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Tetrazole and acylsulfonamide bioisosteric replacements of the carboxylic acid in a dual MCL-1/BCL-x_L inhibitor are tolerated†

 Lijia Chen,^{‡,a} Brandon Lowe,^{‡,a} and Steven Fletcher  ^{*ab}

Overexpression of the anti-apoptotic protein MCL-1 is associated with a plethora of human cancers, and it reduces the sensitivity of cancer cells to approved chemotherapies. Accordingly, the discovery of MCL-1 inhibitors is an active area of interest. Many inhibitors of the anti-apoptotic MCL-1 protein bear a crucial carboxylic acid that may engage Arg263 in the BH3-binding groove. We previously described the salicylic acid-based dual MCL-1/BCL-x_L inhibitor **17cd**, which is currently undergoing lead optimization. As part of that process, we wished to investigate bioisosteric replacement of **17cd**'s key carboxylic acid. Herein we describe the synthesis of a variety of analogues of a simpler analogue of **17cd** presenting carboxylic acid surrogates. The acylsulfonamide and tetrazole motifs, which exhibit comparable pK_{a} s to the carboxylic acid function, displayed similar, or better, binding affinities to MCL-1 and BCL-x_L as the corresponding carboxylic acid-containing lead. Our best compound was acylsulfonamide **7d** with a K_i of 800 nM against MCL-1 and 1.82 mM against BCL-x_L and demonstrated an improved effect on the viability of the HL60 acute myeloid leukemia cell line relative to the parent carboxylic acid-containing dual inhibitor from which it was derived.

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1 Introduction

Myeloid cell leukemia-1 (MCL-1) is an anti-apoptotic member of the BCL-2 family of proteins that regulates apoptosis.¹ Overexpression of MCL-1 is associated with a wide range of human cancers, and protects cancer cells from apoptosis, which inherently also reduces the sensitivity of these cells to standard chemotherapies, as well as to radiotherapy.^{2–4}

Currently, there are several on-going clinical trials investigating the therapeutic efficacy of a variety of MCL-1 inhibitors. However, some trials have been terminated or suspended owing to serious adverse effects.⁵ The majority of MCL-1 inhibitors bind in the BH3-binding groove on the surface of the protein, mimicking the BH3 domain of the pro-apoptotic counterparts, thereby relinquishing these to activate cell death.^{5–7} It is important that research into MCL-1 inhibitors bearing novel chemotypes is continued to increase the likelihood of the discovery of a clinically effective and safe therapeutic. However, selective MCL-1 inhibitors may suffer from resistance due to concomitant anti-apoptotic sister protein upregulation, and *vice versa*.^{8,9} Previously, we

described the small molecule “**17cd**” (Fig. 1) that is a dual inhibitor of MCL-1 ($K_i = 0.629 \mu\text{M}$) and the sister anti-apoptotic BCL-2 protein BCL-x_L ($K_i = 1.67 \mu\text{M}$). We reasoned that a dual MCL-1/BCL-x_L inhibitor may be a more promising therapeutic than a selective inhibitor through co-targeting two anti-apoptotic BCL-2 proteins.¹⁰ However, as predicted, the impact of **17cd** on the viability of human leukemia HL60 cells is modest with an IC_{50} of 18.5 μM . We are thus continuing our optimization of **17cd** in order to gain greater affinities to both MCL-1 and BCL-x_L, and that work will be published in due course. The carboxylic acid of **17cd** is paramount to its biological activity, and it cannot be discarded,¹¹ as has been observed with other carboxylic acid featuring MCL-1 inhibitors^{12,13} that (may) form a salt bridge with Arg263 in the BH3-binding groove on MCL-1.¹⁴

Since lipophilic drugs containing carboxylic acids poorly penetrate cell membranes and/or become bound to serum proteins, which reduces their bioavailability,¹⁵ we wanted to investigate if ester prodrugs would enhance cell killing, and if carboxylic acid bioisosteres of **17cd** would retain/improve the activity of the parent compound, as well as improve cell killing. Herein, we describe the synthesis and biological evaluation of some ester prodrugs of our salicylic acid-based dual MCL-1/BCL-x_L inhibitors as well as some classical and non-classical bioisosteres.

2 Experimental

2.1 Chemistry

2.1.1 General procedures. All reactions were performed in oven-dried glassware under an inert (N_2) atmosphere, unless

^aUniversity of Maryland School of Pharmacy, 20N Pine St, Baltimore, MD 21201, USA.
 E-mail: steven.fletcher@rx.umd.edu

^bUniversity of Maryland Greenebaum Cancer Center, 20 S. Greene St., Baltimore, MD, 21201, USA

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‡ These authors contributed equally.

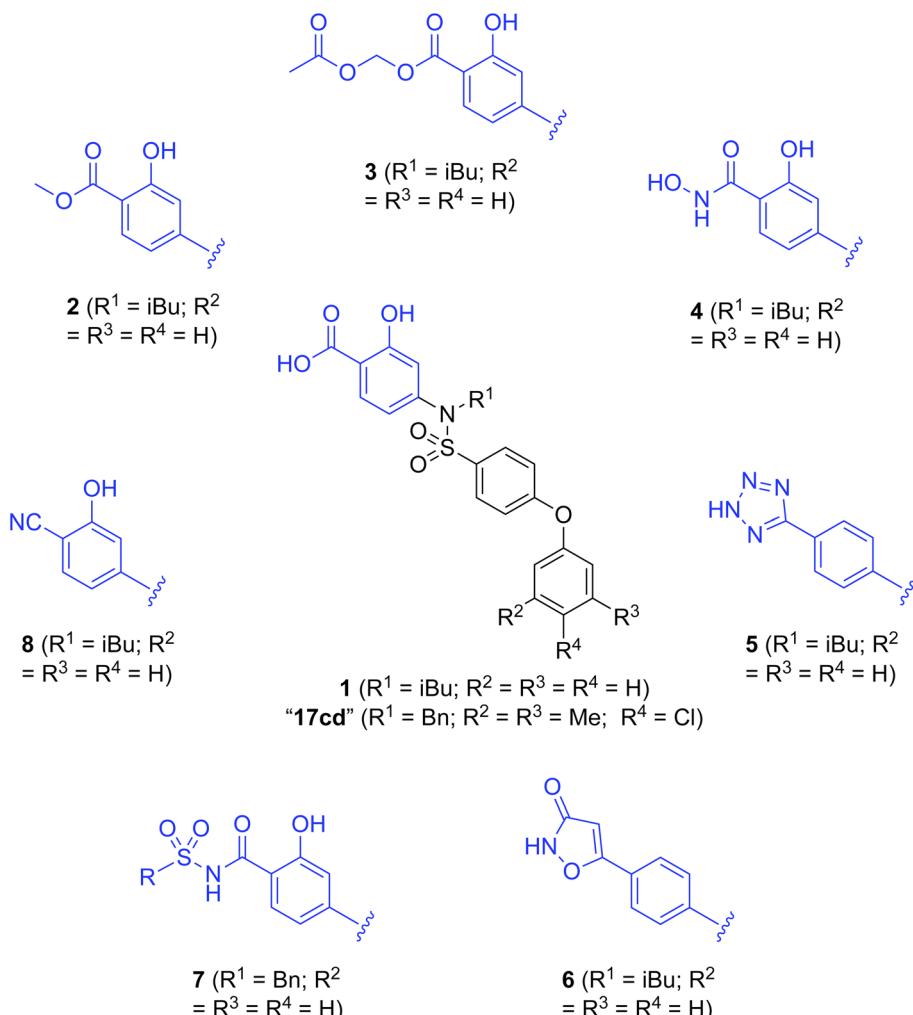


Fig. 1 Target esters and bioisosteres of 1 and "17cd".¹¹

otherwise stated. Anhydrous solvents were used as supplied without further purification. ^1H and ^{13}C NMR spectra were recorded on a Varian 400 MHz NMR spectrometer at 25 °C. Chemical shifts are reported in parts per million (ppm) and are referenced to residual non-deuterated solvent peak (CHCl_3 : δ_{H} 7.26, δ_{C} 77.2; DMSO: δ_{H} 2.50, δ_{C} 39.5); coupling constants are in Hz. High resolution mass spectra (HRMS) were recorded on an Agilent 6560 Quadrupole time of flight (QTOF) mass spectrometer using electrospray ionization (ESI).

2.1.1.1 General procedure A: methyl esterification. The acid (1 eq.) was suspended in MeOH (0.5 M), cooled to 0 °C, and H_2SO_4 (7 eq.) was added dropwise. The reaction was slowly warmed up to room temperature and then refluxed overnight. TLC indicated the reaction was complete. The volatiles were evaporated and the residue was poured into ice. The pH was adjusted to 7 using 1 M NaOH solution. The precipitate was filtered and washed with deionized water, then dried in the vacuum oven overnight to yield the methyl ester as a white solid.

2.1.1.2 General procedure B: O-benzylation. The phenol (1 eq.) was dissolved in anhydrous DMF (0.1 M), cooled to 0 °C, followed by addition of $\text{K}'\text{OBu}$ (1.1 eq.). Benzyl bromide (1.1 eq.)

was then added and the reaction was stirred at room temperature overnight. TLC indicated the reaction was complete, which was then partitioned between EtOAc and H_2O . The organic layer was collected, washed repeatedly with H_2O (4×), then dried over Na_2SO_4 , filtered, concentrated and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 1 : 1 to give the product.

2.1.1.3 General procedure C: reductive amination. The aniline (1 eq.) was dissolved in dichloroethane (0.1 M), then the corresponding aldehyde/ketone (1.3 eq.) and acetic acid (1.2 eq.) were added. Next, $\text{NaBH}(\text{OAc})_3$ (2.5 eq.) was added batchwise into the reaction and stirred at room temperature overnight. TLC indicated the reaction was complete. Saturated NaHCO_3 (aq) was poured into the reaction mixture and bubbled for 30 min. The mixture was then collected and partitioned between water and DCM (3×). The combined organic layers were dried over Na_2SO_4 , filtered, concentrated and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4 : 1 to give the product.

2.1.1.4 General procedure D: sulfonamide synthesis. The aniline (1 eq.) was dissolved in anhydrous CHCl_3 (0.1 M),



followed by the addition of the desired sulfonyl chloride (1.5 eq.), DIPEA (3 eq.) and DMAP (0.1 eq.). The reaction was heated at 65 °C overnight under N₂ atmosphere. TLC indicated the reaction was complete. The volatiles were evaporated and the residual was reconstituted in EtOAc and washed with 1 M HCl. The organic layer was collected and dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4:1 to give the product.

