Supplementary Material

Discovery and Biological Characterization of Potent Myeloid Cell Leukemia-1 (Mcl-1) Inhibitors

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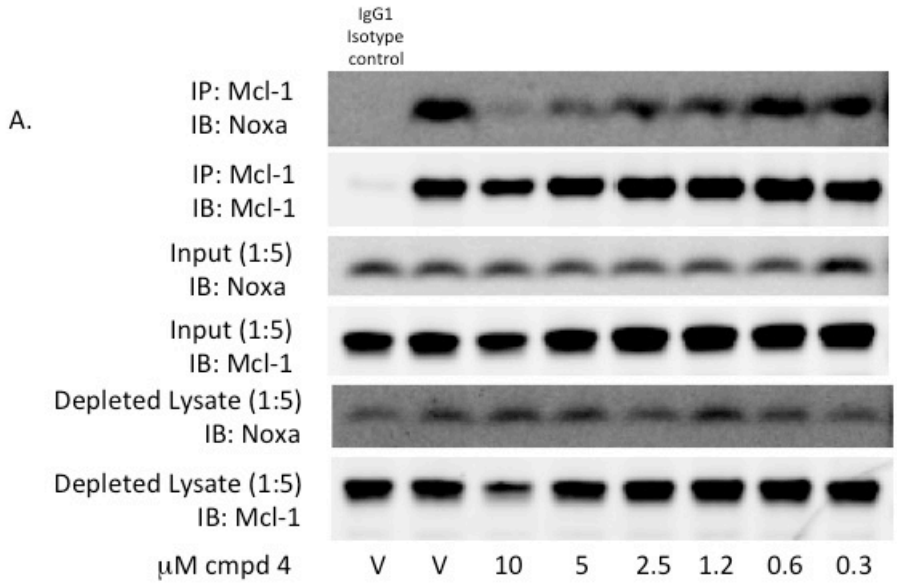
Table 1 X-Ray Data collection and refinement statistics (molecular replacement)

	Comp. 2	Comp. 5
Data collection		
Space group	$P2_1$	$P2_1$
Cell dimensions		
a, *b*, *c* (Å)	39.91, 136.04, 63.49	39.74, 87.84, 54.07
α, β, γ (°)	90.00, 101.62, 90.00	90.00, 99.68, 90.00
Resolution (Å)	2.6 (2.6-2.76) *	2.4 (2.4-2.48)
R_{sym} or R_{merge}	0.109/0.047 (0.709/0.312)	0.105/0.058 (0.367/0.215)
I / σI	24.23 (2.93)	14.55 (2.49)
Completeness (%)	90.8 (82.6)	94.1 (89.5)
Redundancy	2.5 (2.1)	2.8 (2.2)
Refinement		
Resolution (Å)	2.6-30.0	2.4-30.0
No. reflections	18533	13642
R_{work} / R_{free}	0.19/0.27	0.15/0.21
No. atoms		
Protein	4761	2425
Ligand	172	100
Water	94	114
B-factors	(Ask for input)	
Protein	41	39
Ligand	40	32
Water	33	42
R.m.s. deviations		
Bond lengths (Å)	0.009	0.008
Bond angles (°)	1.213	1.070

*One crystal was used for each structure. *Values in parentheses are for highest-resolution shell.

Figure S1. Dose-dependent disruption of Mcl-1/Noxa in AMO-1 cells.



