Discovery of a First-in-Class Inhibitor of the Histone Methyltransferase SETD2 Suitable for Preclinical Studies

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Synthetic Procedures

Proton NMR spectra were recorded on Agilent 400 or 300 MHz spectrometers. Proton chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using the residual solvent resonance (DMSO- d6, δ 3.33 ppm; methanol- d4, δ 3.31 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of triplets (dt), doublet of triplets of doublets of triplets (dtdd), triplet of triplets (tt), triplet of triplets of doublets (tdt), triplet of triplets of triplets of triplets (tdtd), triplet of quartets (tq), quartet of doublets (qd), quartet of triplets (qt), pentet (p), pentet of doublets (pd), septet (sept), multiplet (m)], coupling constants [Hz], integration, specific proton assignment).

Unless otherwise noted, all NMR spectra were acquired at ambient temperature. Analytical thin-layer chromatography (TLC) was performed using EMD Millipore silica gel 60 F254 precoated plates (0.25 mm thickness) and were visualized by irradiation with UV light (254 nm) and staining with KMnO4. TLC Rf values are reported. Normal phase flash chromatography was performed using Silicycle silica gel (particle size 32-63 μm). Reversed phase chromatography used C-18 silica and was performed on a Biotage Isolera One purification system. All chemicals were purchased commercially and used as received unless otherwise noted. Building blocks that are commercially available from local vendors were purchased and used as received.

**LC-MS analytical method:** The instrument used for LC-MS analysis was a Shimadzu LCMS-2000 equipped with 2 LC-30AD pumps for a binary gradient, degasser, autosampler, PDA model SPD-M20A, D2 lamp, 190-400 nm, MS parameter: pos/neg 90-900, nebulizing gas flow: 1.5 mL/min, gas temp: 250 °C.

**Method 1:** Kinetex EVO C18, 50x2.1 mm, 2.6 μm, Temp 35 °C, flow 1.0 mL/min, gradient: t0 = 10% B, t2.0min = 95% B, t2.70min = 95% B, t2.80min = 10% B. Mobile Phase A: Water +6.5 mM NH4HCO3 (pH = 10)/Mobile Phase B: MeCN.

**Method 2:** Shim-pack XR-ODS, 50x3.0 mm, 2.2 μm, Temp 35 °C, flow 1.2 mL/min, gradient: t0 = 5% B, t2.0min = 95% B, t2.70min = 95% B, t2.80min = 10% B. Mobile Phase A: Water + 0.05% TFA/Mobile Phase B: MeCN + 0.05% TFA.

**Method 3:** Halo C18, 50x3.0 mm, 2.7 μm, Temp 45 °C, flow 1.5 mL/min, gradient: t0 = 5% B, t3.25min = 45% B, t3.00min = 100% B, t4.00min = 100% B, t4.70min = 5% B Mobile Phase A: Water + 0.05% TFA/Mobile Phase B: MeCN + 0.05% TFA.

**Method 4:** Cortecs C18, 50x2.1 mm, 2.7 μm, Temp 40 °C, flow 1.0 mL/min, gradient: t0 = 10% B, t1.00min = 100% B, t1.50min = 100% B, t1.60min = 10% B, Mobile Phase A: Water + 0.05% FA/Mobile Phase B: MeCN + 0.05% FA.
Overview for Table 1 Analogs: Analogs of the early hit 3 were generally prepared by coupling of commercially available indole acids with benzylic amines which are also commercially available. The preparation of 4-fluoro-7-methyl-1H-indole-2-carboxylic acid 36 is as follows:

Supplementary Scheme S1: Preparation of 4-fluoro-7-methyl-1H-indole-2-carboxylic acid 36.

To a reaction vessel containing a solution of (5-fluoro-2-methylphenyl)hydrazine hydrochloride 34 (100 g, 572.7 mmol, 1.0 equiv) in ethanol (400 mL) was added sulfuric acid (10 mL) followed by ethyl 2-oxopropanoate (66 g, 1.2 equiv) under a nitrogen atmosphere at 25 °C. The resulting mixture was aged with stirring for 2 hours, at which time the reaction was shown to be complete by LC-MS. The product mixture was then concentrated under reduced pressure (12 mbar), and the solids were collected by filtration furnishing ethyl (2E)-2-[2-(5-fluoro-2-methylphenyl)hydrazin-1-ylidene]propanoate S1 (120 g, 88% yield) as a yellow solid. 

LCMS: Method 2 (ESI, m/z): RT = 1.40 min, m/z = 239.0 [M+H]+ (calc m/z = 238.1); ¹H NMR (400 MHz, DMSO-d₆) δ 11.96 (d, J = 2.0 Hz, 1H), 7.15 (m, 2H), 6.62 (m, 1H), 4.25 (q, J = 7.1 Hz, 2H), 2.12 (d, J = 9.3 Hz, 6H), 1.29 (t, J = 7.1 Hz, 3H).

To a reaction vessel containing a solution of ethyl (2E)-2-[2-(5-fluoro-2-methylphenyl)hydrazin-1-ylidene]propanoate S1 (40 g, 167.9 mmol, 1.0 equiv) in toluene (400 mL) was added 4-methylbenzene-1-sulfonic acid (50 g, 290.4 mmol, 1.70 equiv) under a nitrogen atmosphere. The resulting mixture was aged for 18 hours at 100 °C with stirring, at which time the reaction was shown to be complete by LC-MS. The product mixture was then concentrated under reduced pressure (12 mbar), diluted with ethyl acetate (100 mL) followed by saturated aqueous ammonium chloride (300 mL) and transferred to a separatory funnel. The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 150 mL). The organic layers were combined and dried over magnesium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar). The residue obtained was purified by flash-column chromatography (eluting with 20% ethyl acetate/petroleum ether), fractions collected and concentrated, and the product residue was recrystallized from boiling ethanol to afford ethyl 4-fluoro-7-methyl-1H-indole-2-carboxylate 35 (9.0 g, 24% yield) as a yellow solid.

LCMS: Method 2 (ESI, m/z): RT = 1.35 min, m/z = 222.0 [M+H]+ (calc m/z = 221.1); ¹H NMR (400 MHz, DMSO-d₆) δ 12.07 (s, 1H), 7.17 (d, J = 2.1 Hz, 1H), 7.00 (m, 1H), 6.77 (m, 1H), 4.36 (q, J = 7.1 Hz, 2H), 2.49 (d, J = 1.0 Hz, 3H), 1.35 (t, J = 7.1 Hz, 3H).

Sodium hydroxide (8 g, 200.0 mmol, 5.0 equiv) dissolved in water (50 mL) was added to a solution of ethyl 4-fluoro-7-methyl-1H-indole-2-carboxylate 35 (9.1 g, 41.1 mmol, 1.0 equiv) in tetrahydrofuran (150 mL) and methanol (2 mL). The reaction mixture was stirred at 21 °C for 6 hours. The product mixture was then concentrated under reduced pressure (12 mbar). The residue solution obtained was adjusted to pH = 4 with hydrochloric acid (3 M). The product was collected by filtration to afford 4-fluoro-7-methyl-1H-indole-2-carboxylic acid 36 (8.0 g, 81%) as a brown solid. LCMS: Method 4 (ESI, m/z): RT = 0.65 min, m/z = 192.1 [M-H]- (calc m/z = 193.1); ¹H NMR (400 MHz, DMSO-d₆) δ 13.12 (br s, 1H), 11.96 (s, 1H), 7.11 (s, 1H), 6.98 (dd, J = 4.8, 7.6 Hz, 1H), 6.75 (dd, J = 7.8, 10.6 Hz, 1H), 2.45 (s, 3H).

Analogs 3-15 were prepared via the following from the appropriate carboxylic acid:
Supplementary Scheme S2: Preparation of analogs in Table 1.

**General reaction procedure (GP1):** To a reaction vessel containing the appropriate carboxylic acid (1.0 equiv), N-ethyl-N-isopropylpropan-2-amine (5.0 equiv), and cyclopropyl(3-methylpyridin-2-yl)methanamine (2 equiv) in DMF (5 mL) was added HATU (1.3 equiv) at 21 °C. The reaction mixture was aged for 18 h with stirring, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with deionized water (10 mL) and transferred to a separatory funnel containing ethyl acetate (25 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 25 mL). The organic layers were combined and dried over magnesium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar). The residue was purified by Prep-HPLC with the following conditions: Waters 2767-2; Column: XBridge Shield Prep OBDA C18 Column, 19×150 mm 5 um; Mobile Phase A: Water (10 mmol/L NH₄HCO₃), Mobile Phase B: MeCN; Flow rate: 10 mL/min; Gradient: 10% B to 30% in 2 min, to 46% B in 10 min, to 100% B in 1 min; 254 nm.

Prepared by using GP1 with 7-methyl-1H-indole-2-carboxylic acid (100 mg, 0.57 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (111 mg, 0.68 mmol, 1.2 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-methyl-1H-indole-2-carboxamide 3 (95 mg, 52% yield) as a white solid. Spectral data: **LCMS:** Method 2 (ESI, m/z): RT=1.38 min, m/z =320.21 [M +H]⁺ (calc m/z = 319.17); **¹H NMR** (300 MHz, methanol-d4) δ 8.40 (dd, J = 4.8, 1.2 Hz, 1H), 7.62 (dd, J = 6.9, 0.9 Hz, 1H), 7.44 (dd, J = 7.8, 0.6 Hz, 1H), 7.22 (dd, J = 7.8, 4.8 Hz, 1H), 7.14 (s, 1H), 7.03 (m, 1H), 6.97 (m, 1H), 4.99 (d, J = 8.7 Hz, 1H), 2.52 (s, 3H), 2.49 (s, 3H), 1.49-1.52 (m, 1H), 0.43-0.68 (m, 4H).
Prepared by using GP1 with 1H-indole-2-carboxylic acid (400 mg, 2.5 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (441 mg, 2.7 mmol, 1.1 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-1H-indole-2-carboxamide 5 (257 mg, 25% yield) as a white solid. **Prep-HPLC**: XSelect CSH Prep C18 OBD Column, 19×250 mm 5 um; Mobile Phase A: Water + 0.05% TFA, Mobile Phase B: MeCN; Flow rate: 25 mL/min; Gradient: 17% B to 47% in 7 min, 254 nm, RT = 6.63 min; Spectral data: **LCMS**: Method 3 (ESI, m/z): RT=2.60 min, m/z =306.10 [M +H]⁺ (calc m/z = 305.15); **¹H NMR** (400 MHz, DMSO-d6) δ 11.58 (s, 1H), 9.01 (d, J = 6.4 Hz, 1H), 8.54 (d, J = 4.4 Hz, 1H), 7.87 (brs, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.46 (s, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.28 (brs, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 4.74 (t, J = 8.0 Hz, 1H), 2.47 (s, 3H), 1.62-1.53 (m, 1H), 0.65-0.38 (m, 4H).

Prepared by using GP1 with 7-propyl-1H-indole-2-carboxylic acid (200 mg, 0.98 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (200 mg, 1.23 mmol, 1.2 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-propyl-1H-indole-2-carboxamide 6 (99 mg, 22% yield) as a white solid. **Prep-HPLC**: XSelect CSH Prep C18 OBD Column, 19×250 mm 5 um; Mobile Phase A: Water + 0.05% TFA, Mobile Phase B: MeCN; Flow rate: 25 mL/min; Gradient: 17% B to 47% in 7 min, 254 nm; Spectral data: **LCMS**: Method 2 (ESI, m/z): RT=1.89 min, m/z =348.05 [M +H]⁺ (calc m/z = 347.20); **¹H NMR** (300 MHz, methanol-d4) δ 8.49 (d, J = 4.2 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.51 (dd, J = 7.5, 5.1 Hz, 1H), 7.45 (dd, J = 7.5, 1.5 Hz, 1H), 7.19 (s, 1H), 7.06–6.98 (m, 2H), 4.81 (s, 1H), 2.87 (t, J = 7.5 Hz, 2H), 2.57 (s, 3H), 1.79-1.72 (m, 2H), 1.63-1.52 (m, 1H), 1.00 (t, J = 7.2 Hz, 3H), 0.85–0.48 (m, 4H).
Prepared by using GP1 with 7-cyclopropyl-1H-indole-2-carboxylic acid (120 mg, 0.60 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (340 mg, 0.89 mmol, 1.2 equiv) resulting in 7-cyclopropyl-N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-1H-indole-2-carboxamide 7 (65 mg, 32% yield) as a white solid.

Spectral data: LCMS: Method 1 (ESI, m/z): RT=1.49 min, m/z =346.1 [M +H]+ (calc m/z = 345.2); \(^1\)H NMR (400 MHz, DMSO-d6) δ 11.44 (s, 1H), 8.92 (d, \(J = 7.6\) Hz, 1H), 8.44 (d, \(J = 3.6\) Hz, 1H), 7.59 (d, \(J = 7.2\) Hz, 1H), 7.39 (d, \(J = 8.0\) Hz, 1H), 7.21 (m, 2H), 6.94 (t, \(J = 7.6\) Hz, 1H), 6.72 (d, \(J = 6.8\) Hz, 1H), 4.82 (t, \(J = 8.4\) Hz, 1H), 2.42 (m, 4H), 1.63-1.59 (m, 1H), 1.02-0.98 (m, 2H), 0.73-0.71 (m, 2H), 0.59–0.55 (m, 1H), 0.49-0.43 (m, 2H), 0.38-0.36 (m, 1H).

Prepared by using GP1 with 4-fluoro-7-methyl-1H-indole-2-carboxylic acid 36 (150 mg, 0.78 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (216 mg, 1.33 mmol, 1.7 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-4-fluoro-7-methyl-1H-indole-2-carboxamide 8 (50 mg, 19% yield) as a white solid.

Spectral data: LCMS: Method 1 (ESI, m/z): RT=1.43 min, m/z =338.0 [M +H]+ (calc m/z = 337.2); \(^1\)H NMR (400 MHz, methanol-d4) δ 8.43 (dd, \(J = 3.6\), 1.2 Hz, 1H), 7.65 (dd, \(J = 7.6\), 0.8 Hz, 1H), 7.26–7.23 (m, 2H), 6.99-6.96 (m, 1H), 6.67 (dd, \(J = 10.4\), 8.0 Hz, 1H), 5.00 (d, \(J = 8.8\) Hz, 1H), 2.51 (s, 6H), 1.56–1.50 (m, 1H), 0.70-0.54 (m, 2H), 0.51-0.46 (m, 2H); \(^19\)F NMR (376 MHz, methanol-d4) δ -127.9.
Prepared by using GP1 with 5-fluoro-7-methyl-1H-indole-2-carboxylic acid (270 mg, 1.4 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (390 mg, 2.4 mmol, 1.7 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-5-fluoro-7-methyl-1H-indole-2-carboxamide 9 (76 mg, 16% yield) as an off-white solid. Spectral data: LCMS: Method 1 (ESI, m/z): RT=1.39 min, m/z =338.1 [M +H]+ (calc m/z = 337.2); 1H NMR (400 MHz, methanol-d4) δ 8.42 (dd, J = 3.6, 1.2 Hz, 1H), 7.65 (ddd, J = 7.6, 1.7, 0.8 Hz, 1H), 7.24 (dd, J = 7.7, 4.8 Hz, 1H), 7.13 (s, 1H), 7.11 (d, J = 2.4 Hz, 1H), 6.86 (ddd, J = 10.0, 2.5, 1.0 Hz, 1H), 5.00 (d, J = 8.7 Hz, 1H), 2.55 (s, 3H), 2.51 (s, 3H), 1.54-1.52 (m, 1H), 0.69-0.54 (m, 2H), 0.49–0.45 (m, 2H); 19F NMR (376 MHz, methanol-d4) δ -126.1.