2.1.1.5 General procedure E: nucleophilic aromatic substitution (S_NAr). The fluorobenzene (1 eq.) was dissolved in anhydrous DMSO (0.1 M), followed by adding corresponding phenol (5 eq.), and K₂CO₃ (5 eq.). The reaction was heated at 100 °C overnight. TLC indicated the reaction was complete, which was then partitioned between EtOAc and H₂O. The organic layer was collected, washed repeatedly with H₂O (4×), then dried over Na₂SO₄, filtered, concentrated and then reconstituted with Et₂O. The Et₂O solution was washed with 1 M NaOH (4×), then dried over Na₂SO₄, filtered, concentrated and then purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4:1 to give the product.

2.1.1.6 General procedure F: ester hydrolysis. The ester (1 eq.) was dissolved in a mixed solvent of THF/MeOH/H₂O 3:1:1 (0.1 M). LiOH·H₂O (4 eq.) was added to the reaction, which was stirred at room temperature overnight, or until TLC indicated the reaction was complete. The volatiles were evaporated and the residue was partitioned between EtOAc and 1 M HCl. The organic layer was collected, dried over Na₂SO₄, filtered, and concentrated to yield the product.

2.1.1.7 General procedure G: debenzylation. The benzylated phenol (1 eq.) was dissolved in a solvent mixture of toluene/TFA 2:1 (0.1 M), and stirred at room temperature overnight. TLC indicated the reaction was complete. The volatiles were evaporated and the residue was purified by preparative TLC using an eluent of DCM/MeOH/AcOH 92:7:1 to give the product.

2.1.1.8 Methyl 2-hydroxy-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate (2). Methyl 2-(benzyloxy)-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate¹¹ was debenzylated according to general procedure G on a scale of 0.2 mmol to give the product as a white solid (81 mg, 89%): ¹H NMR (CDCl₃, 400 MHz) δ 10.78 (1H, s, OH), 7.79 (1H, d, *J* = 8.4 Hz, Ar), 7.51 (2H, d, *J* = 8.4 Hz, Ar), 7.41 (2H, t, *J* = 8.0 Hz, Ar), 7.22 (1H, t, *J* = 8.0 Hz, Ar), 7.07 (2H, d, *J* = 7.6 Hz, Ar), 6.97 (2H, d, *J* = 8.4 Hz, Ar), 6.80 (1H, d, *J* = 8.0 Hz, Ar), 6.64 (1H, s, Ar), 3.95 (3H, s, O-CH₃), 3.31 (2H, d, *J* = 7.2 Hz, CH₂), 1.66–1.58 (1H, m, CH), 0.90 (6H, d, *J* = 6.0 Hz, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 169.9, 161.8, 161.6, 155.0, 146.2, 131.5, 130.3, 130.1, 129.7, 124.9, 120.3, 119.7, 117.3, 115.9, 111.3, 57.2, 52.4, 26.8, 19.8.

2.1.1.9 Acetoxymethyl 2-hydroxy-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate (3). Acetoxymethyl 2-(benzyloxy)-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate (14) was debenzylated according to general procedure G on a scale of 0.15 mmol to give the product as a white solid (61 mg, 80%): ¹H NMR (CDCl₃, 400 MHz) δ 10.42 (1H, s, OH), 7.82 (1H, d, *J* = 8.8 Hz, Ar), 7.51 (2H, d, *J* = 8.4 Hz, Ar), 7.41 (2H, t, *J* = 7.6 Hz, Ar), 7.22 (1H, t, *J* = 8.0 Hz, Ar), 7.07 (2H, d, *J* = 8.8 Hz, Ar), 6.97 (2H, d, *J* = 8.4 Hz, Ar), 6.80 (1H, d, *J* = 8.0 Hz, Ar), 6.69

(1H, s, Ar), 5.99 (2H, s, O-CH₂-O), 3.31 (2H, d, *J* = 8.0 Hz, N-CH₂), 2.15 (3H, s, COCH₃), 1.65–1.58 (1H, m, CH), 0.90 (6H, d, *J* = 6.4 Hz, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 162.3, 161.7, 147.0, 131.4, 130.7, 130.1, 129.6, 125.0, 120.3, 119.7, 117.3, 116.1, 110.3, 79.3, 57.1, 26.8, 20.6, 19.8; HRMS-ESI: *m/z* found 512.1343 [M-H]⁻, C₂₆H₂₆NO₈S requires 512.1385.

2.1.1.10 N,2-Dihydroxy-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzamide (4). NH₂OH·HCl (6.1 mmol, 1.12 g, 25 eq.) and NaOH (36.5 mmol, 1.46 g, 57 eq.) was dissolved in H₂O (14 mL) in a flask, followed by dropwise addition of methyl 2-hydroxy-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate (2; 0.64 mmol, 293 mg, 1 eq.) in dioxane (7 mL). Reaction was stirred at room temperature overnight. TLC indicated the reaction was complete. The reaction was cooled to 0 °C, and 1 M HCl was added slowly. The reaction mixture was partition between DCM and H₂O, organic layer was collected and dried over Na₂SO₄, concentrated, and purified by flash column chromatography over silica gel using an eluent of DCM/MeOH/H₂O 79:9:1 to give the product as a light red solid (209 mg, 71%): ¹H NMR (DMSO-d₆, 400 MHz) δ 12.2 (1H, s br, NH), 11.4 (1H, s br, NH-OH), 9.34 (1H, s, Ph-OH), 7.59 (1H, d, *J* = 7.6 Hz, Ar), 7.52–7.42 (4H, m, Ar), 7.22 (1H, s, Ar), 7.10 (2H, d, *J* = 7.2 Hz, Ar), 7.05 (2H, d, *J* = 7.6 Hz, Ar), 6.63–6.58 (2H, m, Ar), 3.28 (2H, d, *J* = 12.0 Hz, N-CH₂), 1.44–1.41 (1H, m, Ar), 0.80 (6H, d, *J* = 6.4 Hz, 2*CH₃); ¹³C NMR (DMSO-d₆, 100 MHz) δ 161.3, 159.7, 155.1, 143.5, 131.8, 130.8, 130.2, 128.0, 125.5, 120.5, 118.5, 118.0, 116.9, 114.0, 109.8, 59.8, 25.8, 20.0; HRMS-ESI: *m/z* found 455.1323 [M-H]⁻, C₂₃H₂₃N₂O₆S requires 455.1288.

2.1.1.11 N-(4-(2H-Tetrazol-5-yl)phenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (5). *N*-(4-Cyanophenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (18; 0.25 mmol, 100 mg, 1 eq.) was dissolved in anhydrous DMF (0.1 M), followed by the addition of NaN₃ (0.74 mmol, 49 mg, 3 eq.) and NH₄Cl (1.0 mmol, 54 mg, 4 eq.). The reaction mixture was heated at 125 °C overnight. TLC indicated the reaction was complete. 1 M HCl was added to the flask to quench the reaction, and the mixture was partitioned between EtOAc and 1 M HCl. Organic layer was collected and washed with 1 M HCl 3 times, dried over Na₂SO₄, concentrated, and purified by prep-TLC using an eluent of DCM/MeOH/AcOH 92:7:1 to give the product as a white solid (86 mg, 76%): ¹H NMR (CDCl₃, 400 MHz) δ 8.13 (2H, d, *J* = 6.4 Hz, Ar), 7.51 (2H, d, *J* = 8.0 Hz, Ar), 7.39 (2H, t, *J* = 6.8 Hz, Ar), 7.30–7.19 (3H, m, Ar), 7.06 (2H, d, *J* = 6.8 Hz, Ar), 6.97 (2H, d, *J* = 7.6 Hz, Ar), 3.35 (2H, d, *J* = 6.4 Hz, N-CH₂), 1.62–1.58 (1H, m, CH), 0.90 (6H, d, *J* = 6.0 Hz, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 161.9, 154.8, 142.2, 130.9, 130.2, 129.8, 129.2, 128.1, 125.1, 123.3, 120.4, 117.3, 57.4, 26.8, 19.8; *t*_R = 12.3 min (100%); HRMS-ESI: *m/z* found 448.1442 [M-H]⁻, C₂₃H₂₂N₅O₃S requires 448.1449.

2.1.1.12 *N*-Isobutyl-*N*-(4-(3-oxo-2,3-dihydroisoxazol-5-yl)phenyl)-4-phenoxybenzenesulfonamide (6). NH₂OH·HCl (0.44 mmol, 31 mg, 4 eq.) and NaOH (0.7 mmol, 28 mg, 6.4 eq.) was dissolved in MeOH (2 mL) in a flask, followed by addition of methyl 3-(4-(N-isobutyl-4-phenoxyphenylsulfonamido)phenyl)propiolate (19; 0.11 mmol, 50 mg, 1 eq.). Reaction was stirred at room temperature overnight. TLC indicated the reaction was



complete. The reaction was cooled to 0 °C, and 1 M HCl was added slowly. The reaction mixture was partition between DCM and H₂O, organic layer was collected and dried over Na₂SO₄, concentrated, and purified by Prep-TLC using an eluent of DCM/MeOH/AcOH 92 : 7 : 1 and then re-purified by Prep-TLC using an eluent of DCM/MeOH/H₂O 79 : 9 : 1 to give the product as a white solid (10 mg, 20%): ¹H NMR (CDCl₃, 400 MHz) δ 7.69 (2H, d, *J* = 8.0 Hz, Ar), 7.49 (2H, d, *J* = 8.0 Hz, Ar), 7.42 (2H, t, *J* = 6.8 Hz, Ar), 7.26–7.20 (3H, m, Ar), 7.07 (2H, d, *J* = 7.6 Hz, Ar), 6.98 (2H, d, *J* = 7.6 Hz, Ar), 6.22 (1H, s, CHCO), 3.34 (2H, d, *J* = 6.8 Hz, N-CH₂), 1.62–1.58 (1H, m, CH), 0.92 (6H, d, *J* = 6.4 Hz, 2^{*}CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 161.7, 154.9, 141.6, 131.4, 130.9, 130.2, 129.7, 128.9, 128.1, 126.4, 125.0, 120.3, 117.2, 91.8, 57.4, 26.9, 19.8; HRMS-ESI: *m/z* found 463.1313 [M-H]⁻, C₂₅H₂₃N₂O₅S requires 463.1333.

