Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

SUPPLEMENTARY DATA

The structure-activity relationships of L3MBTL3 inhibitors: A second series of potent compounds which bind the L3MBTL3 dimer.

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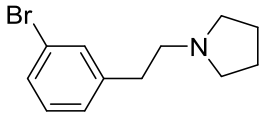
General chemistry procedures

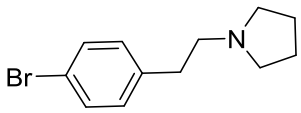
All starting materials were purchased from Sigma Aldrich, with the exception of 2-oxa-6-azaspiro[3.3]heptane (Synthonix) and 2-(4-bromo-2-chlorophenyl)acetic acid (Matrix Scientific). All preparative RP-HPLC was performed on an Agilent Prep 1200 series. Samples were injected onto a Phenomenex Luna 75 × 30 mm, 5 μM, C18 column at room temperature. Unless stated otherwise, a linear gradient from 5-40% MeOH in water, 0.1% TFA over 25 minutes, followed by an increase of 40-100% MeOH in water, 0.1% TFA over 5 minutes was used. The flow rate was 40 mL/min, and the eluent was monitored at 220 and 254 nm. All Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}H NMR) and 100 MHz for carbon (^{13}C NMR). Chemical shifts are reported in ppm (δ) from highest to lowest shifts. LCMS analyses of all compounds were acquired using an Agilent 6110 Series system with a UV detector. Samples were injected onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 μM, C18 column at room temperature. A linear gradient from 10%-100% MeOH in water, 0.1% acetic acid over 5 minutes was used, followed by pumping 100% MeOH for another 2 minutes. The flow rate was 1.0 mL/min, and the eluent was monitored at to 220, 254 and 280 nm. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. LCMS are quoted as retention time, followed by the observed ions from largest to smallest. LCMS and NMR were used to establish the purity of target compounds. All compounds had > 95% purity unless noted otherwise. High-resolution (positive ion) mass spectrum (HRMS) for exemplar compounds were acquired using a Thermo LTqFT mass spectrometer under FT control at 100000 resolution. Unless stated otherwise, product yields were calculated using the product mass.

Experimental procedures for all new compounds

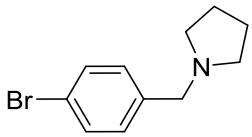
General procedure for nucleophilic substitution: A 20 mL reaction vial equipped with stir bar was charged with the desired alkyl bromide (1 equiv) in CH_3CN or DMF. The desired amine (1 equiv) was added followed by K_2CO_3 (3 equiv). The reaction was stirred at room temperature for 16 – 64 hours before being filtered. The filtrate was concentrated in vacuo and the crude mixture was purified by silica gel chromatography to give the title compound.



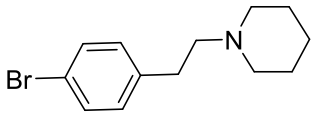
1-(3-Bromophenethyl)pyrrolidine (S1): The general procedure for nucleophilic substitution was followed with 1-bromo-3-(2-bromoethyl)benzene (114 µL, 0.758 mmol), pyrrolidine (62.0 µL, 0.758 mmol) and K_2CO_3 (314 mg, 2.28 mmol) in CH_3CN (6 mL). The reaction was stirred for 64 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (123 mg, 64%) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.42-7.39 (m, 1H), 7.42 – 7.30 (m, 1H), 7.25 – 7.16 (m, 2H), 2.86 – 2.77 (m, 2H), 2.75 – 2.65 (m, 2H), 2.67 – 2.56 (m, 4H), 1.90 – 1.76 (m, 4H). LCMS (General) t_R = 3.04 min, *m/z* = 256 $[M+H]^+$ for ^{81}Br, 254 $[M+H]^+$ for ^{79}Br.



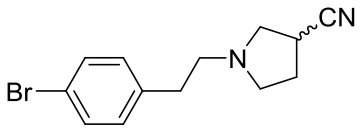
1-(4-Bromophenethyl)pyrrolidine) (S2): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(bromomethyl)benzene (261 mg, 0.987 mmol), pyrrolidine (81.0 µL, 0.987 mmol) and K_2CO_3 (409 mg, 2.96 mmol) in CH_3CN (5 mL). The reaction was stirred for 16 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-10% MeOH in DCM, 1% NH_3) to give the title compound (146 mg, 58%) as a colourless oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.42 (d, *J* = 8.3 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 3.45 – 3.16 (m, 8H), 2.21 – 2.08 (m, 4H). LCMS (General) t_R = 2.74 min, *m/z* = 256 $[M+H]^+$ for ^{81}Br, 254 $[M+H]^+$ for ^{79}Br.



1-(4-Bromobenzyl)pyrrolidine (S3): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(bromomethyl)benzene (300 mg, 1.20 mmol), pyrrolidine (99.0 µL, 1.20 mmol) and K_2CO_3 (498 mg, 3.60 mmol) in CH_3CN. The reaction was stirred for 24 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-10% MeOH in DCM, 1% NH_3) to give the title compound (112 mg, 48%) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 3.60 (s, 2H), 2.58 – 2.49 (m, 4H), 1.87 – 1.76 (m, 4H). LCMS (General) t_R = 0.54 min, *m/z* = 242 $[M+H]^+$ for ^{81}Br, 240 $[M+H]^+$ for ^{79}Br.

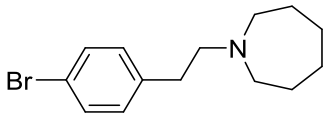


1-(4-Bromophenethyl)piperidine (S4): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (261 mg, 0.987 mmol), pyridine (97.0 µL, 0.987 mmol) and K_2CO_3 (409 mg 2.96 mmol) in CH_3CN. The reaction was stirred for 16 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (131 mg, 50%) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.5 Hz, 2H), 2.83 – 2.73 (m, 2H), 2.59 – 2.42 (m, 6H), 1.64 (p, *J* = 5.6 Hz, 4H), 1.52 – 1.46 (m, 2H). LCMS (General) t_R = 2.80 min, *m/z* = 270 $[M+H]^+$ for ^{81}Br, 268 $[M+H]^+$ for ^{79}Br.

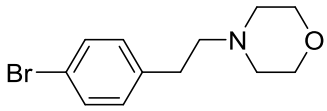


1-(4-Bromophenethyl)pyrrolidine-3-carbonitrile (S5): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (99.5 mg, 0.377 mmol), (*rac*)-pyrrolidine-3-carbonitrile·HCl (50.0 mg, 0.377 mmol) and K_2CO_3 (156 mg, 1.13 mmol) in DMF. The reaction was stirred for 30 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (41 mg, 39%) as a colourless oil. LCMS

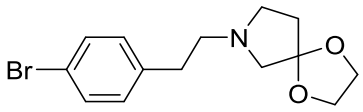
(General) t_R = 3.26 min, *m/z* = 281 $[M+H]^+$ for ^{81}Br, 279 $[M+H]^+$ for ^{79}Br. No further characterisation was performed.



1-(4-Bromophenethyl)azepane (S6): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (150 µL, 1.74 mmol), hexamethylenimine (111 µL, 0.987 mmol) and K_2CO_3 (409 mg, 2.96 mmol) in CH_3CN (5 mL). The reaction was stirred for 23 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (165 mg, 59%) as a colourless oil. ^1H NMR (400 MHz, Methanol-d4) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.3 Hz, 2H), 2.83 – 2.66 (m, 8H), 1.77 – 1.57 (m, 8H). LCMS (General) t_R = 3.53 min, *m/z* = 284 $[M+H]^+$ for ^{81}Br, 282 $[M+H]^+$ for ^{79}Br.

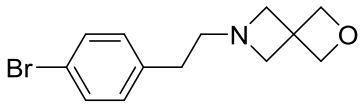


4-(4-Bromophenethyl)morpholine (S7): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (150 µL, 0.987 mmol), morpholine (85.0 µL, 0.987 mmol), K_2CO_3 (409 mg, 2.96 mmol) and KI (491 mg, 2.96 mmol) in DMF (5 mL). The reaction was stirred for 18 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (63 mg, 24%) as a white solid. ^1H NMR (400 MHz, Methanol-d4) δ 7.43 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 3.77 – 3.69 (m, 4H), 2.85 – 2.77 (m, 2H), 2.70 – 2.56 (m, 6H). LCMS (General) t_R = 2.89 min, *m/z* = 272 $[M+H]^+$ for ^{81}Br, 270 $[M+H]^+$ for ^{79}Br.

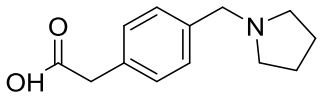


7-(4-Bromophenethyl)-1,4-dioxa-7-azaspiro[4.4]nonane (S8): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (150 µL, 0.987 mmol), 1,4-dioxa-7-

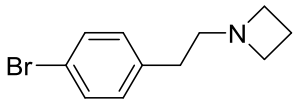
azaspiro[4.4]nonane (78.9 µL, 0.987 mmol) and K_2CO_3 (409 mg, 2.96 mmol) in CH_3CN (5 mL). The reaction was stirred for 19 hours. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (191 mg, 62%) as a yellow oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.3 Hz, 2H), 3.94 – 3.84 (m, 4H), 2.81 – 2.60 (m, 8H), 2.03 (t, *J* = 7.1 Hz, 2H). LCMS (General) t_R = 3.39 min, *m/z* = 314 $[M+H]^+$ for ^{81}Br, 312 $[M+H]^+$ for ^{79}Br.



