Supporting Information for

Single Diastereomer of a Macrolactam Core Binds Specifically to Myeloid Cell Leukemia 1 (MCL1)

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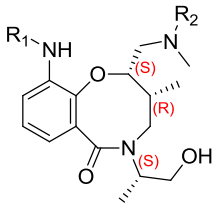
1. Synthetic procedure and compound characterization:

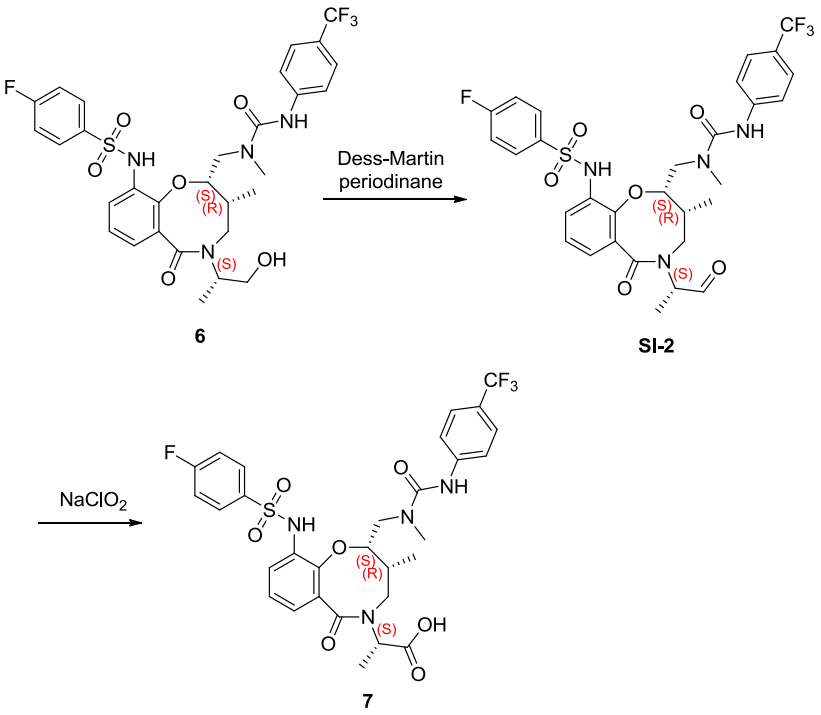
General: All oxygen and/or moisture sensitive reactions were carried out under N_2 atmosphere in oven or flame dried glassware. All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on a Bruker 300 MHz or 400 MHz spectrometer. Proton and carbon chemical shifts are reported in ppm referenced to the NMR solvent. Flash chromatography was performed using 40-60 um silica gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. Tandem liquid chromatography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector, with a Poroshell 120 EC-C18 column. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.5 min at 1.75 mL/min. Purity was measured by UV absorbance at 210 nm. HRMS was obtained by a hybrid quadrupole-orbitrap mass spectrometer (QExactive, Thermo Scientific) set in positive ion mode.

Synthesis of the library members of the structure **SI-1** has been reported previously.[1]



SI-1

Synthesis of 7:

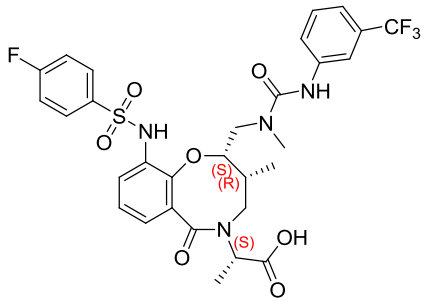


(S)-2-((2S,3R)-10-(4-fluorophenylsulfonamido)-3-methyl-2-((1-methyl-3-(4-(trifluoromethyl)phenyl)ureido)methyl)-6-oxo-3,4-dihydro-2H-benzo[b][1,5]oxazocin-5(6H)-yl)propanoic acid (7): 6 (47.5 mg, 0.073 mmol) was dissolved in DCM (2 mL), and the solution was cooled to 0 °C. Dess-Martin Periodinane (93 mg, 0.218 mmol) was added and then the ice bath was removed. After 20 min, the reaction mixture was diluted with EtOAc (50 mL), and washed with 1N NaOH and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Column chromotography with 0 to 10% MeOH/DCM eluted the product **SI-2** (42 mg, 88% yield) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 8.58 (s, 1H), 7.81 (dd, J = 8.8, 5.0 Hz, 2H), 7.74 - 7.48 (m, 5H), 7.22 (d, J = 7.6 Hz, 1H), 6.93 (dt, J = 27.9, 8.3 Hz, 3H), 6.63 (s, 1H), 4.85 (d, J = 9.6 Hz, 1H), 4.61 (ddd, J = 36.3, 14.9, 8.6 Hz, 2H), 3.32 - 2.97 (m, 5H), 2.82 (d, J = 14.5 Hz, 1H), 1.90 (dt, J = 13.0, 6.8 Hz, 1H), 1.64 - 1.40 (m, 3H), 0.71 (d, J = 6.6 Hz, 3H).

