Supporting information

Discovery of potent myeloid cell leukemia-1 (Mcl-1) inhibitors using fragment-based methods and structure-based design

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Supplementary Table S1

Mcl-l protein sequence of the construct used for X-ray crystallography: Residues 172-327 of the human Mcl-1 gene (Uniprot: *Q07820*).

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        10          20          30          40          50          60
GDELYRQSLE IISRYLREQA TGAKDTKPMG RSGATSRKAL ETLRRVGDGV QRNHETAFQG

        70          80          90         100         110         120
MLRKLDIKNE DDVKSLSRVM IHVFSDGVTN WGRIVTLISF GAFVAKHLKT INQESCIEPL

       130         140         150
AESITDVLVR TKRDWLVKQR GWDGFVEFFH VEDLEGG
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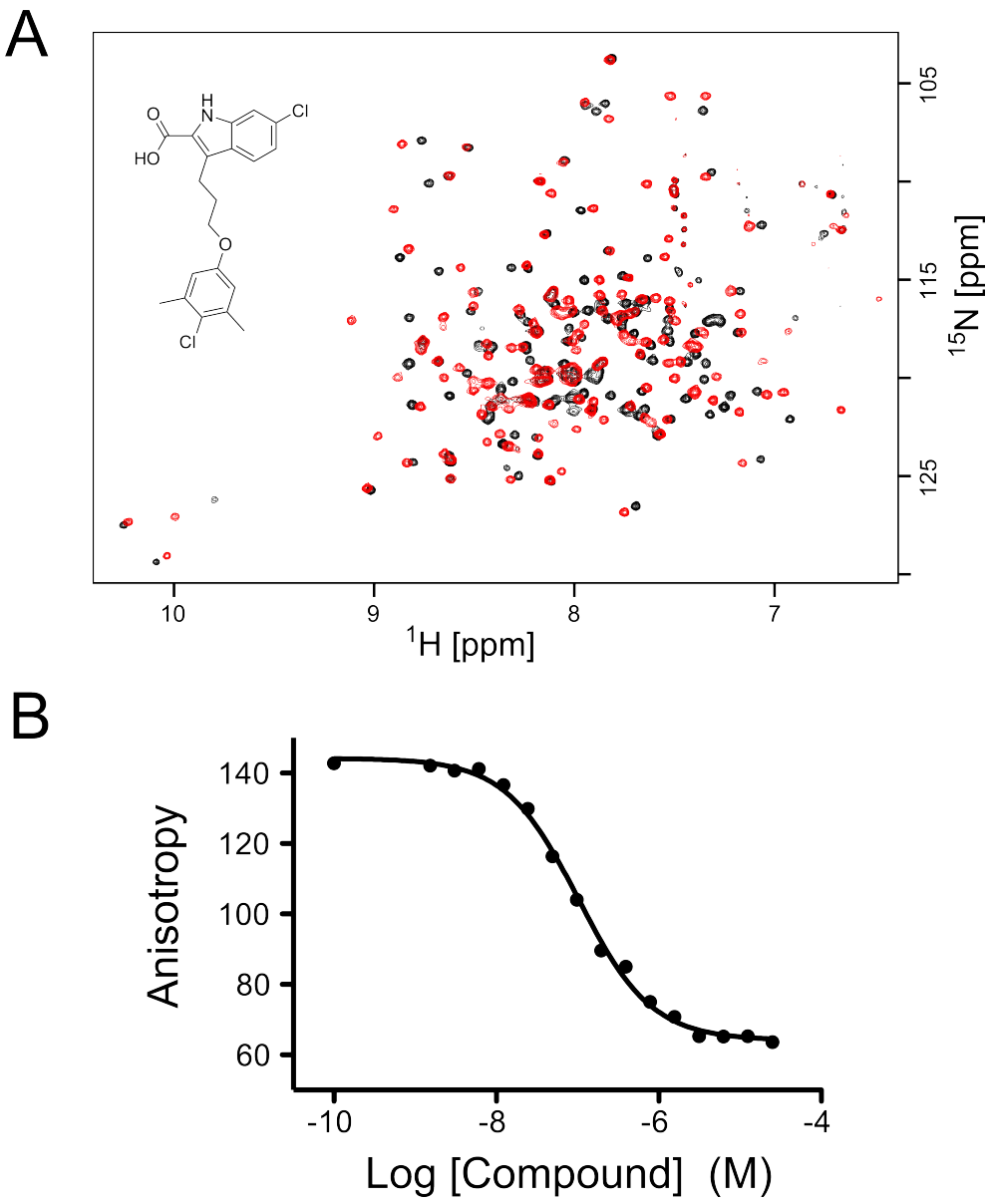
Number of amino acids:	157
Molecular weight:	17822 Da

Note: The expression vector contained a MBP-tag for enhanced solubility. The initial glycine is an artifact left from the TEV protease cleavage. In the Δ5 construct, the last five residues were removed.



Supplementary Figure S1. (A) ^{1}H-^{15}N HMQC spectra of Mcl-1 with (red) and without (black) merged compound **53** (insert). The NMR sample contained 70μM ^{15}N-labeled protein and 100μM ligand. (B) FPA dose-response curve of **53** displacing a FITC-labeled BAK peptide from Mcl-1. K_i was determined to 55±18 nM

Experimental section: Synthesis and characterization of intermediates

4-chloro-3-methylbenzo[b]thiophene-2-carboxylic acid (82a):

Step A. To a solution of 2'-chloro-6'-fluoroacetophenone (1.73 g, 10.0 mmol) in anhydrous toluene (10 mL) was added methyl thioglycolate (0.90 mL, 10 mmol) and DBU (3.0 mL, 20 mmol) in sequence at 0 °C under Ar. The reaction mixture was stirred for 3 hours at 0 °C, slowly warmed to room temperature and stirred additional 15 h. The reaction was quenched by addition of H2O (50 mL) then extracted with EtOAc (2 x 100 mL). The combined organic layer was successively washed with HCl (6N), water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by automated flash column chromatography (Isco, Inc. 100sg Combiflash) to yield methyl 4-chloro-3-methylbenzo[b]thiophene-2-carboxylate as a white solid (1.67 g, 6.95 mmol). MS (ESI) m/z = 241.2 $(M+H)^+$.