2.1.1.13 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-hydroxy-*N*-(methylsulfonyl)benzamide (7a). 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-*N*-(methylsulfonyl)benzamide (21a) was debenzylated according to general procedure G on a scale of 0.09 mmol to give the product as a beige solid (25 mg, 50%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.64–7.58 (3H, m, Ar), 7.45 (2H, t, *J* = 7.6 Hz, Ar), 7.26–7.21 (5H, m, Ar), 7.17–7.08 (5H, m, Ar), 6.58–6.56 (2H, m, Ar), 4.76 (2H, s, N-CH₂), 3.06 (3H, s, CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.6, 154.7, 136.1, 131.4, 130.5, 129.9, 128.4, 127.9, 127.5, 125.1, 120.2, 117.6, 117.4, 116.2, 52.8, 40.8.

2.1.1.14 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-hydroxy-*N*-(phenylsulfonyl)benzamide (7b). 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-*N*-(phenylsulfonyl)benzamide (21b) was debenzylated according to general procedure G on a scale of 0.08 mmol to give the product as a beige solid (25 mg, 50%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.85 (2H, d, *J* = 7.2 Hz, Ar), 7.60 (2H, d, *J* = 8.4 Hz, Ar), 7.54–7.42 (6H, m, Ar), 7.25–7.06 (10H, m, Ar), 6.57–6.51 (2H, m, Ar), 4.73 (2H, s, N-CH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.5, 155.1, 143.3, 136.5, 132.5, 131.7, 130.9, 130.3, 128.9, 128.8, 128.3, 127.8, 127.7, 125.5, 120.6, 118.1, 117.8, 116.5, 53.2; HRMS-ESI: *m/z* found 613.1178 [M-H]⁻, C₃₂H₂₅N₂O₇S₂ requires 613.1109.

2.1.1.15 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-hydroxy-*N*-(3-nitro-4-(((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (7c). 4-(*N*-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-*N*-(3-nitro-4-(((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (21c) was debenzylated according to general procedure G on a scale of 0.08 mmol to give the product as a bright yellow solid (25 mg, 40%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.61 (1H, t, *J* = 5.6 Hz, NH-Ph), 8.57 (1H, s, Ar), 7.90 (1H, d, *J* = 9.6 Hz, Ar), 7.65 (2H, d, *J* = 8.8 Hz, Ar), 7.53–7.47 (3H, m, Ar), 7.29–7.16 (10H, m, Ar), 7.11 (2H, d, *J* = 8.4 Hz, Ar), 6.64 (1H, s, SO₂NHCO), 6.59 (1H, d, *J* = 8.8 Hz, Ar), 4.78 (2H, s, N-CH₂), 3.85–3.82 (2H, m, CH₂), 3.34–3.22 (4H, m, 2^{*}CH₂), 1.91–1.88 (1H, m, CH), 1.60 (2H, d, *J* = 12.0 Hz, CH₂), 1.29–1.20 (2H, m, CH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.6, 155.0, 147.4, 143.5, 136.3, 134.8, 131.7, 130.9, 130.5, 130.3, 129.9, 128.8, 128.3, 127.9, 125.5, 120.6, 118.0, 117.8, 116.6, 115.3, 67.0, 53.2, 48.3, 34.2, 30.6; HRMS-ESI: *m/z* found 771.1800 [M-H]⁻, C₃₈H₃₅N₄O₁₀S₂ requires 771.1693.

2.1.1.16 4-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-hydroxy-*N*-(3-nitro-4-(((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (7d). 4-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)-*N*-(3-nitro-4-(((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (21d) was debenzylated according to general procedure G on a scale of 0.14 mmol to give the product as a bright yellow solid (65 mg, 56%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.60–8.51 (2H, m, Ph-NH and Ar), 7.85 (1H, d, *J* = 8.8 Hz, Ar), 7.59 (2H, d, *J* = 7.6 Hz, Ar), 7.47 (1H, d, *J* = 8.0 Hz, Ar), 7.20–7.06 (8H, m, Ar), 6.99 (2H, s, Ar), 6.56–6.51 (2H, m, Ar), 4.73 (2H, s, N-CH₂), 3.79 (2H, d, *J* = 9.6 Hz, CH₂), 3.28–3.18 (4H, m, 2^{*}CH₂), 2.30 (6H, s, 2^{*}CH₃), 1.87–1.84 (1H, m, CH), 1.56 (2H, d, *J* = 12.4 Hz, CH₂), 1.21–1.18 (2H, m, CH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.4, 153.0, 147.3, 143.3, 138.4, 136.5, 134.8, 131.8, 130.3, 129.8, 128.8, 128.3, 127.8, 120.7, 118.1, 117.8, 116.5, 115.1, 67.0, 53.2, 48.3, 34.2, 30.6, 20.8; HRMS-ESI: *m/z* found 833.1383 [M-H]⁻, C₄₀H₃₈ClN₄O₁₀S₂ requires 833.1723.

2.1.1.17 *N*-(4-Cyano-3-hydroxyphenyl)-*N*-isobutyl-4-phenoxybenzenesulfonamide (8). *N*-(4-Cyano-3-methoxyphenyl)-*N*-isobutyl-4-phenoxybenzenesulfonamide (24; 0.07 mmol, 30 mg, 1 eq.) was dissolved in anhydrous DMF (0.1 M), followed by the addition of LiCl (0.28 mmol, 12 mg, 4 eq.). The reaction mixture was then stirred at 160 °C for 30 min in a microwave vessel. The reaction was allowed to cool, dilute with EtOAc and partition with between EtOAc and H₂O. The organic layers were combined and washed with H₂O, dried over Na₂SO₄, concentrated, and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4 : 1 to give the product as a white solid (11 mg, 40%): ¹H NMR (CDCl₃, 400 MHz) δ 7.50 (2H, d, *J* = 8.0 Hz, Ar), 7.42 (3H, t, *J* = 9.2 Hz, Ar), 7.23 (1H, t, *J* = 7.2 Hz, Ar), 7.06 (2H, d, *J* = 7.6 Hz, Ar), 7.01–6.97 (3H, m, Ar), 6.60 (1H, d, *J* = 7.6 Hz, Ar), 3.29 (2H, d, *J* = 6.8 Hz, Ar), 1.61–1.56 (1H, m, CH), 0.89 (6H, d, *J* = 6.0 Hz, 2^{*}CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 162.0, 159.3, 154.7, 145.0, 133.2, 130.7, 130.2, 129.7, 125.2, 120.4, 119.1, 117.3, 117.2, 115.8, 98.8, 57.1, 26.8, 19.8; *t*_R = 13.3 min (100%); HRMS-ESI: *m/z* found 421.1261 [M-H]⁻, C₂₃H₂₁N₄O₁₀S₂ requires 421.1228.

2.1.1.18 Methyl 4-(*N*-benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoate (11). 4-Amino-2-hydroxybenzoic acid was esterified according to general procedure A on a scale of 26 mmol to give methyl 4-amino-2-hydroxybenzoate as a brown solid (4.1 g, 95%): ¹H NMR (CDCl₃, 400 MHz) δ 10.92 (1H, s, OH), 7.59 (1H, d, *J* = 7.6 Hz, Ar), 6.13 (1H, d, *J* = 7.6 Hz, Ar), 6.11 (1H, s, Ar), 3.85 (3H, s, OCH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 170.5, 163.5, 153.3, 131.6, 106.8, 103.0, 100.7, 51.7; methyl 4-amino-2-hydroxybenzoate was *O*-benzylated according to general procedure B on a scale of 12 mmol to give Methyl 4-amino-2-(benzyloxy)benzoate as a beige solid (2.1 g, 70%): ¹H NMR (CDCl₃, 400 MHz) δ 7.78 (1H, d, *J* = 8.8 Hz, Ar), 7.55–7.46 (2H, m, Ar), 7.37 (2H, t, *J* = 7.2 Hz, Ar), 7.30 (1H, t, *J* = 7.2 Hz, Ar), 6.35–6.24 (2H, m, Ar), 5.12 (2H, s, CH₂), 4.02 (2H, br s, NH₂), 3.83 (3H, s, OCH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 161.6, 155.9, 147.1, 132.1, 129.6, 123.8, 122.9, 121.9, 102.1, 94.7, 65.6, 46.7. Methyl 4-amino-2-(benzyloxy)benzoate underwent reductive amination with benzaldehyde according to general procedure C on a scale



of 3.0 mmol to give methyl 4-(benzylamino)-2-(benzyloxy)benzoate as a pale brown solid (500 mg, 48%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.77 (1H, d, J = 8.4 Hz, Ar), 7.46 (2H, d, J = 8.0 Hz, Ar), 7.37–7.24 (7H, m, Ar), 6.19 (1H, d, J = 8.4 Hz, Ar), 6.14 (1H, s, Ar), 5.26 (2H, s, O-CH₂), 4.46 (1H, s, NH), 4.31 (2H, s, N-CH₂), 3.82 (3H, s, O-CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 166.4, 160.7, 152.8, 138.2, 136.9, 134.2, 128.8, 128.5, 127.6, 127.5, 127.4, 126.7, 108.6, 104.9, 97.4, 70.3, 51.4, 47.7. Methyl 4-(benzylamino)-2-(benzyloxy)benzoate was coupled to 4-phenoxybenzenesulfonyl chloride according to general procedure D on a scale of 1.44 mmol to yield the product as a beige solid (800 mg, 96%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.63 (1H, d, J = 8.4 Hz, Ar), 7.55 (2H, d, J = 8.4 Hz, Ar), 7.42–7.34 (6H, m, Ar), 7.30 (1H, d, J = 6.4 Hz, Ar), 7.22–7.16 (4H, m, Ar), 7.12–7.10 (2H, m, Ar), 7.05 (2H, d, J = 8.0 Hz, Ar), 6.98 (2H, d, J = 8.8 Hz, Ar), 6.75 (1H, s, Ar), 6.58 (1H, d, J = 7.6 Hz, Ar), 4.98 (2H, s, O-CH₂), 4.68 (2H, s, N-CH₂), 3.83 (3H, s, O-CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 166.0, 161.9, 158.2, 154.8, 143.5, 136.2, 135.2, 132.0, 131.5, 130.3, 129.9, 128.6, 128.5, 128.4, 127.9, 127.8, 126.8, 125.1, 120.4, 119.6, 119.3, 117.3, 114.8, 70.6, 54.1, 52.1.