6-(4-Bromophenethyl)-2-oxa-6-azaspiro[3.3]heptane (S9): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (150 µL, 0.987 mmol), 2-oxa-6-azaspiro[3.3]heptane hemioxalate (78.9 µL, 0.987 mmol) and K_2CO_3 (409 mg, 2.96 mmol) in CH_3CN (5 mL). The reaction was stirred for 24 hours and the crude mixture was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (191 mg, 62%) as a yellow oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.41 (d, *J* = 8.3 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.71 (s, 4H), 3.38 (s, 4H), 2.71 – 2.55 (m, 4H). LCMS (General) t_R = 3.39 min, *m/z* = 314 $[M+H]^+$ for ^{81}Br, 312 $[M+H]^+$ for ^{79}Br.

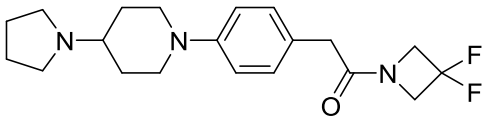


2-(4-(Pyrrolidin-1-ylmethyl)phenyl)acetic acid (S10): A 10 mL microwave vessel was charged with 2-(4-(bromomethyl)phenyl)acetic acid (200 mg, 0.873 mmol), pyrrolidine (71.1 µL, 0.873 mmol) and K_2CO_3 (362 mg, 2.62 mmol). CH_3CN (4 mL) was added and the reaction was heated under microwave irradiation at 100°C (150 W, 250 psi) for 10 minutes. The reaction mixture was filtered and the solvent was removed in vacuo to give the crude solid which was carried on without further purification (yield to be calculated over 2 synthetic steps). LCMS (General) t_R = 2.02 min, *m/z* = 220 $[M+H]^+$. No further characterisation was performed.

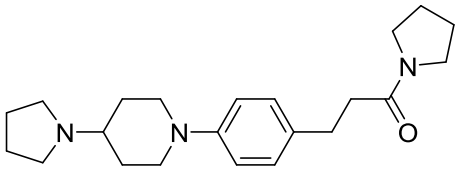


1-(4-bromophenethyl)azetidine (S11): The general procedure for nucleophilic substitution was followed with 1-bromo-4-(2-bromoethyl)benzene (150 µL, 0.987 mmol), azetidine·hydrochloride (184 mg, 0.1.97 mmol) and K_2CO_3 (818 mg, 5.91 mmol) in CH_3CN (10 mL). The reaction was stirred for 16 hours and the crude mixture was purified by silica gel chromatography (Isco, 4g, 0-16% MeOH in DCM, 1% NH_3) to give the title compound (31 mg, 7%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.47 (d, *J* = 8.3 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 3.80 (t, *J* = 7.8 Hz, 4H), 3.20 – 3.13 (m, 2H), 2.81 – 2.74 (m, 2H), 2.34 (p, *J* = 7.8 Hz, 2H). LCMS (General) t_R = 3.27 min, *m/z* = 242 $[M+H]^+$ for ^{81}Br, 240 $[M+H]^+$ for ^{79}Br.

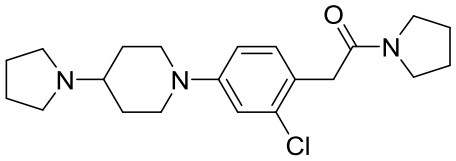
General procedure for Buchwald coupling: A 10 mL microwave vessel equipped with stir bar was charged with the desired aryl bromide (1 equiv), the desired amine (1.2 equiv), RuPhos (0.03-0.1 equiv), RuPhos pre-catalyst (0.03-0.1 equiv), and NaO*t*-Bu (2-2.5 equiv). THF was added and the reaction mixture was bubbled with nitrogen for 10 minutes before being heated to 120°C under microwave irradiation for 10 minutes. The reaction mixture was cooled to room temperature, filtered through a PTFE micron filter and purified by silica gel chromatography or RP-HPLC. The product fractions were combined and the solvent was removed in vacuo to give the title compound.



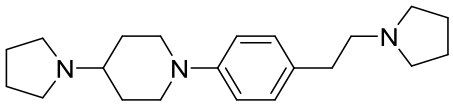
1-(3,3-Difluoroazetidin-1-yl)-2-(4-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)ethan-1-one (S11): The general procedure for Buchwald coupling was followed with aryl bromide **S14** (100 mg, 0.345 mmol), 4-(pyrrolidin-1-yl)piperidine (63.8 mg, 0.414 mmol), RuPhos (16.1 mg, 0.0350 mmol), RuPhos pre-catalyst (28.2 mg, 0.0345 mmol), and NaO*t*-Bu (66.3 mg, 0.690 mmol) in 4 mL of THF. The crude mixture was purified by silica gel chromatography (Isco, 4g, 0-80% MeOH in DCM, 1% NH_3) to give the title compound (79 mg, 63%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 4.51 (t, *J* = 12.0 Hz, 2H), 4.31 (t, *J* = 12.2 Hz, 2H), 3.76 – 3.66 (m, 2H), 3.50 (s, 2H), 2.76 – 2.61 (m, 6H), 2.29 – 2.18 (m, 1H), 2.10 – 2.00 (m, 2H), 1.87 – 1.78 (m, 4H), 1.64 (qd, *J* = 12.4, 4.1 Hz, 2H). LCMS (General) t_R = 3.08 min, *m/z* = 386 $[M+Na]^+$, 364 $[M+H]^+$, 183 $[M+2H]^{2+}$.



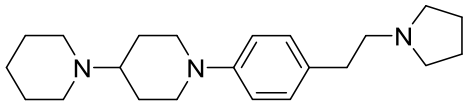
1-(Pyrrolidin-1-yl)-3-(4-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)propan-1-one (S12): The general procedure for Buchwald coupling was followed with aryl bromide **S15** (123 mg, 0.434 mmol), 4-(pyrrolidin-1-yl)piperidine (80.3 mg, 0.521 mmol), RuPhos (10.1 mg, 0.0217 mmol), RuPhos pre-catalyst (17.7 mg, 0.0217 mmol), and NaO*t*-Bu (83.4 mg, 0.868 mmol) in 4 mL of THF. The crude was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (108 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.11 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.6 Hz, 2H), 3.75 – 3.66 (m, 2H), 3.37 (t, *J* = 6.6 Hz, 2H), 3.33 – 3.26 (m, 3H), 3.12 – 3.04 (m, 3H), 2.88 – 2.73 (m, 3H), 2.70 (td, *J* = 12.5, 2.3 Hz, 2H), 2.56 (t, *J* = 7.5 Hz, 2H), 2.19 – 2.10 (m, 2H), 2.04 – 1.92 (m, 4H), 1.91 – 1.68 (m, 6H). ^{13}C NMR (100 MHz, Methanol-d4) δ 173.58 , 150.88 , 134.06 , 130.12 , 118.34 , 63.57 , 52.66 , 48.01 , 46.86 , 37.65 , 31.58 , 30.84 , 26.88 , 25.30 , 23.94 . LCMS (General) t_R = 3.31 min, *m/z* = 378 $[M+Na]^+$, 356 $[M+H]^+$, 178 $[M+2H]^{2+}$.



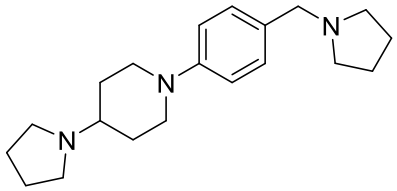
2-(2-Chloro-4-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)-1-(pyrrolidin-1-yl)ethan-1-one (S13): The general procedure for Buchwald coupling was followed with aryl bromide **S18** (125 mg, 0.413 mmol), 4-(pyrrolidin-1-yl)piperidine (63.7 mg, 0.413 mmol), RuPhos (9.70 mg, 0.0207 mmol), RuPhos pre-catalyst (16.9 mg, 0.0207 mmol), and NaO*t*-Bu (79.4 mg, 0.826 mmol) in 4 mL of THF. The crude was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (91 mg, 59%) as an off-white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.15 (d, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 2.5 Hz, 1H), 6.89 (dd, *J* = 8.6, 2.6 Hz, 1H), 3.77 – 3.67 (m, 2H), 3.71 (s, 2H), 3.56 (t, *J* = 6.8 Hz, 2H), 3.46 (t, *J* = 6.9 Hz, 2H), 2.79 – 2.62 (m, 6H), 2.24 (tt, *J* = 11.2, 4.0 Hz, 1H), 2.11 – 1.96 (m, 4H), 1.95 – 1.88 (m, 2H), 1.88 – 1.79 (m, 4H), 1.63 (qd, *J* = 12.3, 4.0 Hz, 2H). LCMS (General) t_R = 3.62 min, *m/z* = 376 $[M+H]^+$, 186 $[M+2H]^{2+}$.