Prepared by using GP1 with 6-fluoro-7-methyl-1H-indole-2-carboxylic acid (200 mg, 1.04 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (201 mg, 1.24 mmol, 1.2 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-6-fluoro-7-methyl-1H-indole-2-carboxamide 10 (76 mg, 16% yield) as an off-white solid. Spectral data: LCMS: Method 1 (ESI, m/z): RT=1.41 min, m/z =338.1 [M +H]+ (calc m/z = 337.2); 1H NMR (400 MHz, methanol-d4) δ 8.42 (dd, J = 4.8, 1.6 Hz, 1H), 7.55 (ddd, J = 7.7, 1.7, 0.8 Hz, 1H), 7.44 (dd, J = 8.7, 5.2 Hz, 1H), 7.24 (dd, J = 7.6, 4.8 Hz, 1H), 7.16 (s, 1H), 6.86 (dd, J = 10.3, 8.8 Hz, 1H), 4.99 (d, J = 8.7 Hz, 1H), 2.51 (s, 3H), 2.45 (d, J = 1.7 Hz, 3H), 1.54-1.50 (m, 1H), 0.73-0.52 (m, 2H), 0.52-0.42 (m, 2H); 19F NMR (376 MHz, methanol-d4) δ -126.6.
Prepared by using GP1 with 7-methyl-1H-1,3-benzodiazole-2-carboxylic acid (300 mg, 1.7 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine*2HCl S2-HCl (518 mg, 2.20 mmol, 1.3 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-methyl-1H-benzo[d]imidazole-2-carboxamide 11 (75 mg, 14% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.22 min, m/z =343.10 [M +Na]+ (calc m/z = 343.15); ¹H NMR (400 MHz, methanol-d4) δ 8.45 (dd, J = 4.9, 1.6 Hz, 1H), 7.63 (dd, J = 8.0, 0.8 Hz, 1H), 7.38 (brs, 1H), 7.24 (m, 2H), 7.13 (brs, 1H), 5.12 (brs, 1H), 2.62 (brs, 3H), 2.49 (s, 3H), 1.54 (s, 1H), 0.63–0.50 (m, 4H).

Prepared by using GP1 with 7-methyl-1-benzothiophene-2-carboxylic acid (170 mg, 0.88 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (246 mg, 1.52 mmol, 1.7 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-methylbenzo[b]thiophene-2-carboxamide 12 (64 mg, 21% yield) as a white solid. Spectral data: LCMS: Method 1 (ESI, m/z): RT=1.50 min, m/z =337.1 [M +H]+ (calc m/z = 336.1); ¹H NMR (400 MHz, DMSO-d6) δ 9.16 (d, J = 7.8 Hz, 1H), 8.44 (dd, J = 4.8, 1.6 Hz, 1H), 8.34 (s, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.28 (d, J = 7.1 Hz, 1H), 7.21 (dd, J = 7.6, 4.7 Hz, 1H), 4.75 (dd, J = 9.0, 7.8 Hz, 1H), 2.50 (s, 3H), 2.40 (s, 3H), 1.64–1.55 (m, 1H), 1.62–0.31 (m, 4H).
Prepared by using GP1 with 7-methyl-1,3-benzothiazole-2-carboxylic acid (190 mg, 0.98 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (160 mg, 0.99 mmol, 1.0 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-methylbenzo[d]thiazole-2-carboxamide 13 (83 mg, 25% yield) as a light yellow solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.69 min, m/z =338.21 [M +H]+ (calc m/z = 337.12); ^1H NMR (400 MHz, methanol-d4) δ 8.46 (d, J = 4.0 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.37 (d, J = 7.3 Hz, 1H), 7.26 (dd, J = 7.7, 4.8 Hz, 1H), 5.11 (d, J = 8.2 Hz, 1H), 2.62 (s, 3H), 2.50 (s, 3H), 1.58-1.50 (m, 1H), 0.67-0.56 (m, 4H).

Prepared by using GP1 with 7-methyl-1H-pyrrolo[3,2-b]pyridine-2-carboxylic acid (160 mg, 0.91 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine S2 (160 mg, 0.99 mmol, 1.1 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-7-methyl-1H-pyrrolo[3,2-b]pyridine-2-carboxamide 14 (51 mg, 18% yield) as a white solid. Prep-HPLC: Kinetex EVO C18 Column, 21.2×150 mm 5 um; Mobile Phase A: Water (10 mmol/L NH₄HCO₃), Mobile Phase B: MeCN Flow rate: 25 mL/min; Gradient: 10% B to 60% in 9 min, 254 nm; Spectral data: LCMS: Method 2 (ESI, m/z): RT=0.92 min, m/z =321.19 [M +H]+ (calc m/z = 320.16); ^1H NMR (300 MHz, DMSO-d6) δ 11.72 (s, 1H), 9.04 (d, J = 7.8 Hz, 1H), 8.44 (d, J = 3.3 Hz, 1H), 8.24 (d, J = 4.5 Hz, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.34 (s, 1H), 7.21 (dd, J = 7.2, 4.8 Hz, 1H), 7.00 (d, J = 4.2 Hz, 1H), 4.80 (t, J = 8.5 Hz, 1H), 2.51 (d, J = 6.3 Hz, 3H), 2.42 (s, 3H), 1.64–1.61 (m, 1H), 0.60-0.35 (m, 4H).

Prepared by using GP1 with 7-methylimidazo[1,2-a]pyridine-2-carboxylic acid (150 mg, 0.85 mmol, 1.0 equiv) and cyclopropyl(3-methylpyridin-2-yl)methanamine*2HCl S2-HCl (219 mg, 0.92 mmol, 1.1 equiv) resulting in N-(cyclopropyl(3-methylpyridin-2-yl)methyl)-5-methylimidazo[1,2-a]pyridine-2-carboxamide 15 (57 mg, 21% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.01 min, m/z =321.20 [M +H]+ (calc m/z = 320.16); ^1H NMR (400 MHz, methanol-d4) δ 8.43 (d, J = 4.9 Hz, 1H), 8.19 (s, 1H), 7.63 (dd, J = 7.6, 0.8 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), 7.35 (dd, J = 9.2, 6.9 Hz, 1H), 7.22 (dd, J = 7.7, 4.8 Hz, 1H), 6.83 (d, J = 6.8 Hz, 1H), 5.16 (d, J = 8.0 Hz, 1H), 2.66 (s, 3H), 2.50 (s, 3H), 1.54-1.49 (m, 1H), 0.61-0.44 (m, 4H).
Overview for Table 2 Analogs: Compounds containing an aryl central core were prepared using a variant of the route shown in Supplementary Scheme S3. Compound 16 was prepared using the commercially available S3 building block.

Supplementary Scheme S3: Preparation of analogs in Table 2.

General reaction procedure (GP2): To a reaction vessel containing the appropriate carboxylic acid (1.0 equiv), N-ethyl-N-isopropylpropan-2-amine (5.0 equiv), and the substituted aniline partner (1.5 equiv) in DMF (5 mL) was added HATU (0.46 mmol, 1.3 equiv) at 21 °C. The reaction mixture was aged for 18 h with stirring, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with deionized water (10 mL) and transferred to a separatory funnel containing ethyl acetate (25 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 25 mL). The organic layers were combined and dried over magnesium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar). The residue was purified by Prep-HPLC with the following conditions: Column: XBridge Prep OBD C18 Column, 30×150 mm 5 um; Mobile Phase A: Water (10 mmol/L NH4HCO3), Mobile Phase B: MeCN; Flow rate: 10 mL/min; Gradient: 35 B to 65 B in 7 min; 254 nm.

Prepared by using GP2 with 7-methyl-1H-indole-2-carboxylic acid (100 mg, 0.57 mmol, 1.0 equiv) and 3-(morpholin-4-yl)aniline (153 mg, 0.86 mmol, 1.5 equiv) resulting in 7-methyl-N-(3-morpholinophenyl)-1H-indole-2-carboxamide 17 (92 mg, 48% yield) as a white solid. Prep-HPLC: Xselect CSH F-Phenyl OBD Column, 19×150 mm 5 um; Mobile Phase A: Water (0.05% TFA), Mobile Phase B: MeCN Flow rate: 25 mL/min; Gradient: 40% B to 83% in 7 min, 254 nm; Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.64 min, m/z =336.10 [M +H]+ (calc m/z = 335.16); 1H NMR (400 MHz, methanol-d4) δ 8.23-8.20 (m, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.31-7.29 (m, 1H), 7.18 (m, 3H), 7.05 (m, 2H), 3.92 (t, J = 4.8 Hz, 4H), 2.95 (t, J = 4.8 Hz, 4H), 2.59 (s, 3H).
Prepared by using GP2 with 7-methyl-1\textit{H}-indole-2-carboxylic acid (100 mg, 0.57 mmol, 1.0 equiv) and 3-\((\text{morpholin-4-yl})\)aniline (153 mg, 0.86 mmol, 1.5 equiv) resulting in 7-methyl-\textit{N}\((\text{3-morpholinophenyl})\)-1\textit{H}-indole-2-carboxamide \textbf{17} (92 mg, 48\% yield) as a white solid. \textbf{Prep-HPLC}: Xselect CSH F-Phenyl OBD Column, 19×150 mm 5 \text{ \textmu}m; Mobile Phase A: Water (0.05\% TFA), Mobile Phase B: MeCN Flow rate: 25 mL/min; Gradient: 40\% B to 83\% in 7 min, 254 nm; Spectral data: \textbf{LCMS}: Method 2 (ESI, \textit{m/z}): RT=1.64 min, \textit{m/z} =336.10 [M +H]\textsuperscript{+} (calc \textit{m/z} = 335.16); \textbf{\textit{1H NMR}} (400 MHz, DMSO-\textit{d}6) \(\delta\) 11.53 (s, 1H), 10.06 (s, 1H), 7.50 (d, \(J = 7.6\) Hz, 1H), 7.43 (t, \(J = 2.2\) Hz, 1H), 7.39 (d, \(J = 2.1\) Hz, 1H), 7.35–7.33 (m, 1H), 7.22 (t, \(J = 8.1\) Hz, 1H), 7.00 (m, 2H), 6.74–6.72 (m, 1H), 3.77 (m, 4H), 3.12 (t, \(J = 4.8\) Hz, 4H), 2.54 (s, 3H).

Prepared by using GP2 with 4-fluoro-7-methyl-1\textit{H}-indole-2-carboxylic acid \textbf{36} (120 mg, 0.62 mmol, 1.0 equiv) and 3-\((\text{morpholin-4-yl})\)aniline (120 mg, 0.67 mmol, 1.1 equiv) resulting in 4-fluoro-7-methyl-\textit{N}\((\text{3-morpholinophenyl})\)-1\textit{H}-indole-2-carboxamide \textbf{18} (41 mg, 19\% yield) as a white solid. Spectral data: \textbf{LCMS}: Method 2 (ESI, \textit{m/z}): RT=1.64 min, \textit{m/z} =354.10 [M +H]\textsuperscript{+} (calc \textit{m/z} = 353.15); \textbf{\textit{1H NMR}} (400 MHz, methanol-\textit{d}4) \(\delta\) 7.43–7.42 (m, 1H), 7.38 (s, 1H), 7.27–7.22 (m, 2H), 6.99–6.96 (m, 1H), 6.82–6.79 (m, 1H), 6.67 (dd, \(J = 10.8, 8.0\) Hz, 1H), 3.85 (m, 4H), 3.18 (m, 4H), 3.12 (t, \(J = 4.8\) Hz, 4H), 2.52 (s, 3H); \textbf{\textit{19F NMR}} (282 MHz, methanol-\textit{d}4) \(\delta\) -127.9.
Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 36 (200 mg, 1.05 mmol, 1.0 equiv) and 3-(4-methylpiperazin-1-yl)aniline (200 mg, 1.05 mmol, 1.1 equiv) resulting in 4-flouro-7-methyl-N-(3-(4-methylpiperazin-1-yl)phenyl)-1H-indole-2-carboxamide 19 (74 mg, 20% yield) as a white solid. Spectral data:

**LCMS:** Method 2 (ESI, m/z): RT=1.64 min, m/z =367.21 [M +H]+ (calc m/z = 366.19);

**1H NMR** (300 MHz, methanol-d4) δ 7.45 (br s, H1), 7.40 (s, 1H), 7.29-7.23 (m, 2H), 7.02-6.97 (m, 1H), 6.84-6.78 (m, 1H), 6.69 (dd, J = 10.2, 8.0 Hz, 1H), 3.27 (m, 4H), 2.66 (m, 4H), 2.54 (s, 3H), 2.38 (s, 3H);

**19F NMR** (376 MHz, methanol-d4) δ -127.9.

**Compound 20** was initially prepared using the following route:

Using the following procedure: 1-(3-Bromophenyl)-N,N-dimethylpiperdin-4-amine (200 mg, 0.71 mmol, 1.0 equiv), (1S,2S)-1-N,2-N-dimethylcyclohexane-1,2-diamine (250 mg, 1.76 mmol, 2.5 equiv), copper iodide (270 mg, 1.42 mmol, 2.0 equiv) and cesium carbonate (600 mg, 2.0 equiv) were added to dry DMF (3 mL) under an atmosphere of nitrogen. The mixture stirred for 10 minutes and then 4-flouro-7-methyl-1H-indole-2-carboxamide 36-NH$_2$ (200 mg, 1.05 mmol, 1.5 equiv) was added. The reaction mixture was aged for 3 h with stirring at 100 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with deionized water (20 mL) and transferred to a separatory funnel containing ethyl acetate (15 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 15 mL). The organic layers were combined and dried over magnesium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under
reduced pressure (12 mbar). The residue obtained was purified by Prep-HPLC with the following conditions: Column: XBridge Prep OBD C18 Column, 30×150 mm 5 μm; Mobile Phase A: Water (10 mmol/L NH₄HCO₃), Mobile Phase B: MeCN; Flow rate: 10 mL/min; Gradient: 35 B to 65 B in 7 min; 254 nm to afford N-(3-(4-(dimethylamino)piperidin-1-yl)phenyl)-4-fluoro-7-methyl-1H-indole-2-carboxamide 20 (36 mg, 13% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.08 min, m/z =395.21 [M +H]+ (calc m/z = 394.22); ¹H NMR (400 MHz, methanol-d4) δ 7.46 (br s, 1H), 7.40 (s, 1H), 7.27-7.23 (m, 2H), 7.01-6.98 (m, 1H), 6.83-6.81 (m, 1H), 6.70 (dd, J = 10.4, 8.0 Hz, 1H), 3.83 (d, J = 12.0 Hz, 2H), 2.77 (t, J = 12.0 Hz, 2H), 2.54 (s, 3H), 2.37 (m, 7H), 2.02 (m, 2H), 1.66 (m, 2H); ¹⁹F NMR (376 MHz, methanol-d4) δ -127.9.

The aryl central core for 21-23 was prepared via the route shown in Supplementary Scheme S4. 3,5-Disubstituted-nitrobenzene S5/S8/S11 was reacted with the N,N-dimethylpiperdin-4-amine to provide the displacement product S6/S9/S12. Reduction of the nitro group was accomplished by either Fe/H⁺ or Pd/C in the presence of hydrogen gas.