SI-2 (36 mg, 0.056 mmol) was dissolved in t-BuOH (1 mL), 2-methyl-2-butene (0.25 mL) and acetonitrile (0.1 mL) and the homogenous solution was treated with $NaClO_2$ (45.5 mg, 0.504 mmol) and NaH_2PO_4 (40.3 mg, 0.336 mmol) in Water (0.5 mL). After 10 min, LCMS indicated the reaction was complete. The reaction mixture was partitioned between EtOAc

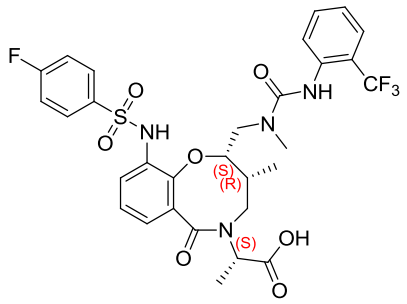
(50 mL) and 1N HCl (10 mL), and the organic layer was washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered, and concentrated. Preparative TLC with 10% MeOH/DCM afforded **7** as white solid (24.2 mg, 65% yield): ^{1}H NMR (400 MHz, MeOD) δ 7.63-7.78 (m, 4H), 7.41-7.53 (m, 3H), 7.02-7.13 (m, 3H), 6.82 (m, 1H), 4.87 (d, J = 9.5 Hz, 1H), 4.66 (d, J = 7.7 Hz, 1H), 4.34 (dd, J = 14.5, 9.7 Hz, 1H), 2.96 (s, 3H), 2.90 - 2.65 (m, 2H), 1.97 (m, 1H), 1.43 (d, J = 7.2 Hz, 3H), 0.80 (m, 1H), 0.51 (d, J = 6.4 Hz, 3H). LCMS t_R = 1.50 min. HRMS (ESI$^+$): calcd for $C_{30}H_{30}F_4N_4O_7S$ (M+H): 667.1844; found: 667.1837.

8-18 were prepared using similar procedure as above. Analytical data for **8-18** follows below:



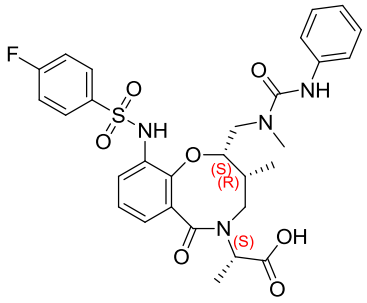
8

^{1}H NMR (400 MHz, MeOD) δ 7.83 (d, J = 8.2 Hz, 1H), 7.75 - 7.64 (m, 3H), 7.49 (d, J = 7.8 Hz, 1H), 7.36 (t, J = 8.1 Hz, 1H), 7.23 (dt, J = 23.4, 7.4 Hz, 4H), 7.10 (d, J = 7.9 Hz, 1H), 7.03 (t, J = 8.6 Hz, 2H), 6.80 (t, J = 8.0 Hz, 1H), 4.94 (d, J = 9.6 Hz, 1H), 4.67 (q, J = 7.3 Hz, 1H), 4.31 (dd, J = 14.4, 9.8 Hz, 1H), 3.21 (s, 4H), 2.95 (s, 3H), 2.84 - 2.71 (m, 2H), 2.10 - 1.97 (m, 1H), 1.38 (d, J = 7.4 Hz, 3H), 0.47 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.49 min. HRMS (ESI$^+$): calcd for $C_{30}H_{30}F_4N_4O_7S$ (M+H): 667.1844; found: 667.1835.