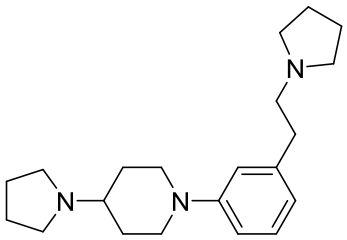
4-(Pyrrolidin-1-yl)-1-(4-(2-(pyrrolidin-1-yl)ethyl)phenyl)piperidine (UNC2533A, 1): A 10 mL microwave vessel equipped with stir bar was charged with aryl bromide **S2** (125 mg, 0.490 mmol, 1 equiv), 4-(pyrrolidin-1-yl)piperidine (190 mg, 1.23 mmol 2.5 equiv), Xphos (28.1 mg, 0.0590 mmol, 0.1 equiv), $Pd_2(dba)_3$ (13.5 mg, 0.0150 mmol, 0.03 equiv) and caesium carbonate (401 mg, 1.23 mmol, 2.5 equiv). Dioxane/water (3:1, 4 mL) was added and the reaction was bubbled with nitrogen for 10 minutes. The mixture was heated in the microwave (110°C for 20 minutes, replenish catalyst, then 125°C for 3 h) cooled to room temperature and concentrated in vacuo. The residue was taken up in methanol, filtered (PTFE micron filter) and purified by RP-HPLC to give the TFA salt of the title compound (31 mg, 12%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.28 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 8.7 Hz, 2H), 3.87 – 3.78 (m, 2H), 3.78 – 3.59 (m, 4H), 3.45 – 3.30 (m, 3H), 3.26 – 3.05 (m, 4H), 3.07 – 2.95 (m, 4H), 2.38 – 2.26 (m, 2H), 2.22 – 2.09 (m, 4H), 2.08 – 1.88 (m, 6H). LCMS (General) t_R = 0.96 min, *m/z* = 328 $[M+H]^+$. HRMS Calculated for $C_{21}H_{33}N_3 + H^+$: 328.2752, Observed: 328.2747 ± 1.7 ppm



1'-(4-(2-(Pyrrolidin-1-yl)ethyl)phenyl)-1,4'-bipiperidine (2): A 10 mL round-bottomed flask equipped with stir bar and condenser was charged with the aryl bromide **S2** (50.0 mg, 0.197 mmol), 1,4'-bipiperidine (39.7 mg, 0.236 mmol), RuPhos (9.19 mg, 0.0197 mmol), RuPhos pre-catalyst (16.1 mg, 0.0197 mmol), and NaO*t*-Bu (47.0 mg, 0.492 mmol). THF (4 mL) was added and the reaction bubbled with nitrogen for 10 minutes. The reaction was heated to 85°C for 3 hours, cooled to room temperature and the solvent was removed in vacuo. The residue was taken up in methanol, filtered (PTFE micron filter) and purified by RP-HPLC to give the TFA salt of the title compound (87 mg, 78%) as a light brown solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.20 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 3.91 – 3.79 (m, 2H), 3.72 – 3.60 (m, 2H), 3.55 (d, *J* = 12.2 Hz, 2H), 3.43 – 3.32 (m, 2H), 3.18 – 2.89 (m, 6H), 2.79 (t, *J* = 12.6 Hz, 2H), 2.25 – 2.08 (m, 4H), 2.08 – 1.95 (m, 4H), 1.94 – 1.65 (m, 6H), 1.60 – 1.44 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d4) δ 149.45 , 131.14 , 130.86 , 119.27 , 64.51 , 57.27 , 55.32 , 51.33 , 50.83 , 32.37 , 27.05 , 24.49 , 24.01 , 22.90 . LCMS (General) t_R = 1.66 min, *m/z* = 343 $[M+H]^+$, 172 $[M+2H]^{2+}$.

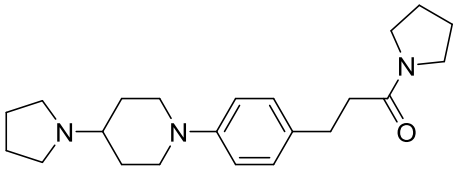


4-(Pyrrolidin-1-yl)-1-(4-(pyrrolidin-1-ylmethyl)phenyl)piperidine (3): A mixture of aryl bromide **S3** (235 mg, 0.980 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (234 mg, 50 mol%), tris(dibenzylideneacetone)dipalladium(0) (224 mg, 25 mol%), and sodium *tert*-butoxide (167 mg, 1.74 mmol) in anhydrous toluene (4 mL) was added to a sealable reaction tube. The solution was bubbled with N_2 for 20 minutes and 4-(1-pyrrolidinyl)piperidine (211 mg, 1.37 mmol) was added subsequently. The reaction tube was tightly sealed and the reaction was stirred at 120°C for 15 hours. The reaction was cooled to room temperature, diluted with dichloromethane, filtered over a thick pad of Celite, and then concentrated in vacuo. The crude mixture was taken up in methanol-water and filtered. This was repeated until there was no longer solid precipitate upon the addition of water. The methanol-water mixture was concentrated under reduced pressure, and the resulting crude material was purified by reverse phase HPLC to give the TFA salt of the title compound (388 mg, 73%) as a light red solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.40 – 7.35 (m, 2H), 7.10 – 7.05 (m, 2H), 4.26 (s, 2H), 3.97 – 3.90 (m, 2H), 3.74 – 3.61 (m, 2H), 3.51 – 3.40 (m, 2H), 3.37 – 3.28 (m, 1H), 3.23 – 3.09 (m, 4H), 2.86 (td, *J* = 13.1, 2.3 Hz, 2H), 2.29 – 1.92 (m, 10H), 1.81 (qd, *J* = 12.3, 4.2 Hz, 2H). LCMS (General) t_R = 0.99 min, *m/z* = 314 $[M+H]^+$.

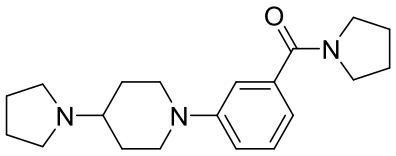


4-(Pyrrolidin-1-yl)-1-(3-(2-(pyrrolidin-1-yl)ethyl)phenyl)piperidine (5): The general procedure for Buchwald coupling was followed with **S1** (50.0 mg, 0.197 mmol), 4-(pyrrolidin-1-yl)piperidine (36.0 mg, 0.236 mmol), RuPhos (7.80 mg, 0.0197 mmol), RuPhos pre-catalyst (16.0 mg, 0.0197 mmol), and NaO*t*-Bu (160 mg, 0.492 mmol) in 4 mL of THF. The crude was purified by RP-HPLC to give the title compound (58 mg, 54%) as an off-white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.25 (t, *J* = 7.8 Hz, 1H), 7.00 – 6.91 (m, 2H), 6.83 (d, *J* = 7.4 Hz, 1H), 3.89 – 3.82 (m, 2H), 3.74 – 3.61 (m, 4H), 3.46 – 3.39 (m, 2H), 3.22 – 3.06 (m, 4H), 3.04 – 2.96 (m, 2H), 2.83 (td, *J* = 12.7, 2.4 Hz, 2H), 2.30 – 2.09 (m, 6H), 2.03 (ddt, *J* = 14.0, 11.1, 4.7 Hz, 4H), 1.83 (qd, *J* = 12.3, 4.1 Hz, 2H). ^{13}C NMR (100 MHz, Methanol-d4)

δ 150.63 , 137.21 , 129.49 , 120.51 , 117.07 , 115.55 , 62.02 , 55.85 , 53.93 , 51.44 , 32.00 , 28.20 , 22.58 , 22.42 . LCMS (General) t_R = 1.32 min, *m/z* = 328 $[M+H]^+$, 165 $[M+2H]^{2+}$. HRMS Calculated for $C_{21}H_{33}N_3$ + H^+: 328.2752, Observed: 328.2762 ± 2.9 ppm.



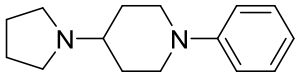
1-(Pyrrolidin-1-yl)-3-(4-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)propan-1-one (8): The general procedure for Buchwald coupling was followed with aryl bromide **S15** (123 mg, 0.434 mmol), 4-(pyrrolidin-1-yl)piperidine (80.0 mg, 0.521 mmol), RuPhos (10.1 mg, 0.0217 mmol), RuPhos pre-catalyst (17.7 mg, 0.0217 mmol), and NaO*t*-Bu (83.0 mg, 0.868 mmol) in 4 mL THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (108 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.11 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.6 Hz, 2H), 3.76 – 3.66 (m, 2H), 3.37 (t, *J* = 6.6 Hz, 2H), 3.33 – 3.27 (obscured by CD_3OD, 2H), 3.13 – 3.04 (m, 4H), 2.88 – 2.75 (m, 3H), 2.70 (td, *J* = 12.5, 2.3 Hz, 2H), 2.56 (t, *J* = 7.5 Hz, 2H), 2.20 – 2.10 (m, 2H), 2.02 – 1.93 (m, 4H), 1.90 – 1.67 (m, 6H). ^{13}C NMR (100 MHz, Methanol-d4) δ 173.58 , 150.88 , 134.06 , 130.12 , 118.34 , 63.57 , 52.66 , 48.01 , 46.86 , 37.65 , 31.58 , 30.84 , 26.88 , 25.30 , 23.94 . LCMS (General) t_R = 3.31 min, *m/z* = 378 $[M+Na]^+$, 356 $[M+H]^+$, 178 $[M+2H]^{2+}$. HRMS Calculated for $C_{22}H_{33}N_3O$ + H^+: 356.2702, Observed: 356.2714 ± 3.5 ppm.