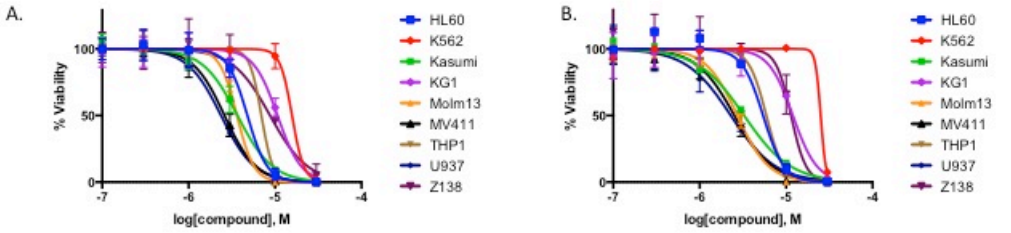
Co-IP of Mcl-1 and Noxa in human AMO-1 cells with and without (V= DMSO vehicle) indicated concentration of compound 4 . (A) Co-IP of Mcl-1 and Noxa. Figure S1 clearly shows a complete and unequivocal dose dependent compound mediated Noxa displacement from Mcl-1 in a co-IP. Based on concentrations of antibodies and other binding partners of indicated proteins little or no change is expected in the depleted lysates. There is an apparent increase in Mcl-1 protein concentration in the input portion (4th gel from top, cells treated with 1.2 μM compound had a 1.5 fold increase in Mcl-1 levels compared to the vehicle).

Methods: co-IP experiment Mcl-1/Noxa

Human AMO-1cells, grown in RPMI-1640 media supplemented with 10% FBS were incubated with 0.1% DMSO or compound for 90 minutes. Whole cell lysates, harvested in low-salt buffer (50mM Tris pH 7.4, 150 mM NaCl, 2.5mM MgCl, 0.5%

NP40 and HALT protease and phosphatase inhibitors, 0.5 at 2.5 uMmM EDTA) are used for Mcl-1 (Y37) Abcam (Cambridge, UK) immunoprecipitation, followed by Western analysis for Noxa (Millipore, USA). Blots were probed with IRDye-conjugated secondary antibodies (Licor, Lincoln, NE, USA) and measured on a Licor Odyssey using Licor Image Studio.

Figure S2.
Dose response data from a three day cell viability studies using compound **4** and 5. Dose response curves done were done in triplicate using methods described in text. Curves with error bars are shown for a panel of AML cell lines. **A.** compound 4, **B**. compound 5.



C. IC_{50} values from three day cell viability studies of compound **4** and **5** in a panel of AML(LHS) and MM (RHS) cell lines.

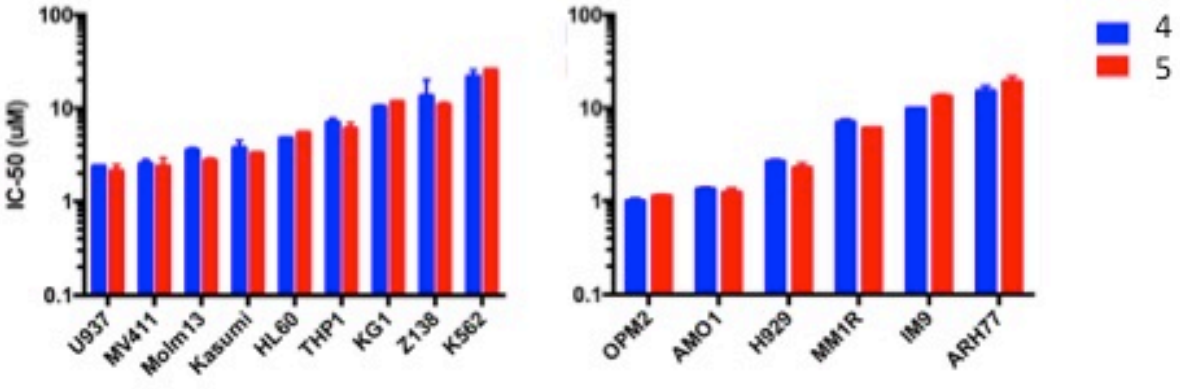
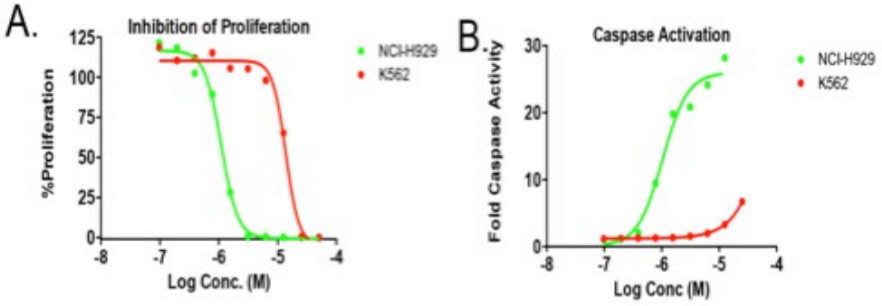


Figure S3.
Additional Cell based characterization of compounds.