2.1.1.19 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoic acid (13). Methyl 4-(N-benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoate was saponified according to general procedure F on a scale of 1.38 mmol to give the product as a beige solid (450 mg, 57%): ^1H NMR (CDCl_3 , 400 MHz) δ 10.5 (1H, s br, COOH), 7.97 (1H, d, J = 8.8 Hz, Ar), 7.54 (2H, d, J = 8.4 Hz, Ar), 7.44–7.34 (6H, m, Ar), 7.24–7.15 (6H, m, Ar), 7.08–7.05 (3H, m, Ar), 6.99 (2H, d, J = 8.4 Hz, Ar), 6.64 (1H, d, J = 8.8 Hz, Ar), 5.14 (2H, s, O-CH₂), 4.72 (2H, s, N-CH₂); ^{13}C NMR (CDCl_3 , 100 MHz) δ 164.5, 162.2, 157.1, 154.7, 144.9, 134.9, 133.9, 131.0, 130.3, 129.8, 129.3, 129.2, 128.6, 128.3, 128.1, 127.9, 125.3, 120.5, 119.7, 117.4, 116.8, 114.8, 72.5, 53.8.

2.1.1.20 Acetoxymethyl 2-(benzyloxy)-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoate (14). 2-(Benzyl)-4-(N-isobutyl-4-phenoxyphenylsulfonamido)benzoic acid¹¹ (0.22 mmol, 116 mg, 1 eq.) was dissolved in anhydrous DMF (0.1 M), followed by the addition of K_2CO_3 (0.44 mmol, 61 mg, 2 eq.), bromomethyl acetate (0.22 mmol, 22 μL , 1 eq.). The reaction was stirred at room temperature overnight. TLC indicated the reaction was completed. The mixture was then partitioned between water (50 mL) and EtOAc (3 \times 50 mL). Organic layer was combined, washed with H_2O , dried over Na_2SO_4 , filtered, concentrated and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4 : 1 to give product as white solid (91 mg, 68%); ^1H NMR (CDCl_3 , 400 MHz) δ 7.78 (1H, d, J = 8.0 Hz, Ar), 7.45 (4H, d, J = 8.4 Hz, Ar), 7.40–7.34 (4H, m, Ar), 7.24 (1H, t, J = 7.2 Hz, Ar), 7.20 (1H, t, J = 6.8 Hz, Ar), 7.03 (2H, d, J = 7.6 Hz, Ar), 6.95–6.90 (3H, m, Ar), 6.58 (1H, d, J = 8.4 Hz, Ar), 5.94 (2H, s, O-CH₂-O), 5.10 (2H, s, O-CH₂-Ph), 3.26 (2H, d, J = 6.8 Hz, N-CH₂), 2.07 (3H, s, COCH₃), 1.52–1.45 (1H, m, CH), 0.83 (6H, d, J = 6.4 Hz, 2*CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 163.7, 161.7, 159.0, 154.9, 145.0, 135.9, 132.6, 131.3, 130.2, 129.7, 128.6, 127.9, 126.9, 125.1, 120.3, 118.8, 117.9, 117.2, 114.8, 79.5, 70.7, 57.3 26.8, 20.7, 19.8.

2.1.1.21 N-(4-Iodophenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (17). 4-Iodo-N-isobutylaniline was

coupled to 4-phenoxybenzenesulfonyl chloride according to general procedure D on a scale of 0.73 mmol to give the product as a beige solid (100 mg, 27%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.61 (2H, d, J = 8.8 Hz, Ar), 7.47 (2H, d, J = 8.8 Hz, Ar), 7.39 (2H, t, J = 7.6 Hz, Ar), 7.20 (1H, t, J = 8.0 Hz, Ar), 7.04 (2H, d, J = 8.0 Hz, Ar), 6.95 (2H, d, J = 8.8 Hz, Ar), 6.81 (2H, d, J = 8.4 Hz, Ar), 3.25 (2H, d, J = 7.2 Hz, N-CH₂), 1.55–1.52 (1H, m, CH), 0.87 (6H, d, J = 6.8 Hz, 2*CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 161.6, 154.9, 139.3, 138.2, 131.4, 130.3, 130.1, 129.8, 125.0, 120.3, 117.2, 93.1, 57.5, 26.8, 19.8.

2.1.1.22 N-(4-Cyanophenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (18). A stirring solution of *N*-(4-iodophenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (17; 0.6 mmol, 300 mg, 1 eq.) in anhydrous DMF (0.1 M) was degassed, and then $\text{Zn}(\text{CN})_2$ (0.6 mmol, 70 mg, 1 eq.), $\text{Pd}(\text{PPh}_3)_4$ (0.3 mmol, 347 mg, 0.5 eq.), Zn (0.03 mmol, 2 mg, 0.05 eq.) and $\text{Zn}(\text{OAc})_2$ (0.03 mmol, 6 mg, 0.05 eq.) were added. The reaction was heated to 120 °C for 2 h. After which reaction was allowed to cool, add water, extract with EtOAc 3 times. The organic layers were combined and washed with NaHCO_3 (aq), dried over Na_2SO_4 , concentrated, and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 6 : 1 to give the product as a brown solid (100 mg, 41%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.60 (2H, d, J = 7.6 Hz, Ar), 7.44–7.38 (4H, m, Ar), 7.22 (3H, d, J = 8.4 Hz, Ar), 7.04 (2H, d, J = 8.0 Hz, Ar), 6.95 (2H, d, J = 7.6 Hz, Ar), 3.32 (2H, d, J = 7.2 Hz, N-CH₂), 1.60–1.51 (1H, m, CH), 0.88 (6H, d, J = 6.4 Hz, 2*CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 161.9, 143.8, 132.8, 130.9, 130.2, 129.6, 128.7, 125.1, 120.4, 118.1, 117.3, 111.2, 57.1, 26.9, 19.8.

2.1.1.23 Methyl 3-(4-(N-isobutyl-4-phenoxyphenylsulfonamido)phenyl)propiolate (19). *N*-(4-Iodophenyl)-N-isobutyl-4-phenoxybenzenesulfonamide (17; 0.6 mmol, 300 mg, 1 eq.) was dissolved in anhydrous toluene (0.1 M), followed by PPh_3 (0.06 mmol, 15.7 mg, 0.1 eq.), CuI (0.24 mmol, 46 mg, 0.4 mmol) and Et_3N (4.8 mmol, 669 μL , 8 eq.). The system was flushed with N_2 for 5 min, then $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.12 mmol, 84 mg, 0.2 eq.) and methyl propionate (2.4 mmol, 213.5 μL , 4 eq.) was added. The reaction was stirred in a microwave reactor at 110 °C for 40 min with high absorption level, then cooled to room temperature and stirred overnight. TLC indicated the reaction was complete. The reaction mixture was partitioned between H_2O and DCM 3 times, organic layer was collected and dried over Na_2SO_4 , concentrated, and purified by flash column chromatography over silica gel using an eluent of hexanes/ethyl acetate 4 : 1 to give the product as a yellow oil (124 mg, 44%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.53 (2H, d, J = 8.4 Hz, Ar), 7.47–7.39 (4H, m, Ar), 7.24 (1H, t, J = 8.0 Hz, Ar), 7.12 (2H, d, J = 8.8 Hz, Ar), 7.06 (2H, d, J = 6.8 Hz, Ar), 6.96 (2H, d, J = 8.4 Hz, Ar), 3.04 (3H, s, O-CH₃), 3.31 (2H, d, J = 8.0 Hz, N-CH₂), 1.59–1.55 (1H, m, CH), 0.89 (6H, d, J = 6.0 Hz, 2*CH₃); ^{13}C NMR (CDCl_3 , 100 MHz) δ 161.7, 154.9, 154.3, 141.6, 133.6, 131.2, 130.2, 129.7, 128.4, 125.1, 120.3, 118.7, 117.2, 85.4, 81.1, 57.3, 52.9, 26.8, 19.8.

2.1.1.24 4-Fluoro-3-nitrobenzenesulfonamide. 2-fluoronitrobenzene (35 mmol, 5 g, 1 eq.) was dissolved in chlorosulfonic acid (11 mL) and heated at 95 °C overnight. The reaction mixture was cooled to room temperature, whilst 110 mL



isopropanol and 30 mL $\text{NH}_3 \cdot \text{H}_2\text{O}$ was prepared in a round bottom flask and cooled to -40°C . The reaction mixture was slowly added into the flask over 1 h, which formed yellow slurry. Upon the completion of addition, TLC indicated the reaction was complete. 3 M HCl was added to the reaction till pH was acidic. The mixture was partitioned between H_2O and EtOAc 3 times, organic layer was collected and combined, dried over Na_2SO_4 , concentrated then reconstituted with H_2O to reform the slurry, filtered, and washed with small amount of H_2O , filter cake was collected and dried in an vacuum oven overnight to yield the product as a beige solid (1.7 g, 22%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.50 (1H, d, $J = 7.2$ Hz, Ar), 8.18–8.15 (1H, m, Ar), 7.81–7.76 (1H, m, Ar), 7.71 (2H, s, NH_2); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 158.0, 155.4, 141.3, 133.9, 133.8, 124.5, 120.5, 120.3.

2.1.1.25 3-Nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino benzenesulfonamide (20). 4-Fluoro-3-nitrobenzenesulfonamide (7.75 mmol, 1.7 g, 1 eq.) was dissolved in THF (0.1 M) followed by TEA (9.3 mmol, 1.3 mL, 1.2 eq.) and 1-tetrahydropyran-4-yl-methylamine (7.75 mmol, 892 mg, 1 eq.). The reaction was stirred at room temperature overnight. TLC indicated the reaction was complete. The reaction was diluted with EtOAc, then washed the organic layer with saturated NaH_2PO_4 solution, brine, then dried over dried over Na_2SO_4 , concentrated and purified by flash column chromatography over silica gel using an eluent of hexanes/ethyl acetate, 1:2 to give the product as a bright yellow solid (2.1 g, 90%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.55 (1H, t, $J = 5.2$ Hz, NH), 8.42 (1H, d, $J = 1.2$ Hz, Ar), 7.78 (1H, d, $J = 9.2$ Hz, Ar), 7.31 (2H, s, NH_2), 7.26 (1H, d, $J = 8.4$ Hz, Ar), 3.83–3.79 (2H, m, NH-CH_2), 3.33–3.19 (4H, m, 2^*CH_2), 1.88–1.585 (1H, m, CH), 1.57 (2H, d, $J = 12.4$ Hz, CH_2), 1.26–1.17 (2H, m, CH_2); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 147.1, 133.2, 130.4, 129.8, 125.2, 116.2, 67.1, 48.2, 39.3, 30.6.