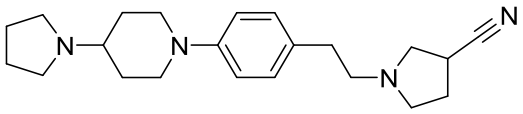
Pyrrolidin-1-yl(3-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)methanone (9): The general procedure for Buchwald coupling was followed with aryl bromide **S16** (91.0 mg, 0.358 mmol), 4-(pyrrolidin-1-yl)piperidine (66.3 mg, 0.430 mmol), RuPhos (16.7 mg, 0.0358 mmol), RuPhos pre-catalyst (29.2 mg, 0.0358 mmol), and NaO*t*-Bu (68.8 mg, 0.716 mmol) in 4 mL of THF. The crude was purified by silica gel chromatography (Isco, 4g, 0-80% EtOAc in hexanes) to give the title compound (99 mg, 85%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.29 (td, *J* = 7.6, 1.1 Hz, 1H), 7.07 (dq, *J* = 9.7, 1.3 Hz, 2H), 6.91 (dt, *J* = 7.5, 1.2 Hz, 1H), 3.83 – 3.71 (m, 2H), 3.57 (t, *J* = 7.0 Hz, 2H), 3.45 (t, *J* = 6.6 Hz, 2H),

2.75 (td, *J* = 12.6, 2.5 Hz, 2H), 2.68 (td, *J* = 5.6, 4.4, 3.0 Hz, 4H), 2.25 (tt, *J* = 11.1, 4.0 Hz, 1H), 2.11 – 2.01 (m, 2H), 1.99 (p, *J* = 6.5 Hz, 2H), 1.89 (p, *J* = 6.7 Hz, 2H), 1.88 – 1.79 (m, 4H), 1.64 (qd, *J* = 12.4, 4.1 Hz, 2H). ^{13}C NMR (100 MHz, Methanol-d4) δ 172.37 , 152.70 , 138.80 , 130.24 , 119.24 , 118.61 , 115.79 , 63.51 , 52.47 , 50.91 , 49.54 , 47.35 , 31.82 , 27.18 , 25.35 , 23.99 . LCMS (General) t_R = 3.25 min, *m/z* = 328 $[M+H]^+$.

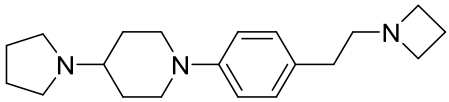
1,4-bis(4-(pyrrolidin-1-yl)piperidin-1-yl)benzene (10): The general procedure for Buchwald coupling was followed with 1-bromo-4-iodobenzene (141 mg, 0.500 mmol), 4-(pyrrolidin-1-yl)piperidine (185 mg, 1.20 mmol), RuPhos (12.0mg, 0.0250 mmol), RuPhos pre-catalyst (20.0 mg, 0.0250 mmol), and NaO*t*-Bu (96.0 mg, 1.00 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (10 mg, 3%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.25 (s, 4H), 3.83 (d, *J* = 12.5 Hz, 4H), 3.77 – 3.58 (m, 4H), 3.42 (t, *J* = 12.8 Hz, 2H), 3.27 – 3.06 (m, 7H), 2.35 (d, *J* = 12.5 Hz, 4H), 2.25 – 1.88 (m, 13H). LCMS (General) t_R = 1.34 min, *m/z* = 383 $[M+H]^+$.



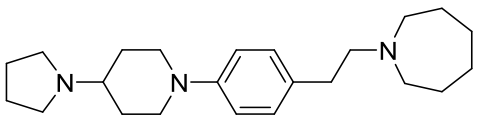
1-phenyl-4-(pyrrolidin-1-yl)piperidine (11): The general procedure for Buchwald coupling was followed with chlorobenzene (91.0 mg, 0.811 mmol), 4-(pyrrolidin-1-yl)piperidine (150 mg, 0.973 mmol), RuPhos (10.0 mg, 0.0200 mmol), RuPhos pre-catalyst (16.0 mg, 0.0200 mmol), and NaO*t*-Bu (156 mg, 1.62 mmol) in 4 mL of THF. The crude mixture was purified twice by silica gel chromatography (Isco, 4g, 0-16% EtOAc in hexanes and 4g, 0-16% EtOAc in hexanes) to give the title compound (63 mg, 34%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.25 – 7.16 (m, 2H), 7.00 – 6.91 (m, 2H), 6.84 – 6.77 (m, 1H), 3.73 – 3.63 (m, 2H), 2.73 – 2.57 (m, 7H), 2.17 (tt, *J* = 11.2, 4.0 Hz, 1H), 2.06 – 1.96 (m, 2H), 1.89 – 1.75 (m, 4H), 1.63 (qd, *J* = 12.6, 4.0 Hz, 2H). LCMS (General) t_R =2.96 min, *m/z* = 231 $[M+H]^+$.



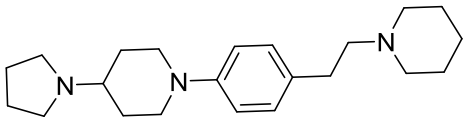
1-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)pyrrolidine-3-carbonitrile (12): The general procedure for Buchwald coupling was followed with aryl bromide **S5** (41.0 mg, 0.147 mmol), 4-(pyrrolidin-1-yl)piperidine (27.0 mg, 0.176 mmol), RuPhos (6.90 mg, 0.0470 mmol), RuPhos pre-catalyst (12.0 mg, 0.0147 mmol), and NaO*t*-Bu (35.0 mg, 0.367 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (15 mg, 18%) as an orange oil which solidified upon standing. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.22 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 2H), 3.90 – 3.55 (m, 8H), 3.53 – 3.42 (m, 2H), 3.25 – 3.08 (m, 2H), 3.03 – 2.93 (m, 2H), 2.85 (t, *J* = 13.5 Hz, 2H), 2.64 – 2.51 (m, 1H), 2.46 – 2.31 (m, 1H), 2.31 – 2.22 (m, 2H), 2.21 – 2.11 (m, 2H), 2.09 – 1.94 (m, 2H), 1.85 (qd, *J* = 12.3, 4.1 Hz, 2H), 1.79 – 1.61 (m, 1H), 1.48 – 1.23 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d4) δ 150.65 , 130.69 , 129.34 , 119.95 , 118.76 , 63.34 , 57.73 , 56.88 , 54.69 , 52.87 , 49.75 , 32.10 , 29.57 , 27.49 , 23.86 . LCMS (General) t_R = 0.94 min, *m/z* = 353 $[M+H]^+$, 177 $[M+2M]^{2+}$.



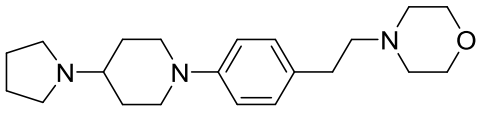
1-(4-(2-(azetidin-1-yl)ethyl)phenyl)-4-(pyrrolidin-1-yl)piperidine (14) The general procedure for Buchwald coupling was followed with aryl bromide **S11** (31.0 mg, 0.129 mmol), 4-(pyrrolidin-1-yl)piperidine (24.0 mg, 0.155 mmol), RuPhos (3.00 mg, 0.00645 mmol), RuPhos pre-catalyst (5.30 mg, 0.00645 mmol), and NaO*t*-Bu (25.0 mg, 0.258 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC and lyophilized to give the TFA salt of the title compound (20 mg, 29%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.31 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H), 4.24 – 4.13 (m, 2H), 4.10 – 4.01 (m, 2H), 3.82 (d, *J* = 12.9 Hz, 2H), 3.77 – 3.60 (m, 2H), 3.47 – 3.40 (m, 3H), 3.28 – 3.05 (m, 4H), 2.89 – 2.82 (m, 2H), 2.63 – 2.49 (m, 1H), 2.46 – 2.30 (m, 3H), 2.25 – 2.10 (m, 2H), 2.10 – 1.94 (m, 4H). ^{13}C NMR (100 MHz, Methanol-d4) δ 149.33, 131.01, 130.90 , 119.34, 119.25 , 116.35, 62.78, 57.20, 56.01, 52.90, 50.56, 31.10, 29.25, 23.86, 17.34. LCMS (General) t_R = 1.02 min, *m/z* = 314 $[M+H]^+$.



1-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)azepane (17): The general procedure for Buchwald coupling was followed with aryl bromide **S6** (82.5 mg, 0.325 mmol), 4-(pyrrolidin-1-yl)piperidine (60.0 mg, 0.389 mmol), RuPhos (7.60 mg, 0.0163 mmol), RuPhos pre-catalyst (13.3 mg, 0.0163 mmol), and NaO*t*-Bu (78.0 mg, 0.813 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (130 mg, 68%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.22 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 3.85 – 3.77 (m, 2H), 3.73 – 3.62 (m, 2H), 3.53 (ddd, *J* = 13.5, 7.6, 2.6 Hz, 2H), 3.36 – 3.32 (m, 2H), 3.29 – 3.11 (m, 5H), 3.03 – 2.96 (m, 2H), 2.85 (td, *J* = 12.8, 2.4 Hz, 2H), 2.31 – 2.20 (m, 2H), 2.17 (s, 2H), 2.08 – 1.69 (m, 12H). LCMS (General) t_R = 1.93 min, *m/z* = 356 $[M+H]^+$, 179 $[M+2H]^{2+}$.

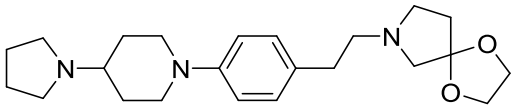


1-(4-(2-(Piperidin-1-yl)ethyl)phenyl)-4-(pyrrolidin-1-yl)piperidine (18): The general procedure for Buchwald coupling was followed with aryl bromide **S4** (76.0 mg, 0.283 mmol), 4-(pyrrolidin-1-yl)piperidine (52.0 mg, 0.340 mmol), RuPhos (6.60 mg, 0.0283 mmol), RuPhos pre-catalyst (11.6 mg, 0.0283 mmol), and NaO*t*-Bu (231 mg, 0.708 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (106 mg, 66%) as an off-white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.24 (d, *J* = 8.7 Hz, 2H), 7.09 (d, *J* = 8.7 Hz, 2H), 3.81 (d, *J* = 13.0 Hz, 2H), 3.76 – 3.63 (m, 2H), 3.60 (d, *J* = 12.4 Hz, 2H), 3.38 – 3.33 (m, 1H), 3.29 – 3.23 (m, 2H), 3.24 – 3.08 (m, 2H), 3.04 – 2.86 (m, 6H), 2.28 (dt, *J* = 12.6, 2.8 Hz, 2H), 2.24 – 1.69 (m, 11H), 1.65 – 1.41 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d4) δ 129.91 , 119.14 , 57.50 , 52.97 , 51.60 , 29.08 , 27.33 , 22.86 , 22.46 , 21.27. LCMS (General) t_R = 1.81 min, *m/z* = 342 $[M+H]^+$, 172 $[M+2H]^{2+}$.

