Synthesis, Optimization, and Evaluation of Novel Small Molecules as Antagonists of WDR5-MLL Interaction

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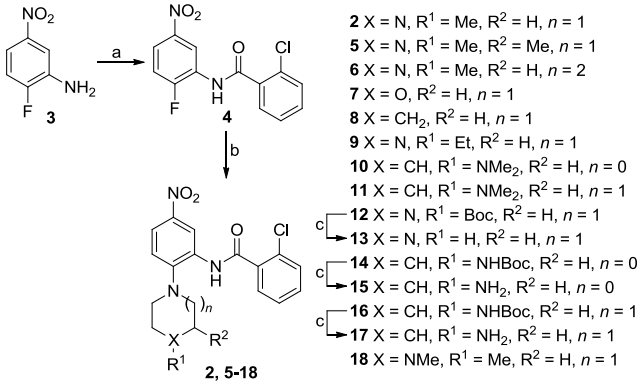
General Information

All oxygen and/or moisture sensitive reactions were carried out under N_2 atmosphere in glassware purged with N_2 prior to use. All reagents and laboratory grade solvents were purchased from commercial vendors and used as received, without further purification. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance-III 500 MHz spectrometer (500 MHz ^{1}H, 125 MHz ^{13}C). Proton chemical shifts are reported in ppm (δ) referenced to the NMR solvent.[1] Data are reported as follows: chemical shifts (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet); coupling constant(s) (*J*) in Hz; integration. Unless otherwise noted, NMR data were collected at 25 °C. Flash column chromatography was performed using a Biotage SP1 system fitted with a KP-SIL SNAP Silica Gel (60 Å mesh) Flash Cartridge (FSKO-1107). Purity determination was conducted by UV absorbance at 254 nm during tandem liquid chromatography/mass spectrometry (LCMS) using a Waters Acquity separations module. Identity was determined via low-resolution mass spectrometry (LRMS) conducted in positive ion mode using a Waters Acquity SQD mass spectrometer (electrospray ionization source) fitted with a PDA detector. Mobile phase A consisted of 0.1% formic acid in water, while mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 5 minutes at 0.5 mL/min. An Acquity CSH C18 (2.1 x 50 mm, 1.7 μm) column was used with column temperature maintained at 25 °C. The sample solution injection volume was 5 μL. Analytical thin-layer chromatography (TLC) was performed on aluminum sheets, silica gel 60 F_{254} (0.2 mm, VWR International, Darmstadt, Germany). Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. High-resolution mass spectrometry (HRMS) was conducted using a Waters Xevo quadrupole-time-of-flight (QTOF) hybrid mass spectrometer system coupled with an Acquity ultra-performance liquid chromatography (UPLC) system. Chromatographic separations were carried out on an Acquity BEH C18 (2.1 x 50 mm, 1.7 μm) column. The mobile phase was 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Leucine Enkephalin was used as lock mass. MassLynx 4.1 was used for data analysis.

[1] Gottlieb, H. E., Kotlyar, V., Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

Experimental Procedures and Compound Characterization Data

Scheme 1. Synthesis of compounds **2**, **4-18**



Step A - Preparation of 2-Chloro-*N*-(2-fluoro-5-nitrophenyl)benzamide (4)

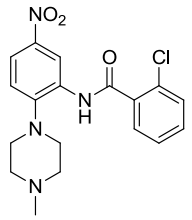
To a solution of commercially available 2-fluoro-5-nitroaniline **3** (0.500 g, 3.20 mmol) in anhydrous dichloromethane (10 mL) was added 2-chlorobenzoyl chloride (0.728 g, 0.527 mL, 4.16 mmol) under N_2 atmosphere at room temperature. This was followed by the addition of pyridine (0.335 mL, 4.16 mmol), and the resulting solution was stirred at room temperature for 4 h. The reaction was subsequently diluted with dichloromethane (10 mL), and washed with water (20 mL). The organic phase was then separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel using 50% EtOAc/hexanes as the eluent, providing **4** as a white solid (1.13 g, 92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 8.92-8.90 (m, 2H), 8.16-8.13 (m, 1H), 8.03-8.00 (m, 1H), 7.66-7.46 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.6, 157.7 (d, $J_{C\text{-}F}$ = 258.1 Hz), 143.6, 135.5, 131.5, 130.0, 129.6, 129.3, 127.1, 126.6 (d, $J_{C\text{-}F}$ = 13.8 Hz), 121.8 (d, $J_{C\text{-}F}$ = 9.4 Hz), 119.9, 117.1 (d, $J_{C\text{-}F}$ = 22.4 Hz).

Step B - General Method for the Preparation of Compounds 2, 5-12, 14, 16, 18 by Addition of Secondary Amines to 2-Chloro-*N*-(2-fluoro-5-nitrophenyl)benzamide (4)

To a solution of 2-chloro-*N*-(2-fluoro-5-nitrophenyl)benzamide **4** (0.200 g, 0.678 mmol) in DMF (4 mL) at room temperature was added the secondary amine of choice (0.881 mmol), followed by *N*,*N*-diisopropylethylamine (0.153 mL, 0.881 mmol). The resulting solution was then heated to 80°C for 1 h, and subsequently cooled to room temperature. Following dilution with EtOAc (40 mL), the organic phase was washed with water (2 x 20 mL), separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes, or by column chromatography on silica gel using a common 5-20% MeOH/EtOAc gradient to afford compounds **2**, **5-12**, **14**, **16**, **18**.

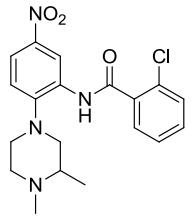
Step C - General Method for the Preparation of Compounds 13, 15, 17 by Deprotection of Compounds 12, 14, 16

To a solution of the Boc-protected amine **12**, **14**, **16** (0.400 mmol) in dichloromethane (5 mL) at room temperature was added trifluoroacetic acid (0.153 mL, 2.00 mmol). The resulting solution was then stirred for 2 h prior to dilution with saturated aqueous sodium bicarbonate solution (10 mL) and dichloromethane (10 mL). The layers were separated, and the organic layer washed with water (2 x 10 mL) before being dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes, or by column chromatography on silica gel using a common 5-20% MeOH/EtOAc gradient to afford compounds **13**, **15**, **17**.



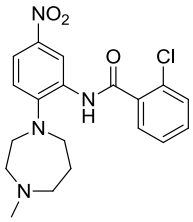
2-Chloro-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (2)

^{1}H NMR (500 MHz, MeOD-d_4) δ 7.76 (d, J = 2.4 Hz, 1H), 7.69 (dd, J = 7.4, 1.4 Hz, 1H), 7.58-7.48 (m, 3H), 7.12 (d, J = 8.5 Hz, 1H), 6.57 (dd, J = 8.5, 2.6 Hz, 1H), 3.02 (br s, 8H), 2.65 (s, 3H). ^{13}C NMR (125 MHz, MeOD-d_4) δ 167.1, 147.2, 137.5, 134.9, 133.9, 132.8, 131.5, 131.4, 130.7, 128.7, 123.2, 113.1, 109.1, 56.1, 52.4, 30.7. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}ClN_4O_3$ $[M+H]^+$: 375.1224, found: 375.1220.