**Supplementary Scheme S4:** Preparation of the aryl central core for 21-23.

*Preparation of S7*: 1-Fluoro-3-methyl-5-nitrobenzene S5 (500 mg, 3.22 mmol, 1.0 equiv), N,N-dimethylpiperdin-4-amine (413 mg, 3.22 mmol, 1.0 equiv) and potassium carbonate (890 mg, 6.44 mmol, 2.0 equiv) were added to dry DMSO (5 mL) under an atmosphere of nitrogen. The reaction mixture was aged for 3 hours with stirring at 100 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (50 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing N,N-dimethyl-1-(3-methyl-5-nitrophenyl)piperidin-4-
amine S6 (200 mg, 24% yield) as a yellow solid. **LCMS:** Method 3 (ESI, m/z): RT=0.57 min, m/z =264.2 [M +H]+ (calc m/z = 263.2).

A 2-neck reaction vessel was charged with N,N-dimethyl-1-(3-methyl-5-nitrophenyl)piperidin-4-amine S6 (200 mg, 0.76 mmol, 1 equiv), Pd/C (80 mg), and MeOH (4 mL). The solids were stirred for 1 minute and the vessel was evacuated and backfilled with nitrogen (x3). Then a balloon of hydrogen gas was attached to the reaction vessel with an adapter that allowed for the balloon to be closed off to the reaction flask. With the hydrogen balloon closed off, the reaction vessel was evacuated and then the balloon was opened to the reaction vessel (x2). After 1 hour, the flask was evacuated and filled with nitrogen, and the solids were filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing 1-(3-amino-5-methylphenyl)-N,N-dimethylpiperidin-4-amine S7 (140 mg, 79%) as a colorless oil. **LCMS:** Method 3 (ESI, m/z): RT=0.13 min, m/z =234.2 [M +H]+ (calc m/z = 233.2).

**Preparation of S10:** 1,3-dichloro-5-nitrobenzene S8 (500 mg, 2.6 mmol, 1.0 equiv), N,N-dimethylpiperidin-4-amine (5 mL) and potassium carbonate (710 mg, 5.14 mmol, 1.0 equiv) were added to dry DMSO (5 mL) under an atmosphere of nitrogen. The reaction mixture was aged for 3 hours with stirring at 100 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (50 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing N,N-dimethyl-1-(3-chloro-5-nitrophenyl)piperidin-4-amine S9 (600 mg, 81% yield) as a red oil. **LCMS:** Method 3 (ESI, m/z): RT=1.08 min, m/z =284.6 [M +H]+ (calc m/z = 283.1).

1-(3-Chloro-5-nitrophenyl)-N,N-dimethylpiperidin-4-amine S9 (500 mg, 1.76 mmol, 1.0 equiv) and Fe fillings (989 mg, 10.00 equiv) were added to a reaction vessel containing aqueous HCl (1 mL) and methanol (10 mL), and the resulting solution was stirred for at 21 °C. After the reaction had aged for 2 hours, the product mixture was filtered and concentrated under reduced pressure (12 mbar). The product residue was purified by reverse flash chromatography (C18 silica gel; mobile phase, MeCN in water, 0% to 100% gradient in 30 min; detector, UV 254 nm) furnishing 1-(3-amino-5-chlorophenyl)-N,N-dimethylpiperidin-4-amine S10 (450 mg, 80% yield) as a red solid. **LCMS:** Method 1 (ESI, m/z): RT=0.82 min, m/z =254.6 [M +H]+ (calc m/z = 253.1).

**Preparation of S13:** 1,3-Difluoro-5-nitrobenzene S11 (2.0 g, 12.6 mmol, 1.0 equiv), N,N-dimethylpiperidin-4-amine (1.6 g, 12.5 mmol, 1.0 equiv) and potassium carbonate (1.7 g mg, 12.3 mmol, 1.0 equiv) were added to dry DMSO (9 mL) under an atmosphere of nitrogen. The reaction mixture was aged for 2.5 hours with stirring at 80 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (50 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing 1-(3-fluoro-5-nitrophenyl)-N,N-dimethylpiperidin-4-amine S12 (2.5 g, 74% yield) as a yellow solid. **LCMS:** Method 4 (ESI, m/z): RT=0.55 min, m/z =267.8 [M +H]+ (calc m/z = 267.1).

1-(3-Fluoro-5-nitrophenyl)-N,N-dimethylpiperidin-4-amine S12 (700 mg, 2.62 mmol, 1.0 equiv) and Fe fillings (734 mg, 5.0 equiv) were added to a reaction vessel containing aqueous ammonium chloride (695 mg, 13.0 mmol, 5.0 equiv in 2 mL of water) and ethanol (8 mL), and the resulting solution was stirred for at 80 °C. After the reaction had aged for 2 hours, the product mixture was filtered and concentrated under reduced pressure (12 mbar). The product residue was purified by reverse flash chromatography (IntelFlash-1 Column, C18 silica gel; mobile phase, MeCN/ water =0-100%; Detector, UV 254 nm) furnishing 1-(3-amino-5-fluorophenyl)-N,N-dimethylpiperidin-4-amine S13 (300 mg, 48% yield) as a brown solid. **LCMS:** Method 1 (ESI, m/z): RT=0.72 min, m/z =238.0 [M +H]+ (calc m/z = 237.2).
Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 36 (100 mg, 0.52 mmol, 1.0 equiv) and 1-(3-amino-5-methylphenyl)-N,N-dimethylpiperidin-4-amine S7 (120 mg, 0.51 mmol, 1.00 equiv) resulting in N-[3-[4-(dimethylamino)piperidin-1-yl]-5-methylphenyl]-4-flouro-7-methyl-1H-indole-2-carboxamide 21 (58 mg, 28% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=2.61 min, m/z =409.2 [M +H]+ (calc m/z = 408.2); ¹H NMR (300 MHz, methanol-d4) δ 7.40 (s, 1H), 7.26 (s, 1H), 7.05 (d, J = 3.3 Hz, 2H), 6.69 – 6.66 (m, 2H), 3.79 (d, J = 2.1 Hz, 2H), 2.72 – 2.69 (m, 2H), 2.54 (s, 3H), 2.34 (s, 10H), 1.99 (d, J = 2.1 Hz, 2H), 1.79 – 1.58 (m, 2H); ¹⁹F NMR (282 MHz, methanol-d4) δ -127.8.

Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 36 (150 mg, 0.78 mmol, 1.0 equiv) and 1-(3-amino-5-chlorophenyl)-N,N-dimethylpiperidin-4-amine (200 mg, 0.79 mmol, 1.0 equiv) S10 (185 mg, 0.78 mmol, 1.0 equiv) resulting in N-[3-chloro-5-[4-(dimethylamino)piperidin-1-yl]phenyl]-4-flouro-7-methyl-1H-indole-2-carboxamide 22 (28 mg, 8% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, m/z): RT=2.77 min, m/z =429.2 [M +H]+ (calc m/z = 428.2); ¹H NMR (300 MHz, methanol-d4) δ 7.42 (s, 1H), 7.38 (t, J = 1.8 Hz, 1H), 7.33 (t, J = 1.8 Hz, 1H), 7.03-6.98 (m, 1H), 6.78 (t, J = 1.8 Hz, 1H), 6.70 (d, J = 10.4, 7.8 Hz, 1H), 3.85 (d, J = 13.3 Hz, 2H), 2.80 (t, J = 12.7 Hz, 2H), 2.55 (s, 3H), 2.38 (m, 7H), 2.06-2.01 (m, 2H), 1.67-1.61 (m, 2H); ¹⁹F NMR (282 MHz, methanol-d4) δ -127.8.
Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid \(36\) (150 mg, 0.78 mmol, 1.0 equiv) and 1-(3-amino-5-fluorophenyl)-N,N-dimethylpiperidin-4-amine \(S13\) (185 mg, 0.78 mmol, 1.0 equiv) resulting in N-[3-[4-(dimethylamino)piperidin-1-yl]-5-fluorophenyl]-4-fluoro-7-methyl-1H-indole-2-carboxamide \(23\) (41 mg, 19% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, \(m/z\)): RT=1.20 min, \(m/z = 413.0 \ [M +H]^+\) (calc \(m/z = \) 412.2); \(^1\)H NMR (300 MHz, methanol-\(d_4\)) \(\delta\) 7.41 (s, 1H), 7.15 (m, 2H), 7.04-6.96 (m, 1H), 6.70 (dd, \(J = 10.3, 7.9\) Hz, 1H), 6.51 (dt, \(J = 12.2, 2.2\) Hz, 1H), 3.84 (d, \(J = 12.8\) Hz, 2H), 2.78 (t, \(J = 12.7\) Hz, 2H), 2.54 (s, 3H), 2.36 (m, 7H), 2.00 (d, \(J = 12.7\) Hz, 2H), 1.61 (qd, \(J = 12.3, 4.0\) Hz, 2H); \(^{19}\)F NMR (282 MHz, methanol-\(d_4\)) \(\delta\) -113.9, -127.8.

The aryl central core for \(24\) and \(25\) is prepared via the route shown in Supplementary Scheme S5. 3-bromo-nitrobenzene \(S14\) was coupled via a Pd-catalyzed Buchwald reaction with the \(N\)-Boc-protected aminopyrrolidine to provide \(S15\). Elaboration of the terminal amine by removal of the \(N\)-Boc protecting group followed by acetylation provided the fully elaborated substituted nitrobenzene \(S16\). Reduction of the nitro group with \(\text{Fe/H}^+\) furnished \(S17\).

3,5-Difluoro-nitrobenzene \(S18\) was reacted with the \(N\)-Boc-protected aminopyrrolidine to provide the displacement product \(S19\). Elaboration of the terminal amine by removal of the \(N\)-Boc protecting group followed by acetylation provided the fully elaborated substituted nitrobenzene \(S20\). Reduction of the nitro group with \(\text{Pd/C}\) in the presence of hydrogen gas furnished \(S21\).

**Supplementary Scheme S5:** Preparation of the aryl central core for \(24\) and \(25\).

**Preparation of \(S17\):** 1-Bromo-3-nitrobenzene \(S14\) (500 mg, 2.48 mmol, 1.0 equiv), potassium carbonate (680 mg, 4.92 mmol, 2.0 equiv), \(\text{tert-butyl pyrrolidine-3-yl N-methylcarbamate}\) (590 mg, 2.93 mmol, 1.2 equiv), and \(\text{Ru-Phos}\) (600 mg, 1.0 equiv) were added to dry DMSO (5 mL) under an atmosphere of nitrogen. The reaction mixture was aged for 2 h with stirring at 100 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (5 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL) and additional aqueous sodium chloride (45 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing \(\text{tert-butyl 1-[3-nitrophenyl]pyrrolidine-3-yl N-methylcarbamate}\) \(S15\) (500 mg, 63% yield) as a yellow solid. LCMS: Method 4 (ESI, \(m/z\)): \(RT=1.16 \text{ min, } m/z = 322.2 \ [M +H]^+\) (calc \(m/z = 321.2\)).

**Tert-butyl \(N\)-methyl-N-[1-(3-nitrophenyl)pyrrolidin-3-yl]carbamate \(S15\)** (200 mg, 0.62 mmol, 1.00 equiv) was added to a reaction vessel containing trifluoroacetic acid (2 mL) and dichloromethane (5 mL). The resulting solution was aged for 2 hours with stirring at 21 °C at which time the resulting product mixture was concentrated under reduced pressure (12 mbar) furnishing \(\text{N-methyl-1-[3-nitrophenyl]pyrrolidin-3-amine}\) (150 mg, crude) as a white solid. LCMS: Method 1 (ESI, \(m/z\)): \(RT=0.83 \text{ min, } m/z = 222.2 \ [M +H]^+\) (calc \(m/z = 221.1\)).
nitrophenyl)pyrrolidin-3-amine (500 mg, 2.26 mmol, 1.0 equiv), acetic anhydride (2 mL), and triethylamine (2 mL) were added to dry dichloromethane (5 mL). After the reaction mixture aged for 2 hours with stirring, the product mixture was concentrated under reduced pressure (12 mbar) furnishing N-methyl-N-[1-(3-nitrophenyl)pyrrolidine-3-yl]acetamide S16 (450 mg crude, 76% yield) as a white solid. **LCMS:** Method 2 (ESI, m/z): RT=0.92 min, m/z =264.0 [M +H]^+ (calc m/z = 263.1).

N-Methyl-N-[1-(3-nitrophenyl)pyrrolidin-3-yl]acetamide (20 mg, 0.08 mmol, 1.00 equiv) and Fe fillings (42 mg, 10.00 equiv) were added to a reaction vessel containing aqueous HCl (0.5 mL) and methanol (1 mL), and the resulting solution was stirred for at 21 °C. After the reaction had aged for 2 hours, the product mixture was filtered and concentrated under reduced pressure (12 mbar). The product residue was purified by reverse flash chromatography (C18 silica gel; mobile phase, MeCN in water, 0% to 100% gradient in 30 min; detector, UV 254 nm) furnishing N-[1-(3-aminophenyl)pyrrolidin-3-yl]-N-methylacetamide S17 (15 mg, 85% yield) as a white solid. **LCMS:** Method 2 (ESI, m/z): RT=0.80 min, m/z =233.95 [M +H]^+ (calc m/z = 233.15).

Preparation of S21: 1,3-Difluoro-5-nitrobenzene S18 (2 g, 12.6 mmol, 1.0 equiv), cesium carbonate (13 g, 39.9 mmol, 3.2 equiv), and tert-butyl pyrrolidine-3-yl N-methylcarbamate (2.6 g, 12.0 mmol, 1.03 equiv) were added to dry DMSO (30 mL) under an atmosphere of nitrogen. The reaction mixture was aged for 2 h with stirring at 100 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (50 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL) and additional aqueous sodium chloride (50 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar). The residue was purified via flash chromatography (silica; EtOAc/PE 50/50), collected fractions combined, and concentrated under reduced pressure (12 mbar) furnishing tert-butyl N-[1-(3-fluoro-5-nitrophenyl)pyrrolidin-3-yl]-N-methylcarbamate S19 (680 mg, 16% yield) as a light yellow solid. **LCMS:** Method 2 (ESI, m/z): RT=1.49 min, m/z =340.1 [M +H]^+ (calc m/z = 339.2).

Tert-butyl N-[1-(3-fluoro-5-nitrophenyl)pyrrolidin-3-yl]-N-methylcarbamate S19 (650 mg, 1.92 mmol, 1.00 equiv) was added to a reaction vessel containing trifluoroacetic acid (3 mL) and dichloromethane (7 mL). The resulting solution was aged for 1 hour with stirring at 21 °C at which time the resulting product mixture was concentrated under reduced pressure (12 mbar) furnishing 1-(3-fluoro-5-nitrophenyl)-N-methylpyrrolidin-3-amine (350 mg, crude) as a light yellow solid. **LCMS:** Method 2 (ESI, m/z): RT=0.92 min, m/z =239.75 [M +H]^+ (calc m/z = 239.1). 1-(3-fluoro-5-nitrophenyl)-N-methylpyrrolidin-3-amine (1.0 g, 4.18 mmol, 1.0 equiv), acetic anhydride (2 mL), and triethylamine (2 mL) were added to dry dichloromethane (10 mL). After the reaction mixture aged for 2 hours with stirring, the product mixture was concentrated under reduced pressure (12 mbar). The product residue was purified by flash chromatography (silica; EtOAc/PE 50/50) furnishing N-[1-(3-fluoro-5-nitrophenyl)pyrrolidin-3-yl]-N-methylacetamide S20 (900 mg, 77% yield) as a white solid. **LCMS:** Method 2 (ESI, m/z): RT=1.20 min, m/z =281.65 [M +H]^+ (calc m/z = 281.12).