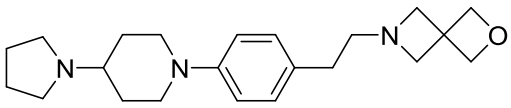


4-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)morpholine (19): The general procedure for Buchwald coupling was followed with aryl bromide **S7** (57.0 mg, 0.211 mmol), 4-(pyrrolidin-1-yl)piperidine (39.0 mg, 0.252 mmol), RuPhos (2.95 mg, 0.00633 mmol), RuPhos pre-catalyst (5.17 mg, 0.00633 mmol), and NaO*t*-Bu (40.6 mg, 0.422 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (51 mg, 43%) as a white solid. ^{1}H NMR (400

MHz, Methanol-d4) δ 7.19 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 4.12 – 4.02 (m, 2H), 3.85 – 3.62 (m, 6H), 3.61 – 3.48 (m, 2H), 3.37 – 3.33 (m, obscured by CD_3OD, 2H), 3.30 – 3.08 (m, 5H), 3.02 – 2.95 (m, 2H), 2.79 (td, *J* = 13.1, 1.7 Hz, 2H), 2.28 – 2.09 (m, 4H), 2.01 (d, *J* = 6.1 Hz, 2H), 1.88 – 1.75 (m, 2H). ^{13}C NMR (100 MHz, Methanol-d4) δ 149.69 , 130.84 , 130.61 , 119.17 , 65.06 , 62.97 , 59.34 , 53.22 , 52.91 , 50.34 , 30.12 , 29.38 , 23.87 . LCMS (General) t_R = 0.93 min, *m/z* = 344 $[M+H]^+$, 173 $[M+2H]^{2+}$.



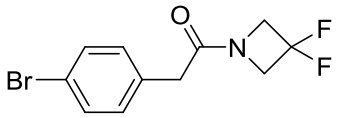
7-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)-1,4-dioxa-7-azaspiro[4.4]nonane (20): The general procedure for Buchwald coupling was followed with aryl bromide **S8** (95.5 mg, 0.306 mmol), 4-(pyrrolidin-1-yl)piperidine (56.6 mg, 0.367 mmol), RuPhos (16.8 mg, 0.0306 mmol), RuPhos pre-catalyst (24.9 mg, 0.0306 mmol), and NaO*t*-Bu (58.8 mg, 0.612 mmol) in 4 mL of THF. The crude mixture was purified by RP-HPLC to give the TFA salt of the title compound (184 mg, 98%) as an off-white solid. ^1H NMR (400 MHz, Methanol-d4) δ 7.19 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 4.10 – 3.92 (m, 4H), 3.81 (d, *J* = 13.1 Hz, 2H), 3.59 (s, 3H), 3.45 – 3.39 (m, 2H), 3.33 – 3.11 (m, obscured by CD_3OD, 5H), 2.99 – 2.90 (m, 2H), 2.79 (td, *J* = 12.6, 2.1 Hz, 2H), 2.43 – 2.09 (m, 6H), 2.08 – 1.94 (m, 2H), 1.83 (qd, *J* = 12.2, 3.9 Hz, 3H). 13C NMR (100 MHz, Methanol-d4) δ 149.59 , 129.17 , 127.61 , 117.17 , 112.26 , 64.99 , 62.06 , 59.32 , 56.74 , 51.42 , 33.56 , 30.37 , 28.25 , 22.42 . LCMS (General) t_R = 1.53 min, *m/z* = 386 $[M+H]^+$, 194 $[M+2H]^{2+}$.



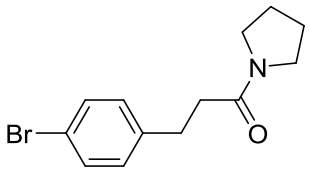
6-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)-2-oxa-6-azaspiro[3.3]heptane (21): The general procedure for Buchwald coupling was followed with aryl bromide **S9** (174 mg, 0.617 mmol), 4-(pyrrolidin-1-yl)piperidine (114 mg, 0.740 mmol), RuPhos (14.4 mg, 0.0309 mmol), RuPhos pre-catalyst (25.2 mg, 0.0309 mmol), and NaO*t*-Bu (118 mg, 1.23 mmol) in 4 mL of THF. The crude was purified by C18 RP-chromatography (Isco, 26 g, 5-100% MeOH in water) to give the free base of the title compound (107 mg, 49%) as a white solid. 1H NMR (400 MHz, Methanol-d4) δ 7.06 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 4.70 (s, 4H), 3.64 (dt, J = 13.4, 3.4 Hz, 2H), 3.42 – 3.33 (m, 4H), 2.76 – 2.48 (m, 10H), 2.17 (tt, J = 11.0, 3.9 Hz, 1H), 2.08 – 1.96 (m, 2H), 1.89 – 1.75 (m, 4H), 1.63 (qd, J = 12.3, 4.0 Hz, 2H).

13C NMR (100 MHz, Methanol-d4) δ 151.36 , 132.01 , 130.24 , 118.27 , 82.19 (d, J = 3.9 Hz), 64.41 , 63.52 , 61.99 , 52.42 , 50.47 , 40.43 , 34.15 , 32.14 , 24.01 . LCMS (General) t_R = 0.772 min, *m/z* = 356 $[M+H]^+$, 179 $[M+2H]^{2+}$.

General procedure for amide coupling: To a mixture of the acid (1 equiv) and TBTU (1.5 equiv) in DMF, the desired amine (1.2 equiv) was added followed by triethylamine (3 - 4 equiv). The mixture was stirred at room temperature for 17 – 21 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with either EtOAc or CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with brine (10 mL) and dried over $MgSO_4$. After filtration, the solvent was removed in vacuo and the crude mixture purified by silica gel chromatography to give the title compound.

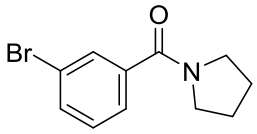


2-(4-Bromophenyl)-1-(3,3-difluoroazetidin-1-yl)ethan-1-one (S14): The general procedure for amide coupling was followed with 2-(4-bromophenyl)acetic acid (86.0 mg, 0.400 mmol), 3,3-difluoroazetidine·hydrochloride (62.2 mg, 0.480 mmol), TBTU (154 mg, 0.481 mmol), triethylamine (121 µL, 1.20 mmol) in DMF (5 mL). The reaction was stirred for 21 hours. The crude product was purified by silica gel chromatography (Isco, 4g, 0-80% EtOAc in hexane) to give the title compound (100 mg, 86%) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.46 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 4.35 (dt, *J* = 17.9, 11.9 Hz, 4H), 3.48 (s, 2H). LCMS (General) t_R = 5.11 min, *m/z* = 312 $[M+Na]^+$, 290 $[M+H]^+$.

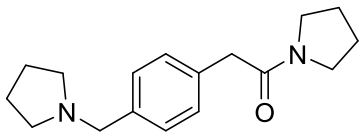


3-(4-Bromophenyl)-1-(pyrrolidin-1-yl)propan-1-one (S15): The general procedure for amide coupling was followed with 3-(4-bromophenyl)propanoic acid (226 mg, 0.987 mmol), pyrrolidine (81.1 µL, 0.987 mmol), TBTU (379 mg, 1.18 mmol), triethylamine (164 µL, 1.18 mmol) in DMF (5 mL). The crude product was purified by silica gel chromatography (Isco, 4g, 0-100% EtOAc in hexane) to give the

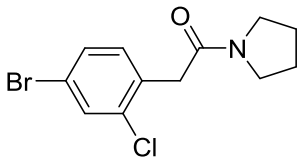
title compound (245 mg, 88%) as a white solid. 1H NMR (400 MHz, Chloroform-d) δ 7.38 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 3.45 (t, J = 6.8 Hz, 2H), 3.29 (t, J = 6.7 Hz, 2H), 2.92 (d, J = 7.9 Hz, 2H), 2.57 – 2.48 (m, 2H), 1.94 – 1.78 (m, 4H). LCMS (General) t_R = 5.50 min, *m/z* = 284 $[M+H]^+$ for ^{81}Br, 282 $[M+H]^+$ for ^{79}Br.



(3-Bromophenyl)(pyrrolidin-1-yl)methanone (S16): The general procedure for amide coupling was followed with 3-bromobenzoic acid (150 mg, 0.746 mmol), pyrrolidine (73.5 µL, 0.895 mmol), TBTU (287 mg, 0.895 mmol), triethylamine (313 µL, 2.24 mmol) in DMF (5 mL). The reaction was stirred for 17 hours. The crude product was purified by silica gel chromatography (Isco, 4g, 0-100% EtOAc in hexane) to give the title compound (182 mg, 96%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.38 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 3.29 (t, *J* = 6.7 Hz, 2H), 2.92 (d, *J* = 7.9 Hz, 2H), 2.56 – 2.47 (m, 2H), 1.96 – 1.74 (m, 4H). LCMS (General) t_R = 5.08 min, *m/z* = 256 $[M+H]^+$ for ^{81}Br, 254 $[M+H]^+$ for ^{79}Br.