2.1.1.26 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-N-(methylsulfonyl)benzamide (21a). 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoic acid was coupled to methanesulfonamide according to general procedure H on a scale of 0.18 mmol to give the product as a beige solid (56 mg, 56%); ^1H NMR (CDCl₃, 400 MHz) δ 10.14 (1H, s, NH), 8.01 (1H, d, $J = 8.8$ Hz, Ar), 7.56 (2H, d, $J = 8.4$ Hz, Ar), 7.45–7.36 (7H, m, Ar) 7.26–7.17 (6H, m, Ar), 7.08 (2H, d, $J = 8.0$ Hz, Ar), 7.03–7.00 (3H, m, Ar), 6.68 (1H, d, $J = 8.0$ Hz, Ar), 5.12 (2H, s, O-CH₂), 4.76 (2H, s, N-CH₂), 3.27 (3H, s, CH₃); ^{13}C NMR (CDCl₃, 100 MHz) δ 162.6, 162.2, 157.0, 154.7, 145.3, 134.9, 133.9, 132.9, 131.1, 130.3, 129.8, 129.2, 128.3, 127.9, 127.8, 125.3, 120.5, 119.6, 117.7, 117.4, 114.7, 72.3, 53.8, 41.6.

2.1.1.27 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-N-(phenylsulfonyl)benzamide (21b). 4-(N-benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoic acid (13) was coupled to benzenesulfonamide according to general procedure H on a scale of 0.18 mmol to give the product as a beige solid (55 mg, 44%); ^1H NMR (CDCl₃, 400 MHz) δ 10.24 (1H, s, NH), 7.93 (2H, d, $J = 8.0$ Hz, Ar), 7.84 (1H, d, $J = 8.0$ Hz, Ar), 7.56 (1H, t, $J = 8.0$ Hz, Ar), 7.52–7.38 (10H, m, Ar), 7.21–7.13 (7H, m, Ar), 7.05 (2H, d, $J = 8.0$ Hz, Ar), 6.98–6.95 (3H, m, Ar), 6.57 (1H, d, $J = 8.8$ Hz, Ar), 5.09 (2H, s, O-CH₂), 4.70 (2H, s, N-CH₂); ^{13}C NMR (CDCl₃, 100 MHz) δ 184.0, 178.4, 162.1, 161.5, 156.8, 144.9, 138.8, 134.9, 134.1, 133.7, 132.8, 130.9, 130.3,

129.7, 129.2, 128.8, 128.6, 128.4, 128.3, 128.0, 127.9, 125.3, 120.5, 119.3, 118.1, 117.3, 114.8, 72.2, 53.8.

2.1.1.28 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)-N-((3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (21c). 4-(N-Benzyl-4-phenoxyphenylsulfonamido)-2-(benzyloxy)benzoic acid (13) was coupled to 3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)benzenesulfonamide (20) according to general procedure H on a scale of 0.27 mmol to give the product as bright yellow solid (66 mg, 30%); ^1H NMR (CDCl₃, 400 MHz) δ 10.28 (1H, s, SO₂NH), 8.73 (1H, s, NHCH₂), 8.49 (1H, t, $J = 5.2$ Hz, Ar), 8.01 (1H, d, $J = 8.8$ Hz, Ar), 7.84 (1H, d, $J = 8.0$ Hz, Ar), 7.52–7.38 (8H, m, Ar), 7.24–7.12 (7H, m, Ar), 7.05 (2H, d, $J = 7.6$ Hz, Ar), 6.97–6.95 (3H, m, Ar), 6.85 (1H, d, $J = 10.0$ Hz, Ar), 6.59 (1H, d, $J = 8.8$ Hz, Ar), 5.09 (2H, s, O-CH₂), 4.70 (2H, s, N-CH₂), 4.02–3.98 (2H, m, CH₂), 3.38 (2H, t, $J = 12.4$ Hz, CH₂), 3.23 (2H, t, $J = 6.0$ Hz, CH₂), 1.93–1.90 (1H, m, CH), 1.70 (2H, d, $J = 12.4$ Hz, CH₂), 1.45–1.35 (2H, m, CH₂); ^{13}C NMR (CDCl₃, 100 MHz) δ 162.1, 161.7, 156.9, 154.6, 147.9, 145.1, 135.5, 134.9, 133.9, 132.7, 130.9, 130.3, 129.7, 129.4, 129.3, 129.1, 128.6, 128.3, 127.9, 125.3, 124.8, 120.5, 119.4, 117.8, 117.3, 114.6, 113.7, 72.3, 67.4, 53.8, 49.1, 34.6, 30.7.

2.1.1.29 4-(N-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)-N-((3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonyl)benzamide (21d). 4-(N-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)benzoic acid¹¹ was coupled to 3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)benzenesulfonamide (20) according to general procedure H on a scale of 0.32 mmol to give the product as a bright yellow solid (132 mg, 43%); ^1H NMR (CDCl₃, 400 MHz) δ 10.28 (1H, s, SO₂NH), 8.72 (1H, d, $J = 2.4$ Hz, Ar), 8.49 (1H, t, $J = 5.6$ Hz, Ar), 8.00 (1H, d, $J = 8.8$ Hz, Ar), 7.84 (1H, d, $J = 8.8$ Hz, Ar), 7.52–7.42 (5H, m, Ar), 7.39 (2H, d, $J = 6.8$ Hz, Ar), 7.21–7.12 (5H, m, Ar), 6.98 (1H, s, NHCH₂), 6.94 (2H, d, $J = 8.4$ Hz, Ar), 6.85 (1H, d, $J = 8.8$ Hz, Ar), 6.78 (2H, s, Ar), 6.58 (1H, d, $J = 8.4$ Hz, Ar), 5.09 (2H, s, O-CH₂), 4.70 (2H, s, N-CH₂), 4.01–3.98 (2H, m, CH₂), 3.41–3.21 (4H, m, 2^*CH_2), 2.35 (6H, s, 2^*CH_3), 1.93–1.91 (1H, m, CH), 1.70 (2H, d, $J = 12.4$ Hz, CH₂), 1.45–1.37 (2H, m, CH₂); ^{13}C NMR (CDCl₃, 100 MHz) δ 162.1, 161.7, 156.9, 152.3, 147.9, 145.1, 138.4, 135.5, 134.9, 133.9, 132.7, 131.1, 130.7, 129.7, 129.4, 129.3, 139.1, 128.6, 128.3, 127.9, 124.8, 120.3, 119.4, 117.8, 117.2, 114.7, 117.2, 114.7, 113.7, 97.8, 72.3, 67.4, 53.8, 49.1, 34.6, 30.7, 20.9.

2.1.1.30 *N*-(4-Bromo-3-methoxyphenyl)-*N*-isobutyl-4-phenoxybenzenesulfonamide (23). 4-Bromo-3-methoxyaniline (22) underwent reductive amination with isobutyraldehyde according to general procedure C on a scale of 4.95 mmol to give 4-bromo-*N*-isobutyl-3-methoxyaniline as a brown oil (1 g, 78%); ^1H NMR (CDCl₃, 400 MHz) δ 6.96 (1H, d, $J = 8.4$ Hz, Ar), 5.88 (1H, s, Ar), 5.82 (1H, d, $J = 7.6$ Hz, Ar), 3.56 (3H, s, O-CH₃), 3.49 (1H, s br, NH), 2.62 (2H, d, $J = 6.4$ Hz, N-CH₂), 1.63–1.56 (1H, m, CH), 0.90 (6H, d, $J = 6.0$ Hz, 2^*CH_3); ^{13}C NMR (CDCl₃, 100 MHz) δ 165.8, 156.4, 149.2, 133.2, 105.9, 97.3, 55.9, 51.8, 28.1, 20.5. 4-Bromo-*N*-isobutyl-3-methoxyaniline was coupled to 4-phenoxypyrene sulfonyl chloride according to general procedure D on a scale of 3.8 mmol to give the product as a beige solid (1.6 g, 89%); ^1H NMR (CDCl₃, 400 MHz) δ 7.49 (2H, d, $J = 6.8$ Hz, Ar),



7.39 (3H, t, J = 8.4 Hz, Ar), 7.20 (1H, t, J = 7.6 Hz, Ar), 7.04 (2H, d, J = 7.6 Hz, Ar), 6.96 (2H, d, J = 8.0 Hz, Ar), 6.72 (1H, s, Ar), 6.40 (1H, d, J = 8.0 Hz, Ar), 3.81 (3H, s, O-CH₃), 3.26 (2H, d, J = 6.8 Hz, N-CH₂), 1.61–1.55 (1H, m, CH), 0.89 (6H, d, J = 6.4 Hz, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 161.6, 156.1, 154.9, 139.9, 133.0, 131.3, 130.2, 129.8, 125.0, 120.6, 120.3, 117.2, 113.5, 111.0, 57.8, 56.3, 26.8, 19.9.

2.1.1.31 *N*-(4-Cyano-3-methoxyphenyl)-*N*-isobutyl-4-phenoxybenzenesulfonamide (24). A stirring solution of *N*-(4-bromo-3-methoxyphenyl)-*N*-isobutyl-4-phenoxybenzenesulfonamide (23; 0.2 mmol, 100 mg, 1 eq.) in anhydrous DMF (0.1 M) was degassed, and then Zn(CN)₂ (0.2 mmol, 24 mg, 1 eq.), Pd(PPh₃)₄ (0.1 mmol, 120 mg, 0.5 eq.), Zn (0.1 mmol, 10 mg, 0.5 eq.) and Zn(OAc)₂ (0.1 mmol, 20 mg, 0.5 eq.) were added. The reaction was heated to 120 °C for 2 h. After which reaction was allowed to cool, add water, extract with EtOAc 3 times. The organic layers were combined and washed with NaHCO₃ (aq), dried over Na₂SO₄, concentrated, and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 4:1 to give the product as a light yellow solid (30 mg, 34%): ¹H NMR (CDCl₃, 400 MHz) δ 7.69–7.59 (4H, m, Ar), 7.46–7.41 (2H, m, Ar), 7.25 (2H, d, J = 7.6 Hz, Ar), 7.17 (2H, d, J = 8.0 Hz, Ar), 7.11 (1H, s, Ar), 6.77 (1H, d, J = 8.0 Hz, Ar), 4.09 (3H, s, O-CH₃), 3.51 (2H, d, J = 6.8 Hz, CH₂), 1.81–1.77 (1H, m, CH), 1.10 (6H, d, J = 6.4 Hz, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 161.9, 161.5, 154.8, 145.4, 133.7, 130.9, 130.2, 129.7, 125.1, 120.3, 118.8, 117.2, 115.8, 112.7, 100.8, 57.2, 56.2, 26.9, 19.9.