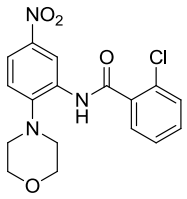
2-Chloro-*N*-(2-(3,4-dimethylpiperazin-1-yl)-5-nitrophenyl)benzamide (5)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.87 (br s, 1H), 8.66 (d, J = 2.2 Hz, 1H), 8.05 (dd, J = 9.0, 2.8 Hz, 1H), 7.67-7.57 (m, 2H), 7.56-7.47 (m,2H), 7.28 (d, J = 9.0 Hz, 1H), 3.33-3.28 (m, 1H), 3.24 (br dt, 1H), 2.95 (td, J = 11.5, 2.6 Hz, 1H), 2.78 (br dt, 1H), 2.56-2.53 (m, 1H), 2.33 (td, J = 11.3, 2.8 Hz, 1H), 2.26-2.19 (m, 1H), 2.20 (s, 3H), 1.00 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 151.0, 141.3, 136.1, 131.4, 130.1, 129.7, 128.9, 127.3, 121.5, 119.9, 119.6 (2), 56.8, 54.5, 50.3 (2), 42.1, 16.5. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_4O_3$ $[M+H]^+$: 389.1380, found: 389.1362.



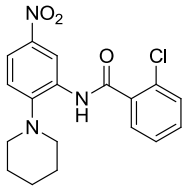
2-Chloro-*N*-(2-(4-methyl-1,4-diazepan-1-yl)-5-nitrophenyl)benzamide (6)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.40 (br s, 1H), 8.34 (d, J = 2.8 Hz, 1H), 7.98 (dd, J = 9.3, 2.8 Hz, 1H), 7.65-7.55 (m, 2H), 7.55-7.42 (m, 2H), 7.16 (d, J = 9.4 Hz, 1H), 3.58-3.53 (m, 2H), 3.50-3.46 (m, 2H), 2.90-2.84 (m, 2H), 2.75-2.70 (m, 2H), 2.50 (s,3H), 1.77-1.69 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 152.2, 138.0, 136.3, 131.2, 129.9, 129.7, 128.7, 127.2, 125.7, 123.8, 122.3, 118.3, 54.7 (2), 51.1, 48.4, 48.2, 30.0. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_4O_3$ $[M+H]^+$: 389.1380, found: xxxx.



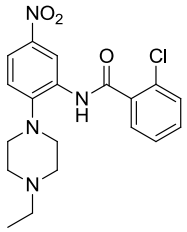
2-Chloro-*N*-(2-morpholino-5-nitrophenyl)benzamide (7)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.70 (s, 1H), 8.07 (dd, J = 9.0, 2.8 Hz, 1H), 7.66-7.63 (m, 1H), 7.61-7.57 (m, 1H), 7.56-7.46 (m, 2H), 7.30 (d, J = 9.0 Hz, 1H), 3.76 (br t, 4H), 3.10 (br t, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 151.0, 141.6, 136.2, 131.3, 130.4, 129.7 (2), 128.9, 127.3, 121.4, 119.9, 119.7, 65.8 (2), 50.6 (2). HRMS (ESI) *m/z* calcd for $C_{17}H_{16}ClN_3O_4$ $[M+H]^+$: 362.0908, found: 362.0942.



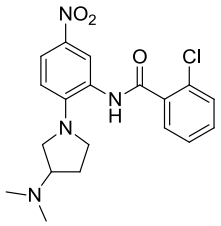
2-Chloro-*N*-(5-nitro-2-(piperidin-1-yl)phenyl)benzamide (8)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.69 (d, J = 2.2 Hz, 1H), 8.05 (dd, J = 9.0, 2.8 Hz, 1H), 7.66-7.63 (m, 1H), 7.61-7.57 (m, 1H), 7.56-7.47 (m, 2H), 7.27 (d, J = 9.0 Hz, 1H), 3.04 (br t, 4H), 1.70-1.62 (m, 4H), 1.59-1.52 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 152.1, 141.1, 136.2, 131.3, 130.3, 129.8 (2), 128.9, 127.3, 121.4, 119.7, 119.4, 51.6 (2), 25.3 (2), 23.5. HRMS (ESI) *m/z* calcd for $C_{18}H_{18}ClN_3O_3$ $[M+H]^+$: 360.1115, found: 360.1101.



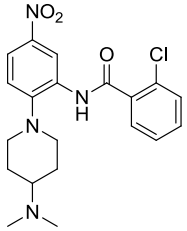
2-Chloro-*N*-(2-(4-ethylpiperazin-1-yl)-5-nitrophenyl)benzamide (9)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.48 (d, J = 2.0 Hz, 1H), 9.23 (br s, 1H), 8.03 (dd, J = 8.8, 2.7 Hz, 1H), 7.87 (dd, J = 7.4, 1.5 Hz, 1H), 7.53-7.44 (m, 3H), 7.32 (d, J = 8.8 Hz, 1H), 3.04 (br s, 4H), 2.65 (br m, 4H), 2.52 (q, J = 6.6 Hz, 2H), 1.15 (q, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.3, 147.4, 145.0, 134.6, 133.8, 132.2, 130.9, 130.6, 130.3, 127.5, 120.8, 119.7, 115.3, 52.9 (2), 52.3 (2), 52.0, 11.9. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_4O_3$ $[M+H]^+$: 389.1380, found: 389.1356.



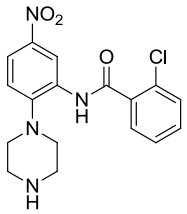
2-Chloro-*N*-(2-(3-(dimethylamino)pyrrolidin-1-yl)-5-nitrophenyl)benzamide (10)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.05-7.99 (m, 2H), 7.66-7.62 (m, 1H), 7.60-7.57 (m, 1H), 7.55-7.46 (m, 2H), 6.84 (d, J = 9.3 Hz, 1H), 3.72-3.67 (m, 1H), 3.66-3.56 (m, 2H), 3.47-3.41 (m, 1H), 2.66-2.63 (m, 1H), 2.15 (s, 6H), 2.20-2.04 (m, 1H), 1.80-1.69 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.1, 150.1, 136.2, 135.9, 131.2, 130.1, 129.9, 128.8, 127.2, 126.6, 123.9, 120.9, 114.1, 64.7, 54.9, 54.4, 49.3, 43.9, 29.4. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_4O_3$ $[M+H]^+$: 389.1380, found: 389.1384.