(A) Three day cell viability studies of compound **4** in the Mcl-1 sensitive cell line (H929) and the Mcl-1 insensitive line (K562)
(B) Caspase activation studies three hours after dosing with compound **4** in the Mcl-1 sensitive cell line (H929) and the Mcl-1 insensitive line (K562), showing dose dependent fold caspase activation over vehicle treated cells.
Cell based studies to verify the on target, on mechanism action of **4** or **5** against human Mcl-1.



Additional Methods Details.

Chemistry

All NMR spectra were recorded at room temperature on a 500 MHz AMX Bruker spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constant (Hz). Low-resolution mass spectra were obtained on an Agilent 1200 series 6140 mass spectrometer with electrospray ionization. All samples were of ≥95% purity as analyzed by LC–UV/vis-MS. Analytical HPLC was performed on an Agilent 1200 series with UV detection at 214 and 254 nm along with ELSD detection. LC/MS parameters were as follows: Phenomenex-C18 Kinetex column, 50 Å~ 2.1 mm, 2 min gradient, 5% (0.1% TFA/MeCN)/95% (0.1% TFA/H2O) to 100% (0.1% TFA/MeCN). Preparative purification was performed on a Gilson HPLC (Phenomenex-C18, 100 Å~ 30 mm, 10 min gradient, 5 → 95% MeCN/H2O with 0.1% TFA) or by automated flash column chromatography (Isco, Inc. 100sg Combiflash). All reagents were purchased from chemical suppliers and used without purification.

Preparation of 3-(6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxamido)benzoate (2).

Ethyl 6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylate. A solution of ethyl 7-bromo-6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1H-indole-2-carboxylate (1.0 g, 2.0 mmol), 1,3,5-trimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (520 mg, 2.2 mmol, 1.1 eq), $Pd(PPh_3)_4$ (116 mg, 0.100 mmol, 5 mmol%) and K_2CO_3 (3.0 mL, 6.0 mmol) in 1,4-Dioxane (6 mL) was heated to 120 °C in Biotage Initiator for 90 min. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (CombiFlash Rf, 12 gram column, 0-70% EtOAc/Hex) to give the title compound (850 mg, 1.61 mmol, 80%) as yellow foam. ^{1}H NMR (500 MHz in $CDCl_3$): δ 8.66 (s, 1H), 7.64 (d, *J* = 8.6 Hz, 1H), 7.29 - 7.19 (m, 1H), 6.63 (s, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.99 (s, 3H), 3.97 (d, *J* = 5.9 Hz, 2H), 3.27 (t, *J* = 7.3 Hz, 2H), 2.34 (s, 6H), 2.21-2.05 (m, 8H), 1.40 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C): δ 161.8, 156.7, 145.7, 140.9, 137.0, 136.1, 132.0, 126.7, 126.1, 124.6, 124.5, 121.9, 121.8, 114.4, 113.6, 112.9, 67.3, 61.0, 35.4, 30.2, 21.1, 20.9, 14.3, 11.1, 10.1; LCMS: R_T 2.117 min, >98% purity at 215 nm and 254 nm, MS (ES) 628.0 (M+H).