N-[1-(3-Fluoro-5-nitrophenyl)pyrrolidin-3-yl]-N-methylacetamide S20 (300 mg, 1.07 mmol, 1.00 equiv) and Raney Ni (300 mg) were added to a reaction vessel containing methanol (10 mL). Hydrogen gas was introduced into the reaction vessel and the resulting solution was stirred for at 21 °C. After the reaction had aged for 1 hour, the product mixture was filtrated and concentrated under reduced pressure (12 mbar) furnishing N-[1-(3-amino-5-fluorophenyl)pyrrolidin-3-yl]-N-methylacetamide S21 (220 mg, 82% yield) as an off-white solid. **LCMS:** Method 2 (ESI, m/z): RT=0.85 min, m/z =251.85 [M +H]^+ (calc m/z = 251.14).
Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 36 (165 mg, 0.85 mmol, 1.0 equiv) and \(N\)-[1-(3-aminophenyl)pyrrolidin-3-yl]-N-methylacetamide S17 (200 mg, 0.86 mmol, 1.0 equiv) resulting in 4-fluoro-7-methyl-\(N\)-[3-[3-(N-methylacetamido)pyrrolidin-1-yl]phenyl]-1H-indole-2-carboxamide 24 (111 mg, 32% yield) as a white solid. Spectral data: LCMS: Method 2 (ESI, \(m/z\)): RT=2.30 min, \(m/z = 430.95 \ [M +Na]^+ \) (calc \(m/z = 431.19\)); \(^1\)H NMR (300 MHz, methanol-\(d_4\)) \(\delta 7.40 \) (s, 1H), 7.28 – 7.10 (m, 2H), 7.10 – 7.00 (m, 2H), 6.99 (d, \(J = 5.6 \) Hz, 1H), 6.70-6.60 (m, 1H), 5.38 – 5.28 (m, 1H), 3.59-3.50 (m, 1H), 3.50-3.40 (m, 1H), 3.40 – 3.25 (m, 1H), 3.04 (s, 2H), 2.92 (s, 1H), 2.55 (d, \(J = 1.1 \) Hz, 3H), 2.38-3.26 (m, 1H), 2.25 (s, 2H), 2.16 (s, 2H); \(^{19}\)F NMR (376 MHz, methanol-\(d_4\)) \(\delta -127.9\).

Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 36 (250 mg, 1.29 mmol, 1.0 equiv) and \(N\)-[1-(3-amino-5-fluorophenyl)pyrrolidin-3-yl]-N-methylacetamide S21 (330 mg, 1.31 mmol, 1.00 equiv) resulting 4-fluoro-\(N\)-[3-fluoro-5-[3-(N-methylacetamido)pyrrolidin-1-yl]phenyl]-7-methyl-1H-indole-2-carboxamide 25 (47 mg, 8% yield) as a light yellow solid. Spectral data: LCMS: Method 1 (ESI, \(m/z\)): RT=3.21 min, \(m/z = 426.95 \ [M+H]^+ \) (calc \(m/z = 426.19\)); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta 11.92 \) (s, 1H), 10.19 (s, 1H), 7.51 (d, \(J = 1.5 \) Hz, 1H), 7.19 – 7.08 (m, 1H), 7.03 – 6.93 (m, 1H), 6.87 (d, \(J = 2.1 \) Hz, 1H), 6.82 – 6.72 (m, 1H), 6.23 – 6.10 (m, 1H), 5.23 – 4.63 (m, 1H), 3.54 – 3.33 (m, 2H), 3.26 – 3.16 (m, 2H), 2.90 (s, 2H), 2.76 (s, 1H), 2.61 – 2.51 (m, 3H), 2.24 – 2.00 (m, 5H); \(^{19}\)F NMR (376 MHz, DMSO-\(d_6\)) \(\delta -112.1, -125.6\).

Overview for Table 3 analogs: **Supplementary Scheme S6** shows the method used for the preparation of compounds with a saturated central core. We determined early in the project that the only active isomer in this series of compounds was the cis isomer with the absolute configuration as drawn in the Scheme 1. Since we knew the desired absolute configuration, we began our syntheses by coupling the necessary indole acid with \((1R,3R)\)-3-aminocyclohexan-1-ol to provide the amide, which was oxidized to the corresponding ketone with PCC. Analogs in Table 4 are derived from this intermediate.
**Supplementary Scheme S6:** Preparation of analogs in Table 4 through intermediate 37.

**Preparation of 37:** To a reaction vessel containing 4-fluoro-7-methyl-1H-indole-2-carboxylic acid 36 (4.5 g, 23.3 mmol, 1.0 equiv) was added HATU (10.6 g, 28 mmol, 1.2 equiv), (1R,3R)-3-aminocyclohexan-1-ol (2.7 g, 23.3 mmol, 1.0 equiv) in DMF (50 mL) followed by N,N'-diisopropylethylamine (9.0 g, 69.9 mmol, 3.0 equiv) at 21 °C. After stirring for 2 hours, the reaction mixture was diluted with deionized water (50 mL) and transferred to a separatory funnel containing ethyl acetate (100 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 100 mL). The organic layers were combined and dried over sodium sulfate, filtered, and concentrated under reduced pressure (12 mbar). The residue obtained was purified by flash-column chromatography (eluting with 50% ethyl acetate/petroleum ether to afford 4-fluoro-N-((1R,3R)-3-hydroxycyclohexyl)-7-methyl-1H-indole-2-carboxamide (6.5 g, 96%) as a light yellow solid. **LCMS:** (ESI, m/z): RT=0.651 min, m/z =291.3 [M+H]+ (calc m/z = 290.1); **1H NMR** (400 MHz, methanol-d4) δ 7.21 (s, 1H), 6.99 – 6.93 (m, 1H), 6.66 (dd, J = 10.4, 7.8 Hz, 1H), 4.42 – 4.32 (m, 1H), 4.20 – 4.135 (m, 1H), 2.51 (s, 3H), 2.07 – 1.78 (m, 3H), 1.79 – 1.26 (m, 5H).

Pyridinium chlorochromate (PCC, 5.9 g, 27.6 mmol, 2.0 equiv) was added in a single portion under nitrogen to a round bottom flask equipped with a magnetic stir bar. Ethyl acetate (50 mL) was added to the vessel followed by 4-fluoro-N-((1R,3R)-3-hydroxycyclohexyl)-7-methyl-1H-indole-2-carboxamide (4.0 g, 13.8 mmol, 1.0 equiv). After stirring for 18 hours at 21 °C, the reaction mixture was diluted with aqueous sodium bicarbonate (50 mL) and transferred to a separatory funnel containing ethyl acetate (100 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (2 x 100 mL). The organic layers were combined and dried over sodium sulfate, filtered, and concentrated under reduced pressure (12 mbar). The residue obtained was purified by flash-column chromatography (eluting with 50% ethyl acetate/petroleum ether to afford 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide (2.9 g, 73% yield) as a yellow solid. **LCMS:** (ESI, m/z): RT=0.729 min, m/z =289.25 [M+H]+ (calc m/z = 288.13); **1H NMR** (400 MHz, DMSO-d6) δ 11.71 (s, 1H), 8.53 (d, J = 7.7 Hz, 1H), 7.25 (d, J = 2.1 Hz, 1H), 6.90 (dd, J = 7.9, 5.1 Hz, 1H), 6.70 (dd, J = 10.4, 7.8 Hz, 1H), 4.25 – 4.08 (m, 1H), 2.58 – 2.51 (m, 2H), 2.45 (s, 3H), 2.41 – 2.15 (m, 2H), 2.06 – 1.92 (m, 2H), 1.82 – 1.56 (m, 2H).

With intermediate 37 in hand, reductive amination with the requisite amine afforded the desired product as a mixture of cis and trans isomers. A cis/trans ratio of 2/1 to 1/1 was typically obtained. The reaction scheme is outlined in **Supplementary Scheme S7.**

**Supplementary Scheme S7:** Preparation of analogs 26, 28-33 in Table 4.
**General reaction procedure (GP3):** To a reaction vessel containing the amine hydrochloride (0.70 mmol, 2 equiv) suspended in 1,2-DCE (5.0 mL) was added N-N'-diisopropylethylamine (270 mg, 2.1 mmol, 6.0 equiv) at 21 °C. After the reaction had aged 2 hours, the mixture was concentrated under reduced pressure. To this residue, 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide (100 mg, 0.35 mmol, 1.0 equiv) was added to the flask followed by methanol (5.0 mL). Sodium cyanoborohydride was added in one portion to the reaction mixture and stirred at 21 °C. After the reaction mixture had aged 18 hours, the product mixture was concentrated under reduced pressure (12 mbar). The residue was purified by Prep-HPLC (conditions listed).

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (264 mg, 0.92 mmol, 1.0 equiv) and morpholine (160 mg, 1.84 mmol, 2.0 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-morpholinocyclohexyl)-1H-indole-2-carboxamide 26 (51 mg, 13% yield) as a white solid. **Prep-HPLC conditions:** XBridge Shield RP18 OBD Column, 30*150 mm, 5μm; Mobile Phase A: Water(10MMOL/L NH4HCO3), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 31% B to 55% B in 7 min, 55% B; Wave Length: 254/220 nm; RT= 6.67 min; Spectral data: **LCMS:** Method 2 (ESI, m/z): RT=1.14 min, m/z =360.12 [M +H]+ (calc m/z = 359.20); **1H NMR** (400 MHz, methanol-d4) δ 7.22 (s, 1H), 6.98 (dd, J = 7.2, 5.1 Hz, 1H), 6.68 (dd, J = 10.3, 7.9 Hz, 1H), 4.11-4.00 (m, 3H), 3.79 (m, 2H), 3.50 (m, 2H), 3.41 (m, 2H), 3.30 (m, 2H), 2.51 (m, 4H), 2.22 (d, J = 10.8 Hz, 1H), 2.10-2.01 (m, 2H), 1.63-1.40 (m, 4H); **19F NMR** (282 MHz, methanol-d4) δ -128.0.

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (200 mg, 0.71 mmol, 1.0 equiv) and 1-methylpiperazin-2-one (161 mg, 1.41 mmol, 2.0 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 28 (15 mg, 10% yield) as a white solid. **Prep-HPLC conditions:** XBridge Shield RP18 OBD Column, 19*150 mm, 5μm; Mobile Phase A: Water(10 mmol/L NH4HCO3), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 26% B to 42% B in 10 min,
42% B; Wave Length: 254/220 nm; RT=9.27 min; Spectral data: **LCMS**: Method 2 (ESI, m/z): RT=1.27 min, m/z =408.85 [M +Na]^+ (calc m/z = 409.20); **^1^H NMR** (400 MHz, methanol-d4) δ7.17 (s, 1H), 6.95 (dd, J = 5.2, 1.2 Hz, 1H), 6.64 (dd, J = 10.4, 7.6 Hz, 1H), 3.97-3.94 (m, 1H), 3.83 (t, J = 5.5 Hz, 2H), 3.30 (s, 3H), 2.96 (s, 3H), 2.49-2.85 (m, 2H), 2.60-2.56 (m, 1H), 2.51 (s, 3H), 2.22-2.19 (m, 1H), 2.03-1.90 (m, 3H), 1.50-1.25 (m, 4H); **^1^H NMR** (376 MHz, methanol-d4) δ -128.0.

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (100 mg, 0.35 mmol, 1.0 equiv) and 1-methanesulfonyl piperazine (85 mg, 0.52 mmol, 1.5 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-(4-(methylsulfonyl)piperazin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 29 (15 mg, 10% yield) as a white solid. **Prep-HPLC conditions**: Shim-pack XR-ODS C18, Column, 50*3.0 mm, 2.2μm; Mobile Phase A: water/0.05%TFA, Mobile Phase B: ACN/0.05%TFA; Flow rate: 1.2 mL/min; Wave Length: 220 nm; RT=1.42 min; Spectral data: **LCMS**: Method 2 (ESI, m/z): RT=1.42 min, m/z =437.3 [M +H]^+ (calc m/z = 436.2); **^1^H NMR** (400 MHz, methanol-d4) δ 7.21 (s, 1H), 6.96 (dd, J = 4.8, 0.8 Hz, 1H), 6.66 (dd, J = 10.4, 8.0 Hz, 1H), 3.99-3.92 (m, 1H), 3.20 (t, J = 4.7 Hz, 4H), 2.82 (s, 3H), 2.68 (m, 4H), 2.49 (m, 4H), 2.18 (d, J = 11.6 Hz, 1H), 1.97 (d, J = 11.6 Hz, 1H), 1.92-1.88 (m, 2H), 1.43-1.23 (m, 4H); **^1^F NMR** (376 MHz, methanol-d4) δ -127.9.

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (100 mg, 0.35 mmol, 1.0 equiv) and N-methyl-N-[(3S)-pyrrolidin-3-yl]acetamide (74 mg, 0.52 mmol, 1.5 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-(3S)-(N-methylacetamido)pyrrolidin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 30 (16 mg, 11% yield) as an off-white solid. **Prep-HPLC conditions**: XBridge Shield RP18 OBD Column, 30*150 mm, 5μm; Mobile Phase A: Water (10 mmol/L NH4HCO3), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 34% B to 53% B in 7 min, 53% B; Wave Length: 254 nm; RT=5.98 min; Spectral data: **LCMS**: Method 2
(ESI, m/z): RT=2.43 min, m/z =415.20 [M +H]+ (calc m/z = 414.24); ^1^H NMR (400 MHz, methanol-d4) δ 7.20 (s, 1H), 6.96 (dd, J = 8.0, 6.0 Hz, 1H), 6.67 (dd, J = 10.4, 8.0 Hz, 1H), 4.61–4.53 (m, 1H), 4.02–3.96 (m, 1H), 3.01–2.84 (m, 5H), 2.72–2.64 (m, 2H), 2.51 (s, 3H), 2.30–2.14 (m, 4H), 2.09–1.77 (m, 6H), 1.47–1.26 (m, 4H); ^19^F NMR (282 MHz, methanol-d4) δ -128.0.

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (100 mg, 0.35 mmol, 1.0 equiv) and N-methyl-N-((3R,5S)-pyrrolidin-3-yl)methanesulfonamide hydrochloride (149 mg, 0.69 mmol, 2.0 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S,3-(3S)-3-(N-methanesulfonyl)-pyrrolidin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 31 (14 mg, 10% yield) as an off-white solid. **Prep-HPLC conditions:** XBridge Shield RP18 OBD Column, 30*150 mm, 5μm; Mobile Phase A: Water (10mmol/L NH₄HCO₃), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 34% B to 44% B in 7 min, 44% B; Wave Length: 220 nm; RT=4.78 min; Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.38 min, m/z =415.30 [M +H]+ (calc m/z = 414.24); ^1^H NMR (400 MHz, methanol-d4) δ 7.20 (s, 1H), 6.96 (dd, J = 8.0, 6.0 Hz, 1H), 6.67 (dd, J = 10.4, 8.0 Hz, 1H), 4.61–4.53 (m, 1H), 4.02–3.96 (m, 1H), 3.01–2.84 (m, 5H), 2.72–2.64 (m, 2H), 2.51 (s, 3H), 2.30–2.14 (m, 4H), 2.09–1.77 (m, 6H), 1.47–1.26 (m, 4H); ^19^F NMR (282 MHz, methanol-d4) δ -127.7.

Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (100 mg, 0.35 mmol, 1.0 equiv) and N-methyl-N-((3R)-pyrrolidin-3-yl)acetamide (74 mg, 0.52 mmol, 1.5 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-((3R)-3-(N-methylacetamido)pyrrolidin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 31 (14 mg, 10% yield) as an off-white solid. **Prep-HPLC conditions:** XBridge Shield RP18 OBD Column, 30*150 mm, 5μm; Mobile Phase A: Water (10mmol/L NH₄HCO₃), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 34% B to 44% B in 7 min, 44% B; Wave Length: 220 nm; RT=4.78 min; Spectral data: LCMS: Method 2 (ESI, m/z): RT=1.38 min, m/z =415.30 [M +H]+ (calc m/z = 414.24); ^1^H NMR (400 MHz, methanol-d4) δ 7.20 (s, 1H), 6.96 (dd, J = 8.0, 6.0 Hz, 1H), 6.67 (dd, J = 10.4, 8.0 Hz, 1H), 4.61–4.53 (m, 1H), 4.02–3.96 (m, 1H), 3.01–2.84 (m, 5H), 2.72–2.64 (m, 2H), 2.51 (s, 3H), 2.30–2.14 (m, 4H), 2.09–1.77 (m, 6H), 1.47–1.26 (m, 4H); ^19^F NMR (282 MHz, methanol-d4) δ -127.7.
Prepared by using GP3 with 4-fluoro-7-methyl-N-((1R)-3-oxocyclohexyl)-1H-indole-2-carboxamide 37 (150 mg, 0.52 mmol, 1.0 equiv) and N-methyl-N-[(3R)-pyrrolidin-3-yl]methanesulfonamide (185 mg, 1.04 mmol, 2.0 equiv) resulting in 4-fluoro-7-methyl-N-((1R,3S)-3-((3R)-3-(N-methylmethanesulfonamido)pyrrolidin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 33 (31 mg, 13% yield) as an off-white solid. **Prep-HPLC conditions:** XBridge Prep OBD C18 Column, 30*150 mm, 5μm; Mobile Phase A: Water(10 mmol/L NH4HCO3), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 37% B to 67% B in 7 min, 67% B; Wave Length: 254 nm; RT: 6.13 min; Spectral data: 

**LCMS:** Method 2 (ESI, m/z): RT=1.38 min, m/z =451.25 [M +H]+ (calc m/z = 450.21); 

**1H NMR** (300 MHz, methanol-d4) δ 7.22 (s, 1H), 6.95 – 6.91 (m, 1H), 6.70 – 6.66 (m, 1H), 4.56 – 4.30 (m, 2H), 3.14 – 3.04 (m, 1H), 2.93 – 2.81 (m, 7H), 2.63 – 2.47 (m, 4H), 2.44 – 2.35 (m, 1H), 2.32 – 2.06 (m, 3H), 2.03 – 1.74 (m, 4H), 1.71 – 1.38 (m, 4H); 

**19F NMR** (282 MHz, methanol-d4) δ 128.0.

Supplementary Scheme S8: Preparation of reaction intermediates leading to Compound 27.

Preparation of S25: Cyclohexenone S23 (1.0 g, 10.4 mmol, 1.0 equiv), potassium carbonate (8.83 g, 41.6 mmol, 4.0 equiv), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (4.27 g, 20.8 mmol, 2.0 equiv), and [Rh(COD)OH]2 (173 mg, 0.26 mmol, 0.025 equiv) were added to dioxane/water (10 mL; 1/1) under an atmosphere of nitrogen. The reaction mixture was aged for 6 h with stirring at 60 °C, at which time the reaction was shown to be complete by TLC. The product mixture was then diluted with aqueous sodium chloride solution (5 mL) and transferred to a separatory funnel containing ethyl acetate (50 mL) and additional aqueous sodium chloride (45 mL). The layers were separated, and the aqueous layer was re-extracted with additional ethyl acetate (3 x 50 mL). The organic layers were combined and dried over sodium sulfate. The dried solution was filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar). The residue was purified by reverse flash chromatography (C18 silica gel, mobile phase, MeOH in water, 10% to 50% gradient in 10 min; detector, UV 254 nm) furnishing 3-(pyridin-4-yl)cyclohexan-1-one S24 (1.2 g, 65% yield) as a light yellow oil. **LCMS:** Method 4 (ESI, m/z): RT=1.16 min, m/z =176.1 [M +H]+ (calc m/z = 175.1).

A 2-neck reaction vessel was charged with 3-(pyridin-4-yl)cyclohexan-1-one S24 (1.2 g, 6.85 mmol, 1 equiv), ammonium formate (4.32 g, 68.5 mmol, 10 equiv), Pd/C (3.6 g), and MeOH (20 mL). The solids were stirred for 1 minute and the vessel was evacuated and backfilled with nitrogen (x3). Then a balloon of hydrogen gas was attached to the reaction vessel with an adapter that allowed for the balloon to be closed off to the reaction flask. With the hydrogen balloon closed off, the reaction vessel was evacuated and then the balloon was opened to the
reaction vessel (x2). After 1 hour, the flask was evacuated and filled with nitrogen, and the solids were filtered through a plug of celite and the filtrate was concentrated under reduced pressure (12 mbar) furnishing 3-(pyridin-4-yl)cyclohexan-1-amine S25 (800 mg, 66%) as a light yellow oil. **LCMS:** Method 2 (ESI, m/z): RT=0.32 min, m/z =177.2 [M +H]⁺ (calc m/z = 176.1).

Prepared by using GP2 with 4-flouro-7-methyl-1H-indole-2-carboxylic acid 37 (219 mg, 1.14 mmol, 1.0 equiv) and 3-(pyridin-4-yl)cyclohexan-1-amine S25 (200 mg, 1.13 mmol, 1.0 equiv) resulting 4-fluoro-7-methyl-N-[3-(pyridin-4-yl)cyclohexyl]-1H-indole-2-carboxamide (300 mg, 75% yield) as a light yellow oil. **Prep-HPLC conditions:** Xselect CSH C18 OBD Column 30*150mm 5μm, n; Mobile Phase A: Water(0.05%TFA ), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 15% B to 41% B in 7 min, 41% B; Wave Length: 220 nm; RT= 6.14 min;

**Chiral-prep HPLC conditions:** Chiral ADH, 4.6*100mm, 5um; Mobile Phase A: Hex(0.1%DEA): EtOH=70: 30; Flow rate: 1 mL/min; Gradient: 0% B to 0% B; Injection Volume: 5μl mL; retention time: 6.394 min
After chiral prep-HPLC, 4-fluoro-7-methyl-N-[3-(pyridin-4-yl)cyclohexyl]-1H-indole-2-carboxamide (100 mg) yielded 4-fluoro-7-methyl-N-((1R,3S)-3-(pyridin-4-yl)cyclohexyl)-1H-indole-2-carboxamide (15 mg, 15% yield) as an off-white solid. Spectral data: **LCMS:** Method 2 (ESI, m/z): RT=1.71 min, m/z =352.2 [M +H]+ (calc m/z = 351.2); **1H NMR** (400 MHz, methanol-d4) δ 8.45-8.44 (m, 2H), 7.37-7.36 (m, 2H), 7.21 (s, 1H), 6.95 (dd, J = 7.8, 5.2 Hz, 1H), 6.66 (dd, J = 10.3, 7.9 Hz, 1H), 4.14-4.08 (m, 1H), 2.85-2.79 (m, 1H), 2.50 (s, 3H), 2.22-1.92 (m, 4H), 1.68-1.44 (m, 4H); **19F NMR** (282 MHz, methanol-d4) δ 128.0.

**Stereoisomer Assignment of 4-fluoro-7-methyl-N-((1R,3S)-3-(3S)-3-(N-methylmethanesulfonamido)pyrrolidin-1-yl)cyclohexyl)-1H-indole-2-carboxamide 32:**

For determination of the depicted stereoisomer of compd 32, 2D NMR (gCOSY, HSQCAD, and 2D NOESY experiments) was used and assignment was made based on the analysis of the data for the two diaxial methines. The 1H NMR spectrum of 32 showed 31 proton signals, including 9 methyl protons, 14 methylene protons, 6 methine protons, and 2 active protons (N-H).

N-H assignments: The 1H NMR signal at 11.61 ppm (s, 1H) showed no correlation in the HSQC spectrum and was assigned to NH-8 according to its chemical shift, COSY correlation (H10), and NOESY correlation (H -7). The 1H NMR signal at 8.31 ppm (d, 1H) also showed no correlation in the HSQC spectrum and was assigned to NH-13 according to its chemical shift, COSY correlation (H-14), and NOESY (H-15).

Methyl assignments: The 1H NMR signal at 2.84 ppm (s, 3H) was associated with the 13C NMR signal at 36.8 ppm (HSQC) and is assigned H-27 according to its chemical shift, COSY correlation (H-22/24), and NOESY correlation (H-22/24 and H-23). The 1H NMR signal at 2.72 ppm (s, 3H) was associated with the 13C NMR signal at 29.4 ppm (HSQC) and is assigned to H-26 according to its chemical shift, COSY correlation (H-22/24), and NOESY correlation (H-22/24 and H-23). The 1H NMR signal at 2.46 ppm (s, 3H) was associated with the 13C NMR signal...
at 17.2 ppm (HSQC) and is assigned to H-7 according to its chemical shift, COSY correlation (H-1), and NOESY (H-1).

Aryl methine assignments: The 1H NMR signal at 7.19 ppm (s, 1H) was associated with the 13C NMR signal at 100.0 ppm (HSQC) and is assigned H-10 according to its chemical shift. The 1H NMR signal at 6.90 ppm (dd, 1H) was associated with the 13C NMR signal at 124.4 ppm (HSQC) and is assigned to H-1 according to its chemical shift, COSY correlation (H-7), and NOESY correlation (H-7). The 1H NMR signal at 6.70 (dd, 1H) was associated with the 13C NMR signal at 104.6 ppm (d, split by F) and is assigned H-2 according to its chemical shift.

Aliphatic methine assignments: The 1H NMR signal at 4.31 ppm (dt, 1H) was associated with the 13C NMR signal at 55.4 ppm (HSQC) and is assigned H-23 according to its chemical shift, COSY correlation (H-26, H22/24), and NOESY correlation (H-26, H22/24). The 1H NMR signal at 3.85 ppm (td, 1H) was associated with the 13C NMR signal at 47.5 ppm (HSQC) and is assigned H-14 according to its chemical shift, COSY correlation (H-13, H-15), and NOESY correlation (H-15, H-16). The 1H NMR signal at 2.12 ppm (m, 2H) was associated with the 13C NMR signal at 61.9 ppm (HSQC) and is assigned H-16 according to its chemical shift, COSY correlation (H-15, H-17), and NOESY (H-14, H-15).

1H NMR (400 MHz), DMSO-d6 (32):
13C NMR (100 MHz)
DMSO-d6 (32):

HSQC (400/100 MHz)
DMSO-d6 (32):
gCOSY (400 MHz)
DMSO-d6 (32):

NOESY (400 MHz)
DMSO-d6 (32):
SETD2 (1434-1711) Assay

The biochemical assay monitored the incorporation of the tritiated methyl group from S-adenosyl-methionine (SAM) into a biotinylated histone 3 peptide corresponding to residues 26-40. The sequence of the substrate peptide is biotin-Ahx-RKSAPAGKGKPHR-NH2 (Biopeptide Co., Inc., San Diego, CA) and 3H-SAM was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). For the assay, 40 µL of enzyme was incubated with 1 µL of compound or DMSO for 30 minutes before initiating the reaction with 10 µL of substrate solution in a 384-well assay plate. The assay was performed at room temperature in assay buffer composed of 25 mM bicine, pH 8.0, 7.5 mM β-mercaptoethanol, 0.002 % Tween-20, and 0.01 % bovine skin gelatin (BSG). The reaction was quenched during the linear portion of product formation with 10 µL of 1 mM S-adenosyl-homocysteine (SAH) and 1 mM SAM. From the quenched reaction, 50 µL was transferred to a streptavidin-coated Flashplate (Perkin Elmer) and incubated for at least 2h before washing once with 0.1 % Tween-20. Signal from the 3H-labeled peptide captured by the streptavidin-coated plates was counted by a Topcount plate reader (Perkin Elmer). Percent inhibition (%I) and IC50 values were calculated using equations 1 and 2 respectively.

\[
%I = \left(1 - \left(\frac{S_{\text{min}}}{S_{\text{max}} - S_{\text{min}}}\right)\right)
\]

(eq 1)

\[
%I = (100 - \text{bottom}) \left(\frac{1}{1 + \left(\frac{IC50}{I}\right)^n}\right) + \text{bottom}
\]

(eq 2)

Min is the signal from fully inhibited SETD2 from wells with a final concentration of 20 uM SAH and max is the signal from wells with DMSO instead of compound. For IC50 calculation, bottom is the theoretical minimum %I, I is the concentration of inhibitor, and n is the Hill slope. Compound IC50 determination was performed by testing 10 concentrations of compound diluted 3-fold in duplicate at final concentrations of 4 nM enzyme and substrate concentrations equal to their K_M values of 0.7 µM peptide and 2 µM SAM. Geometric mean IC50 values from at least 2 replicates are reported for all active compounds.

The mechanism of inhibition with respect to substrate was tested by measuring the dependence of compound IC50 on substrate concentration. When the concentration of SAM was varied (10, 5, 2.5, 1.25, 0625, and 0.3125 µM), peptide concentration was fixed at 0.7 µM. When the concentration of peptide was varied (5, 2.5, 1.25, 0.625, 0.3125, and 0.156 µM), the concentration of SAM was fixed at 2 µM. The inhibition constant (Ki) was determined by the fit of the data to the Cheng-Pruschoff equations for either uncompetitive (eq 3) or noncompetitive (eq 4) inhibition.

\[
IC50 = K_i \left(1 + \frac{K_M}{S}\right)
\]

(eq 3)

\[
IC50 = K_i
\]

(eq 4)
**Supplementary Figure S1:** Dependence of EPZ-719 (32) IC₅₀ values on the concentration of (A) SAM and (B) peptide. The solid line shows the fit of the data to eq 3 for uncompetitive inhibition vs SAM (A) and eq 4 for noncompetitive inhibition vs peptide (B).

Inhibition of SETD2 by EPZ-719 (32) is dependent on the concentration of SAM with its potency increasing with increasing SAM concentration (Supplementary Figure S1A). This was best described by uncompetitive inhibition producing a Kᵢ value of 3.3 ± 0.1 nM. In contrast, inhibition by EPZ-719 (32) is independent of peptide concentration and is best described by noncompetitive inhibition with a Kᵢ value of 8.8 ± 1.2 nM (Supplementary Figure S1B). Uncompetitive inhibition vs SAM means that the maximal potency by EPZ-719 (32) requires saturating concentrations of SAM. Since the experiment with varied peptide uses a sub-saturating concentration of SAM, the Kᵢ value determined will be overestimated. As EPZ-719 (32) inhibition is noncompetitive vs peptide, its potency is best determined from the experiment with varied SAM. Therefore, the more accurate Kᵢ for EPZ-719 (32) is 3.3 nM.