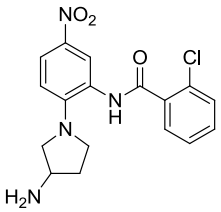
2-Chloro-*N*-(2-(4-(dimethylamino)piperidin-1-yl)-5-nitrophenyl)benzamide (11)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.68 (d, J = 2.1 Hz, 1H), 8.04 (dd, J = 9.0, 2.8 Hz, 1H), 7.67-7.63 (m, 1H), 7.62-7.58 (m, 1H), 7.56-7.48 (m, 2H), 7.27 (d, J = 9.0 Hz, 1H), 3.47-3.39 (m, 2H), 2.74 (br t, 2H), 2.25-2.16 (m, 1H), 2.18 (s, 6H), 1.88-1.80 (m, 2H), 1.65-1.55 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 151.6, 141,1, 136.2, 131.3, 130.2, 129.8, 129.7, 128.9, 127.3, 121.3, 119.6 (2), 61.0, 50.0 (2), 41.6 (2), 28.0 (2). HRMS (ESI) *m/z* calcd for $C_{20}H_{23}ClN_4O_3$ $[M+H]^+$: 402.1537, found: 402.1519.



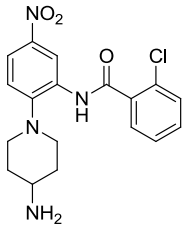
2-Chloro-*N*-(5-nitro-2-(piperazin-1-yl)phenyl)benzamide (13)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.68 (s, 1H), 8.06 (dd, J = 9.0, 2.8 Hz, 1H), 7.67-7.63 (m, 1H), 7.61-7.58 (m, 1H), 7.56-7.47 (m, 2H), 7.27 (d, J = 9.0 Hz, 1H), 3.30 (br s, 1H), 3.05-3.00 (m, 4H), 2.90-2.86 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 150.7, 141.3, 136.2, 131.3, 130.3, 129.7 (2), 128.9, 121.4, 119.7 (2), 119.4, 51.2 (2), 45.0 (2). HRMS (ESI) *m/z* calcd for $C_{17}H_{17}ClN_4O_3$ $[M+H]^+$: 361.1067, found: 361.1089.



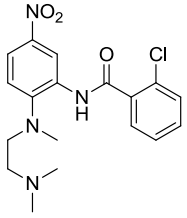
(*S*)-*N*-(2-(3-Aminopyrrolidin-1-yl)-5-nitrophenyl)-2-chlorobenzamide (15)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, *J* = 2.6 Hz, 1H), 8.19 (br s, 1H), 7.94 (dd, *J* = 2.6, 9.2 Hz, 1H), 7.75 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.41-7.31 (m, 3H), 6.75 (d, *J* = 9.2 Hz, 1H), 3.69-3.52 (m, 3H), 3.40-3.35 (m, 1H), 3.10 (dd, *J* = 4.4, 10.0 Hz, 1H), 2.14-2.07 (m, 1H), 1.72-1.66 (m, 1H), 1.5 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4, 150.0, 136.3, 135.9, 131.5, 130.3, 129.8, 128.8, 127.2, 126.6, 123.9, 120.9, 114.1, 64.7, 55.0, 49.7, 29.6. HRMS (ESI) *m/z* calcd for $C_{17}H_{17}ClN_4O_3$ $[M+H]^+$: 361.1067, found: 361.1092.



***N*-(2-(4-Aminopiperidin-1-yl)-5-nitrophenyl)-2-chlorobenzamide (17)**

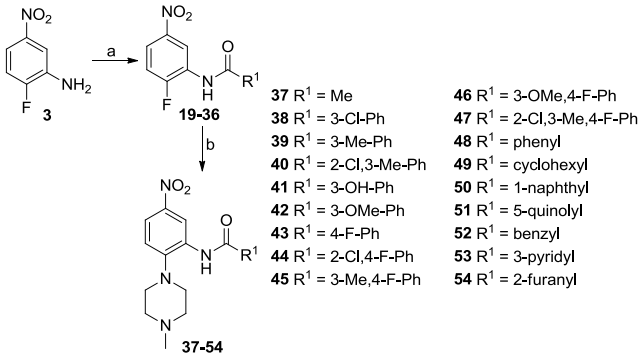
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.93 (br s, 1H), 8.68 (d, *J* = 2.5 Hz, 1H), 8.07 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.70-7.66 (m, 1H), 7.61-7.57 (m, 1H), 7.56-7.51 (m, 1H), 7.50-7.46 (m, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 3.45-3.38 (br t, 2H), 3.32 (br s, 2H), 3.21-3.13 (m, 1H), 2.90-2.84 (m, 2H), 2.00-1.95 (m, 2H), 1.75-1.61 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 151.2, 141.5, 136.2, 131.3, 130.4, 129.7, 128.9, 127.3, 121.4, 120.0, 118.5, 116.1, 48.8 (2), 47.4, 30.0 (2). HRMS (ESI) *m/z* calcd for $C_{18}H_{19}ClN_4O_3$ $[M+H]^+$: 375.1224, found: 375.1235.



2-Chloro-*N*-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-nitrophenyl)benzamide (18)

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.22 (br s, 1H), 9.13 (d, *J* = 2.7 Hz, 1H), 8.00 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.67-7.64 (m, 1H), 7.61-7.58 (m, 1H), 7.56-7.47 (m, 2H), 7.34 (d, *J* = 9.1 Hz, 1H), 3.06 (br t, 2H), 2.85 (s, 3H), 2.37 (br t, 2H), 1.77 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.6, 149.6, 140.9, 136.6, 131.4, 130.9, 130.1, 129.8, 129.1, 127.4, 120.1, 119.9, 117.2, 56.0, 54.7, 45.6 (2), 40.1. HRMS (ESI) *m/z* calcd for $C_{18}H_{21}ClN_4O_3$ $[M+H]^+$: 377.138, found: 377.1372.

Scheme 2. Synthesis of compounds **37-54**

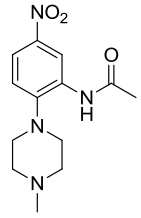


Step A - General Method for the Preparation of Compounds 19-36 by Acylation of 2-fluoro-5-nitroaniline (3)

To a solution of commercially available 2-fluoro-5-nitroaniline **3** (0.500 g, 3.20 mmol) in dry dichloromethane (10 mL) was added the benzoyl or acetyl chloride (4.16 mmol) under N_2 atmosphere at room temperature. This was followed by the addition of pyridine (0.335 mL, 4.16 mmol), and the resulting solution was stirred at room temperature for 4 h. The reaction was subsequently diluted with dichloromethane (10 mL), and washed with water (20 mL). The organic phase was then separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel using 50% EtOAc/hexanes as the eluent to provide **19-36**.

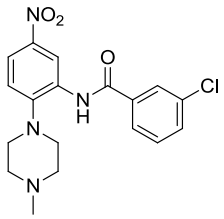
Step B - General Method for the Preparation of Compounds 37-54 by Addition of 1-Methylpiperazine to Compounds 19-36

To a solution of the benzamide **19-36** (0.500 mmol) in DMF (4 mL) at room temperature was added 1-methylpiperazine (0.072 mL, 0.650 mmol), followed by *N*,*N*-diisopropylethylamine (0.113 mL, 0.881 mmol). The resulting solution was then heated to 80°C for 1 h, and subsequently cooled to room temperature. Following dilution with ethyl acetate (40 mL), the organic phase was washed with water (2 x 20 mL), separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes, or by column chromatography on silica gel using a common 5-20% MeOH/EtOAc gradient to afford compounds **37-54**.