6-Chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylic acid. To a solution of ethyl 6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylate (150 mg, 0.28 mmol) in a mixture of EtOH (2.0 mL) and THF (0.5 mL) was added LiOH (aq. 1.0 mL, 2N, 2.0 mmol). The resulting mixture was stirred at 40 °C for 20 h then cooled to ambient temperature. The reaction mixture was concentrated *in vacuo*, and the residue was purified by the reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 30-80% CH_3CN, 0.1% TFA) to give the title compound (133 mg, 0.27 mmol, 95%) as colorless oil. ^{1}H NMR: (500 MHz in $CDCl_3$) δ 9.80 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.64 (s, 2H), 3.99 (t, *J* = 6.0 Hz, 2H), 3.81 (s, 3H), 3.31 (t, *J* = 7.0 Hz, 2H), 2.34 (s, 6H), 2.21-2.19 (m, 2H), 2.06 (s, 3H), 2.04 (s, 3H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 164.4, 156.8, 149.0, 140.5, 137.5, 136.9, 131.7, 126.3, 126.0, 124.9, 123.9, 121.4, 121.2, 116.1, 114.5, 113.1, 67.3, 35.9, 30.4,

21.1, 20.9, 12.2, 10.3; LCMS: R_T = 1.865 min; > 98% purity at 215 nm and 254 nm; MS (ES) 499.90 (M+H).

Methyl 3-(6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxamido)benzoate. A solution of 6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylic acid (40 mg, 0.080 mmol), methyl 3-aminobenzoate (15 mg, 0.096 mmol), DMAP (12 mg, 0.096 mmol) and HATU (37 mg, 0.096 mmol) in DMF (1 mL) was stirred at ambient temperature for 6 h. The reaction mixture was concentrated, and the residue was purified by the reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 30-90% CH_3CN, 0.1% TFA) to give the title compound (45 mg, 0.071 mmol, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C): δ 10.24 (s, 1H), 9.27 (s, 1H), 8.08 (s, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 7.36 (t, *J* = 7.7 Hz, 1H), 7.26 (d, *J* = 3.9 Hz, 1H), 6.61 (s, 2H), 4.05 - 4.01 (m, 2H), 4.00 (s, 3H), 3.83 (s, 3H), 3.40 (t, *J* = 7.2 Hz, 2H), 2.28 (s, 6H), 2.27-2.24 (m, 2H), 2.23 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C): δ 166.6, 159.9, 156.5, 145.2, 142.5, 138.2, 136.9, 136.1, 131.2, 130.7, 128.9, 127.9, 127.0, 126.3, 125.4, 125.1, 122.4, 121.8, 121.6, 121.4, 114.8, 113.9, 112.5, 67.3, 52.0, 35.3, 29.9, 21.2, 20.8, 10.9, 10.1; LCMS: R_T 2.104 min, >98% purity at 215 nm and 254 nm, MS (ES) 633.0 (M+H).

Compound 2. A solution of methyl 3-(6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxamido)benzoate (42 mg, 0.066 mmol) in EtOH (0.6 mL), THF (0.15 mL) and LiOH (0.1 mL) was stirred at 40 °C for 16 h. The reaction mixture was concentrated, and the residue was purified by the reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 30-80% CH_3CN, 0.1% TFA) to give the title compound (37 mg, 0.06 mmol, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C): δ 10.51 (s, 1H), 9.48 (s, 1H), 8.18 (s, 1H), 8.03 (s, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 6.60 (s, 2H), 3.98 (t, *J* = 5.2 Hz, 2H), 3.92 (s, 3H), 3.38 (t, *J* = 5.2 Hz, 2H), 2.27 (s, 6H), 2.24-2.20 (m, 2H), 2.19 (s, 3H), 2.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C): δ 170.4, 160.2, 156.6, 145.4, 142.7, 138.5, 137.0, 136.9, 136.3, 131.3, 130.3, 129.1, 127.7, 127.0, 126.2, 125.5, 125.4, 123.3, 121.8, 121.6,

114.7, 114.4, 113.9, 112.7, 67.3, 35.3, 29.9, 20.8, 10.9, 10.0; LCMS: R_T 1.948 min, >98% purity at 215 nm and 254 nm, MS (ES) 618.9 (M+H).

Preparation of 3-(8-Chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)benzoic acid (3).