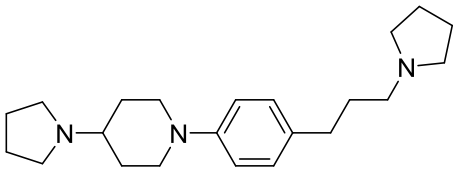


1-(Pyrrolidin-1-yl)-2-(4-(pyrrolidin-1-ylmethyl)phenyl)ethan-1-one (S17): The general procedure for amide coupling was followed with **S10** (191 mg, 0.873 mmol), pyrrolidine (86.2 µL, 1.05 mmol), TBTU (337 mg, 1.05 mmol), triethylamine (486 µL, 3.49 mmol) in DMF (4 mL). The reaction was stirred for 17 hours. The crude product was purified by silica gel chromatography (Isco, 4g, 0-20% MeOH in DCM, 1% NH_3) to give the title compound (124 mg, 52% over 2 synthetic steps) as a yellow oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.31 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 3.69 (s, 2H), 3.66 (s, 2H), 3.51 (t, *J* = 6.7 Hz, 2H), 3.43 (t, *J* = 6.9 Hz, 2H), 2.61 – 2.54 (m, 4H), 2.00 – 1.78 (m, 8H). LCMS (General) t_R = 2.74 min, *m/z* = 273 $[M+H]^+$.



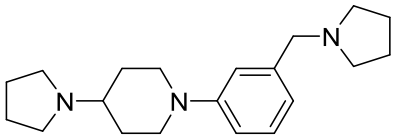
2-(4-Bromo-2-chlorophenyl)-1-(pyrrolidin-1-yl)ethan-1-one (S18): The general procedure for amide coupling was followed with 2-(4-bromo-2-chlorophenyl)acetic acid (375 mg, 1.50 mmol), pyrrolidine (148 µL, 1.80 mmol), TBTU (579 mg, 1.80 mmol), triethylamine (630 µL, 4.50 mmol) in DMF (10 mL). The reaction was stirred for 19 hours. The crude product was purified by silica gel chromatography (Isco, 12 g, 0-50% EtOAc in hexane) to give the title compound (quantitative, accurate yield to be calculated over 2 synthetic steps) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.54 (d, *J* = 2.1 Hz, 1H), 7.36 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 3.70 (s, 2H), 3.49 (dt, *J* = 10.3, 6.9 Hz, 5H), 1.97 (p, *J* = 6.8 Hz, 2H), 1.87 (p, *J* = 6.9 Hz, 2H). LCMS (General) t_R = 5.60 min, *m/z* = 304 $[M+H]^+$ for ^{81}Br, 302 $[M+H]^+$ for ^{79}Br.

General procedure for amide reduction: A solution of the amide (1 equiv) in THF was added drop-wise to a cooled (0°C) suspension of $LiAlH_4$ (2.0 equiv.) in THF. The reaction was refluxed until completion was observed by LCMS. The mixture was cooled to 0°C, diluted with Et_2O (40 mL) and quenched by the successive addition of H_2O (150 µL), 15% aq. NaOH (150 µL), and H_2O (450 µL). The precipitated aluminium salts were filtered and the combined filtrate and washings were dried in vacuo. The crude product was purified by RP-HPLC to give the title compound.

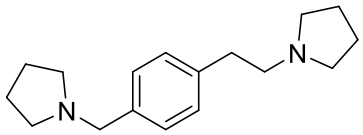


4-(Pyrrolidin-1-yl)-1-(4-(3-(pyrrolidin-1-yl)propyl)phenyl)piperidine (4): The general procedure for amide reduction was followed with amide **S12** (78.0 mg, 0.455 mmol) and 2M $LiAlH_4$ in THF (220 µL, 0.439 mmol) in 14 mL of THF. The reaction was refluxed for 18 hours. The crude was purified by RP-HPLC to give the TFA salt of the title compound (78 mg, 62%) as an off-white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.17 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.2 Hz, 2H), 3.82 – 3.73 (m, 2H), 3.73 – 3.58 (m, 4H), 3.22 – 3.11 (m, 4H), 3.09 – 2.97 (m, 2H), 2.86 (t, *J* = 12.4 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 2.32 – 2.23 (m, 2H), 2.22 – 1.93 (m, 11H), 1.87 (q, *J* = 11.6 Hz, 2H). ^{13}C NMR (100 MHz, Methanol-d4) δ

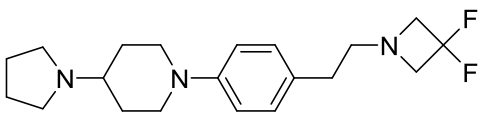
148.49 , 135.88 , 130.46 , 119.39 , 62.73 , 55.74 , 55.16 , 52.94 , 50.97 , 32.69 , 29.33 , 28.81 , 23.94 , 23.87 . LCMS (General) t_R = 2.32 min, *m/z* = 342 $[M+H]^+$, 172 $[M+2H]^{2+}$.



4-(Pyrrolidin-1-yl)-1-(3-(pyrrolidin-1-ylmethyl)phenyl)piperidine (6): The general procedure for amide reduction was followed with amide **9** (49.5 mg, 0.151 mmol) and 2M $LiAlH_4$ in THF (151 µL, 0.302 mmol) in 10 mL of THF. The reaction was refluxed for 20 hours. The crude was purified by RP-HPLC to give the TFA salt of the title compound (15 mg, 18%) as a brown oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.32 (t, *J* = 7.9 Hz, 1H), 7.11 (s, 1H), 7.07 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 3.93 – 3.83 (m, 2H), 3.74 – 3.59 (m, 2H), 3.54 – 3.40 (m, 2H), 3.25 – 3.06 (m, 5H), 2.82 (td, *J* = 12.8, 2.3 Hz, 2H), 2.27 – 1.90 (m, 12H), 1.80 (qd, *J* = 12.3, 4.2 Hz, 2H). 13C NMR (100 MHz, Methanol-d4) δ 152.55 , 133.11 , 122.30 , 63.57 , 59.64 , 54.83 , 52.83 , 29.60 , 23.85 . LCMS (General) t_R = 1.01 min, *m/z* = 314 $[M+H]^+$, 158 $[M+2H]^{2+}$.

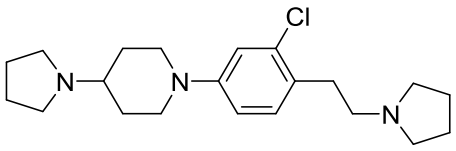


1-(4-(2-(Pyrrolidin-1-yl)ethyl)benzyl)pyrrolidine (7): The general procedure for amide reduction was followed with amide **S17** (124 mg, 0.455 mmol) and 2M $LiAlH_4$ in THF (455 µL, 0.910 mmol) in 20 mL of THF. The reaction was refluxed for 22.5 hours. The crude was purified by C18Aq RP-chromatography (Isco, 12g, 0-100% MeOH in water, 0.1% TFA) to give the TFA salt of the title compound (14 mg, 6%) as a white solid. 1H NMR (400 MHz, Methanol-d4) δ 7.51 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 4.36 (s, 2H), 3.78 – 3.62 (m, 2H), 3.55 – 3.41 (m, 4H), 3.23 – 3.01 (m, 6H), 2.27 – 2.10 (m, 4H), 2.11 – 1.92 (m, 4H). LCMS (General) t_R = 0.59 min, *m/z* = 260 $[M+H]^+$, 130 $[M+2H]^{2+}$.

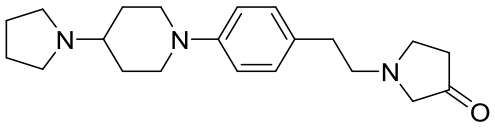


1-(4-(2-(3,3-Difluoroazetidin-1-yl)ethyl)phenyl)-4-(pyrrolidin-1-yl)piperidine (15): The general procedure for amide reduction was followed with amide **S11** (79.0 mg, 0.217 mmol) and 2M $LiAlH_4$ in

THF (217 µL, 0.435 mmol) in 14 mL of THF. The reaction was refluxed for 24 hours. The crude was purified by RP-HPLC to give the TFA salt of the title compound (22 mg, 18%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 4.51 (t, *J* = 12.0 Hz, 2H), 4.31 (t, *J* = 12.2 Hz, 2H), 3.75 – 3.66 (m, 2H), 3.50 (s, 2H), 3.39 – 3.28 (m, obscured by CD_3OD, 2H), 2.76 – 2.62 (m, 6H), 2.29 – 2.17 (m, 1H), 2.10 – 1.99 (m, 2H), 1.87 – 1.79 (m, 4H), 1.64 (qd, *J* = 12.4, 4.1 Hz, 2H). LCMS (General) t_R =2.10 min, *m/z* = 350 $[M+H]^+$, 176 $[M+2H]^{2+}$.

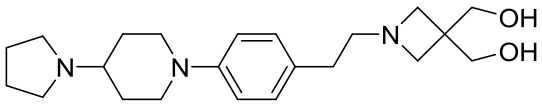


1-(3-chloro-4-(2-(Pyrrolidin-1-yl)ethyl)phenyl)-4-(pyrrolidin-1-yl)piperidine (22): The general procedure for amide reduction was followed with amide **S13** (89.0 mg, 0.237 mmol) and 2M $LiAlH_4$ in THF (237 µL, 0.473 mmol) in 10 mL of THF. The reaction was refluxed for 7 hours. The crude was purified by RP-HPLC to give the TFA salt of the title compound (87 mg, 62%) as a light orange solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.24 (d, *J* = 8.6 Hz, 1H), 7.06 (d, *J* = 2.5 Hz, 1H), 6.95 (dd, *J* = 8.5, 2.5 Hz, 1H), 3.86 – 3.75 (m, 2H), 3.75 – 3.58 (m, 4H), 3.39 – 3.24 (m, 3H), 3.20 – 3.01 (m, 6H), 2.85 (td, *J* = 12.7, 2.3 Hz, 2H), 2.30 – 1.91 (m, 10H), 1.83 (qd, *J* = 12.3, 4.1 Hz, 2H). LCMS (General) t_R = 2.36 min, *m/z* = 362 $[M+H]^+$ for ^{35}Cl, 364 $[M+H]^+$ for ^{37}Cl, 183 $[M+2H]^{2+}$ for ^{35}Cl, 182 $[M+2H]^{2+}$ for ^{37}Cl.