2.1.1.32 5-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-hydroxybenzoic acid (27). Methyl 5-amino-2-hydroxybenzoate was reductive aminated by benzaldehyde according to general procedure C on a scale of 6.0 mmol to give methyl 5-(benzylamino)-2-hydroxybenzoate as a brown oil (1.26 g, 81%): ¹H NMR (CDCl₃, 400 MHz) δ 10.18 (1H, s, OH), 7.35–7.24 (5H, m, Ar), 7.08 (1H, s, Ar), 6.83 (2H, s, Ar), 4.26 (2H, s, N-CH₂), 3.90 (3H, s, O-CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 170.5, 154.4, 140.6, 139.1, 128.7, 127.7, 127.4, 122.8, 118.2, 112.1, 111.7, 52.2, 49.2. Methyl 2-hydroxy-5-(isobutylamino)benzoate was coupled to 4-fluorobenzenesulfonyl chloride according to general procedure D on a scale of 4.9 mmol to yield methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-hydroxybenzoate as a beige solid (1.85 g, 90%): ¹H NMR (CDCl₃, 400 MHz) δ 10.76 (1H, s, OH), 7.69–7.65 (2H, m, Ar), 7.45 (1H, d, J = 3.2 Hz, Ar), 7.25–7.16 (7H, m, Ar), 6.94 (1H, d, J = 9.2 Hz, Ar), 6.79 (1H, d, J = 9.2 Hz, Ar), 4.67 (2H, s, N-CH₂), 3.00 (3H, s, O-CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 169.7, 166.5, 163.9, 161.0, 135.9, 135.3, 130.8, 130.4, 130.3, 129.6, 128.6, 128.5, 127.9, 118.2, 116.4, 116.1, 112.5, 54.8, 52.5. Methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-hydroxybenzoate was *O*-benzylated according to general procedure B on a scale of 4.45 mmol to give methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-(benzyloxy)benzoate as a white solid (1.21 g, 54%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.71–7.67 (2H, m, Ar), 7.46–7.39 (4H, m, Ar), 7.35 (2H, t, J = 7.2 Hz, Ar), 7.29–7.14 (8H, m, Ar), 7.07 (1H, d, J = 8.8 Hz, Ar), 5.09 (2H, s, O-CH₂), 4.73 (2H, s, N-CH₂), 3.71 (3H, s, O-CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 166.4, 165.5, 163.8, 156.9, 136.9, 136.4, 134.2, 133.8, 131.6, 131.0, 130.9, 128.9, 128.8, 128.6, 128.2, 127.9, 127.4, 120.6, 117.2, 116.9, 114.6, 70.2, 53.9, 52.5.

Methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-hydroxybenzoate was *O*-benzylated according to general procedure B on a scale of 4.45 mmol to give methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-(benzyloxy)benzoate as a white solid (1.21 g, 54%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.71–7.67 (2H, m, Ar), 7.46–7.39 (4H, m, Ar), 7.35 (2H, t, J = 7.2 Hz, Ar), 7.29–7.14 (8H, m, Ar), 7.07 (1H, d, J = 8.8 Hz, Ar), 5.09 (2H, s, O-CH₂), 4.73 (2H, s, N-CH₂), 3.71 (3H, s, O-CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 166.4, 165.5, 163.8, 156.9, 136.9, 136.4, 134.2, 133.8, 131.6, 131.0, 130.9, 128.9, 128.8, 128.6, 128.2, 127.9, 127.4, 120.6, 117.2, 116.9, 114.6, 70.2, 53.9, 52.5. Methyl 5-(*N*-benzyl-4-fluorophenylsulfonamido)-2-(benzyloxy)benzoate was coupled to 3,5-dimethyl-4-chloro-phenol according to general procedure E on a scale of 2.4 mmol to yield the product as an ivory solid (778 mg, 50%): ¹H NMR (CDCl₃, 400 MHz) δ 7.58 (2H, d, J = 8.8 Hz, Ar), 7.44 (2H, d, J = 7.6 Hz, Ar), 7.39–7.35 (3H, m, Ar), 7.31 (1H, d, J = 7.2 Hz, Ar), 7.22–7.17 (5H, m, Ar), 7.16–7.13 (1H, m, Ar), 7.00 (2H, d, J = 9.6 Hz, Ar), 6.87–6.84 (3H, m, Ar), 5.10 (2H, s, O-CH₂), 4.68 (2H, s, N-CH₂), 3.84 (3H, s, O-CH₃), 2.39 (6H, s, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 165.7, 161.6, 157.6, 152.7, 138.3, 136.2, 135.5, 135.2, 131.8, 131.5, 131.3, 130.7, 129.9, 128.6, 128.5, 127.9, 127.7, 126.7, 120.6, 120.1, 117.4, 113.9, 70.6, 51.7, 52.1, 20.9. Methyl 5-(*N*-benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)benzoate was saponified according to general procedure F except the temperature was raised to 60 °C on a scale of 1.21 mmol to afford 5-(*N*-benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)benzoic acid as a beige solid (645 mg, 85%): ¹H NMR (CDCl₃, 400 MHz) δ 7.58 (1H, d, J = 2.4 Hz, Ar), 7.52 (2H, d, J = 8.8 Hz, Ar), 7.41–7.38 (5H, m, Ar), 7.21–7.16 (5H, m, Ar), 6.99–6.96 (3H, m, Ar), 6.84 (2H, s, Ar), 5.18 (2H, s, O-CH₂), 4.68 (2H, s, N-CH₂), 2.36 (6H, s, 2*CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 164.6, 161.9, 156.6, 152.5, 138.3, 137.8, 135.2, 133.8, 133.2, 131.6, 130.8, 129.8, 129.3, 129.2, 128.6, 128.3, 128.0, 127.8, 127.6, 120.3, 117.4, 114.5, 113.7, 72.5, 54.4, 20.9. 5-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)benzoic acid was debenzylated according to general procedure G on a scale of 0.16 mmol to give 5-(*N*-benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-hydroxybenzoic acid (27) as a beige solid (69 mg, 80%): ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.59 (2H, d, J = 8.4 Hz, Ar), 7.29 (1H, s, Ar), 7.23–7.19 (5H, m, Ar), 7.16–7.06 (3H, m, Ar), 7.00 (2H, s, Ar), 6.75 (1H, d, J = 8.8 Hz, Ar), 4.68 (2H, s, N-CH₂), 2.31 (6H, s, 2*CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.4, 153.1, 138.4, 136.5, 135.8, 131.9, 130.9, 130.4, 130.1, 129.7, 128.8, 128.6, 127.9, 120.7, 118.0, 117.9, 54.2, 20.8; HRMS-ESI: *m/z* found 536.0945 [M–H][–], C₂₈H₂₃ClNO₆S requires 536.0940.

2.1.1.33 5-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-hydroxy-*N*-(3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)phenylsulfonylbenzamide (29). 5-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)benzoic acid (27) was coupled to 3-nitro-4-((tetrahydro-2H-pyran-4-yl)methyl)amino)benzenesulfonamide (20) according to general procedure H on a scale of 0.24 mmol to give 5-(*N*-benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-



(benzyloxy)-*N*-((3-nitro-4-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)phenyl)sulfonyl)benzamide (**29**) as a bright yellow solid (53 mg, 25%): ^1H NMR (CDCl_3 , 400 MHz) δ 10.32 (1*H*, s, NHSO_2), 8.70 (1*H*, s, Ar), 8.49 (1*H*, t, J = 5.2 Hz, Ar), 7.98 (1*H*, d, J = 9.6 Hz, Ar), 7.61 (1*H*, d, J = 2.4 Hz, Ar), 7.52–7.40 (7*H*, m, Ar), 7.31 (1*H*, d, J = 8.4 Hz, Ar), 7.24–7.16 (5*H*, m, Ar), 6.95–6.92 (3*H*, m, Ar and NHCH_2), 6.86–6.83 (3*H*, m, Ar), 5.49 (2*H*, s, $\text{O}-\text{CH}_2$), 4.62 (2*H*, s, $\text{N}-\text{CH}_2$), 4.00 (2*H*, d, J = 11.6 Hz, CH_2), 3.38 (2*H*, t, J = 10.8 Hz, CH_2), 3.22 (2*H*, t, J = 6.4 Hz, CH_2), 2.38 (6*H*, s, 2* CH_3), 2.00–1.92 (1*H*, m, CH), 1.69 (2*H*, d, J = 12.4 Hz, CH_2), 1.42–1.36 (2*H*, m, CH₂); ^{13}C NMR (CDCl_3 , 100 MHz) δ 161.8, 161.4, 156.2, 152.5, 147.9, 138.4, 137.1, 135.3, 135.2, 133.9, 133.0, 131.5, 131.0, 130.7, 129.7, 129.5, 129.3, 128.9, 128.5, 128.4, 127.9, 127.8, 124.7, 120.3, 119.4, 117.4, 113.7, 113.6, 72.5, 67.4, 54.3, 49.1, 34.6, 30.7, 20.9. 5-(*N*-Benzyl-4-(4-chloro-3,5-dimethylphenoxy)phenylsulfonamido)-2-(benzyloxy)-*N*-((3-nitro-4-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)phenyl)sulfonyl)benzamide was debenzylated according to general procedure G on a scale of 0.06 mmol to give the product as a bright yellow solid (22 mg, 45%): ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 8.51 (2*H*, m, Ph-*NH* and Ar), 7.85 (1*H*, d, J = 8.8 Hz, Ar), 7.59 (2*H*, d, J = 7.6 Hz, Ar), 7.47 (1*H*, d, J = 8.0 Hz, Ar), 7.19–7.07 (8*H*, m, Ar), 6.99 (2*H*, s, Ar), 6.56–6.51 (2*H*, m, Ar), 4.73 (2*H*, s, $\text{N}-\text{CH}_2$), 3.79 (2*H*, d, J = 9.6 Hz, CH_2), 3.28–3.18 (4*H*, m, 2* CH_3), 2.97 (6*H*, s, 2* CH_3), 1.87–1.84 (1*H*, m, CH), 1.55 (2*H*, d, J = 12.4 Hz, CH_2), 1.21–1.18 (2*H*, m, CH₂); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ 161.4, 153.0, 147.3, 143.4, 138.4, 136.5, 134.8, 131.8, m 130.3, 129.8, 128.8, 128.3, 127.8, 120.7, 118.1, 117.8, 116.5, 115.1, 67.0, 53.2, 48.3, 34.2, 30.6, 20.7; HRMS-ESI: *m/z* found 833.1886 [$\text{M}-\text{H}$][–], $\text{C}_{40}\text{H}_{38}\text{ClN}_4\text{O}_{10}\text{S}_2$ requires 833.1723.