Ethyl 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylate. To a flame dried round bottom flask (50 mL) equipped with magnetic stir bar was added ethyl 6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-2-carboxylate (360 mg, 0.683 mol) and anhydrous DMF (2 mL) and the solution was stirred in ice bath under nitrogen atmosphere. Sodium hydride (60 %) (25 mg, 0.62 mol) was added and after 3 min *tert*-butyl 2-oxo-1,3-oxazinane-3-carboxylate was added. The reaction mixture was stirred in the ice bath for 20 min and then at ambient temperature overnight. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with brine (2 x 30 mL), dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (Combi-flash Rf, eluent 20% ethyl acetate in hexanes) to give the title compound (305 mg, 65%). ^{1}H NMR: δ 7.58 (d, J = 10.7 Hz, 1H), 7.23 (d, J = 10.7 Hz, 1H), 6.64 (s, 2H), 4.42-4.35 (m, 3H), 4.32-4.24 (m, 1H), 4.18-4.08 (m, 1H), 3.98 (t, J = 7.5 Hz, 2H), 3.88 (s, 3H), 3.24 (t, J = 9.0 Hz, 2H), 2.76 (q, J = 7.0 Hz, 2H), 2.34 (s, 6H), 2.13 (t, J = 7.0 Hz, 2H), 2.09 (s, 3H), 2.03 (s, 3H), 1.48 (t, J = 7.0 Hz, 2H), 1.43 (t, J = 9.0 Hz, 3H), 1.42 (s, 9H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 162.3, 156.7, 155.6, 146.4, 138.0, 137.2, 136.9, 134.2, 126.8, 126.1, 126.0, 125.0, 121.7, 120.9, 116.4, 114.4, 113.5, 78.9, 67.4, 60.7, 42.8, 37.9, 36.2, 31.2, 30.5, 28.3, 21.8, 20.9, 14.3, 12.3, 10.3; LCMS: R_T = 2.320 min; > 98% purity at 215 nm and 254 nm; MS (ES) 685.00 (M+H).

8-Chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1H-pyrazol-4-yl)-2,3,4,5-tetrahydro-1H-[1,4]diazepino[1,2-a]indol-1-one. A solution of ethyl 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-6-chloro-3-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxylate (218 mg, 0.32 mol) in anhydrous CH_2Cl_2 was cooled at 0 °C in ice bath. TFA (1.5 mL) was added dropwise, and the reaction mixture was stirred at ambient temperature for 2 h.

The solvent was removed *in vacuo* and anhydrous ethanol (10 mL) was added followed by anhydrous K_2CO_3 (829 mg, 1.92 mmols). The reaction mixture was stirred at RT for overnight. The reaction mixture was diluted with ethyl acetate (60 mL) and washed with brine (2 x 30 mL). The organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (Combi-flash Rf, CH_2Cl_2/methanol = 0-10% gradient) to give the title compound (150 mg, 87%). ^{1}H NMR: (500 MHz in$CDCl_3$) δ 7.62 (d, *J* = 10.7 Hz, 2H), 7.25 (d, *J* = 10.7 Hz, 2H), 6.65 (s, 2H), 6.23 (t, *J* = 7.5 Hz, 1H), 4.06-3.93 (m, 4H), 3.90 (s, 3H), 3.22-3.15 (m, 4H), 2.35 (s, 6H), 2.20 (quint., *J* = 8.7 Hz, 2H), 2.06 (s, 3H), 2.04 (s, 3H), 1.82-1.66 (m, 2H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 166.4, 156.8, 145.8, 138.1, 136.9, 135.8, 132.9, 131.6, 126.7, 125.9, 121.7, 121.2, 120.8, 115.1, 114.5, 113.5, 67.4, 41.0, 38.5 36.1, 30.2, 30.1, 20.9, 20.4, 12.1, 10.0; LCMS: R_T = 1.896 min., > 98% purity at 215 nm and 254 nm; MS (ES) 539.00 (M+H).