1-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)pyrrolidin-3-one (13): A solution of the acetal **20** (695 mg, 1.80 mmol) in THF (15 mL) and 4N HCl (16 mL) was heated at 60 °C for 6 hours. The solution was cooled and the solvent was removed in vacuo. The crude mixture was purified by RP-HPLC (5-40% CH_3CN in water, 0.1% TFA over 25 minutes, flow rate = 40 mL/min) to give the title compound (54 mg, 7%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.87 (s, 1H), 7.15 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 8.6 Hz, 2H), 3.79 (d, *J* = 12.9 Hz, 7H), 3.61 – 3.36 (m, obscured by CD_3OD), 3.31 – 3.19 (m, 1H), 3.16 – 3.02 (m, 2H), 2.89 (t, *J* = 8.1 Hz, 2H), 2.71 – 2.60 (m, 4H), 2.16 – 2.06 (m, 2H), 2.08 – 1.94 (m, 2H), 1.93 – 1.79 (m, 2H), 1.67 (qd, *J* = 12.1, 4.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ 206.44 ,

158.49 , 158.16 , 149.15 , 129.33 , 126.90 , 116.27 , 61.09 , 57.44 , 56.07 , 50.84 , 47.20 , 35.26 , 29.94 , 27.67 , 22.55 , 9.87 . LCMS (General) t_R = 1.17 min, *m/z* = 342 $[M+H]^+$, 181 $[M+2H]^{2+}$.



(1-(4-(4-(Pyrrolidin-1-yl)piperidin-1-yl)phenethyl)azetidine-3,3-diyl)dimethanol (16): To a solution of **21** (205 mg, 0.351 mmol) in water was added TFA (1 mL). The solution was stirred at 60°C for 16 hours. The reaction was cooled and the solvent was removed in vacuo and the residue lyophilised from water to give the TFA salt of the title compound (147 mg, 70%) as a white solid. 1H NMR (400 MHz, Methanol-d4) δ 7.22 – 7.14 (m, 2H), 7.00 (d, J = 8.6 Hz, 2H), 4.14 – 4.05 (m, 2H), 3.95 – 3.87 (m, 2H), 3.85 – 3.76 (m, 2H), 3.67 (s, 2H), 3.58 (s, 2H), 3.46 (s, 2H), 3.41 – 3.33 (m, 2H), 3.33 – 3.23 (m, 1H, obscured by CD_3OD), 3.15 (d, J = 16.3 Hz, 2H), 2.87 – 2.73 (m, 4H), 2.29 – 2.21 (m, 2H), 2.19 – 2.11 (m, 2H), 2.08 – 1.94 (m, 2H), 1.82 (qd, J = 12.3, 11.8, 3.4 Hz, 2H). LCMS (General) t_R = 1.04 min, *m/z* = 374 $[M+H]^+$, 188 $[M+2H]^{2+}$.

Protein expression and purification

L3MBTL1 and L3MBTL3 were purified essentially as described previously.[1] Briefly, cell pellets from 2 L cultures expressing His-tagged proteins were lysed with BugBuster protein extraction reagent (EMD Millipore, Darmstadt, Germany) containing 20 mM imidazole. The cell lysate was clarified by centrifugation and loaded onto a 5 mL HisTrap HP column (GE Healthcare, Piscataway, NJ) equilibrated with binding and wash buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 30 mM imidazole) using an ÄKTA FPLC (GE Healthcare, Piscataway, NJ) at 1 mL/min. His-tagged protein was eluted using a linear gradient of elution buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 500 mM imidazole) over 20 column volumes. Fractions containing the desired protein were confirmed by SDS-PAGE, pooled and loaded at 2 mL/min onto a HiLoad 26/60 Superdex 200 prep grade size exclusion column (GE Healthcare, Piscataway, NJ) using an ÄKTA FPLC. A constant flow of 2 mL/min size exclusion buffer (25 mM Tris·HCl pH 8.0, 250 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.02% Tween 20) was used to elute proteins. Fractions containing the desired protein were identified by SDS-PAGE, pooled and subjected to simultaneous concentration and buffer exchange using an Amicon Ultra-15 centrifugal filter unit (Millipore, Billerica, MA) and storage buffer (20 mM Tris·HCl pH 8.0, 150 mM NaCl and 2 mM DTT for L3MBTL1 and 20 mM Tris HCl, pH 8.0, 250 mM NaCl and 2 mM DTT for L3MBTL3).

A pET28-mhl vector containing the coding region for residues 130-566 of MBTD1 (reference sequence NP_060113) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. MBTD1 protein was purified essentially as described for L3MBTL1 and L3MBTL3.

A pET28-mhl vector containing the coding region for residues 1485-1611 of 53BP1 (reference sequence (NP_001135452) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. 53BP1 protein was purified essentially as described above except for the use of a buffer consisting of 25 mM Tris HCl pH 7.5, 150 mM NaCl, and 2 mM DTT for size exclusion chromatography and protein storage.

A pET28 vector containing the coding region for residues 121-286 of UHRF1 (reference sequence (NP_001041666) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression

was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. UHRF1 protein was purified exactly as described for 53BP1.

A pET28 vector containing the coding region for residues 8-62 of CBX7 (reference sequence (NP_783640) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. CBX1 protein was purified exactly as described for 53BP1.

The identity of all expression constructs was verified by DNA sequencing and all proteins were at least 95% pure as determined by Coomassie staining. Protein concentration was determined by absorbance at 280 nm using the Edelhoch method. PHF23 and JARID1A proteins were provided by Greg Wang (UNC).

AlphaScreen®

The AlphaScreen® assay was performed as described elsewhere.[2] In brief, compound plates (1 µL at 30 mM highest concentration; 3-fold, 20-point dilutions in DMSO) were diluted in 1X assay buffer (20 mM TRIS pH 8.0, 25 mM NaCl, 2 mM DTT and 0.05% Tween-20) over two steps to 3 mM (L3MBTL1, MBTD1) or 300 µM (all other proteins) using a Multimek robotic pipettor (Nanoscreen) and 1 µL was spotted into the wells of 384-well low-volume Proxiplates (PerkinElmer). To these plates 9 µL of protein-peptide mix in 1X assay buffer was added by Multidrop (Thermo) and incubated for 30 min at room temperature. Next, 2 µL of a 1:1 mixture of streptavidin-conjugate donor and nickel-chelate acceptor beads (45 µg/mL in 1X assay buffer) were added and the plates were allowed to incubate for an additional 30 min in the dark at room temperature. After incubation, the plates were read on an EnVision multi-label reader equipped with an HTS AlphaScreen® laser (Perkin Elmer). The screens reported were performed up to 300 µM (L3MBTL1 and MBTD1) or 30 µM (all other proteins), and therefore it should be noted that those compounds referred to as inactive are indeed inactive only within the concentration range tested. PHF23 and JARID1A were GST tagged and consequently for these assays GST-acceptor beads were used.

The IC_{50} values reported are the average of at least 2 values ± the standard deviation. When IC_{50} values for a single compound were not all active (< 30 µM or < 300 µM) or inactive (> 30 µM or > 300 µM) the IC_{50} values were calculated from replicate runs by averaging data points for each compound concentration and plotted using 4-paramter curve fitting (GraphPad Prism 5).

Supplementary Table 1. Alphascreen proteins and corresponding peptide substrates.

Protein	Peptide	Peptide sequence	Final [Protein] (nM) in 10 µL	Final [Peptide] (nM) in 10 µL
L3MBTL1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	150	250
L3MBTL3	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH	150	200
MBTD1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	120	200
CBX7	H3K9Me3	ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH2	150	200
53BP1	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH	130	200
$UHRF1_{TTD}$	H3K9Me3	Biotin-AHA-ARTKQTARK(Me3)STGGKA-COOH	150	250
PHF23	H3K4Me3	NH2-ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)	10	250
JARID1A	H3K4Me3	NH2-ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)	12.5	25

