92 (s, 3H), 3.80 (s, 3H), 3.54 (t, $J = 4.9$ Hz, 1H), 2.38–1.95 (m, 3H), 1.88–1.77 (m, 4H), 1.67–1.60 (m, 2H), 1.25–0.96 (m, 4H). LC-MS m/z 630.2 (M+H); method A; RT = 2.90 min.

2-(3-((5-cyclopropyl-3-(2-(trifluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-methoxybenzo[d]thiazole-6-carboxylic acid (**14**). Compound **14** was synthesized from **14h** following the same procedure as was used for compound **1**. Purification of the final compound was done using silica gel chromatography with MeOH/DCM (1–5 %, 20 minute gradient on 40 g size column). Crystalline samples of the final compound were obtained from isopropanol-water (3:1, 1.5 mL, 50 mg scale). ^1H NMR (DMSO- d_6 , 400 MHz) δ 12.99 (br s, 1H), 7.99 (d, $J = 1.5$ Hz, 1H), 7.75–7.61 (m, 2H), 7.60–7.51 (m, 2H), 7.39 (d, $J = 1.5$ Hz, 1H), 4.43 (br s, 2H), 4.20 (s, 2H), 3.89 (s, 3H), 3.54 (t, $J = 4.9$ Hz, 1H), 2.34 (tt, $J = 8.3, 5.1$ Hz, 1H), 1.98 (dt, $J = 14.8, 4.4$ Hz, 2H), 1.88–1.77 (m, 4H), 1.73 (d, $J = 14.6$ Hz, 2H), 1.25–0.96 (m, 4H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 171.26, 167.09, 164.76, 149.21, 145.90, 145.41, 131.73, 131.60, 131.06, 127.72, 123.93, 122.73, 121.34, 120.01 (q, $J = 260$ Hz), 115.79, 113.95, 111.62, 108.50, 71.89, 59.16, 56.66, 55.48, 32.97, 27.43, 7.90, 6.99. ^{19}F (DMSO- d_6 , 376 MHz) δ 56.4. LC-MS m/z 616.1 (M + 1); method B; RT = 2.31 min. LC-MS purity >99 %. M.p. = 230–233 °C, broad (uncorrected).

Synthesis of methyl 2-(3-((5-cyclopropyl-3-(2-(difluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylate, **15**

Methyl 2-(3-((5-cyclopropyl-3-(2-(difluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylate (**15h**). Compound **15h** was made using the same procedure as **1h** and on the same scale starting with **22e** and **25a**. ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.24 (s, 1H), 7.63–7.60 (m, 2H), 7.51 (d, $J = 7.5$ Hz, 1H), 7.43–7.37 (m, 2H), 7.21 (t, $J = 79.0$ Hz, CF $_2$ H, 1H), 4.33 (s, 2H), 4.23 (s, 2H), 3.84 (s, 3H), 3.57 (t, $J = 4.1$ Hz, 1H), 2.51–2.32 (m, 1H), 1.97 (d, $J = 14.7$ Hz, 2H), 1.88–1.69 (m, 6H), 1.23–0.96 (m, 4H). LC-MS m/z 600.2 (M+H); method A; RT 3.30 min.

2-(3-((5-cyclopropyl-3-(2-(difluoromethoxy)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylic acid (**15**). Compound **15** was synthesized from **15h** following the same procedure as was used for compound **1**. Purification of final compound was done using silica gel chromatography with MeOH-DCM (1–5 %, 20 minute gradient on 40 g size column). Crystalline samples of final material were obtained from isopropanol-water (4:1, 1 mL, 50 mg scale). Data collected for the hemihydrate (C $_{29}$ H $_{27}$ F $_3$ N $_3$ O $_{5.5}$ S): C 58.58, H 4.58, N 7.07; Found: C 58.27, H 4.84, N 6.84. ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.14 (d, $J = 1.5$ Hz, 1H), 7.59–7.48 (m, 2H), 7.45 (dd, $J = 7.8, 1.8$ Hz, 1H), 7.35–7.25 (m, 2H), 7.09 (d, $J = 73.6$ Hz, 1H), 4.27 (s, 2H), 4.23–4.05 (m, 2H), 3.47 (t, $J = 4.8$ Hz, 1H), 2.26 (tt, $J = 8.3, 5.2$ Hz, 1H), 1.91 (dt, $J = 14.8, 4.3$ Hz, 2H), 1.86–1.70 (m, 5H), 1.70–1.62 (m, 2H), 1.09–0.96 (m, 4H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 170.87, 166.29, 165.84, 159.62, 151.25 (d, $J = 248$ Hz), 148.59 (t, $J = 4.0$ Hz), 144.58 (d, $J = 13$ Hz), 133.12, 131.43, 125.22, 123.70, 120.72, 119.26, 118.30, 116.28 (t, $J = 259$ Hz), 112.88 (d, $J = 19$ Hz), 112.79, 111.89, 71.72, 59.34, 56.84, 33.20, 27.43, 7.86, 7.03. ^{19}F (DMSO- d_6 , 376 MHz) δ 126.3, 82.2 ppm. LC-MS m/z 586.3 (M+H); method B, RT = 2.25 min. LC-MS purity >99 %. M.p. = 220–223 °C, broad (uncorrected).