Methyl 3-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)benzoate. In a 4 dram vial equipped with a stir bar, 8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-2,3,4,5-tetrahydro-1*H*-[1,4]diazepino[1,2-*a*]indol-1-one (70 mg, 0.13 mmol, 1.0 eq), methyl 3-bromobenzoate (55 mg, 0.26 mmol, 2.0 eq), copper (I) iodide (10 mg, 0.052 mmol, 0.40 eq), *rac-N,N'*-dimethylcyclohexane-1,2-diamine (15 mg, 0.105 mmol, 0.81 eq), and potassium carbonate (55 mg, 0.40 mmol, 3.0 eq) were dissolved in toluene (2 mL). The reaction was sealed with a teflon-coated septum cap and then purged with argon for 5 minutes. The reaction was heated to 110 °C for 16 h, after which time the reaction was monitored by LCMS. The reaction was cooled to ambient temperature and diluted into DCM/water (1:1 20 mL). The organic layer was separated and the aqueous layer was extracted with DCM (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (CombiFlash Rf, 4 gram column, eluent 0 to 100% EtOAc in hexanes) to give the title compound (66 mg, 76%). ^{1}H NMR: (500 MHz in $CDCl_3$) δ 7.98-7.94 (m, H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.51-7.46 (m, 2H), 7.24 (d, *J* = 8.5 Hz, 1H), 6.61 (s, 2H), 4.16-4.06 (m, 2H), 3.96 (t, *J* = 6.0, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 3.76-3.67 (m, 2H), 3.16 (oct, *J* = 7.5

Hz, 2H), 2.29 (s, 6H), 2.23 -2.16 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 1.92-1.80 (m, 2H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 166.2, 164.0, 156.8, 146.0, 142.3, 137.7, 136.9, 135.2, 133.0, 132.0, 131.4, 130.7, 129.3, 127.8, 126.7, 126.6, 125.9, 121.5, 121.2, 120.7, 115.5, 114.5, 113.2, 67.3, 52.2, 48.2, 40.7, 36.2, 30.2, 29.5, 20.8, 20.6, 12.3, 10.0; LCMS: R_T = 2.092 min, > 98% purity at 215 nm and 254 nm; MS (ES) 673.00 (M+H).

Compound 3. In a scintillation vial equipped with a stir bar, methyl 3-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)benzoate (40 mg, 0.060 mmol, 1.0 eq) was massed and dissolved in THF/MeOH/H_2O (3.0 mL/0.5 mL/0.5 mL). Lithium hydroxide (5 mg, 0.21 mmol, 3.5 eq) was added, and the reaction was heated to 50 °C for 2 h, after which time the reaction was determined to be complete by LCMS. The reaction was concentrated *in vacuo*, and the crude residue was dissolved in DMSO (2 mL) and filtered. The DMSO solution was purified by reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 30-90% CH_3CN, 0.1% TFA) to yield the title compound (28 mg, 72%). ^{1}H NMR: (500 MHz in $CDCl_3$) δ 8.01-7.98 (m, 2H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.56-7.49 (m, 2H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.63 (s, 2H), 4.13-4.06 (m, 2H), 3.99 (s, 5H), 3.76-3.68 (m, 2H), 3.22 -3.14 (m, 2H), 2.30 (s, 6H), 2.21 (t, *J* = 6.0 Hz, 2H), 2.15 (s, 3H), 2.14 (s, 3H), 1.97-1.89 (m, 2H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 169.6, 164.2, 156.7, 145.7, 142.1, 140.1, 136.9, 135.0, 133.1, 132.0, 131.3, 131.1, 129.4, 128.5, 127.2, 126.9, 126.0, 122.0, 121.6, 114.5 114.3, 113.1, 67.2, 48.4, 40.9, 35.6, 30.2, 29.4, 20.8, 20.6, 11.2, 9.9; LCMS: R_T = 1.944 min, > 98% purity at 215 nm and 254 nm; MS (ES) 658.90 (M+H).

Preparation of 6-(8-Chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-4-carboxylic acid (4).