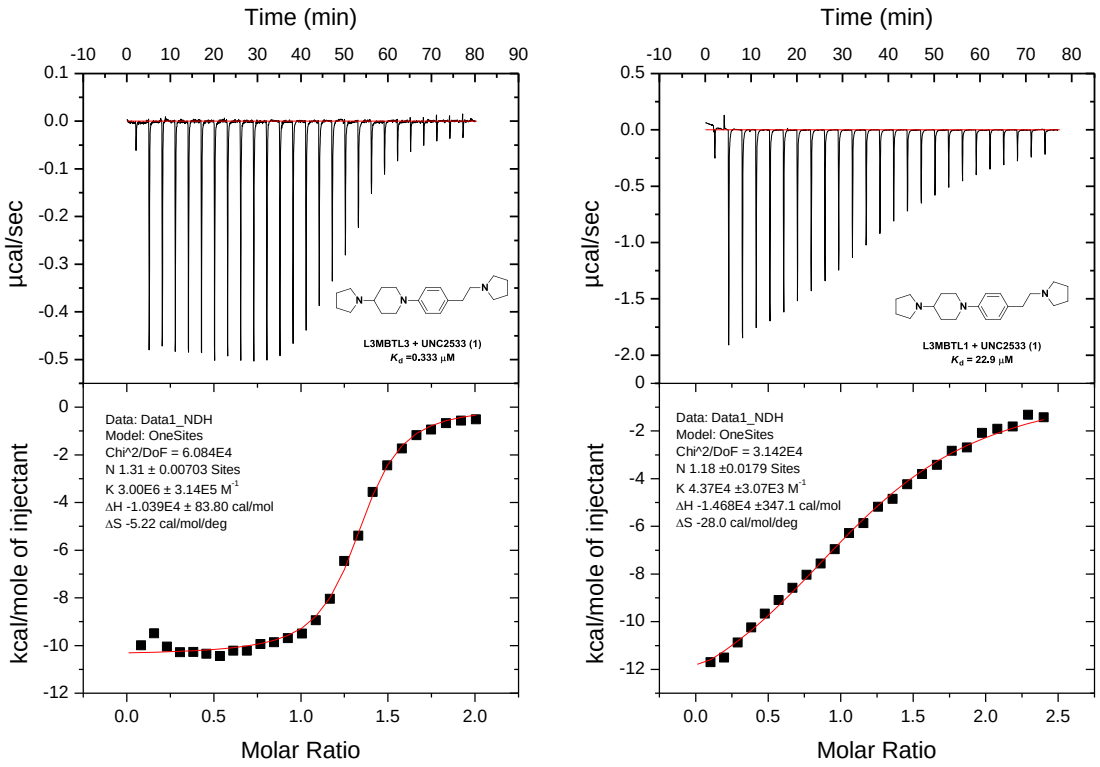
ITC experiments

All ITC experiments were carried out as described by James et al.[3]

Supplementary Table 2. ITC for compounds with L3MBTL3, *Literature values[3]

#	Mean K_d (µM)	n	SD
***UNC1215**	0.12	5	0.11
UNC2533 (1)	0.37	3	0.052
2	2.4	2	0.13
3	1.4	2	0.13
4	1.8	3	0.38
5	2.7	3	0.33
8	38	2	22
11	44	2	4.9
16	0.40	2	0.17
18	0.61	2	0.13

Figure 1) Representative ITC binding curves. UNC2533 (1) is 50 fold more selective for L3MBTL3 over L3MBTL1.



Supplementary Table 3. ITC for compounds with L3MBTL1, *Literature values[3]

#	Mean K_d (µM)	n	SD
UNC1215*	9.4	3	1.7
UNC2533 (1)	19	2	1.4
11	48	1	NA
16	30	1	NA
18	3.3	3	0.15

X-ray crystallography

L3MBTL3 was cloned into pET28-MHL which is a pET expression vector with N-terminal Hexa-His tag (GenBank GI number 5729993). L3MBTL3 was expressed in E.coli BL21 (DE3) strain (EMD Biosciences) in Terrific Broth (TB) and was purified using metal affinity chromatography on a Ni-chelating open column followed by a cation-exchange chromatography on a HiTrap SP HP column and a size exclusion chromatography on a pre-packed HiLoad 16/60 Superdex 200 pg size exclusion column (GE Life Sciences). The N-terminal Hexa-His tag was removed by TEV protease produced in house. The L3MBTL3 protein was crystallized at concentration 10 mg/mL with 5-fold molar excess UNC2533 using the sitting drop vapor diffusion method at 18 °C. The reservoir solution contained 1.5M sodium citrate, 0.1M sodium cacodylic at pH 5.5. Using a nylon loop, crystals were passed through the reservoir solution containing 10% ethyl glycol, flash-frozen and stored in liquid nitrogen until data collection. Diffraction data for native L3MBTL3 + UNC2533 were collected at APS beamline 19ID (Argonne National Laboratory). Data were processed with HKL3000[4] and scaled in space group $P3_121$ to 2.3 Å resolution. Molecular replacement was performed with the program PHASER[5] using a PDB code 4FL6 as search model. The model was refined against native diffraction data to 2.3 Å resolution to an Rcryst of 21.8.0% (Rfree 25.1%) by iteration of interactive rebuilding, restrained refinement and validation using COOT[6], REFMAC[7] and MOLPROBITY[8], respectively, and deposited to the Protein Data Bank (PDB ID: 4L59). Bond lengths and angles deviate from the dictionary values by RMSDs of 0.011 Å and 1.4°, respectively. MOLPROBITY shows 95.7% of amino acid residues are in most favored Ramachandran conformations and 0.0% outliers. The MOLPROBITY score is 100th percentile (100th percentile is the best among structures of comparable resolution). Data collection and processing details are listed in Supplementary Table 4.

Supplementary Table 4. Data collection and refinement statistics (molecular replacement).

PDB ID: 4L59	
Data collection	L3 2533
Space group	$P3_121$
Cell dimensions	
a, *b*, *c* (Å)	104.80, 104.80, 110.64
α, β, γ (°)	90.00, 90.00, 120.00
Resolution (Å)	50.0-2.30(2.34-2.30) *
R_{sym} or R_{merge}	0.073(0.872)
$I / \sigma I$	33.9(2.2)
Completeness (%)	98.9(99.7)
Redundancy	8.6(8.3)
Refinement	
Resolution (Å)	47.40-2.29
No. reflections work/free	30414/1000
R_{work} / R_{free}	0.212/0.250
No. atoms	
Protein	2431
Ligand/ion	24
Water	80
B-factors	
Protein	51.7
Ligand/ion	47.5
Water	47.1
R.m.s. deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.390

*Values in parentheses are for highest-resolution shell.

Supplementary Table 5. Distance and energy measurements for the interactions between UNC2533 and L3MBTL3 (4L59) compared with UNC1215 and L3MBTL3 (4FL6).

UNC2533	**L3MBTL3**				
Atom	Atom	Residue	Interaction	Distance	E (kcal/mol)
Pyrrolidine N	COO-	ASP381	H-donor	2.61	-9.1
C aliphatic	SG	CYS282	H-donor	3.93	-1.1
Pyrrolidine N	COO-	ASP274	H-donor	2.83	-10.7
Pyrrolidine C	O	HOH35	H-donor	3.33	-0.7
Pyrrolidine N	COO-	ASP381	ionic	2.61	-7.7
Pyrrolidine N	COO-	ASP274	ionic	2.83	-5.7
Pyrrolidine C	6-ring	TYR301	H-pi	3.84	-1.4

UNC1215	**L3MBTL3**				
Atom	Atom	Residue	Interaction	Distance	E (kcal/mol)
Pyrrolidine N	COO-	ASP274	H-donor	2.97	-8.6
Piperidine C	COO-	ASP274	H-donor	3.05	-1.3
Pyrrolidine N	COO-	ASP381	H-donor	2.63	-6.1
Carbonyl	Cβ	SER411	H-acceptor	3.54	-0.9
Pyrrolidine N	COO-	ASP274	ionic	2.97	-4.7
Pyrrolidine N	COO-	ASP381	ionic	2.63	-7.5
C6	6-ring	TRP408	H-pi	3.76	-0.7

Supplementary Table 6. Measured distances between the alpha carbons of residue pairs in the binding site of UNC1215-L3MBTL3 complex and the UNC2533-L3MBTL3 complex. Distances were measured manually using the distance function in MOE.

	Residue	Distance between α-carbons (Å)
Domain 1	D274	2.78
	H277	2.98
	V280	3.58
	Y305	2.41
	Y301	3.47
	F298	3.15
	H330	4.29
	E410	3.73
	C282	3.73
Domain 2	D381	3.34
	F387	4.34
	F405	4.32
	C389	4.62
	W408	4.39
	Y412	3.28
	N384	3.34

References

1. *J. M. Herold, T. J. Wigle, J. L. Norris, R. Lam, V. K. Korboukh, C. Gao, L. A. Ingerman, D. B. Kireev, G. Senisterra, M. Vedadi, A. Tripathy, P. J. Brown, C. H. Arrowsmith, J. Jin, W. P. Janzen and S. V. Frye, J. Med. Chem., 2011, **54**, 2504-2511.*
2. *T. J. Wigle, J. M. Herold, G. A. Senisterra, K. Vedadi, D. B. Kireev, C. H. Arrowsmith CH, S. V. Frye and W. P. Janzen, J Biomol Screen, 2010, 62-71.*
3. *L. I. James, D. Barsyte-Lovejoy, N. Zhong, L. Krichevsky, V. K. Korboukh, J. M. Herold, C. J. MacNevin, J. L. Norris, C. A. Sagum, W. Tempel, E. Marcon, H. Guo, C. Gao, X. P. Huang, S. Duan, A. Emili, J. F. Greenblatt, D. B. Kireev, J. Jin, W. P. Janzen, P. J. Brown, M. T. Bedford, C. H. Arrowsmith and S. V. Frye, Nat Chem Biol, 2013, **9**, 184-191.*
4. *Z. Otwinowski and W. Minor, Methods Enzymol., 1997, **276**, 472-494.*
5. *A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, J. Appl. Cryst., 2007, **40**, 658-674.*
6. *P. Emsley and K. Cowtan, Acta Crystallogr D Biol Crystallogr, 2004, **60**, 2126-2132.*
7. *G. N. Murshudov, A. A. Vagin and E. J. Dodson, Acta Crystallogr D Biol Crystallogr, 1997, **53**, 240-255.*
8. *I. W. Davis, L. W. Murray, J. S. Richardson and D. C. Richardson, Nucleic Acids Res., 2004, **32**, W615-W619.*