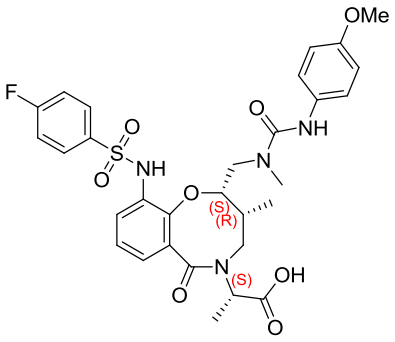
9

^{1}H NMR (400 MHz, MeOD) δ 7.75 - 7.63 (m, 3H), 7.56 (dd, J = 8.0, 4.5 Hz, 3H), 7.25 (dq, J = 17.3, 9.5, 8.6 Hz, 3H), 7.15 (d, J = 8.0 Hz, 1H), 7.08 (q, J = 8.5, 7.4 Hz, 2H), 6.84 (t, J = 8.0 Hz, 1H), 4.95 (d, J = 9.7 Hz, 1H), 4.66 (q, J = 7.4 Hz, 1H), 4.28 (dd, J = 14.4, 9.9 Hz, 1H), 3.26 - 3.12 (m, 4H), 2.95 (s, 3H), 2.82 - 2.65 (m, 2H), 2.04 (td, J = 13.3, 12.1, 6.9 Hz, 1H), 1.36 (d, J = 7.4 Hz, 3H), 0.39 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.44 min. HRMS (ESI$^+$): calcd for $C_{30}H_{30}F_4N_4O_7S$ (M+H): 667.1844; found: 667.1835.



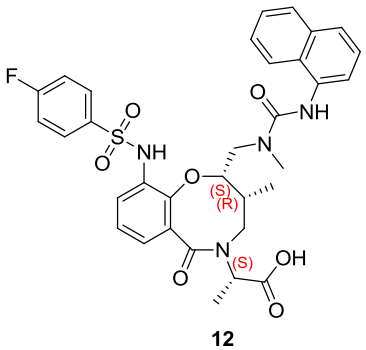
10

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 2H), 8.47 (s, 1H), 7.77 (dd, J = 8.3, 4.7 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 7.42 (d, J = 8.1 Hz, 1H), 7.27 (q, J = 7.9, 6.3 Hz, 3H), 7.18 (dd, J = 10.9, 6.7 Hz, 2H), 7.15 - 6.97 (m, 3H), 6.91 (t, J = 8.5 Hz, 2H), 6.79 (t, J = 8.1 Hz, 1H), 4.96 (q, J = 7.2 Hz, 1H), 4.76 (d, J = 9.2 Hz, 1H), 4.44 (dd, J = 14.6, 9.3 Hz, 1H), 3.27 - 2.98 (m, 2H), 2.86 (s, 3H), 2.68 (d, J = 14.5 Hz, 1H), 2.38 (s, 1H), 1.89 (dt, J = 13.8, 7.1 Hz, 1H), 1.50 (s, 3H), 0.63 (d, J = 6.5 Hz, 3H). LCMS t_R = 1.35 min. HRMS (ESI$^+$): calcd for $C_{29}H_{31}F_4N_4O_7S$ (M+H): 599.1970; found: 599.1965.



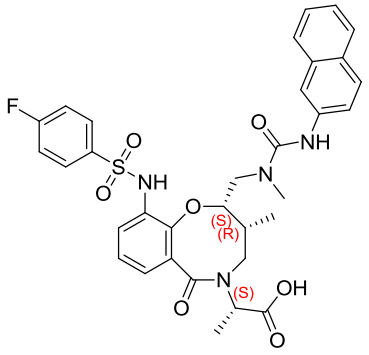
11

^{1}H NMR (400 MHz, DMSO-d6) δ 12.63 (s, 1H), 9.09 (s, 1H), 8.16 (s, 1H), 7.73 (dd, J = 8.7, 4.9 Hz, 2H), 7.43 (dd, J = 8.6, 4.3 Hz, 3H), 7.30 (t, J = 8.6 Hz, 2H), 7.17 - 7.05 (m, 1H), 6.99 - 6.87 (m, 1H), 6.86 - 6.78 (m, 2H), 4.68 (d, J = 8.5 Hz, 1H), 4.49 (q, J = 7.0 Hz, 1H), 4.11 (dt, J = 14.8, 6.6 Hz, 1H), 3.71 (d, J = 3.6 Hz, 3H), 3.30 - 3.22 (m, 1H), 3.17 (s, 1H), 2.99 (d, J = 13.7 Hz, 1H), 2.93 (s, 3H), 2.85 - 2.72 (m, 2H), 1.98 (s, 1H), 1.42 (d, J = 7.1 Hz, 3H), 0.54 (d, J = 6.5 Hz, 3H). LCMS t_R = 1.33 min. HRMS (ESI$^+$): calcd for $C_{30}H_{33}FN_4O_8S$ (M+H): 629.2076; found: 629.2071.