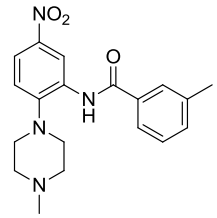
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)acetamide (37)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.13 (br s, 1H), 8.58 (br s, 1H), 7.96 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 3.02 (br t, 4H), 2.54 (br t, 4H), 2.25 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.7, 150.2, 141.5, 130.9, 120.3, 119.3, 118.6, 54.1 (2), 50.0 (2), 45.7, 23.7. HRMS (ESI) *m/z* calcd for $C_{13}H_{18}N_4O_3$ $[M+H]^+$: 279.1457, found: 279.1464.



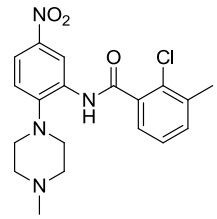
3-Chloro-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (38)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.41 (d, *J* = 2.7 Hz, 1H), 9.18 (br s, 1H), 7.95-7.94 (m, 1H), 7.84-7.82 (m, 1H), 7.62-7.60 (m, 1H), 7.52 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 3.05 (t, *J* = 4.8 Hz, 4H), 2.69 (br s, 4H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.4, 147.1, 145.0, 136.0, 135.3, 133.5, 132.3, 130.4, 127.4, 125.0, 120.6, 119.6, 114.9, 55.7 (2), 51.8 (2), 46.1. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}ClN_4O_3$ $[M+H]^+$: 375.1224, found: 375.1227.



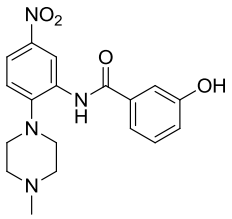
3-Methyl-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (39)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (d, *J* = 2.7 Hz, 1H), 9.16 (br s, 1H), 8.00 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.79 (s, 1H), 7.74-7.72 (m, 1H), 7.47-7.42 (m, 3H), 3.05 (d, *J* = 4.8 Hz, 4H), 2.68 (br s, 4H), 2.49 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 147.1, 145.0, 138.9, 134.2, 133.8, 133.1, 128.9, 127.8, 123.9, 120.4, 119.2, 114.9, 55.7 (2), 51.7 (2), 46.2, 21.5. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_4O_3$ $[M+H]^+$: 355.1770, found: 355.1749.



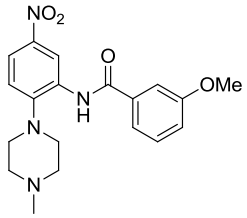
2-Chloro-3-methyl-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (40)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (d, *J* = 2.5 Hz, 1H), 8.99 (br s, 1H), 8.00 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.40-7.28 (m, 3H), 7.11 (dd, *J* = 1.6, 8.1 Hz, 1H), 3.98 (s, 3H), 3.00 (t, *J* = 4.8 Hz, 4H), 2.58 (br s, 4H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.6, 155.5, 147.5, 144.8, 136.5, 133.6, 128.1, 121.5, 120.6, 119.7, 119.1, 115.3, 114.1, 56.6, 55.2 (2), 52.0 (2), 46.0. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_4O_3$ $[M+H]^+$: 389.1380, found: 389.1385.



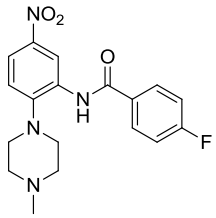
3-Hydroxy-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (41)

^{1}H NMR (500 MHz, MeOD-d_4) δ 8.88 (d, J = 2.7 Hz, 1H), 7.97 (dd, J = 2.7, 8.9 Hz, 1H), 7.32-7.26 (m, 4H), 6.96-6.94 (m, 1H), 3.00 (t, J = 4.8 Hz, 4H), 2.58 (br s, 4H), 2.28 (s, 3H). ^{13}C NMR (125 MHz, MeOD-d_4) δ 167.7, 159.5, 150.9, 145.1, 136.7, 133.6, 131.1, 121.7, 121.5, 120.6, 118.9, 118.8, 115.3, 56.3 (2), 51.9 (2), 46.1. HRMS (ESI) *m/z* calcd for $C_{18}H_{20}N_4O_4$ $[M+H]^+$: 357.1563, found: 357.1581.



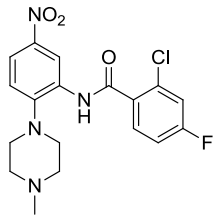
3-Methoxy-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (42)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.42 (d, J = 2.6 Hz, 1H), 9.14 (s, 1H), 7.99 (dd, J = 2.6, 8.8 Hz, 1H), 7.52-7.53 (m, 1H), 7.43-7.47 (m, 2H), 7.28 (d, J = 8.8 Hz, 1H), 7.15 (dt, J = 2.5, 6.8 Hz, 1H), 3.91 (s, 3H), 3.04 (t, J = 4.6 Hz, 4H), 2.68 (bs, 4H), 2.42 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 160.4, 147.3, 145.1, 135.8, 133.9, 130.1, 120.6, 119.5, 118.9, 118.6, 115.2, 112.7, 55.9 (2), 55.8, 51.9 (2), 46.3. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_4O_4$ $[M+H]^+$: 371.1719, found: 371.1715.



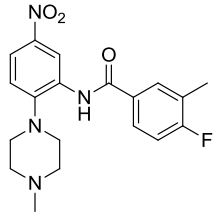
4-Fluoro-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (43)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (br s, 1H), 8.61 (d, J = 2.8 Hz, 1H), 8.10-8.03 (m, 3H), 7.42 (t, J = 8.9 Hz, 2H), 7.31 (d, J = 9.0 Hz, 1H), 3.08 (br t, 4H), 2.48 (br t, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3 (d, $J_{C\text{-}F}$ = 248.2 Hz), 163.9, 151.4, 141.4, 130.5, 130.4, 130.2, 130.1, 121.3, 120.1, 119.7, 115.7, 115.6, 54.5 (2), 50.0 (2), 45.7. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}FN_4O_3$ $[M+H]^+$: 359.1513, found: 359.1519.



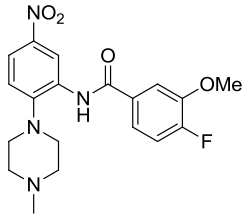
2-Chloro-4-fluoro-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (44)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.46 (d, *J* = 1.8 Hz, 1H), 9.26 (br s, 1H), 8.03 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.93 (dd, *J* = 8.7, 6.1 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.26 (d, *J* = 2.5 Hz, 1H) 7.20-7.16 (m, 1H), 3.03 (br s, 4H), 2.62 (br s, 4H), 2.40 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8 (d, $J_{C\text{-}F}$ = 255.4 Hz), 163.3, 147.3, 145.0, 133.7, 133.0 (d, $J_{C\text{-}F}$ = 9.3 Hz), 131.6 (d, $J_{C\text{-}F}$ = 10.5 Hz), 130.8 (d, $J_{C\text{-}F}$ = 3.7 Hz), 120.9, 119.8, 118.0 (d, $J_{C\text{-}F}$ = 9.3 Hz), 115.3, 115.0, 55.2 (2), 52.0 (2), 46.0. HRMS (ESI) *m/z* calcd for $C_{18}H_{18}ClFN_4O_3$ $[M+H]^+$: 393.113, found: 393.1115.