2.2 Biology

2.2.1 Protein production. A His6-MBP tagged recombinant human Mcl-1 residues 172 to 327 was produced in *E. coli* in either LB or minimal media supplemented with $^{15}\text{NH}_4\text{Cl}$ to produce unlabeled or ^{15}N -labeled Mcl-1. The tagged protein was initially purified from the crude cell lysate by IMAC chromatography (GE Healthcare Life Sciences), and after dialysis to remove the imidazole the affinity tag was cleaved using PreScission Protease (GE Healthcare Life Sciences). A Sephadryl S-200 size exclusion column was used as a final purification step before the protein was concentrated with a 10 000 MWCO centrifugal filter concentrator (Millipore). The protein purity was shown to be >98% by Coomassie Brilliant Blue (Bio-Rad) stained SDS-PAGE gel and the final concentration was determined using the Bradford protein assay (Bio-Rad) with BSA standards (Pierce).

BCL-xL was purchased from SinBiological.

2.2.2 Peptide synthesis and purification. A 6-amino-hexanoic acid linker was conjugated to the N-terminus of the Bak BH3 peptide (GQVGRQLAIIGDDINR), capped with fluorescein (on the amino group of the linker), and the peptide was amidated on the C-terminus to give FITC-Ahx-GQVGRQLAIIGDDINR-CONH₂, hereafter referred to as “FITC-Bak” (synthesized by Neo BioScience in >95% purity).

2.2.3 Fluorescence polarization experiments. Fluorescence polarization experiments were conducted using a BMG

PHERAstar FS multimode microplate reader equipped with two PMTs for simultaneous measurements of the perpendicular and parallel fluorescence emission. The assays were performed in black polypropylene 384-well microplate (Costar) with a final volume of 20 mL. The affinity (K_d) of the FITC-Bak peptide was determined by titrating Mcl-1^{172–327} into 10 nM FITC-Bak peptide in 20 mM HEPES, pH 6.8, 50 mM NaCl, 3 mM DTT, 0.01% Triton X-100 and 5% DMSO at room temperature while monitoring the perpendicular and parallel fluorescence emission with a 485 nm excitation and 520 nm emission filters. The fluorescence polarization competition assay (FPCA) was performed using 100 nM Mcl-1^{172–327} (or 15 nM Bcl-x_L^{2–212} (R&D Systems)) in the same buffer (thus, 10 nM FITC-Bak) with varying concentrations of either unlabeled peptide or 2,6-disubstituted nicotinate. Regression analysis was carried out using Origin (OriginLab, Northampton, MA) to fit the data to the Hill equation to determine the IC_{50} , which delivered K_d s for the FITC-Bak peptide to Mcl-1 of 33.8 ± 0.50 nM and to Bcl-x_L of 6.67 ± 0.05 nM. For the fluorescence polarization competition titrations, an equation derived by Nikolovska-Coleska *et al.*¹⁶ was used to calculate the K_i from the IC_{50} data. All experiments were run in three biological replicates, each in triplicate.

2.2.4 Cell viability assay. Cell viability studies were conducted using the CellTiter-Blue® (Promega, Madison, WI) assay. Briefly, HL60 cells, were seeded in a 96-well plate and maintained at 37 °C with 5% CO₂ for 24 h. The cells were then exposed to test compounds at 11 concentrations ranging from 0 to 20 mM. After 72 h, cell viabilities were then determined according to the manufacturer's instructions.

3 Results and discussion

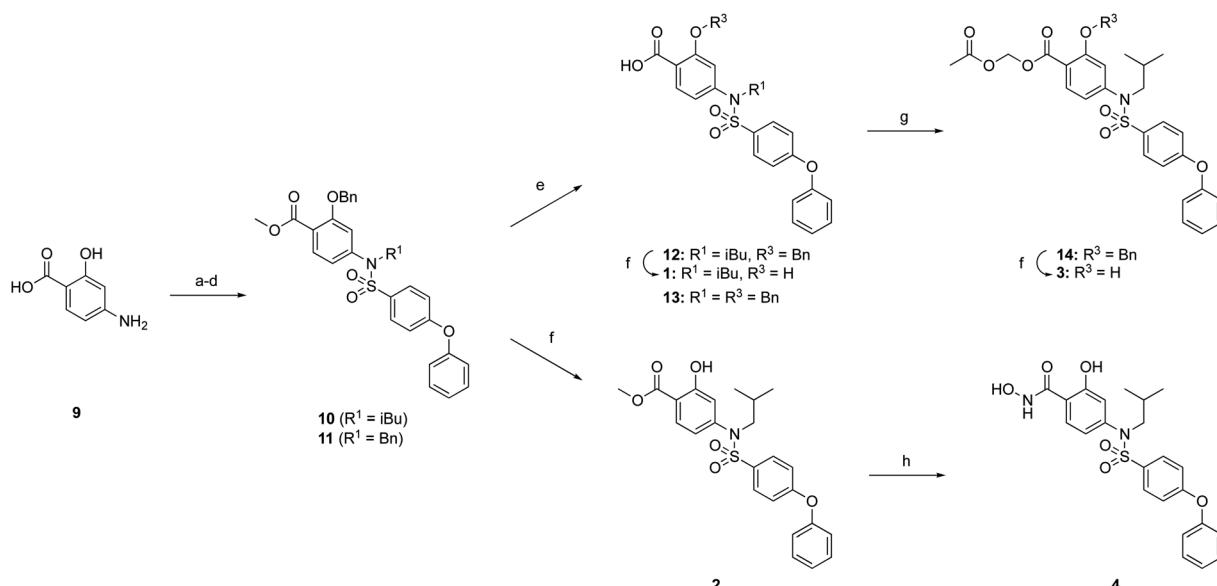
Fig. 1 depicts the target molecules of this work, which vary in the salicylic acid portion in blue (and in some cases the R¹ group is iBu instead of Bn). Although the lead compound was “17cd” (K_i s = 0.629 (MCL-1) and 1.67 (BCL-x_L) μM), for this work, we initially developed analogues of the simpler compound **1**.¹¹ Methyl ester **2** and acetoxyethyl ester **3** are proposed as prodrugs of **1**,¹¹ similarly to how we improved the cell permeability of our lead Myc inhibitor JY-3-094.¹⁷ Classical bioisosteric targets include the hydroxamic acid **4**, which has a pK_a of around 8–9,¹⁸ as well as tetrazole **5**, 3-hydroxyisoxazole **6** (both of **5** and **6** lack the phenyl hydroxyl group owing to synthetic challenges), and acylsulfonamides **7**, all of which have pK_a values of around 4–5,¹⁸ *i.e.* akin to that of the parent carboxylic acid. Cyanophenol **8**, bearing a pK_a of about 7, is an unconventional/putative bioisostere. It is noteworthy that the acylsulfonamide functionality may be further developed beyond the group itself (R² group in **7**), without compromising the acidity, to gain additional interactions with the target. Indeed, Fesik's group previously expanded their MCL-1 inhibitors in this fashion,¹⁹ and it is of interest to elucidate if a similar modification of our inhibitors is tolerated for targeting not only MCL-1 but BCL-xL as well. Likewise, the NH of hydroxamic acids, such as in **4**,²⁰ and the CH alkene carbon of **6**, may be further elaborated whilst retaining the acidic NH.



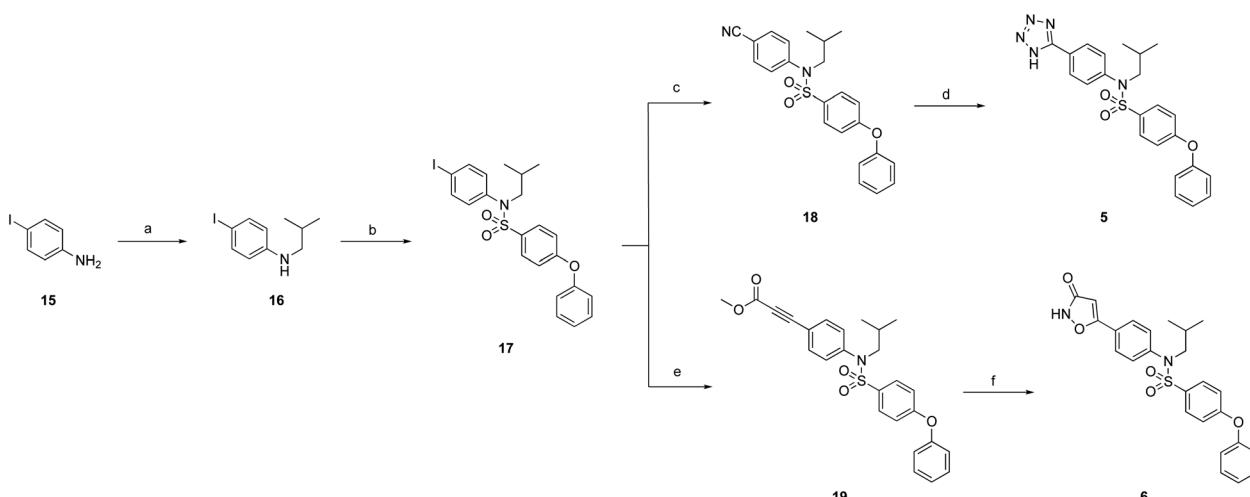
The ester prodrugs **2** and **3** were prepared from 4-amino-salicylic acid (**9**), as shown in Scheme 1. As previously described,¹¹ esterification, *O*-benzylation, followed by reductive amination with either isobutyraldehyde or benzaldehyde, and lastly sulfonylation of the furnished secondary amines delivered tertiary sulfonamides **10** (ref. 11) and **11**, respectively. Saponification of esters of **10** and **11** yielded acids **12** and **13**, and then TFA-mediated debenzylation²¹ of **12** furnished salicylic acid lead compound **1**. Re-esterification of **12** with acetoxyethyl bromide followed again by an uneventful debenzylation

afforded the acetoxyethyl ester prodrug **3**. Meanwhile, debenzylation of **10** yielded methyl ester prodrug **2** again in excellent yield. Finally, hydroxamic acid **4** was acquired by treatment of **2** with hydroxylamine under basic conditions.