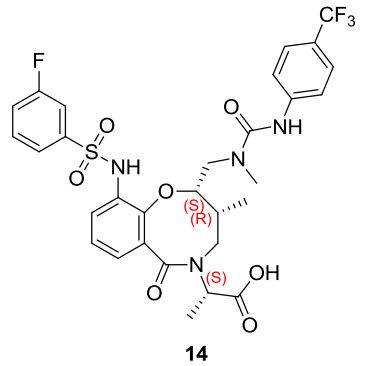
12

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 2H), 8.17 (s, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.75 - 7.55 (m, 5H), 7.41 (dq, J = 40.4, 7.7 Hz, 5H), 7.27 (s, 2H), 6.93 (d, J = 7.8 Hz, 1H), 6.83 (t, J = 8.3 Hz, 2H), 6.60 (t, J = 7.8 Hz, 1H), 4.95 - 4.73 (m, 2H), 4.22 (t, J = 11.5 Hz, 1H), 3.10 (dd, J = 15.5, 4.8 Hz, 1H), 2.95 (d, J = 14.7 Hz, 4H), 2.81 (d, J = 14.1 Hz, 1H), 1.87 (q, J = 9.0, 6.0 Hz, 1H), 1.41 (s, 3H), 0.60 (d, J = 6.5 Hz, 3H). LCMS t_R = 1.39 min. HRMS (ESI$^+$): calcd for $C_{33}H_{33}FN_4O_7S$ (M+H): 649.2127; found: 649.2125.



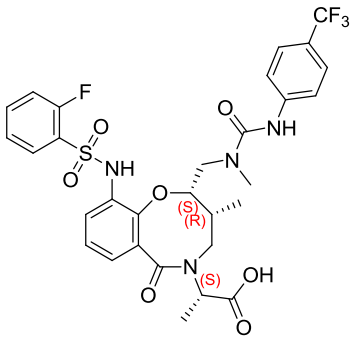
13

^{1}H NMR (400 MHz, MeOD) δ 8.17 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.75 - 7.64 (m, 4H), 7.48 (dd, J = 13.1, 8.4 Hz, 2H), 7.32 (t, J = 7.5 Hz, 1H), 7.29 - 7.19 (m, 4H), 7.11 (d, J = 7.9 Hz, 1H), 6.97 (t, J = 8.6 Hz, 2H), 6.82 (t, J = 8.0 Hz, 1H), 4.88 (d, J = 9.7 Hz, 1H), 4.81 (s, 1H), 4.67 (d, J = 7.6 Hz, 1H), 4.41 (dd, J = 14.6, 9.9 Hz, 1H), 2.98 (s, 3H), 2.86 (t, J = 14.2 Hz, 2H), 2.80 - 2.71 (m, 2H), 2.04 - 1.91 (m, 2H), 1.45 (d, J = 7.4 Hz, 3H), 0.53 (d, J = 6.5 Hz, 3H). LCMS t_R = 1.49 min. HRMS (ESI$^+$): calcd for $C_{33}H_{33}FN_4O_7S$ (M+H): 649.2127; found: 649.2126.



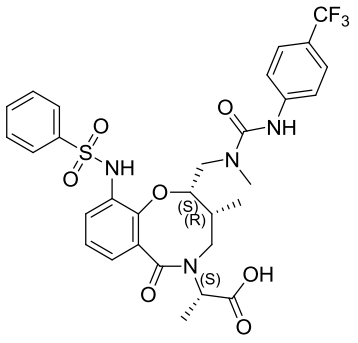
14

^{1}H NMR (400 MHz, MeOD) δ 7.80 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 7.6 Hz, 4H), 7.47 (td, J = 8.0, 4.7 Hz, 1H), 7.34 (pd, J = 8.4, 5.5, 3.3 Hz, 1H), 7.23 (t, J = 9.4 Hz, 1H), 7.19 - 7.09 (m, 1H), 6.95 (t, J = 8.0 Hz, 1H), 4.78 (q, J = 7.4 Hz, 1H), 4.47 (dd, J = 14.5, 9.8 Hz, 1H), 3.07 (s, 3H), 2.99 - 2.81 (m, 2H), 2.34 (s, 1H), 2.08 (dt, J = 12.6, 6.4 Hz, 1H), 1.55 (d, J = 7.4 Hz, 3H), 0.61 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.50 min. HRMS (ESI$^+$): calcd for $C_{30}H_{30}F_4N_4O_7S$ (M+H): 667.1844; found: 667.1843.