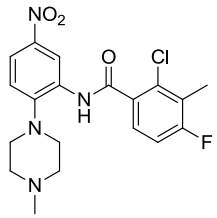
4-Fluoro-3-methyl-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (45)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.68 (br s, 1H), 8.61 (br s, 1H), 8.04 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.93 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.89-7.85 (m, 1H), 7.38-7.31 (m, 2H), 3.10-3.06 (m, 4H), 2.50-2.46 (m, 4H), 2.34 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.1, 162.9 (d, $J_{C\text{-}F}$ = 237.5 Hz), 151.2, 141.5, 131.3 (d, $J_{C\text{-}F}$ = 6.2 Hz), 130.7, 130.0 (d, $J_{C\text{-}F}$ = 3.3 Hz), 127.3 (d, $J_{C\text{-}F}$ = 9.3 Hz), 124.7 (d, $J_{C\text{-}F}$ = 17.8 Hz), 121.1, 119.8, 119.7, 115.3 (d, $J_{C\text{-}F}$ = 22.7 Hz), 54.6 (2), 50.0 (2), 45.7, 14.2 (d, $J_{C\text{-}F}$ = 2.9 Hz). HRMS (ESI) *m/z* calcd for $C_{19}H_{21}FN_4O_3$ $[M+H]^+$: 373.1676, found: 373.1676.



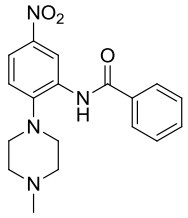
4-Fluoro-3-methoxy-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (46)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38 (d, *J* = 2.7 Hz, 1H), 9.11 (br s, 1H), 7.99 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.68 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.41-7.38 (m, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.23 (dd, *J* = 8.4, 10.5 Hz, 1H), 4.01 (s, 3H), 3.04 (t, *J* = 4.7 Hz, 4H), 2.67 (br s, 4H), 2.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 154.9 (d, $J_{C\text{-}F}$ = 253.8 Hz), 148.4 (d, $J_{C\text{-}F}$ = 11.0 Hz), 147.1, 144.9, 133.6, 130.8 (d, $J_{C\text{-}F}$ = 3.5 Hz), 120.5, 119.4, 118.7 (d, $J_{C\text{-}F}$ = 7.9 Hz), 116.2 (d, $J_{C\text{-}F}$ = 19.2 Hz), 114.9, 113.2 (d, $J_{C\text{-}F}$ = 3.0 Hz), 56.4, 55.7 (2), 51.8 (2), 46.1. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}FN_4O_4$ $[M+H]^+$: 389.1625, found: 389.1612.



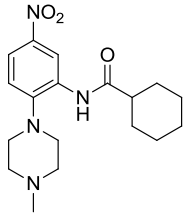
2-Chloro-4-fluoro-3-methyl-*N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (47)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (br s, 1H), 8.99 (br s, 1H), 8.00 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.40-7.33 (m, 2H), 7.30-7.27 (m, 1H), 7.10 (dd, *J* = 10.0, 5.0 Hz, 1H), 3.00 (t, *J* = 5.0 Hz, 4H), 2.58 (br s, 4H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.6, 155.5, 147.5, 144.8, 136.5, 133.6, 128.1, 121.5, 120.6, 119.7, 119.1, 115.3, 114.1, 56.6, 55.2 (2), 52.0 (2), 46.0. HRMS (ESI) *m/z* calcd for $C_{19}H_{20}ClFN_4O_3$ $[M+H]^+$: 407.1286, found: 407.1280.



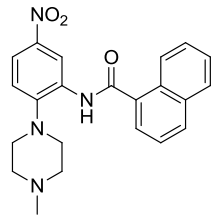
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)benzamide (48)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (br s, 1H), 8.68 (d, *J* = 2.8 Hz, 1H), 8.06 (dd, *J* = 9.0, 2.8 Hz, 1H), 8.04-7.90 (m, 2H), 7.67-7.57 (m, 3H), 7.34 (d, *J* = 9.0 Hz, 1H), 3.09 (br t, 4H), 2.49 (br t, 4H), 2.23 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 151.0, 141.7, 133.8, 132.1, 130.9, 128.7, 128.5, 127.6, 127.3, 121.0, 119.8, 119.3, 54.6 (2), 50.1 (2), 45.7. HRMS (ESI) *m/z* calcd for $C_{18}H_{20}N_4O_3$ $[M+H]^+$: 341.1614, found: 341.1613.



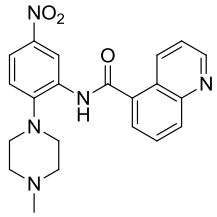
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)cyclohexanecarboxamide (49)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (br s, 1H), 8.58 (d, *J* = 2.7 Hz, 1H), 7.97 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 1H), 3.00 (br t, 4H), 2.53 (br t, 4H), 2.26 (s, 3H), 1.87-1.84 (m, 2H), 1.79-1.73 (m, 2H), 1.68-1.63 (m, 1H), 1.48-1.38 (m, 2H), 1.35-1.15 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.3, 150.3, 141.7, 131.1, 120.2, 119.5, 118.6, 54.2 (2), 50.0 (2), 45.7, 44.2, 29.0 (2), 25.4, 25.1 (2). HRMS (ESI) *m/z* calcd for $C_{18}H_{26}N_4O_3$ $[M+H]^+$: 347.2083, found: 347.2086.



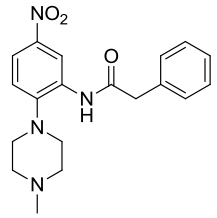
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)-1-naphthamide (50)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.79 (br s, 1H), 8.33 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.14-8.03 (m 3H), 7.84 (d, *J* = 6.7 Hz, 1H), 7.67-7.60 (m, 3H), 7.33 (d, *J* = 9.0 Hz, 1H), 3.13 (br t, 4H), 2.45 (br t, 4H), 2.19 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.3, 151.4, 141.6, 133.7, 133.3, 130.9, 130.7, 129.7, 128.4, 127.1, 126.5, 125.3, 125.2, 125.0, 121.3, 119.8 (2), 54.3 (2), 50.2 (x2), 45.6. HRMS (ESI) *m/z* calcd for $C_{22}H_{22}N_4O_3$ $[M+H]^+$: 391.1770, found: 391.1754.