Methyl 6-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-4-carboxylate. To a solution of 8-Chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1H-pyrazol-4-yl)-2,3,4,5-tetrahydro-1H-[1,4]diazepino[1,2-a]indol-1-one (225 mg, 0.42 mmol) and methyl 6-bromo-1-methyl-1*H*-indole-4-carboxylate (168 mg, 0.63 mmol) in toluene (6.5 mL) was added K_2CO_3

(173 mg, 1.25 mmol), CuI (39.8 mg, 0.21 mmol) and (*rac*)-*trans*-dimethyl cyclohexyldiamine (66 mL, 59.5 mg, 0.42 mmol). The reaction mixture was flushed with Ar for 5 mins and heated to 100 °C. After 16h, the reaction was quenched with aq. saturated NH_4Cl solution and extracted with EtOAc (3 x 5 mL). The combined organic layer was washed with aq. saturated NaCl solution then concentrated *in vacuo*. The crude was purified by flash chromatography (Combi-flash Rf, 4 gram column, eluent 0 to 100% ethyl acetate in hexanes) to provide the title compound (302 mg, 0.41 mmol, 98%) as colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C): δ 7.78 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.52 (s, 1H), 7.28 (s, 1H), 7.25 (d, *J* = 3.0 Hz, 1H), 7.11 (d, *J* = 2.5 Hz, 1H), 6.64 (s, 2H), 4.11-4.15 (m, 2H), 4.00 (s, 4H), 3.98 (s, 4H), 3.82 (s, 3H), 3.76-3.82 (m, 2H), 3.12-3.22 (m, 2H), 2.31 (s, 6H), 2.22 (d, *J* = 6.5 Hz, 2H), 2.17 (s, 3H), 2.15 (s, 3H), 1.93 (br s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C): δ 167.2, 164.5, 156.8, 145.7, 140.0, 137.6, 136.9, 135.2, 134.9, 132.9, 132.32, 132.25, 127.2, 127.0, 126.0, 122.3, 121.58, 121.56, 121.2, 120.8, 114.6, 114.4, 113.0, 112.3, 102.5, 67.5, 51.9, 49.1, 41.0, 35.7, 33.1, 30.3, 29.3, 20.8, 20.7, 11.2, 10.0; LCMS: R_T 2.133 min, >98% purity at 215 nm and 254 nm, MS (ES) 726.3 (M+H).

Compound 4. To a solution of methyl 6-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-4-carboxylate (302 mg, 0.41 mmol) in 5 mL of solvent [THF/H_2O/MeOH (6:1:1)] was added LiOH (107 mg, 4.7 mmol) at room temperature. After 12 h, the reaction was warmed to 40 °C. After 4 h, the reaction was quenched with saturated aq. NH_4Cl solution and extracted with EtOAc. The combined organic layer was concentrated *in vacuo* and the crude was purified using the reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 50-95% CH_3CN, 0.1% TFA) to provide the title compound (220 mg, 0.31mmol, 76%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C): δ 7.82 (s, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.6 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 7.13 (s, 1H), 6.64 (s, 2H), 4.17-4.18 (m, 2H), 4.01 (t, *J* = 6.0 Hz, 2H), 3.95 (s, 3H), 3.80 (s, 5H), 3.17-3.21 (m, 2H), 2.30 (s, 6H), 2.25 (quintet, *J* = 6.5 Hz, 2H), 2.14 (s, 3H), 2.12 (s, 3H), 1.92-1.95 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C): δ 171.0, 164.5, 156.8, 145.9, 138.9, 137.6, 136.9, 135.5, 135.0, 132.8, 132.34, 132.29, 127.5, 126.8, 125.9, 121.8, 121.37, 121.31, 121.17, 121.11, 114.6, 114.3,

113.8, 113.0, 102.6, 67.5, 49.2, 41.0, 35.9, 33.1, 30.3, 29.4, 20.8, 20.7, 11.7, 10.0; LCMS: R_T 1.976 min, >98% purity at 215 nm and 254 nm, MS (ES) 712.0 (M+H).

Preparation of 4-(8-Chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-6-carboxylic acid (5).