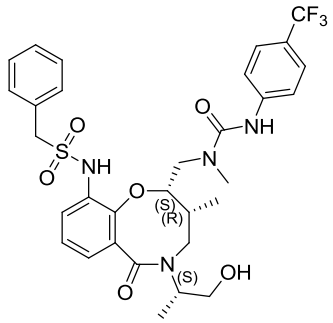
15

^{1}H NMR (400 MHz, MeOD) δ 7.75 (t, J = 7.7 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.53 - 7.37 (m, 4H), 7.29 - 7.19 (m, 1H), 7.10 (ddt, J = 21.5, 13.8, 7.7 Hz, 4H), 6.76 (t, J = 7.9 Hz, 1H), 4.91 (d, J = 9.5 Hz, 1H), 4.70 - 4.61 (m, 1H), 4.40 (dd, J = 14.5, 9.7 Hz, 1H), 3.27 (d, J = 4.6 Hz, 0H), 3.21 (s, 5H), 2.97 (s, 4H), 2.91 - 2.73 (m, 2H), 2.22 (s, 1H), 2.03 (dt, J = 12.8, 6.1 Hz, 1H), 1.45 (d, J = 7.4 Hz, 3H), 0.71 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.46 min. HRMS (ESI$^+$): calcd for $C_{30}H_{30}F_4N_4O_7S$ (M+H): 667.1844; found: 667.1843.



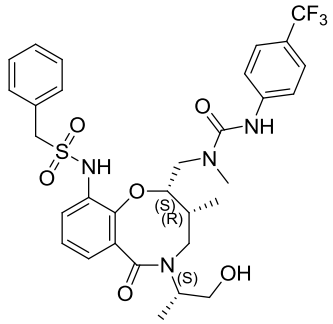
16

^{1}H NMR (400 MHz, MeOD) δ 7.79 (t, J = 9.0 Hz, 4H), 7.62 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 8.3 Hz, 3H), 7.43 (t, J = 7.7 Hz, 2H), 7.27 - 7.09 (m, 3H), 6.92 (t, J = 7.9 Hz, 1H), 4.76 (q, J = 7.3 Hz, 1H), 4.47 (dd, J = 14.5, 9.8 Hz, 1H), 3.39 - 3.23 (m, 3H), 3.06 (s, 3H), 2.97 - 2.79 (m, 2H), 2.33 (s, 1H), 2.06 (dq, J = 12.3, 6.2 Hz, 1H), 1.54 (d, J = 7.4 Hz, 3H), 0.60 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.46 min. HRMS (ESI$^+$): calcd for $C_{30}H_{31}F_3N_4O_7S$ (M+H): 649.1938; found: 649.1939.



17

^{1}H NMR (400 MHz, MeOD) δ 7.70 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.40 - 7.08 (m, 7H), 6.85 (t, J = 8.0 Hz, 1H), 5.02 (d, J = 9.5 Hz, 1H), 4.76 (q, J = 7.2 Hz, 1H), 4.50 (dd, J = 14.5, 9.7 Hz, 1H), 4.38 - 4.17 (m, 2H), 3.76 (d, J = 6.8 Hz, 2H), 3.48 (dd, J = 15.4, 5.7 Hz, 1H), 3.27 - 3.14 (m, 1H), 3.05-3.15 (m, 4H), 2.26 (m, 1H), 1.25 (d, J = 6.8 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H). LCMS t_R = 1.45 min. HRMS (ESI$^+$): calcd for $C_{31}H_{35}F_3N_4O_6S$ (M+H): 649.2302; found: 649.2302.



18

^{1}H NMR (400 MHz, MeOD) δ 7.61 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 7.7 Hz, 1H), 7.38 (d, J = 8.5 Hz, 2H), 7.04-7.14 (m, 4H), 6.94 - 6.79 (m, 3H), 4.91 (d, J = 9.6 Hz, 1H), 4.66 (q, J = 6.8 Hz, 1H), 4.43 (m, 1H), 3.63 (d, J = 6.9 Hz, 2H), 2.75-3.40 (m, 10H), 2.12 (m, 1H), 1.12 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H). LCMS t_R = 1.52 min. HRMS (ESI$^+$): calcd for $C_{32}H_{37}F_3N_4O_6S$ (M+H): 663.2459; found: 663.2459.