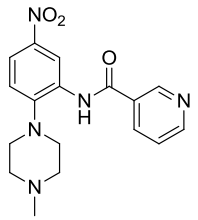
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)quinoline-5-caboxamide (51)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.97 (br s 1H), 9.00 (dd, *J* = 4.1, 1.7 Hz, 1H), 8.80-8.75 (m, 2H), 8.21 (d, *J* = 8.3 Hz, 1H), 8.09 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.99-7.88 (m, 2H), 7.66 (q, *J* = 4.2 Hz, 1H), 7.32 (d, *J* = 9.0 Hz, 1H), 3.14 (br t, 4H), 2.46 (br t, 4H), 2.19 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.3, 151.8, 151.0, 147.7, 141.5, 133.8, 133.7, 131.8, 130.6, 128.6, 125.9, 125.2, 122.3, 121.6, 120.4, 119.8, 54.3 (2), 50.2 (2), 45.7. HRMS (ESI) *m/z* calcd for $C_{21}H_{21}N_5O_3$ $[M+H]^+$: 392.1723, found: 392.1728.



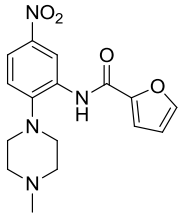
***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)-2-phenylacetamide (52)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (d, *J* = 2.7 Hz, 1H), 8.23 (bs, 1H), 7.93 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.50-7.53 (m, 2H), 7.42-7.46 (m, 3H), 7.22 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 2H), 2.95 (bs, 4H), 2.54 (s, 3H), 2.45 (bs, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.9, 145.4, 145.3, 134.6, 133.6, 130.4 (2), 129.7 (2), 128.1, 121.0, 119.4, 114.8, 53.8 (2), 49.7 (2), 45.3, 44.3. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_4O_3$ $[M+H]^+$: 355.1770, found: 355.1782.



***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)nicotinamide (53)**

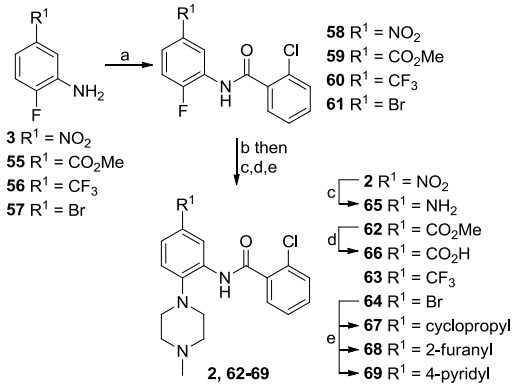
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (d, *J* = 2.7 Hz, 1H), 9.23 (br s, 1H), 9.17 (d, *J* = 1.9 Hz, 1H), 8.86 (dd, *J* = 1.7, 4.8 Hz, 1H), 8.37-8.35 (m, 1H), 8.05 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.56 (ddd, *J* = 0.8, 4.8, 8.0 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 3.06 (d, *J* = 4.7 Hz, 4H), 2.69 (br s, 4H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.0, 153.1, 147.3, 147.2, 145.0, 135.7, 133.3, 130.0, 124.1, 120.7, 119.8, 115.1, 55.6 (2), 51.9 (2), 46.0. HRMS (ESI) *m/z* calcd for $C_{17}H_{19}N_5O_3$ $[M+H]^+$: 342.1566, found: 342.1581.



***N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)furan-2-carboxamide (54)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (d, *J* = 2.6 Hz, 1H), 8.97 (bs, 1H), 8.00 (dd, *J* = 2.6, 8.7 Hz, 1H), 7.57 (d, *J* = 0.9 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 3.7 Hz, 1H), 6.66 (dd, *J* = 1.5, 3.7 Hz, 1H), 3.57 (bs, 4H), 3.25 (bs, 4H), 2.88 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.51, 147.53, 145.76, 144.59, 132.92, 124.86, 121.10, 119.70, 116.67, 115.56, 113.39, 54.18 (2), 53.40 (2), 48.42. HRMS (ESI) *m/z* calcd for $C_{16}H_{18}N_4O_4$ $[M+H]^+$: 331.1406, found: 331.1421.

Scheme 3. Synthesis of compounds **2**, **62-69**



Step A - General Method for the Preparation of Compounds 58-61 from 5-Substituted-2-fluoro-anilines 3, 55-57

To a solution of the commercially available 5-substituted-2-fluoro-aniline **3**, **55-57** (3.20 mmol) in dry dichloromethane (10 mL) was added 2-chlorobenzoyl chloride (0.527 mL, 4.16 mmol) under N_2 atmosphere at room temperature. This was followed by the addition of pyridine (0.335 mL, 4.16 mmol), and the resulting solution was stirred at room temperature for 4 h. The reaction was subsequently diluted with dichloromethane (10 mL), and washed with water (20 mL). The organic phase was then separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel using 50% EtOAc/hexanes as the eluent, providing **58-61**.

Step B - General Method for the Preparation of Compounds 2, 62-64 by Addition of 1-Methylpiperazine to Benzamides 58-61

To a solution of the benzamides **58-61** (0.500 mmol) in DMF (4 mL) at room temperature was added 1-methylpiperazine (0.072 mL, 0.650 mmol), followed by *N*,*N*-diisopropylethylamine (0.113 mL, 0.881 mmol). The resulting solution was then heated to 80°C for 1 h, and subsequently cooled to room temperature. Following dilution with ethyl acetate (40 mL), the organic phase was washed with water (2 x 20 mL), separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes, or by column chromatography on silica gel using a common 5-20% MeOH/EtOAc gradient to afford compounds **2**, **62-64**.

Step C - Preparation of *N*-[5-Amino-2-(4-methylpiperazin-1-yl)phenyl]-2-chlorobenzamide (65)

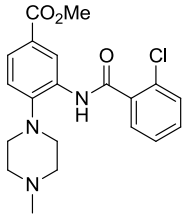
To a solution of 2-chloro-*N*-[2-(4-methylpiperazin-1-yl)-5-nitrophenyl]benzamide **2** (0.150 g, 0.441 mmol) in EtOH/H_2O (2:3, 10 mL) at room temperature was added iron powder (<212 μm, 0.123 g, 2.21 mmol) and copper(II) chloride (0.024 g, 0.216 mmol). The resulting solution was then heated to 100 °C with vigorous stirring for 2 h, and subsequently cooled to room temperature. Following dilution with brine (10 mL) and extraction with EtOAc (2 x 20 mL), the organic phase was separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel using a 0-20% MeOH/CH_2Cl_2 gradient to afford compound **65** as a white solid (0.125 g, 91%).

Step D - Preparation of 3-(2-Chlorobenzamido)-4-(4-methylpiperazin-1-yl)benzoic acid (66)

To a solution of methyl 3-(2-chlorobenzamido)-4-(4-methylpiperazin-1-yl)benzoate **62** (0.410 g, 1.06 mmol) in THF/MeOH/H_2O (1:0.5:1, 10 mL) at room temperature was added LiOH·H_2O (0.133 g, 3.18 mmol). The resulting solution was then heated to 45°C for 1 h, and subsequently cooled to room temperature. Following acidification to pH = 6 with 1 *N* HCl, dilution with brine (20 mL), and extraction with ethyl acetate (2 x 40 mL), the organic phase was separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes to afford compound **66** as a white solid (0.348 g, 88%).