Synthesis of 2-(3-((5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylic acid, **16**

Methyl 2-(3-((5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazol-4-yl)methyl)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylate (**16h**). Compound **16h** was made according to the same procedures and on the same scale as **1h** by starting with **22d** and **25a**. ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.26 (d, $J = 1.6$ Hz, 1H), 7.92 (d, $J = 8.0$ Hz, 1H), 7.84–7.74 (m, 2H), 7.63–7.60 (m, 2H), 4.26 (bs, 4H), 3.84 (s, 3H), 3.52 (t, $J = 4.1$ Hz, 1H), 2.39–2.31 (m, 1H), 2.01–1.94 (m, 2H), 1.85–1.74 (m, 6H), 1.18–1.06 (m, 4H). LC-MS m/z 602.3 (M+H); method A; RT = 3.40 min.

2-(3-((5-cyclopropyl-3-(2-(trifluoromethyl)phenyl)isoxazol-4-yl)methoxy)-8-azabicyclo[3.2.1]octan-8-yl)-4-fluorobenzo[d]thiazole-6-carboxylic acid (**16**). Compound **16** was prepared from **16h** following the same procedure as was used for compound **1**. Purification of final compound was achieved by silica gel chromatography MeOH-DCM (1 % to 5 %, 20 minute gradient on 40 g size column). Crystalline samples of final compound were obtained from isopropanol-water (3:1, 18 mL, 500 mg scale). Data collected for the solvate-free material (C $_{29}$ H $_{25}$ F $_4$ N $_3$ O $_4$ S): C 59.28, H 4.29, N 7.15; Found: C 59.25, H 4.36, N 7.11. ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.20 (d, $J = 2.0$ Hz, 1H), 7.78 (dd, $J = 9.0, 1.0$ Hz, 1H), 7.57–7.50 (m, 2H), 7.43–7.36 (m, 2H), 4.39 (s, 2H), 4.38 (bs, 2H), 3.59 (t, $J = 4.8$ Hz, 1H), 2.19–2.09 (m, 3H), 2.03–1.90 (m, 4H), 1.83 (app d, $J = 12.8$ Hz, 2H), 1.24–1.21 (m, 2H), 1.13–1.08 (m, 2H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 170.93, 166.28, 165.84, 161.10, 151.3 (d, $J = 247$ Hz), 144.60 (d, $J = 13$ Hz), 133.14, 132.35, 131.71, 130.17, 127.45 (q, $J = 20$ Hz), 126.48 (q, $J = 5.0$ Hz), 124.97, 123.60 (d, $J = 11$ Hz), 123.55 (q, $J = 271$ Hz), 119.24, 112.88 (d, $J = 20$ Hz), 111.71, 71.78, 58.75, 56.77, 33.10, 27.39, 7.92, 6.95. ^{19}F (DMSO- d_6 , 376 MHz) δ 126.3, 57.1 ppm. LC-MS m/z 588.2 (M+H); method B; RT = 2.16 min. LC-MS purity >99 %. M.p. = 218 °C sharp, corrected.

BIOLOGICAL ASSAYS

FXR coactivator interaction assay

The FXR coactivator interaction assay was performed in 384 well black solid bottom plates. A mixture of purified, GST-tagged, human FXR ligand binding domain (GST-FXR LBD) protein (Life Technologies) and biotinylated SRC-1 peptide (Biotin-CPSSHSSLTERHKILHRLQLQEGSPS-COOH) in HTRF assay buffer was incubated with test compounds in dose response. After incubation for 1 hour, Anti-GST Eu³⁺ cryptate conjugated antibody, Streptavidin conjugated XL665 and KF were added to the mixture. Plates were incubated for 1 hour at room temperature and HTRF was read on an Envision (Perkin Elmer). Each compound is tested in triplicate with the average shown. The efficacy of the positive control agonist (**2**, GW4064) is arbitrarily set at 100%.

BSEP-luciferase reporter gene assay

A 1531 base pair fragment of the human BSEP proximal promoter was amplified from a BAC clone containing the upstream genomic region of human BSEP (GenBank Accession #AC008177) and cloned into the pGL3 Basic vector create a BSEP promoter-reporter plasmid (named pGL3-hBSEP-luc). HEK293T cells were transfected with pGL3-hBSEP-luc and expression vectors encoding human FXR (NR1H4; GenBank locus NM_001206977.1) and human RXRa (GenBank locus NM_002957) using Fugene 6 (Promega, Cat. #E2691) according to manufacturer instructions. Following transfection, test compounds were added in a 10 point dose response and cells were incubated at 37 °C with 5% CO₂ incubator for 24 hours. Promoter activity was determined by using Steady-Glo reagent (Promega). The maximum efficacy of the positive control agonist (**2**, GW4064) is arbitrarily set at 100%.