**In-cell western (ICW) assay of SETD2 Inhibitors (A549 assay)**

1. **Reagents and Consumables:**

| Reagent Number | Reagent Name                        | Reagent Source | Catalog Number |
|----------------|-------------------------------------|----------------|----------------|
| 1              | A549                                | ATCC           | CCL-185        |
| 2              | F12K                                | Hyclone        | SH30526.01     |
| 3              | FBS                                 | Gbico          | 10099-141      |
| 4              | Penicillin-Streptomycin             | Gbico          | 15140-122      |
| 5              | Poly-D-Lysine Black/Clear Microtest (TM) | Tissue-Culture Treated Polystyrene, 384-well plate | Corning 356663 |
| 6              | Methanol                            | Concord        | R266           |
| 7              | PBS powder                          | Solarbio       | P1010          |
2. Tools and Equipment:

| Tool Number | Tool Name                  | Brand       | Model                          |
|-------------|----------------------------|-------------|--------------------------------|
| 1           | Cell Counter               | Invitrogen  | Countess® Automated Cell Counter |
| 2           | CO₂ incubator              | Thermo Scientific | 371                              |
| 3           | Biological Safety Cabinet (Class II) | Thermo Scientific | 1389                           |
| 4           | Centrifuge                 | Eppendorf   | 5810R                           |
| 5           | ELx405 plate washer        | BioTek      | ELx405 Select CW                |
| 6           | Echo 550                   | Echo        | Echo 550                        |
| 7           | Plate Shaker               | Eppendorf   | MixMate                         |
| 8           | ODYSSEY CLx                | LI-COR      | ODYSSEY CLx                     |
| 9           | Multiflo                   | Biotek      | MultiFlo FX                     |
| 10          | Liquid handle system       | Tecan       | Freedom EVO200                  |

Supplementary Table S2: Tools and equipment for in-cell western assay.

3. Screening Method

Prepare plates with compounds. Seed A549 cells to the plates with compounds, 4,000 cells/well, 50 μL/well. Put the plates at room temperature on a non-vibrating surface for 20 minutes before placing in the incubator. Incubate the plate at 37 °C incubator with CO₂ for 3 days. Remove plate from incubator and bring to room temperature. Blot media out of the plate and add 50 μL of ice cold 100% methanol. Incubate 30 minutes at room temperature. Remove methanol by aspiration. Wash 3X with PBST. Add 50 μL of Blocking Buffer + 0.1% Tween 20. Incubate 1 hour. Remove Blocking Buffer, wash 3X with PBST. Add 20 μL of H3K36me3 (1:1000 dilution). (Cell Signaling Technologies mAb #4909). Incubate overnight at 4 °C. Wash plate 5X with PBS-T. Add 20 μL of DRAQ5 (1:1000) and IRDye 800CW Goat anti-Rabbit IgG (1:500). Incubate for 1 hour in the dark. Wash plate 5X with PBS-T. Wash plate 3X with water. Dry by blotting on paper towel, then centrifuge plate upside down on a thin layer of paper towels for 1 minute at 1000 rpm to remove excess reagent. Leave plate uncovered for ~10 minutes out of direct light.
before reading. Read on Licor instrument. Geometric mean IC_{50} values from at least 2 biological replicates are reported for all compounds.

4. Licor Instrument Settings:

Preset: Plate; Resolution: 84 µm; Quality: low; Focus offset: 4.0; 700 channel intensity: 2; and 800 channel intensity: 3.

14 Day KMS-34 Long Term Proliferation (LTP) Assay

1. Reagents, Consumables, and Equipment:

| Materials                  | Source       | Cat. No. | Lot. No. |
|---------------------------|--------------|----------|----------|
| KMS-34                    | JCRB         | JCRB1195 |          |
| KMS11                     | JCRB         | JCRB1179 |          |
| RPMI 1640                 | Invitrogen   | 11875-119| 1694256  |
| FBS                       | BI           | 04-002-1A| 1609758  |
| 96-well plate, flat       | Corning      | 3599     |          |
| Poly-D-Lysine 96-well     | BD BIOCOAT   | 356640   |          |
| Microplates, black/clear  |              |          |          |
| Calcein-AM                | Invitrogen   | C3099    | 1887153  |

Supplementary Table S3: Reagents for 14-day LTP.

2. Screening method

Day 0: In a flat bottom 96-well plate, add 100 µL of cells per well at 1.25 X 10^5 cells/ml density (Note: Only internal wells. PBS placed in all outer wells to avoid evaporation of the internal wells.) Add 50 µL of compounds to each well. Final volume in each well is 150 µL. Incubate plates for 96 hrs.

Day 1, 2, 3: wait

Day 4: Pipette cells up and down to mix in each well. Aspirate 20 µL of cell suspension in the V-bottom plate and add to a poly-D-lysine coated 96-well plate. Add 30 µL HBSS and 50 µL of HBSS containing 2 µm Calcein-AM. The final concentration is 1 µm. Let cells sit at RT for 10 mins followed by a quick spin to get cells settled on the bottom of the wells. Incubate the plate for additional 40 mins in the incubator (to load Calcein AM and to give cells more time to attach). Take out the plate and read by Acumen. Calculate the cell numbers taken into account the dilution factors. Split the master plate by taking the total viable cell count calculated. Pipette cells up and down to mix in each well. Aspirate 1.1* of calculated cell suspension from each well and add to a V-bottom plate. Spin the plate at 1100 rpm for 5 minutes. Following the spin remove the media, careful not to disturb the cell pellet. Resuspend pellet in 110 µL fresh media. Pipette cells up and down to mix in each well. Aspirate 100 µL of cell suspension from each well and add to a new 96-well flat bottom plate. Add 50 µL of compound solution. Incubate plates for 72 hrs.
Day 7: Pipette cells up and down to mix in each well. Aspirate 20 µL of cell suspension in the V-bottom plate and add to a poly-D-lysine coated 96-well plate. Add 30 µL HBSS and 50 µL of HBSS containing 2 µm Calcein-AM. The final concentration is 1 µm. Let cells sit at RT for 10 mins followed by a quick spin to get cells settled on the bottom of the wells. Incubate the plate for additional 40 mins in the incubator (to load Calcein AM and to give cells more time to attach). Take out the plate and read by Acumen. Calculate the cell numbers taken into account the dilution factors. Split the master plate by taking the total viable cell count calculated. Pipette cells up and down to mix in each well. Aspirate 1.1* of calculated cell suspension from each well and add to a V-bottom plate. Spin the plate at 1100 rpm for 5 minutes. Following the spin remove the media, careful not to disturb the cell pellet. Resuspend pellet in 110µL fresh media. Pipette cells up and down to mix in each well. Aspirate 100 µL of cell suspension from each well and add to a new 96-well flat bottom plate. Add 50 µL of compound solution. Incubate plates for 96 hrs.

Day 11: Pipette cells up and down to mix in each well. Aspirate 20 µL of cell suspension in the V-bottom plate and add to a poly-D-lysine coated 96-well plate. Add 30 µL HBSS and 50 µL of HBSS containing 2 µm Calcein-AM. The final concentration is 1 µm. Let cells sit at RT for 10 mins followed by a quick spin to get cells settled on the bottom of the wells. Incubate the plate for additional 40 mins in the incubator (to load Calcein AM and to give cells more time to attach). Take out the plate and read by Acumen. Calculate the cell numbers taken into account the dilution factors. Split the master plate by taking the total viable cell count calculated. To reduce the variation that may be caused by pipetting: Pipette cells up and down to mix in each well. Aspirate all the cell suspension from B1-2, C1-2, D1-2 and add to a V-bottom plate. Aspirate 1.1* of calculated cell suspension from the other wells and add to a V-bottom plate. Spin the plate at 1100 rpm for 5 minutes. Following the spin remove the media, careful not to disturb the cell pellet. Resuspend pellet in B1-2, C1-2, D1-2 in 100µL fresh media. Pipette cells up and down to mix in each well. Resuspend pellet in the other wells in 110 µL fresh media. Pipette cells up and down to mix in each well. Aspirate 100 µL of cell suspension from each well and add to a new 96-well flat bottom plate.

Day 14: Pipette cells up and down to mix in each well. Aspirate 20 µL of cell suspension in the V-bottom plate and add to a poly-D-lysine coated 96-well plate. Add 30 µL HBSS and 50 µL of HBSS containing 2 µm Calcein-AM. The final concentration is 1 µm. Let cells sit at RT for 10 mins followed by a quick spin to get cells settled on the bottom of the wells. Incubate the plate for additional 40 mins in the incubator (to load Calcein AM and to give cells more time to attach). Take out the plate and read by Acumen. Calculate the cell numbers taken into account the dilution factors.

To calculate growth for days 4,7,11, and 14:

1. Calculate the split factor for day 4 to 7, day 7 to 11, and day 11-14. The split factor is the viable cells/mL on Day X (either 4, 7, or 11) divided by the density the cells are being split back to 2. For growth of cells from day 4 to 7, multiply the day 7 viable cells/mL density by the split factor from day 4.

3. For growth of cells from day 7 to 11, multiply the day 11 viable cells/mL density by the days 4, and 7 split factors.

4. For growth of cells from Day 11 to 14, multiply the Day 14 viable cells/mL density by the days 4, 7, and 11 split factors. 5. Plot growth on semi-log chart (viable cells/mL on Y axis, in log, and days on X axis).

Compound preparation:

Compounds were dissolved in DMSO at 10 mM and were stored at -20 °C. Compounds were diluted with DMSO in 3-fold serial dilution. From 5 mM to 0.25 µm. Transfer 1.2 µL of compounds from compound plate and add to a 96-well plate with 200 µL of media in each well. Mix well by pipetting up and down. Transfer 50 µL of compound-containing media to the cell plate.

Off-Target Screening

1. Histone Methyl Transferases
Compound 32 was tested in a panel of 14 histone methyltransferases to assess its biochemical selectivity across this enzyme class. Other than SETD2, compound 32 displayed at best high micromolar inhibitory potency against these enzymes. These assays were performed as described in Knutson et al\textsuperscript{2}.

| HMT        | IC\textsubscript{50}, μM |
|------------|--------------------------|
| DOT1L      | >200                     |
| EZH1       | >200                     |
| EZH2       | 162                      |
| PRMT3      | >200                     |
| PRMT7      | >200                     |
| PRMT8      | >200                     |
| SETD7      | >200                     |
| SETDB1     | >200                     |
| SMYD2      | >200                     |
| WHSC1      | >200                     |
| EHMT1      | 65.4                     |
| EHMT2      | 66.8                     |
| PRMT6      | 63.6                     |
| SMYD3      | >200                     |

**Supplementary Table S4:** Panel of related histone methyl transferases.

2. Off-Target Receptor Panel

Compound 32 was tested in the Eurofins Safety 47 panel, a panel of 47 common off-targets of concern. A set of ten enzymes, 4 kinase and 6 non-kinase (listed below), showed no inhibition >22% at 10 μM.

Enzymes tested: AChE, COX1, COX2, INSR, LCK, MAOA, PDE3A, PDE4D2, ROCK1, VEGFR2

Three transporters, DAT, NET, and SERT, showed no blockade >15% at 10 μM. Eight ion channels were tested, both as openers and blockers except where indicated below. None of the channels reached an IC\textsubscript{50} or EC\textsubscript{50} below the 10 μM top screening concentration.

Ion channels tested: hERG (blocker only), CAV1.2 (blocker only), NAV1.5 (blocker only), GABAA, HTR3A, KvLQT1/minK, nAChR(a4/b2), NMDAR (1A/2B)

The largest category in the panel is the receptors. These are primarily GPCRs, with some NHRs included. These were tested both in agonist and antagonist modes. Only the three targets listed in the table reached an IC\textsubscript{50} or EC\textsubscript{50} below 10 μM, the remainder failed to meet this threshold.
Supplementary Table S5: Relevant off-target receptor hits.

All receptors tested: ADORA2A, ADRA1A, ADRA2A, ADRB1, ADRB2, AR, AVPR1A, CCKAR, CHRM1, CHRM2, CHRM3, CNR1, CNR2, DRD1, DRD2S, EDNRA, GR, HRH1, HRH2, HTR1A, HTR1B, HTR2A, HTR2B, OPRD1, OPRK1, OPRM1

3. Kinases

Compound 32 was tested against a panel of 45 kinases, listed below (Eurofins). No kinase was inhibited >28% at 10 μM in this panel. Abl(h), Aurora-A(h), CaMKIIα(h), CDK1/cyclinB(h), CDK2/cyclinA(h), CHK1(h), CHK2(h), c-Raf(h), c-SRC(h), EGFR(h), EphA2(h), EphA3(h), EphB4(h), FGFR1(h), FGFR2(h), FGFR3(h), GSK3β(h), IKKa(h), IR(h), IRAK4(h), JAK3(h), JNK1α1(h), KDR(h), Lck(h), MAPK4(h), MAP2K2(h), MAPKAP-K2(h), MARK1(h), Met(h), Mnk2(h), NEK2(h), PAK2(h), PAK4(h), PDK1(h), Pim-2(h), PKA(h), PKBα(h), PKCβII(h), Plk1(h), ROCK-I(h), SAPK2a(h), SGK(h), SIK(h), TAO2(h), TrkA(h)
In Vitro ADME and Pharmacokinetic Assays

Stability in Hepatocytes (Pharmaron, Beijing, China)

1. Materials and Reagents

| Items                  | Supplier                        |
|------------------------|---------------------------------|
| William’s E Medium     | Life Technologies               |
| Human recombinant insulin | Life Technologies               |
| GlutaMAX               | Life Technologies               |
| Isotonic Percoll       | General Electric                |
| Fetal bovine serum     | Corning                         |
| HEPES                  | Life Technologies               |
| Dexamethasone          | Local suppliers (Beijing, China) |
| Mouse Hepatocytes      | BioreclamationIVT               |
| Human Hepatocytes      | BioreclamationIVT               |

Supplementary Table S6: Materials for hepatocyte stability.

2. Study Design

2.1 Preparation of Working Solutions

1) Prepare 10 mM stock solutions of test compound(s) and positive control in appropriate solvent (DMSO). Prepare working solutions of test compounds and reference compound in 50% acetonitrile/50% water at the concentration of 50 μM.

2.2 Preparation of Hepatocytes

1) Place incubation medium (William’s E Medium supplemented with GlutaMAX) and hepatocyte thawing medium in a 37°C water bath, and allow warming for at least 15 minutes prior to use. Remove a vial of cryopreserved hepatocytes from storage, ensuring that vials remain at cryogenic temperatures until thawing process ensues. Thaw the cells by placing the vial in a 37°C water bath and gently shaking the vials for 2 minutes. After thawing is completed, spray vial with 70% ethanol, transfer the vial to a biosafety cabinet.

2) Use wide-bore pipette tip to transfer hepatocytes into 50 mL conical tube containing thawing medium. Place the 50 mL conical tube into a centrifuge and spin at 100 × g for 10 minutes. Upon completion of spin, aspirate thawing medium and resuspend hepatocytes in enough incubation medium to yield ~1.5 × 10⁶ cells/mL.

3) Using AOPI (acridine orange / propidium iodide) staining solution, count cells and determine the viable cell density. Cells with poor viability (<75% viability) are not acceptable for use. Dilute cells with incubation medium to a working cell density of 0.5 × 10⁶ viable cells/mL.

2.3 Procedure for Stability Determination

1) Pipette 198 μL of hepatocytes into each wells of a 96-well non-coated plate. Place the plate in the incubator on an orbital shaker to allow the hepatocytes to warm for 10 minutes. Pipette 2 μL of working solution to mix with 198 μL
of mouse or human hepatocytes to achieve final concentration of 0.5 μM. Return the plate to the incubator and place on an orbital shaker.