2. Materials and methods for biological assays:

a) Differential Scanning Calorimetry (DSC): Human MCL1 (173-329) protein was dialyzed overnight at 4 °C into 25mM HEPES buffer, pH 7.4, 100mM NaCl, and 0.1mM TCEP for all DSC experiments. Experiments were conducted on a MicroCal VP-Capillary DSC instrument (Malvern Instruments Ltd.). Each run consisted of a reference cell containing 400µL of 250µM compound in dialysis buffer with 4% DMSO and a protein cell containing 400µL of 0.5 mg/mL MCL1 (~ 25µM) and 250µM compound in dialysis buffer with 4% DMSO. Melting curves were acquired at a scan rate of 200 °C/hour, between 35-100 °C. Buffer scans were subtracted from all runs and analyzed in Origin software, version 7.0552. ΔT_m values were determined by subtracting an average T_m value determined for runs containing apo MCL1 protein from the MCL1 T_m in the presence of compound.

b) Isothermal Titration Calorimetry (ITC): Human MCL1 (173-329) protein was dialyzed overnight at 4 °C into 25mM HEPES buffer, pH 7.4, 100mM NaCl, and 0.1mM TCEP for all ITC experiments. Compounds were prepared in this dialysis buffer at 400µM or 1200µM (fragments) with 4% DMSO added. 40µL of compound was titrated into 200µL of 25mM MCL1, also in dialysis buffer containing 4% DMSO. Reference experiments (titration of compound into buffer alone) were subtracted to account for compound heat of dilution effects. Experiments were conducted at 25 °C with a MicroCal Auto-ITC200 instrument (Malvern Instruments Ltd), using a 1000 rpm stirring speed and a reference power of 10 µcal/sec. ITC curves were analyzed using Origin software, version 7.0552, and the binding affinity (K_D), enthalpy (ΔH), and entropy (ΔS) were calculated for each compound.

c) ^{19}F NMR: MCL1 protein was dialyzed overnight at 4 °C into 50 mM HEPES buffer containing 100 mM NaCl, 0.1 mM TCEP, 0.0025% TFA (adjusted to pH 7.4). Reference samples of ligand alone were prepared with 50 uL D_2O, 430 uL of dialysis buffer, and 20 uL of compound (dissolved in DMSO-d6) to afford a final ligand concentration of 60 or 120 uM. The final concentration or DMSO-d6 in all samples was 4% v/v. Identical samples were prepared with MCL1 protein to yield a final protein concentration of 10 uM.

^{19}F NMR spectra were acquired with ^{1}H decoupling on a 400 MHz Bruker console, temperature = 300K, relaxation delay = 1 sec., 256 scans. With TFA reference set to -76.500

ppm, chemical shifts were recorded for the CF3 resonance. The chemical shift Δν was calculated using the equation below:

$\Delta\nu = (\nu 2 - \nu 1) * 376.32$ (Hz) (Eq. 1)

Using Eq. 1, the following chemical shifts were measured for ligand **7**:

Ligand concentration (uM)	δ (ppm) with protein	δ (ppm) without protein	Δν	1/Δν
60	-62.5739	-62.5882	5.3768	0.18598423
120	-62.5801	-62.5876	2.8200	0.35450993

Kd was then calculated based on linear fit using Eq. 2 below:

$[L]_0 = [P]_0\Delta_{max}/\Delta_{obs} - K_d$ (Eq. 2)

d) Fluorescence Polarization: A competitive fluorescence polarization (FP) assay was used to determine IC_{50} values for compounds that bind the recombinant MCL1 (173-329) BH3 groove and displace Tetramethylrhodamine (TAMRA)- labeled Noxa peptide (TAMRA-GELEVEFATQLRRFGDKLNF-amide; 21st Century Biochemical, Marlborough, MA). Compound stocks were prepared at 10 mM in 100% DMSO and pipetted into column 1 of a 384 well "compound plate" (Thermo Scientific, Cat # AB-1056). Compounds were then serially diluted in DMSO starting at 10 mM using a Janus Automated Work Station (Perkin Elmer, Waltham, MA). In addition, DMSO and 1 mM unlabeled Noxa peptide in DMSO were added to each well of columns 11 and 12 respectively. DMSO was used as a negative control, while 1 mM (5 μM final) of unlabeled Noxa peptide was used as a positive control. Next, MCL1 recombinant protein was prepared in buffer (25 mM Hepes, 100 mM NaCl, 0.005% Tween 20, 3% DMSO, pH 7.4) at 400 nM (2X) and 10 μL was added to a 384 well black "assay plate" (Corning, Cat # 3573). The assay plate was centrifuged for 1 min at 800 RPM, and 100 nL of serially diluted compound was delivered from the compound plate to the assay plate using a 384W 100 nL Pin Tool (V & P Scientific; San Diego, CA). The assay plate was incubated at room temperature for 30 minutes. After incubation, 25 nM TAMRA-labeled Noxa peptide was prepared in buffer (25 mM Hepes, 100 mM NaCl, 0.005% Tween 20, pH 7.4) at 50 nM (2X) and 10 μL added to each well of the assay plate. The assay plate