Induction of FXR target genes in primary hepatocytes

Primary rat hepatocytes (Thermo Fisher Scientific) were plated in 24 well plates and incubated with a 5 point dose response of test compounds for 24 hours. RNA was harvested from the cells using the RNeasy 96 kit (Qiagen), following the manufacturers' protocol and quantitated using the Quantit RNA assay kit. Quantitative PCR was performed using the SuperScript III One-Step RT-PCR System (Thermo Fisher Scientific) using primers and probe specific to the target (BSEP and SHP or control ribosomal phosphoprotein P0; 36B4). QPCR reactions were performed on the 7900HT (Applied Biosystems) with Ct values were analyzed on the same instrument. The fold change of the transcript over no stimulation was calculated using the $\Delta\Delta C_t$ method, with DMSO (vehicle control) being no stimulation, and 36B4 expression levels as the control.

Induction of FXR target genes in rat liver and intestine

All animal studies were reviewed and approved by the GNF IACUC (Institutional Animal Care and Use Committee) and strictly followed the NIH guidelines for humane treatment of animals. All procedures in this study were in compliance with Animal Welfare Act regulations 9 CFR Part 1, 2, and 3 and US regulations (Guide for the Care and Use of laboratory animals, 1995). Adult male wild-type Sprague-Dawley rats (Harlan Laboratories) were fasted for 3 hours before oral dosing with compound 1, compound 16 or with vehicle. Animals were sacrificed seven hours after dosing using CO₂ according to IACUC approved standard procedures and liver, ileum and whole blood (in heparinized tubes) samples were collected for analysis. For gene expression analysis, the left lobe of the liver and 2-3 cm of the ileum were flash frozen in liquid nitrogen and then stored at -80 °C. Frozen tissues were homogenized in Trizol (Thermo Fisher; performed according to manufacturer instructions for total RNA isolation) and total RNA was extracted using a Qiagen RNeasy Mini Kit (Thermo Fisher Scientific) and quantified using NanoDrop spectrophotometer. Real time quantitative PCR (QPCR) was performed on the RNA using the Applied Biosystems Instrument ViiA7 Sequence Detection System (Foster City, California) using one-step QPCR SuperScriptTM III Platinum[®] reagent (Invitrogen; Carlsbad, California). Pre-made primers and probes were purchased from Applied Biosystems Instruments (Foster City, California) for detection of rat SHP, rat BSEP, rat Cyp8b1, rat FGF15 and rat 36B4 (as a loading control). Each sample was run in duplicate and the fold change of the transcript over no stimulation was calculated using the $\Delta\Delta C_t$ method, with DMSO (vehicle control) being no stimulation, and 36B4 expression levels as the control.

Quantification of FGF15 protein and triglyceride levels

After blood collection, serum was isolated and samples were transferred to the appropriate lab plates for analysis. Triglyceride levels were measured using the Olympus Triglyceride Reagent Test Kit (catalog # OSR601180; Beckman Coulter) on a Beckman Coulter AU400 clinical analyzer. Plasma FGF15 levels were quantified using FGF15 Meso Scale Discovery (MSD) assay developed in-house. Mouse FGF15 antibody from R&D Systems (AF6755) was used both as capture and detection antibody in the assay. For detection, MSD SULFO-TAG NHS-Ester was combined with the detection FGF15 anti-

body. MSD standard 96-well plates were coated with FGF15 capture antibody overnight at 4 °C. The plates were blocked with MSD Blocker A (R93AA-2) and shaken for 1 h at room temperature. After washing the plate with PBS + 0.05% Tween 20 three times, MSD diluent 4 was dispensed into each well and incubated with samples (or purified rat FGF15 as a positive control) at room temperature. After washing three times, detection antibody (diluted with 1% BSA / PBS / 0.05% Tween 20) was added and incubated with shaking at room temperature. After washing and the addition 1X MSD Read buffer (R92TC-2), the plate was read immediately on an MSD SECTOR Imager 6000. Plots of the standard curve of purified rat FGF15 protein and unknown samples were calculated using MSD data analysis software.

Pharmacokinetics studies

Pharmacokinetics studies were performed according to the procedures previously reported in: Li, C.; Liu, B.; Chang, J.; Groessl, T.; Zimmerman, M.; He, Y.; Isbell, J.; Tuntland, T. A Modern *in vivo* Pharmacokinetic Paradigm: Combining Snapshot, Rapid, and Full PK Approaches to Optimize and Expedite Early Drug Discovery. *Drug Discov. Today* **2013**, *18*, 71-78.