2) Remove well contents in 25 μL aliquots at time points of 0 and 120 minutes for human hepatocytes and 0 and 60 minutes for mouse hepatocytes. The aliquots are then mixed with 6 volumes (150 μL) of cold acetonitrile with IS (100 nM alprazolam, 200 nM labetalol, 200 nM caffeine and 2 μM ketoprofen) to terminate the reaction. Centrifuge for 30 minutes at 3,220 × g. Aliquots of 100 μL of the supernatants will be used for LC/MS/MS analysis. The supernatant may be diluted with ultrapure water according to the LC-MS/MS signal response and peak shape. All incubations will be performed in duplicate.

2.4 Data analysis

All calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. Determine the in vitro half-life (t\textsubscript{1/2}) of parent compound by regression analysis of the percent parent disappearance vs. time curve.

a. The in vitro t\textsubscript{1/2} was determined from the slope value: \( t_{1/2} = \frac{0.693}{k} \)

b. Conversion of the in vitro t\textsubscript{1/2} (in min) into the scale-up intrinsic clearance (Scaled-up CL\textsubscript{int}, in mL/min/kg) was done using the following equation (mean of duplicate determinations):

\[
\text{Scaled-up CL}\textsubscript{int} = kV/N \times \text{scaling factor},
\]

where \( V = \) incubation volume (0.2 mL); \( N = \) number of hepatocytes per well (0.1 × 10\textsuperscript{6} cells).

Scaling factors for in vivo intrinsic clearance prediction using different species of hepatocytes are listed below:

| Species | Liver Weight (g liver/kg body weight) | Hepatocyte Concentration (10\textsuperscript{6} cells/g liver) | Scaling Factor |
|---------|-------------------------------------|---------------------------------|---------------|
| Human   | 25.7                                | 99                              | 2544          |
| Mouse   | 87.5                                | 135                             | 11812         |

2.5 LC-MS/MS analysis

All samples were analyzed using similar methodology, however different instruments were used over course of time to gather all data. The table below lists the instruments that were used to analyze the samples:

| Instrument Type | Instrument (Manufacturer) |
|-----------------|---------------------------|
| HPLC            | Shimadzu (Shimadzu Corporation; Kyoto, Japan) |
| Related Details | Mobile Phase: 0.1% formic acid in Water (A) or Acetonitrile (B), elution by gradient |
|                 | Column(s): Waters XSelect HSS T3 C18, 2.5μm (2.1 x 30mm) - or - Phenomenex Kinetex 2.6 μm C18 100R (2.1 x 50 mm) |
|                 | Column Temperature: 40°C |
| MS/MS           | API|4000|TripleQuad 5500|TripleQuad6500+ (Sciex; Framingham, MA, USA) |
| Related Details | Ionization source: Electron Spray Ionization (ESI) |
CYP Inhibition in Human Liver Microsomes (Pharmaron, Beijing, China)

1. Study Procedure

The master solution was prepared according to table below, and then 1 μL of compound solution or 1 μL of DMSO was added to the above master solution. The final concentration of control compounds was 10 μM. The final concentration of test compounds was 50 μM.

| Reagent                     | Stock Concentration | Volume | Final Concentration |
|-----------------------------|---------------------|--------|--------------------|
| MgCl₂ solution              | 50 mM               | 20 μL  | 5 mM               |
| Phosphate buffer            | 200 mM              | 100 μL | 100 mM             |
| Ultra-pure H₂O              | -                   | 56 μL  | -                  |
| Human liver microsomes      | 20 mg/mL            | 2 μL   | 0.2 mg/mL          |

For CYP1A2 inhibition, 1 μL of specific drug substrate (Phenacetin: 8 mM) was added at the final concentration of 40 μM to the above solution. For CYP2B6 inhibition, 1 μL of specific drug substrate (Bupropion: 10 mM) was added at the final concentration of 50 μM to the above solution. For CYP2C8 inhibition, 1 μL of specific drug substrate (Paclitaxel: 1 mM) was added at the final concentration of 5 μM to the above solution. For CYP2C9 inhibition, 1 μL of specific drug substrate (Tolbutamide: 40 mM) was added at the final concentration of 200 μM to the above solution. For CYP2C19 inhibition, 1 μL of specific drug substrate ((s)-Mephénytoïn: 10 mM) was added at the final concentration of 50 μM to the above solution. For CYP2D6 inhibition, 1 μL of specific drug substrate (Dextrométhorphan: 2 mM) was added at the final concentration of 10 μM to the above solution. For CYP3A4 inhibition, 1 μL of specific drug substrate (Midazolam: 1 mM) was added at the final concentration of 5 μM to the above solution.

The mixture was pre-warmed at 37°C for 5 min. The reaction was started by the addition of 20 μL of 10 mM NADPH solution at the final concentration of 1 mM and carried out at 37°C. The reaction was stopped by addition of 400 μL of cold quench solution (methanol containing internal standards [IS: 100 nM alprazolam, 500 nM labetalol and 2 μM ketoprofen]) at the designated time points (Phenacetin: 20 min; Bupropion: 20 min; Paclitaxel: 10 min; Tolbutamide: 20 min; (s)-Mephénytoïn: 20 min; Dextrométhorphan: 20 min; Midazolam: 5 min). Samples were vortexed for 5 minutes and centrifuged at 3220 g for 40 minutes at 4 °C. And then 100 μL of the supernatant was transferred to a new 96-well plate with 100 μL water (depends on the LC-MS signal response and peak shape) for LC-MS/MS analysis. All experiments were performed in duplicate.

2 LC-MS/MS analysis

All samples were analyzed using traditional methodology. For data interpretation, select metabolites of probe substrates noted above were monitored. Selected LC-MS/MS information is noted below:

| Instrument Type | Instrument (Manufacturer) |
|-----------------|---------------------------|
| HPLC            | Shimazu (Shimadzu Corporation; Kyoto, Japan) |
SUPPLEMENTARY INFORMATION

**Related Details**
- Mobile Phase: 0.1% formic acid in Water (A) or Acetonitrile (B), elution by gradient (0.6mL/min)
- Column: XSelect Hss T3 2.5µ (2.1×50 mm) Column XPColumn
- Temperature: 40°C
- 1 or 2 µL injection volume

**MS/MS Related Details**
- API4000|TripleQuad 5500|TripleQuad6500+ (Sciex; Framingham, MA, USA)
- Ionization source: Electron Spray Ionization (ESI)
- Ionization mode: positive (+)

| Marker Metabolite     | Q1 (m/z) | Q3 (m/z) | DP (v) | EP (v) | CE (v) | CXP (v) |
|-----------------------|----------|----------|--------|--------|--------|---------|
| Acetaminophen         | 152.3    | 110.2    | 80     | 8      | 25     | 10      |
| OH Bupropion          | 256.1    | 238.2    | 51     | 10     | 10     | 12      |
| OH Paclitaxel         | 870.3    | 286.2    | 70     | 10     | 20     | 15      |
| OH Tobutamide         | 287.2    | 188.1    | 51     | 10     | 17     | 12      |
| OH-mephenytoin        | 235.1    | 150.1    | 50     | 10     | 26     | 12      |
| Dextrorphan           | 258.1    | 157.1    | 60     | 10     | 35     | 15      |
| OH-midazolam          | 342.0    | 203.0    | 95     | 10     | 37     | 15      |

2. Data Analysis

Percent inhibition was calculated by comparing peak area of marker metabolite in DMSO control samples to that of test samples. Percent inhibition was then used to estimate an IC50 using the following equation:

\[
\text{Estimated IC50} = \frac{[\text{Drug}] \times (100\% - \text{Obs'd Inh}\%)}{\text{Obs'd Inh}\%}
\]

3. Results

| Compound 32 | CYP Isoform | [Drug], µM | %Inh | Est. IC50 |
|-------------|-------------|------------|------|-----------|
| 1A2         | 50          | 21         | 188  |
| 2B6         | 50          | 28         | 129  |
| 2C8         | 50          | 76         | 16   |
| 2C9         | 50          | 58         | 36   |
| 2C19        | 50          | 38         | 82   |
| 2D6         | 50          | 42         | 69   |
| 3A4         | 50          | 48         | 54   |

Caco-2 Permeability *(Pharmaron, Beijing, China)*

1. Material and Reagents

| Items     | Supplier  |
|-----------|-----------|
| DMEM      | Corning   |
2. Study Design

2.1 Preparation of Caco-2 Cells

50 μL and 25 mL of cell culture medium were added to each well of the Transwell insert and reservoir, respectively. And then the HTS transwell plates were incubated at 37 °C, 5% CO₂ for 1 hour before cell seeding. Caco-2 cells were diluted to 6.86x10⁵ cells/mL with culture medium and 50 μL of cell suspension were dispensed into the filter well of the 96-well HTS Transwell plate. Cells were cultivated for 14-18 days in a cell culture incubator at 37 °C, 5% CO₂, 95% relative humidity. Cell culture medium was replaced every other day, beginning no later than 24 hours after initial plating.

2.2 Preparation of Stock Solutions

10 mM stock solutions of test compounds were prepared in DMSO. The stock solutions of positive controls were prepared in DMSO at the concentration of 10 mM. Digoxin and propranolol were used as control compounds in this assay.

2.3 Assay Procedures

The Caco-2 plate was removed from the incubator and washed twice with pre-warmed HBSS (10 mM HEPES, pH 7.4), and then incubated at 37 °C for 30 minutes. The stock solutions of control compounds were diluted in DMSO to get 1 mM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) to get 5 μM working solutions. The stock solutions of the test compounds were diluted in DMSO to get 1 mM solutions, then diluted with HBSS (10 mM HEPES, pH 7.4) to get 5 μM working solutions. The final concentration of DMSO in the incubation system was 0.5%. To determine the rate of drug transport in the apical to basolateral direction. 75 μL of working solution of test compound and control compounds was added to the Transwell insert (apical compartment) and the wells in the receiver plate (basolateral compartment) were filled with 235 μL of HBSS (10 mM HEPES, pH 7.4). To determine the rate of drug transport in the basolateral to apical direction, 235 μL of working solution of test compound and control compounds was to the receiver plate wells (basolateral compartment) and then the Transwell inserts (apical compartment) were filled with 75 μL of HBSS (10 mM HEPES, pH 7.4). Time 0 samples were prepared by transferring 10 μL of working solution to 40 μL HBSS (10 mM HEPES, pH 7.4) in a new 96-well plate, followed by the addition of 200 μL cold acetonitrile or methanol containing appropriate internal standards (IS). The plates were incubated at 37 °C for 2 hours. At the end of the incubation, 10 μL samples from donor sides (apical compartment for A→B flux, and basolateral compartment for B→A) to 40 μL HBSS (10 mM HEPES, pH 7.4) and 50 μL samples from receiver sides (basolateral compartment for A→B flux, and apical compartment for B→A) were transferred to wells of a new 96-well plate, followed by the addition of 4 volume of cold acetonitrile or methanol containing appropriate internal standards (IS). Samples were vortexed for 5 minutes and then centrifuged at 3,220 g for 40 minutes. An aliquot of 100 μL of the supernatant was mixed with an appropriate volume of ultra-pure water before LC-MS/MS analysis.

2.4 Data Analysis

The apparent permeability coefficient (Papp), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

\[
Papp = \frac{(VA \times [\text{drug}]_{\text{acceptor}})}{(\text{Area} \times \text{Time} \times [\text{drug}]_{\text{initial, donor}})}
\]

Where VA is the volume (in mL) in the acceptor well, Area is the surface area of the membrane (0.143 cm² for Transwell-96 Well Permeable Supports), and time is the total transport time in seconds.
The efflux ratio will be determined using the following equation:

\[ \text{Efflux Ratio} = \frac{\text{Papp}(B-A)}{\text{Papp}(A-B)} \]

Where Papp (B-A) indicates the apparent permeability coefficient in basolateral to apical direction, and Papp (A-B) indicates the apparent permeability coefficient in apical to basolateral direction.

**Pharmacokinetics in Mice**

1. **Study Design**

   A single intravenous (IV) dose was administered to mice (tail vein). Fasted mice were also dosed orally (PO). At the designated time points, blood was collected via the dorsal metatarsal vein in mice. Blood was transferred into collection tubes containing K₂-EDTA. For plasma analysis, blood was immediately processed for plasma by centrifugation and stored in a freezer set to be maintained at approximately -80°C until analysis.

2. **Sample Preparation**

   The desired serial concentrations of working solutions were achieved by diluting stock solution (1 mg/mL in DMSO) of analyte with 50% acetonitrile in water. Ten microliters of working solutions were added to 10 μL of the blank mouse plasma to achieve calibration standards of 0.5-1000 ng/mL in a total volume of 20 μL. The resulting 20 μL standard samples were added to 200 μL of acetonitrile for protein precipitation. All samples were then vortexed for 30 seconds. After centrifugation at 4°C and 4000 rpm (ca. 3740 x g) for 15 minutes, the supernatant was diluted with water.

3. **Analytical Method**

   Concentrations in extracted samples were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) using reversed-phase liquid chromatography. Analytes were monitored using Electron Spray Ionization (ESI) with multiple reaction monitoring in positive ion mode. Peak areas were integrated by Analyst® (Sciex) where concentrations were determined by a weighted \((1/x^2)\) linear or quadratic regression of peak area ratios (peak area of analyte/peak area of IS) versus the theoretical concentrations of the plasma calibration standards.

**Instruments and Analytical Details**

| HPLC          | Instrument: Shimadzu (Degasser DGU-20A5; LC-30AD Pumps, CBM-20A Communication Bus; CTO-30A Column oven; Rack Changer II) |
|---------------|--------------------------------------------------------------------------------------------------|
| MS            | API 4000 or API5500                                                                                  |
| Column        | Phenomenex Kinetex 5μ C18 100A (50×2.1 mm)                                                        |
| Polarity      | Positive ion                                                                                         |
| Mobile Phase  | Mobile Phase A: Water (0.1% formic acid, 5% Acetonitrile)                                             |
| (0.6 mL/min)  | Mobile Phase B: Acetonitrile (0.1% formic acid, 5 % Water)                                          |

4. **Pharmacokinetic Analysis**
Individual plasma concentration-time data of mice were analyzed by non-compartmental methods using the Linear/Log trapezoidal method (IV) or the Linear-up/Log-down trapezoidal method (PO) (Phoenix WinNonlin 6.1, Certara, Princeton, NJ). After IV dosing, clearance (CL), steady-state volume of distribution (Vss), terminal elimination half-life (t1/2), area under the curve from time zero to infinity (AUCINF), mean resonance time from time zero to infinity (MRTINF), and terminal phase volume of distribution (Vz) were calculated. After PO dosing, maximum observed concentration (Cmax), time of Cmax (tmax), terminal elimination half-life (t1/2), area under the curve from time zero to infinity (AUCINF), mean resonance time from time zero to infinity (MRTINF), apparent total clearance (CL/F), bioavailability (F), and estimated fraction absorbed (Fa) were calculated.