was centrifuged for 1 min at 800 RPM, and then incubated at room temperature for 30 minutes. The fluorescence polarization (in mP units) was measured at room temperature with excitation wavelength of 544 nm and emission wavelength of 579 nm using an Envision plate reader (Perkin Elmer, Waltham, MA). Each compound was assayed in duplicates run on two separate assay plates. The percentage inhibition was calculated by % inhibition = $\left(\frac{(\{\bar{X}_{min}\}-\{Sample\})}{(\bar{X}_{min}-\bar{X}_{high})}\right) * 100$

Where:

$\bar{X}_{min}$	Average High mP Signal (DMSO; wells A11 – P11 & A23 – A24)
$\{Sample\}$	Raw mP value
$\bar{X}_{high}$	Average Low mP Signal (5 μM unlabeled Noxa; wells A12 – P12 & A24 – P24)

Based on percentage inhibition, the IC_{50} (inhibitor concentration at which 50% of bound TAMRA-labeled Noxa peptide is displaced) was obtained by fitting the inhibition data using a Levenberg-Marquardt curve-fitting algorithm, with finite difference gradients (Dotmatics, San Diego, CA).

3. **X-ray information:** Crystals were grown in the presence of 0.5mM **7** and flash frozen using liquid nitrogen. Data were collected at 100K on a Rigaku FR-E+ SuperBright rotating anode generator with VariMax optics and a Saturn 944 detector. All data were reduced with XDS/XSCALE.[2] The structure was phased by molecular replacement using Phaser from the CCP4 suite of programs with PDB ID 1ANF used as a search model.[3,4] The structure was completed using multiple cycles of refinement in Phenix[5] followed by manual rebuilding of the structure using Coot.[6] The structure was quality checked by Molprobity.[7] The structure has been deposited in the Protein Data Bank (PDB ID 4WGI).

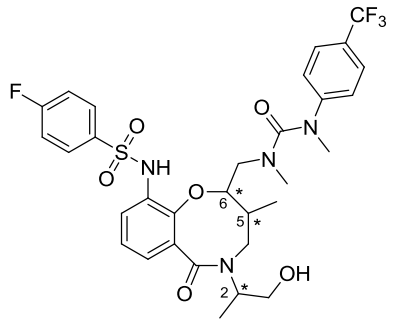
Table SI-1: Structure refinement statistics

Dataset	**7**
a (Å)	99.30
b (Å)	135.87
c (Å)	37.69
α, β, γ	90, 90, 90
Space group	$P2_12_12$
Wavelength (Å)	1.54
Resolution limit (Å)	50-1.85 (1.9-1.85)
Number of observations	44464 (3279)*
Completeness (%)	97.7 (99.9)*
Rmerge (%)	0.062 (0.508)*
Mean I/σI	16.2 (2.12)*
Refinement	
Resolution Range (Å)	50-1.85
Rcryst	0.172
Rfree	0.219
Mean B-factor (Å)	23.43
Rmsd bond lengths (Å)	0.007
Rmsd bong angles (˚)	1.030
Ramachandran statistics (%)	
Favored	98.24

Allowed	1.56
Outlier	0.2
Molprobity score	0.97 (100^{th} percentile)

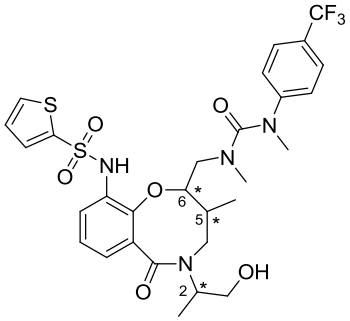
*** Highest resolution shell shown in parentheses**

Figure SI-1: DSF screening data for **6** and related analog **SI-3** (red: binding hit, blue: non-binding, yellow: inconclusive).