Methyl 4-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-6-carboxylate. In a 4 dram vial equipped with a stir bar, 8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-2,3,4,5-tetrahydro-1*H*-[1,4]diazepino[1,2-*a*]indol-1-one (70 mg, 0.13 mmol, 1.0 eq), methyl 4-bromo-1-methyl-1*H*-indole-6-carboxylate (70 mg, 0.26 mmol, 2.0 eq), copper (I) iodide (10 mg, 0.052 mmol, 0.40 eq), *rac-N,N'*-dimethylcyclohexane-1,2-diamine (15 mg, 0.105 mmol, 0.81 eq), and K_2CO_3 (55 mg, 0.40 mmol, 3.0 eq) were dissolved in toluene (2 mL) then purged with argon for 5 minutes. The reaction was heated at 110 °C for 16 h. The reaction was cooled to ambient temperature and diluted into DCM/water (1:1 20 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (CombiFlash Rf, 4 gram column, eluent 0 to 100% EtOAc in hexanes) to give the title compound (67 mg, 71%). ^{1}H NMR: (500 MHz in $CDCl_3$) δ 8.09 (s, 1H), 7.70 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.21 (s, 1H), 6.61 (s, 2H), 6.34 (s, 1H), 4.24-4.20 (m, 2H), 3.99 (t, *J* = 5.5 Hz, 2H), 3.85 (s, 3H), 3.83-3.79 (m, 8H), 3.16 (q, *J* = 4.5 Hz, 2H), 2.32 (s, 6H), 2.27-2.19 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 1.90-1.81 (m, 2H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 167.4, 163.8, 156.8, 146.1, 137.7, 137.3, 136.8, 135.3, 134.4, 132.8, 132.6, 132.4, 128.8, 126.7, 125.8, 123.8, 121.5, 121.1, 120.7, 118.2, 15.5, 114.6, 113.3, 111.3, 99.6, 67.7, 52.0, 48.5, 40.9, 36.2, 33.2, 30.4, 29.5, 20.8, 20.7, 12.4, 10.1; LCMS: R_T = 2.101 min, > 98% purity at 215 nm and 254 nm; MS (ES) 726.00 (M+H).

Compound 5. To a solution of methyl 4-(8-chloro-11-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1-oxo-7-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-[1,4]diazepino[1,2-*a*]indol-2(3*H*)-yl)-1-methyl-1*H*-indole-6-carboxylate (40 mg, 0.055

mmol, 1.0 eq) in THF/MeOH/H_2O (3.0 mL/0.5 mL/0.5 mL) was added LiOH (5 mg, 0.21 mmol, 3.8 eq). The reaction was heated to 50 °C for 2 h, cooled to ambient temperature then concentrated *in vacuo*. The residue was purified by reverse phase HPLC (Phenomenex Gemini C18, H_2O/CH_3CN gradient from 30-90% CH_3CN, 0.1% TFA) to afford the title compound (33 mg, 85%) as a white solid. ^1H NMR: (500 MHz in $CDCl_3$) δ 8.16 (s, 1H), 7.74 (s, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.62 (s, 2H), 6.37 (s, 1H), 4.22 (t, *J* = 5.0 Hz, 2H), 4.00-3.98 (m, 5H), 3.88-3.82 (m, 5H), 3.18 (sex., *J* = 7.0 Hz, 2H), 2.31 (s, 6H), 2.22 (t, *J* = 6.5 Hz, 2H), 2.18 (s, 3H), 2.17 (s, 3H), 1.91 (s, 2H); ^{13}C NMR: (125 MHz in $CDCl_3$) δ 171.5, 164.1, 156.8, 145.7, 140.3, 137.3, 136.9, 135.1, 134.0, 133.1, 132.9, 132.3 129.3, 127.0, 125.9, 123.1, 122.1, 121.7, 121.3, 118.6, 114.6, 114.5, 112.9, 112.2, 99.6, 67.6, 48.7, 41.1, 35.6, 33.3, 30.4, 29.3, 20.8, 20.7, 11.1, 9.9; LCMS: RT = 1.969 min. > 98% purity at 215 and 254 nm; MS (ES) 711.90 (M+H).