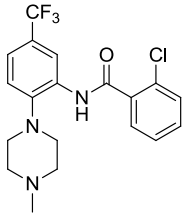
Step E - General Methods for the Preparation of Compounds 67-69 from *N*-(5-Bromo-2-(4-methylpiperazin-1-yl)phenyl)-2-chlorobenzamide (64)

To a solution of *N*-(5-bromo-2-(4-methylpiperazin-1-yl)phenyl)-2-chlorobenzamide **64** (6.82 mmol) in toluene/EtOH (1:1, 30 mL) at room temperature and under a N_2 atmosphere was added the requisite boronic acid (7.64 mmol), sodium carbonate (13.2 mmol, 1.40 g) and tetrakis(triphenylphosphine)palladium(0) (0.200 mmol, 0.231 g). The resulting solution was then heated to 115°C in a pressure vessel for 3 h, and subsequently cooled to room temperature. Following dilution with brine (20 mL) and extraction with EtOAc (2 x 40 mL), the organic phase was separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes, or by column chromatography on silica gel using a common 5-20% MeOH/EtOAc gradient to afford compounds **67-69**.



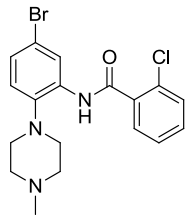
Methyl 3-(2-chlorobenzamido)-4-(4-methylpiperazin-1-yl)benzoate (62)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, *J* = 1.7 Hz, 1H), 9.17 (br s, 1H), 7.99 (dd, *J* = 2.0, 8.3 Hz, 1H), 7.83 (dd, *J* = 1.7, 7.4 Hz, 2H), 7.53-7.42 (m, 3H), 3.94 (s, 3H), 2.99 (t, *J* = 4.8 Hz, 4H), 2.58 (br s, 4H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 164.3, 145.9, 135.3, 133.2, 131.8, 130.6, 130.5, 130.4, 127.4, 127.2, 126.2, 121.0, 120.6, 55.4 (2), 52.1 (3), 46.1. HRMS (ESI) *m/z* calcd for $C_{20}H_{22}ClN_3O_3$ $[M+H]^+$: 388.1428, found: 388.1417.



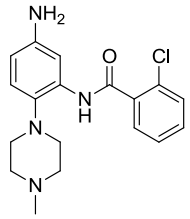
2-Chloro-*N*-(2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)benzamide (63)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 (br s, 1H), 8.28 (br s, 1H), 7.69-7.65 (m, 1H), 7.62-7.59 (m, 1H), 7.55-7.50 (m, 2H), 7.46-7.41 (m, 1H), 7.39-7.37 (m, 1H), 2.98 (br t, 4H), 2.47 (br t, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.4, 148.0, 135.8, 131.5, 130.4, 129.8, 129.4, 129.1, 127.9, 127.6, 127.4, 122.3, 120.8, 119.7, 54.5 (2), 50.7 (2), 45.6. HRMS (ESI) *m/z* calcd for $C_{19}H_{19}ClF_3N_3O$ $[M+H]^+$: 398.1247, found: 398.1222.



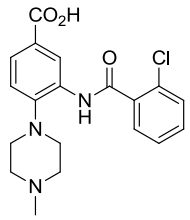
***N*-(5-Bromo-2-(4-methylpiperazin-1-yl)phenyl)-2-chlorobenzamide (64)**

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.26 (bs, 1H), 8.85 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.44-7.50 (m, 3H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 3.27 (bm, 8H), 2.77 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3, 140.0, 135.2, 135.1, 132.1, 131.0, 130.7, 130.3, 127.7, 127.4, 123.1, 122.8, 119.8, 55.4 (2), 51.9 (2), 45.6. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}BrClN_3O$ $[M+H]^+$: 408.0478, found: 408.0458.



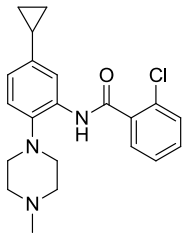
***N*-(5-Amino-2-(4-methylpiperazin-1-yl)phenyl)-2-chlorobenzamide (65)**

^{1}H NMR (500 MHz, MeOD-d_4) δ 7.76 (d, *J* = 2.4 Hz, 1H), 7.68 (dd, *J* = 1.4, 7.4 Hz, 1H), 7.58-7.48 (m, 3H), 7.12 (d, *J* = 8.5 Hz, 1H), 6.57 (dd, *J* = 2.6, 8.5 Hz, 1H), 3.333 (m, 3H), 3.02 (br s, 8H), 2.66 (s, 3H). ^{13}C NMR (125 MHz, MeOD-d_4) δ 167.1, 147.2, 137.5, 134.9, 133.9, 132.8, 131.5, 131.4, 130.7, 128.7, 123.2, 113.1, 109.1, 56.1 (2), 52.4 (2), 30.7. HRMS (ESI) *m/z* calcd for $C_{18}H_{21}ClN_4O$ $[M+H]^+$: 345.1482, found: 345.1485.



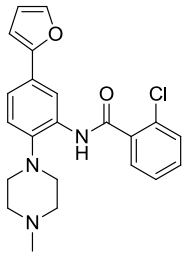
3-(2-Chlorobenzamido)-4-(4-methylpiperazin-1-yl)benzoic acid (66)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (d, *J* = 1.3 Hz, 1H), 7.91 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.66 (dd, *J* = 1.3, 7.5 Hz, 1H), 7.46-7.56 (m, 4H), 7.35-7.37 (m, 1H), 3.29-3.61 (m, 8H), 2.96 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 168.5, 148.7, 137.7, 133.7, 132.8, 131.8, 131.4, 130.3, 129.2, 128.7, 128.0, 127.2, 121.6, 55.1 (2), 50.1 (2), 43.9. HRMS (ESI) *m/z* calcd for $C_{19}H_{20}ClN_3O_3$ $[M+H]^+$: 374.1271, found: 374.1264.



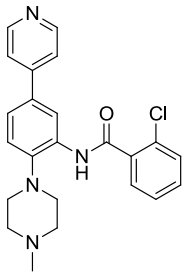
2-Chloro-*N*-(5-cyclopropyl-2-(4-methylpiperazin-1-yl)phenyl)benzamide (67)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.38 (d, J = 1.8 Hz, 1H), 7.81 (dd, J = 1.8, 7.0 Hz, 1H),7.48-7.50 (m, 1H), 7.46-7.40 (m, 2H), 7.18 (d, J = 8.3 Hz, 1H), 6.84 (dd, J = 2.2, 8.1 Hz, 1H), 3.04 (bs, 4H), 2.76 (bs, 4H), 2.49 (s, 3H), 1.96-1.91 (m, 1H), 0.99-0.95 (m, 2H), 0.76-0.73 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.2, 142.7, 138.4, 135.9, 133.9, 131.8, 130.9, 130.7, 130.4, 127.6, 121.6, 121.5, 117.2, 55.5 (x2), 51.9 (x2), 45.5, 15.7, 9.5 (2). HRMS (ESI) *m/z* calcd $C_{21}H_{24}ClN_3O$ for $[M+H]^+$: 370.1686, found: 370.1676.