Step B. To a solution of methyl 4-chloro-3-methylbenzo[b]thiophene-2-carboxylate (1.65 g, 6.85 mmol) in THF (50 mL) and MeOH (12 mL) was added a solution of LiOH (660 mg, 27.4 mmol) in H2O (12 mL) at room temperature and stirred for 4 h. The reaction mixture was acidified with HCl (1N) to pH = 3 then extracted with CH_2Cl_2 (3 x 100 mL). The combined organic solution was concentrated *in vacuo* to give the title compound as a white solid (1.28 g, 5.66 mmol) MS (ESI) m/z = 227.1 $(M+H)^+$.

6-chloro-3-methylbenzo[b]thiophene-2-carboxylic acid (82b):

Step A. The procedure for compound **82a Step A** was followed using 4'-chloro-2'-fluoroacetophenone (1.73 g, 10.0 mmol) to yield methyl 6-chloro-3-methylbenzo[b]thiophene-2-carboxylate as a white solid (2.0 g, 8.4 mmol). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 8.21 (d, J = 1.9 Hz, 1H), 7.99 (d, J = 8.7 Hz, 1H), 7.52 (dd, J = 8.7, 1.9 Hz, 1H), 3.86 (s, 3H), 2.71 (s, 3H); MS (ESI) m/z = 241.2 $(M+H)^+$.

Step B. The procedure for compound **82a Step B** was followed using methyl 6-chloro-3-methylbenzo[b]thiophene-2-carboxylate (2.0 g, 8.4 mmol) to yield the title compound as a white solid (1.9 g, 8.4 mmol). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 8.17 (d, J = 1.9 Hz, 1H), 7.95 (d, J = 8.7 Hz, 1H), 7.50 (dd, J = 8.7, 1.9 Hz, 1H), 2.69 (s, 3H); MS (ESI) m/z = 227.1 $(M+H)^+$.

7-chloro-3-methylbenzo[b]thiophene-2-carboxylic acid (82c):

Step A. The procedure for compound **61 Step A** was followed using using 3'-chloro-2'-fluoroacetophenone (1.73 g, 10.0 mmol) to yield methyl 7-chloro-3-methylbenzo[b]thiophene-2-carboxylate as a white solid (1.61 g, 6.7 mmol) MS (ESI) m/z = 241.2 $(M+H)^+$.

Step B. The procedure for compound **61 Step B** was followed using methyl 7-chloro-3-methylbenzo[b]thiophene-2-carboxylate (1.59 g, 6.6 mmol) to yield 7-chloro-3-methylbenzo[b]thiophene-2-carboxylic acid as a white solid (1.46 g, 6.4 mmol) MS (ESI) m/z = 227.1 $(M+H)^+$.

Ethyl 3-(3-hydroxypropyl)-1*H*-indole-2-carboxylate : To a solution of ethyl 3-(3-ethoxy-3-oxopropyl)-1*H*-indole-2-carboxylate **85a**[37] (1.4 g, 4.8 mmol) in THF (20 mL) was added BH_3 in THF (20 mL, 20 mmol) and stirred for 15h at 20 °C. The reaction was quenched by addition of MeOH then concentrated *in vacuo*. The residue was purified by flash chromatography (Combi-flash Rf Hexane/EtOAc gradient 0-50%) to give the title compound as a white solid in 940 mg (3.8 mmol). >98% @215 nm, MS (ESI) m/z = 248.1 $(M+H)^+$.

Ethyl 1-(3-ethoxy-3-oxopropyl)-1*H*-indole-2-carboxylate (89): To a solution of ethyl 2-indolecarboxylate **88** (1.0 g, 5.2 mmol) in CH_3CN (17 mL) was added ethyl 3-bromopropionate (1.5 mL, 11.8 mmol) followed by K_2CO_3 (1.65g, 12 mmol). The reaction mixture was refluxed for 3 days and the progress of the reaction was monitored by LC-MS. The reaction was quenched by addition of H_2O and extracted with EtOAc. The combined organic layer was successively

washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by automated flash column chromatography (Isco, Inc. 100sg Combiflash, Hexane/EtOAc gradient 0-20%) to give the product as a colorless oil in 1.46 g (97%). ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.69 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.31 – 7.39 (m, 2H), 7.15 - 7.23. (m, 1H), 4.88 (t, *J* = 7.2 Hz, 2H), 4.43 (q, *J* = 6.0 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 7.6 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 3H); >98% @215 nm, MS (ESI) m/z = 290.1 $[M+H]^+$.

Ethyl 1-(3-hydroxypropyl)-1H-indole-2-carboxylate (90): The reduction procedure for preparation of ethyl 3-(3-hydroxypropyl)-1*H*-indole-2-carboxylate was followed using ethyl 1-(3-ethoxy-3-oxopropyl)-1*H*-indole-2-carboxylate (1.40 g, 4.8 mmol) and BH_3 (20.0 mmol, 1M in THF) to produce the alcohol **90** (1.12 g, 4.4 mmol). MS (ESI) m/z = 248.1 $[M+H]^+$.