6

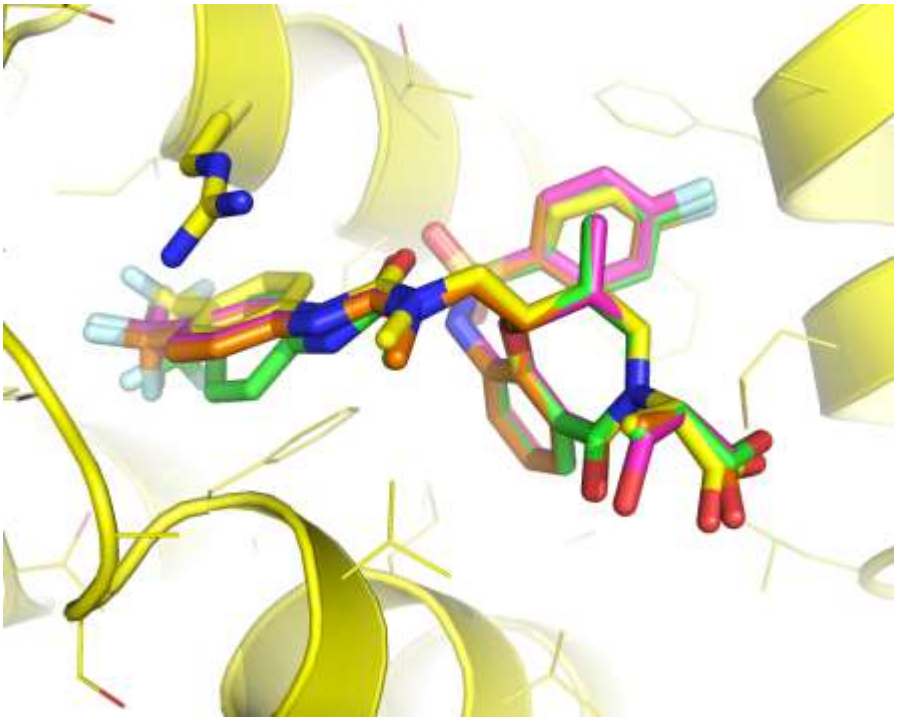
RSS	RRS	RSR	RRR
SSS	SRS	SSR	SRR



SI-3

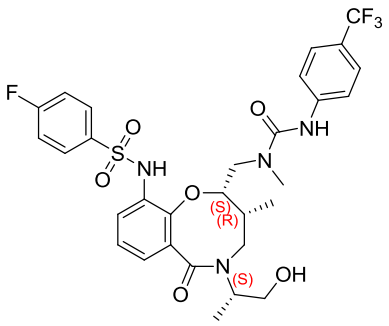
RSS	RRS	RSR	RRR
SSS	SRS	SSR	SRR

Figure SI-2: The lowest energy conformation of the free ligand **6** (green), **7** (orange), and the C2 epimer of **6** (magenta) are aligned on the macrocycle core in the crystal structure of **7** (yellow).



Conformations of compound **7**, compound **6**, and the exocyclic C2 stereoisomer of **6** were generated using the LowModeMD conformational search method in CCG MOE version 2013.08 using the MMFFs force field with Born solvation. The default parameters and charges were used for the force field. The conformational search maintained all conformations within 5 kcal/mol throughout the calculation and terminated after 100 consecutive steps with optimization led to conformers already visited. A cutoff of 0.25Å RMSD on all heavy atoms and energy determined conformer uniqueness. As shown in the figure, out of 100s of conformations within 5 kcal/mol, the lowest-energy conformations matched extremely well with the 1.8Å resolution crystal structure.

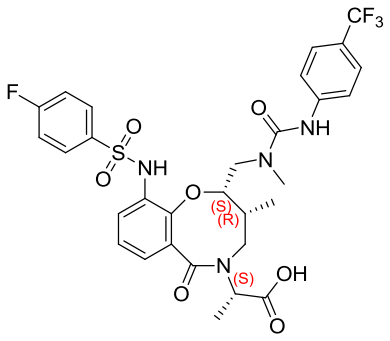
Figure SI-3: DSC data for all eight isomers of **6**



6

$C_2C_5C_6$ stereochemistry	SRS	RRS	SRR	RRR	SSR	RSR	SSS	RSS
DSC ΔT_m/°C	1.9	2.1	0.3	-0.1	-0.2	-0.3	0.0	-0.1

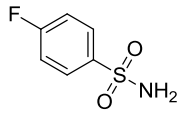
Figure SI-4: FP data of **7** against Bcl-2 and Bcl-xL



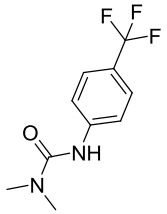
7

	MCL1	Bcl-2	Bcl-xl
FP IC_{50}/ μ M	4.5	>250	>250

Figure SI-5: DSC and ITC data for fragments **19**-**20**



19



20

compound	**19**	**20**
DSC ΔT_m/˚C	0.1	-0.3
ITC	No binding	No binding

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