2-Chloro-*N*-(5-(furan-2-yl)-2-(4-methylpiperazin-1-yl)phenyl)benzamide (68)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.52 (br s, 1H), 8.39 (br s, 1H), 7.75 (d, J = 1.3 Hz, 1H), 7.70-7.64 (m, 2H), 7.63-7.59 (m, 1H), 7.44-7.40 (m, 1H), 7.38-7.34 (m, 1H), 7.26 (d, J = 7.5 Hz, 1H), 6.85 (d, J = 3.2 Hz, 1H), 6.59 (q, J = 1.8 Hz, 1H), 2.92 (br t, 4H), 2.48 (br t, 4H), 2.24 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 152.8, 143.1, 142.7, 136.2, 132.5, 131.5, 130.7, 130.0, 129.3, 127.4, 126.8, 120.9, 120.5, 117.4, 112.0, 105.2, 54.5 (2), 50.9 (2), 45.3. HRMS (ESI) *m/z* calcd for $C_{22}H_{22}ClN_3O_2$ $[M+H]^+$: 396.1479, found: 396.1457.



2-Chloro-*N*-(2-(4-methylpiperazin-1-yl)-5-(pyridin-4-yl)phenyl)benzamide (69)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.60 (br s, 1H), 8.64 (dd, J = 4.6, 1.5 Hz, 2H), 8.40 (br s, 1H), 7.71-7.60 (m, 5H), 7.57-7.48 (m, 2H), 7.34 (d, J = 8.3 Hz, 1H), 2.95 (br t, 4H), 2.48 (br t, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 150.3, 146.5, 145.3, 136.2, 132.5, 132.4, 131.5, 129.9, 129.6, 129.2, 127.4, 126.3, 123.8, 121.0 (2), 120.8 (2), 54.8 (2), 51.1 (2), 45.8. HRMS (ESI) *m/z* calcd for $C_{23}H_{23}ClN_4O$ $[M+H]^+$: 407.1639, found: 407.1637.

Cloning, Expression and Purification of Human WDR5

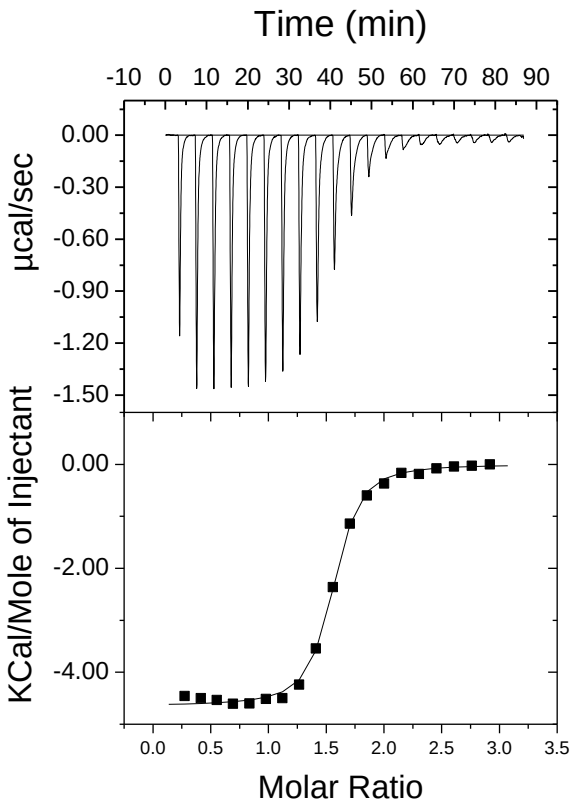
DNA fragment encoding human WDR5 (residues 1-334) was amplified by PCR and sub-cloned into pET28-LIC vector, downstream of the poly-histidine coding region. The protein was expressed in E. coli BL21 (DE3) -V2R- pRARE2 strain by addition of 1 mM isopropyl-1-thio-D-galactopyranoside (IPTG) and incubated overnight at 15 ^{o}C. Harvested cells were resuspended in 20 mM Tris buffer, pH 7.5, supplemented with 500 mM NaCl, 5 mM imidazole, 5% glycerol. The cells were lysed chemically followed by sonication at frequency of 8.5 (10 sec on/10 sec off) for 4min. After clarification of the crude extract by high-speed centrifugation, the lysate was loaded onto DE52 and passed onto the Ni-NTA column. The column was washed and eluted by 20 mM Tris, pH 7.5, 500 mM NaCl, 5% glycerol, 50 mM and 250 mM imidazole respectively. Thrombin was added while the protein was being dialyzed against 20 mM Tris buffer, pH 7.5, and 500 mM NaCl. To remove the cut his-tag and his-tagged protein, dialyzed protein solution was passed through a Ni-NTA column. Flow through was dialyzed again against 20 mM Tris buffer, pH 7.5, and 500 mM NaCl. Pure protein (>95%) was further Concentrating to higher than 20 mg / mL and stored at -80 ^{o}C after flash freezing.

Fluorescence Polarization (FP) Binding Assay

H3(1-15) (ARTKQTARKSTGGKA) peptide for WDR5 was synthesized, N-terminal-labeled with isothiocyanate–fluorescein, and purified by Tufts University Core Services (Boston, MA). Compound binding assays were performed in a 10 μL volume at a constant labeled peptide concentration of 30 nM and protein concentrations of 0.3 μM WDR5. The assay buffer was 80 mM sodium phosphate (pH 6.5), 20 mM KCl, and 0.008% Triton X-100. Fluorescence polarization assays were performed in 384-well Axygen plates using a Synergy 4 microplate reader (BioTek). The excitation wavelength of 485 nm and the emission wavelength of 528 nm were used. To determine K_{dis} values, the data were fit to a hyperbolic function using Sigma Plot software (Systat Software). The K_{dis} values represent the average and standard deviation of quadruplicate measurements. The peptide displacement assay using H3 (1-15) is only accurate for rank ordering compounds with K_{dis} values higher than 200 nM. For more potent compounds, peptides with higher affinity for WDR5 need to be used.

Supplementary Figure 1

Evaluating WDR5-peptide interaction by isothermal titration calorimetry (ITC). Binding of a 15-mer fluorescein labeled peptide corresponding to the first 15 amino acids of histone H3 [FITC-H3 (1-15)] to WDR5 was assessed by ITC (25 injections of 10 µL peptide solution at 1 mM into 50 µM WDR5 in 80 mM Na_3PO_4 pH 6.5, 20 mM KCl in 1.3 mL cell). The experiment was performed in triplicate. The thermodynamic profile of the interaction is presented in the associated table below. The dissociation constant for the peptide was 458 ± 45 nM.



Experiment	dH (cal/mol)	dS (cal/mol deg)	K_D (nM)
1	-4665	13.6	413
2	-4541	13.6	503
3	-4692	13.2	459
Ave	-4633	13.5	458
SD	81	0.2	45