5. Results

### Individual and Mean Plasma Concentration-time Data of Compound 25 after 1 mg/kg IV Administration in CD1 Mice

| Dose (mg/kg) | Dose route | Sampling time (hr) | Concentration (ng/mL) | Mean (ng/mL) | SD (ng/mL) | CV (%) |
|-------------|------------|--------------------|-----------------------|--------------|------------|-------|
| 1           | IV         | 0.05               | Mouse1: 1110 Mouse2: 1240 Mouse3: 1440 | 1263         | 166        | 13.2  |
|             |            | 0.167              | Mouse1: 671 Mouse2: 876 Mouse3: 808 | 785          | 104        | 13.3  |
|             |            | 0.5                | Mouse1: 345 Mouse2: 449 Mouse3: 485 | 426          | 73         | 17.1  |
|             |            | 1                  | Mouse1: 180 Mouse2: 182 Mouse3: 232 | 198          | 29         | 14.9  |
|             |            | 2                  | Mouse1: 49.3 Mouse2: 54.1 Mouse3: 64.8 | 56.1         | 7.9        | 14.2  |
|             |            | 4                  | Mouse1: 5.24 Mouse2: 5.77 Mouse3: 7.80 | 6.27         | 1.35       | 21.6  |
|             |            | 7                  | BLOQ              Mouse1: 0.598 Mouse2: 0.833 | 0.716        | NA         | NA    |
|             |            | 10                 | BLOQ              Mouse1: BLOQ Mouse2: BLOQ Mouse3: BLOQ | NA           | NA         | NA    |
|             |            | 24                 | BLOQ              Mouse1: BLOQ Mouse2: BLOQ Mouse3: BLOQ | NA           | NA         | NA    |

**PK parameters**

| Unit | Mouse1 | Mouse2 | Mouse3 | Mean | SD | CV (%) |
|------|--------|--------|--------|------|----|--------|
| mL/min/kg | 27.8  | 23.6  | 21.2  | 24.2 | 3.4 | 13.9   |
| L/kg  | 1.18   | 0.980 | 0.948 | 1.04 | 0.12| 12.0   |
| hr    | 0.6    | 0.8   | 0.8   | 0.7  | 0.1 | 16.0   |
| hr*ng/mL | 599   | 706   | 787   | 697  | 95  | 13.6   |
| hr    | 0.7    | 0.7   | 0.7   | 0.7  | 0.0 | 3.93   |
| L/kg  | 1.43   | 1.59  | 1.5   | 1.5  | 0.1 | 5.66   |

BLOQ <0.5 ng/mL

### Individual and Mean Plasma Concentration-time Data of Compound 25 after 50 mg/kg PO Administration in CD1 Mice

| Dose (mg/kg) | Dose route | Sampling Time (hr) | Concentration (ng/mL) | Mean (ng/mL) | SD (ng/mL) | CV (%) |
|-------------|------------|--------------------|-----------------------|--------------|------------|-------|
| 50          | POB        | 0.25               | Mouse7: 18.8 Mouse8: 22.5 Mouse9: 7.90 | 16.4         | 7.6        | 46.3  |
|             |            | 0.5                | Mouse7: 31.7 Mouse8: 38.7 Mouse9: 12.3 | 27.6         | 13.7       | 49.6  |
|             |            | 1                  | Mouse7: 35.9 Mouse8: 51.6 Mouse9: 25.3 | 37.6         | 13.2       | 35.2  |
Individual and Mean Plasma Concentration-time Data of Compound 32 after 1 mg/kg IV Administration in CD1 Mice

| Dose (mg/kg) | Dose route | Sampling time (hr) | Concentration (ng/mL) | Mean (ng/mL) | SD (ng/mL) | CV (%) |
|--------------|------------|--------------------|-----------------------|--------------|------------|--------|
| 0.05         | IV         | 0.05               | 234                   | 293          | 4.9        | 12.6   |
| 0.167        |            | 0.167              | 183                   | 220          | 8.6        | 17.0   |
| 0.5          |            | 0.5                | 136                   | 131          | 4.9        | 6.35   |
| 1            |            | 1                  | 71.5                  | 65.3         | 6.5        | 7.35   |
| 2            |            | 2                  | 43.8                  | 29.3         | 6.2        | 20.1   |
| 4            |            | 4                  | 23.9                  | 17.4         | 5.7        | 24.3   |
| 7            |            | 7                  | 8.37                  | 5.45         | 6.73       | 22.2   |
| 10           |            | 10                 | 3.50                  | 2.66         | 1.35       | 13.9   |
| 24           |            | 24                 | 0.519                 | BLOQ         | NA         | NA     |

PK parameters

| PK parameters | Unit       | Mouse1 | Mouse2 | Mouse3 | Mean | SD  | CV (%) |
|---------------|------------|--------|--------|--------|------|-----|--------|
| CL            | mL/min/kg  | 47.9   | 57.2   | 54.8   | 53.3 | 4.8 | 9.09   |
| Vss           | L/kg       | 9.31   | 7.34   | 7.78   | 8.15 | 1.03| 12.7   |
| Terminal t1/2 | hr         | 4.5    | 2.2    | 2.7    | 3.1  | 1.2 | 37.6   |
| AUCINF        | hr*ng/mL   | 348    | 291    | 304    | 314  | 30  | 9.47   |
| MRTINF        | hr         | 3.2    | 2.1    | 2.4    | 2.6  | 0.6 | 22.5   |
| V2            | L/kg       | 18.5   | 11.0   | 13.0   | 14.2 | 3.9 | 27.6   |

BLOQ <0.5 ng/mL; Fa = (%F/100) / (1-[CL/Qh]), where CL is observed blood clearance and Qh is liver blood flow (eg, 90 mL/min/kg for mice).
### Individual and Mean Plasma Concentration-time Data of Compound 32 after 30 mg/kg PO Administration in CD1 Mice

| Dose (mg/kg) | Dose route | Sampling Time (hr) | Concentration (ng/mL) Mouse7 | Concentration (ng/mL) Mouse8 | Concentration (ng/mL) Mouse9 | Mean (ng/mL) | SD (ng/mL) | CV (%) |
|--------------|------------|-------------------|-------------------------------|-------------------------------|-------------------------------|---------------|-----------|--------|
| 30           | PO         | 0.25              | 211                           | 1010                          | 348                           | 523           | 427       | 81.7   |
|              |            | 0.5               | 241                           | 924                           | 604                           | 590           | 342       | 58.0   |
|              |            | 1                 | 309                           | 935                           | 401                           | 548           | 338       | 61.6   |
|              |            | 2                 | 266                           | 918                           | 446                           | 543           | 337       | 62.0   |
|              |            | 4                 | 388                           | 338                           | 427                           | 384           | 45        | 11.6   |
|              |            | 7                 | 87.7                          | 61.6                          | 66.6                          | 72.0          | 13.9      | 19.2   |
|              |            | 10                | 24.3                          | 14.4                          | 15.0                          | 17.9          | 5.6       | 31.0   |
|              |            | 24                | 0.702                         | 0.720                         | 0.735                         | 0.719         | 0.017     | 2.30   |

**PK parameters**

| Unit    | Mouse7 | Mouse8 | Mouse9 | Mean | SD | CV(%) |
|---------|--------|--------|--------|------|----|-------|
| $t_{\text{max}}$ (hr) | 4.0   | 0.3    | 0.5    | 1.6  | 2.1 | 132   |
| $C_{\text{max}}$ (ng/mL) | 388  | 1010   | 604   | 667  | 316 | 47.3  |
| Terminal $t_{1/2}$ (hr) | 2.5   | 2.8    | 2.8    | 2.7  | 0.2 | 5.72  |
| AUC$_{\text{INF}}$ (hr*ng/mL) | 2011 | 3571   | 2462  | 2681 | 803 | 29.9  |
| MRT$_{\text{INF}}$ (hr) | 4.1   | 2.6    | 3.4    | 3.4  | 0.7 | 22.2  |
| CL/F (mL/min/kg) | 249  | 140    | 203   | 197  | 55  | 27.7  |
| F (AUC$_{\text{INF}}$ based) (%) | 21.3  | 37.9   | 26.1  | 28.4 | 8.5 | 29.9  |
| $F_a$ | 0.52   | 0.93   | 0.64   | 0.70 | 0.21 | 30.2  |

BLOQ <0.5 ng/mL; $F_a = (\%F/100) / (1-[CL/Qh])$, where CL is observed blood clearance and Qh is liver blood flow (eg, 90 mL/min/kg for mice).

### Individual and Mean Plasma Concentration-time Data of Compound 32 after 100 mg/kg PO Administration in CD1 Mice

| Dose (mg/kg) | Dose route | Sampling Time (hr) | Concentration (ng/mL) Mouse7 | Concentration (ng/mL) Mouse8 | Concentration (ng/mL) Mouse9 | Mean (ng/mL) | SD (ng/mL) | CV (%) |
|--------------|------------|-------------------|-------------------------------|-------------------------------|-------------------------------|---------------|-----------|--------|
| 100          | PO         | 0.25              | 1720                          | 655                           | 3430                          | 1935          | 1400      | 72.3   |
|              |            | 0.5               | 2750                          | 1890                          | 4860                          | 3167          | 1528      | 48.3   |
|              |            | 1                 | 3770                          | 2230                          | 3780                          | 3260          | 892       | 27.4   |
|              |            | 2                 | 2850                          | 3350                          | 3000                          | 3067          | 257       | 8.37   |
|              |            | 4                 | 2320                          | 2270                          | 3250                          | 2613          | 552       | 21.1   |
|              |            | 7                 | 477                           | 1410                          | 1460                          | 1116          | 554       | 49.6   |
|              |            | 10                | 107                           | 636                           | 522                           | 422           | 278       | 66.0   |
|              |            | 24                | 1.45                          | 6.52                          | 5.82                          | 4.60          | 2.75      | 59.8   |

**PK parameters**

| Unit    | Mouse7 | Mouse8 | Mouse9 | Mean | SD | CV(%) |
|---------|--------|--------|--------|------|----|-------|
| $t_{\text{max}}$ (hr) | 1.0   | 2.0    | 0.5    | 1.2  | 0.8 | 65.5  |
| C<sub>max</sub> | ng/mL | 3770 | 3350 | 4860 | 3993 | 779 | 19.5 |
|----------------|--------|------|------|------|------|-----|------|
| Terminal t<sub>1/2</sub> | hr | 2.1 | 2.2 | 2.1 | 2.1 | 0.0 | 1.67 |
| AUC<sub>INF</sub> | hr*ng/mL | 15400 | 20000 | 24300 | 19900 | 4440 | 22.3 |
| MRT<sub>INF</sub> | hr | 3.3 | 5.0 | 4.3 | 4.2 | 0.9 | 21.1 |
| CL/F | mL/min/kg | 108 | 83.1 | 68.6 | 87 | 20 | 23.0 |
| F (AUC<sub>INF</sub> based) | % | 49.1 | 63.8 | 77.3 | 63.4 | 14.1 | 22.3 |
| F<sub>a</sub> | >1 | >1 | >1 | >1 | NA | NA |

F<sub>a</sub> = (%F/100) / (1-[CL/Qh]), where CL is observed blood clearance and Qh is liver blood flow (eg, 90 mL/min/kg for mice).

### Crystallography Protocols

Crystallographic data collection and refinement statistics

| Compound | 2 | 20 | 32 |
|----------|---|----|----|
| PDB code | 7LZB | 7LZD | 7ZLF |
| Cofactor | SAM | SAM | SAM |
| Space group | P 2<sup>1</sup> 2<sup>1</sup> 2<sup>1</sup> | P 2<sup>1</sup> 2<sup>1</sup> 2<sup>1</sup> | P 2<sup>1</sup> 2<sup>1</sup> 2<sup>1</sup> |
| a, b, c (Å) | 51.55, 76.75, 77.78 | 52.23, 76.38, 76.43 | 49.91, 76.65, 76.19 |
| α, β, γ (°) | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |
| Resolution range (Å) | 54.63-2.28 | 54.03-1.80 | 36.39-2.47 |
| (Highest resolution shell) | 2.34-2.28 | 1.86-1.80 | 2.57-2.47 |
| R<sub>merge</sub>, overall<sup>1</sup> | 0.11 | 0.13 | 0.07 |
| Completeness, overall (%) | 98.7 | 99.3 | 99.4 |
| Reflections, unique | 13698 | 27382 | 10278 |
| Multiplicity | 6.2 | 6.1 | 6.6 |
| I/σ | 2.38 | 2.52 | 1.89 |
| R<sub>value</sub> work (%)<sup>2</sup> | 20.5 | 19.3 | 21.6 |
| R<sub>value</sub> free (%) | 26.4 | 21.8 | 26.3 |
| R.M.S. deviations from ideal Bond lengths (Å) | 0.51 | 0.51 | 0.63 |
| Bond angles (°) | 0.72 | 0.76 | 0.72 |
| Φ,Ψ angle distributions for residues<sup>3</sup> | In preferred regions (%) | 97 | 98 | 97 |
| In allowed regions (%) | 3 | 2 | 3 |
| Outliers (%) | 0 | 0 | 0 |

<sup>1</sup>R<sub>merge</sub> = Σ<sub>k</sub> (|I<sub>k</sub> - <I>|) / Σ<sub>k</sub> I<sub>k</sub>)

<sup>2</sup>R<sub>value</sub> = Σ<sub>k</sub> |F<sub>obs</sub>| - |F<sub>calc</sub>| / Σ<sub>k</sub>|F<sub>obs</sub>|

<sup>3</sup>Ramachandran statistics as defined Engh & Huber (2001)

**Supplementary Table S7:** Crystallographic collection parameters.

Protein Production: SETD2 (1434-1711) was purified from an insect cell expression system. The protein sequence was incorporated in a pFastBacHTb-lic with N-His-TEV and expressed in HF cells for 72 hours. Post-harvest the cells were resuspended in 25mM HEPES, 200mM NaCl, 5mM β-ME, pH 7.5 (buffer A) at a concentration of 1g cell per 8ml buffer. Roche protease inhibitor cocktail and 0.5% CHAPS was added. The mixture was incubated on ice for 30 min. The suspension was sonicated at 200W with a 3sec on, 5sec off cycle seventy times. The sample was centrifuged at 13,000rpm for 30 min at 4°C, the supernatant was collected.
A 20ml CV XK Qiagen Ni affinity column was used. Resin was preincubated with buffer A, then incubated with the sonication supernatant for 2 hours at 4C. The resin was washed with 25mM HEPES, 200mM NaCl, 5mM β-ME, 20mM imidazole, pH 7.5 (buffer B) until no UV signal was observed. The protein was eluted with 10CV of 25mM HEPES, 200mM NaCl, 5mM β-ME, 250mM imidazole, pH 7.5 (buffer C). A 12% SDS-PAGE gel was used to identify the fractions containing the protein.

TEV protease was added at a ratio of 1:20 relative to SETD2 and the sample was incubated overnight.

The cleaved sample was applied to a 10ml CV XK Qiagen Ni affinity column. The resin was pre-equilibrated with buffer A, and the sample was loaded onto the column. The column was washed with 25mM HEPES, 200mM NaCl, 5mM β-ME, 10mM imidazole, pH 7.5 (buffer D) for 6CV and buffer C for 5CV.

The flowthrough from the affinity column was concentrated and applied to a Superdex 200 column preincubated with 25mM HEPES, 200mM NaCl, 5mM β-ME, 10mM imidazole, pH 7.5 (buffer E). Fractions containing the protein were collected, concentrated to 36mg/ml and flash frozen on liquid nitrogen.

X-ray crystallography: SETD2 (1434-1711) protein was concentrated to 10 mg/ml in a protein buffer containing 25mM HEPES, 200mM NaCl, 5mM β-ME, pH 7.5, 20mg/ml SETD2, 3mM SAH and 2mM compound. A crystallization reservoir solution of 0.1 M Potassium thiocyanate, 0.1M Tris pH7.8, 25% w/v PEG2000MME was used. Using a hanging drop method with seeding 1 μL of protein solution was combined with 1 μL of reservoir solution. Crystals were cryo-protected with the addition of 10% glycerol. Diffraction data was collected on BL19U1 at the SSRF or MX2 at AS. Data was reduced with either HKL3000 or XDS and refined with Refmac5. Data collection details and structure details are in the accompanying table.
References:

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