Towards the synthesis of tetrazole **5** (Scheme 2), 4-iodoaniline (**15**) underwent reductive amination with isobutyraldehyde, and then *N*-sulfonylation yielded tertiary sulfonamide **17**. Subsequently, Pd-catalyzed cyanation of **17** with zinc cyanide, doped with zinc dust and zinc acetate,²² delivered nitrile **18**. Finally, a cycloaddition of azide to nitrile **18** delivered the

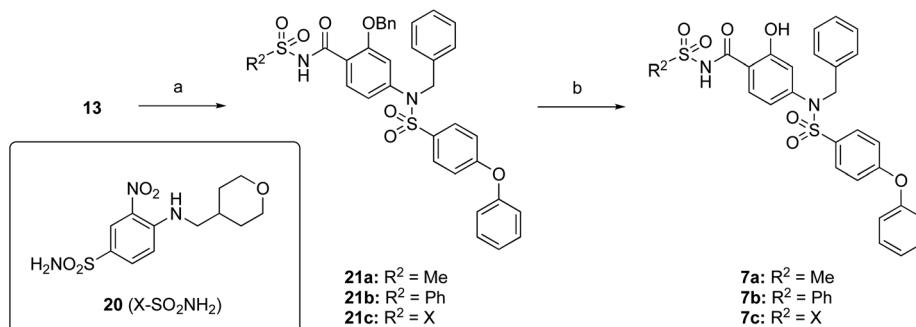


Scheme 1 Reagents and conditions: (a) H_2SO_4 , MeOH, reflux, overnight, 95%; (b) BnBr , $\text{K}'\text{OBu}$, DMF, RT, overnight, 70%; (c) isobutyraldehyde or benzaldehyde, $\text{NaBH}(\text{OAc})_3$, DCE, acetic acid, RT, overnight, 46%; (d) 4-phenoxybenzenesulfonyl chloride, DIPEA, DMAP, CHCl_3 , 60 °C, overnight, 96%; (e) $\text{LiOH}\cdot\text{H}_2\text{O}$, THF-MeOH- H_2O , 3 : 1 : 1, RT, overnight, 57–99%; (f) TFA, toluene, RT, overnight, 80–89%; (g) bromomethyl acetate, K_2CO_3 , DMF, RT, overnight, 68%; (h) $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOH, H_2O , dioxane, RT, overnight, 71%.

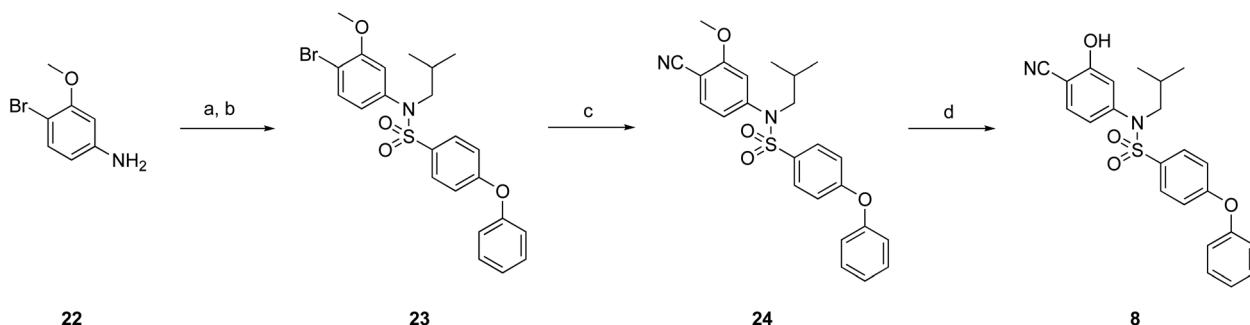


Scheme 2 Reagents and conditions: (a) isobutyraldehyde, $\text{NaBH}(\text{OAc})_3$, DCE, acetic acid, RT, overnight, 78%; (b) 4-phenoxybenzenesulfonyl chloride, DIPEA, DMAP, CHCl_3 , 60 °C, overnight, 89%; (c) $\text{Zn}(\text{CN})_2$, $\text{Pd}(\text{PPh}_3)_4$, $\text{Zn-Zn}(\text{OAc})_2$, 1 : 1, DMF, 120 °C, 2 h, 41%; (d) Na_3N , NH_4Cl , DMF, 125 °C, overnight, 76%; (e) PPh_3 , CuI , Et_3N , $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, methyl propionate, 110 °C, μ waves, 40 min, 44%; (f) $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOH, MeOH, RT, overnight, 20%.





Scheme 3 Reagents and conditions. (a) $R^2SO_2NH_2$, EDCI·HCl, DMAP, CH_2Cl_2 , RT, overnight, 30–56%; (b) TFA, toluene, RT, overnight, 40–50%.



Scheme 4 Reagents and conditions: (a) isobutyraldehyde, $NaBH(OAc)_3$, DCE, acetic acid, RT, overnight, 78%; (b) 4-phenoxybenzenesulfonyl chloride, DIPEA, DMAP, $CHCl_3$, 60 °C, overnight, 89%; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, $Zn-Zn(OAc)_2$, 1:1, DMF, 120 °C, 2 h, 34%; (d) $LiCl$, DMF, 160 °C, μ waves, 30 min, 40%.

Table 1 MCL-1 binding affinities of ester prodrugs and bioisosteres of 1

Number	X	Y	R^1	K_i (μM)	Number	X	Y	R^1	K_i (μM)
1	$\sim -OH$	$\sim -COOH$	$\sim -CH_2-CH_3$	5.77 ± 0.46	6	$\sim -H$	$\sim -CO-NH-$	$\sim -CH_2-CH_3$	>500
2	$\sim -OH$	$\sim -COO-$	$\sim -CH_2-CH_3$	>500	7a	$\sim -OH$	$\sim -CO-NH-$	$\sim -CH_2-C_6H_5$	2.94 ± 0.35
3	$\sim -OH$	$\sim -COO-$	$\sim -CH_2-CH_3$	>500	7b	$\sim -OH$	$\sim -CO-NH-$	$\sim -CH_2-C_6H_5$	1.95 ± 0.22
4	$\sim -OH$	$\sim -CO-NH-OH$	$\sim -CH_2-CH_3$	70.6 ± 5.8	8	$\sim -H$	$\sim -N=N-$	$\sim -CH_2-CH_3$	>500
5	$\sim -OH$	$\sim -CN$	$\sim -CH_2-CH_3$	3.29 ± 0.26					



desired tetrazole **5**. Alternatively, iodide **17** was subjected to a Sonogashira coupling with methyl propionate to furnish the alkyne derivative **19** (moderate yield largely due to incomplete reaction), which was then transformed into 3-hydroxyisoxazole **6** upon treatment with hydroxylamine.

The target acylsulfonamides **7a–c** were prepared by coupling either methanesulfonamide, benzenesulfonamide or **20**, respectively, to carboxylic acid **13**, followed by TFA-driven debenzylation, as depicted in Scheme 3.

As illustrated in Scheme 4, the cyanophenol variant of salicylic acid-based **1**, was accessed from 4-bromo-3-

methoxyaniline (**22**). Briefly, aniline **22** underwent reductive amination with isobutyraldehyde followed by sulfonylation with 4-phenoxybenzenesulfonyl chloride to afford tertiary sulfonamide **23**. Pd-catalyzed cyanation of **23** as before delivered nitrile **24**. Demethylation with LiCl under microwave irradiation then yielded the target cyanophenol **8**.

As shown in Table 1, we initially evaluated these compounds against MCL-1 using a standard fluorescence polarization competition assay (FPCA), as described by us¹¹ and others previously.^{14,19,23} For reference, the previously reported data for compound **1** is included.¹¹ Predictably, esters **2** and **3** were

Table 2 MCL-1 and BCL-x_L binding affinities of acylsulfonamide bioisosteres of **17cd** from ref. 11

Number	X	Y	R ¹	R ⁴	K _i (MCL-1, μ M)	K _i (BCL-x _L , μ M)	Selectivity (K_i MCL-1/ K_i BCL-x _L)
“ 17cd ” ¹¹	~~OH	~~C(=O)OH	~~C ₆ H ₅ CH ₂	~~C ₆ H ₄ Cl-CH ₃	0.629 ± 0.05	1.63 ± 0.18	1 : 2.65
7a	~~OH	~~C(=O)NHOS(=O)(=O)C ₆ H ₅	~~C ₆ H ₅ CH ₂	~~C ₆ H ₅ CH ₂	2.94 ± 0.35	7.22 ± 1.07	1 : 2.45
7b	~~OH	~~C(=O)NHOS(=O)(=O)C ₆ H ₅	~~C ₆ H ₅ CH ₂	~~C ₆ H ₅ CH ₂	1.95 ± 0.22	2.34 ± 0.22	1 : 1.20
7c	~~OH	~~C(=O)NHOS(=O)(=O)C ₆ H ₄ NO ₂ -C ₆ H ₅ CH ₂	~~C ₆ H ₅ CH ₂	~~C ₆ H ₅ CH ₂	1.23 ± 0.15	1.05 ± 0.14	1 : 0.85
7d	~~OH	~~C(=O)NHOS(=O)(=O)C ₆ H ₄ NO ₂ -C ₆ H ₅ CH ₂	~~C ₆ H ₅ CH ₂	~~C ₆ H ₄ Cl-CH ₃	0.800 ± 0.07	1.82 ± 0.18	1 : 2.02
27	~~C(=O)OH	~~OH	~~C ₆ H ₅ CH ₂	~~C ₆ H ₄ Cl-CH ₃	1.40 ± 0.47	1.82 ± 0.23	1 : 1.30
“ 6e-OH-5 ” ¹¹	~~C(=O)OH	~~OH	~~C ₆ H ₅ CH ₂	~~C ₆ H ₄ Cl-CH ₃	0.778 ± 0.041	2.19 ± 0.19	1 : 1.30
29	~~C(=O)NHOS(=O)(=O)C ₆ H ₄ NO ₂ -C ₆ H ₅ CH ₂	~~OH	~~C ₆ H ₅ CH ₂	~~C ₆ H ₄ Cl-CH ₃	1.01 ± 0.08	1.54 ± 0.23	1 : 4.96



inactive, confirming our previous findings that the carboxylic acid, which likely binds Arg263, is crucial to activity.^{11,12} Disappointingly, 3-hydroxyisoxazole **6** and salicylonitrile **8** demonstrated no inhibition of MCL-1. While hydroxamic acid **4** resulted in a significant reduction in affinity relative to control compound **1**, which may be attributed to the reduced pK_a of the hydroxamic acid functional group, the tetrazole (**5**) and acylsulfonamide (**7a** and **7b**) bioisosteres, on the other hand with comparable acidities to carboxylic acids, were around ≥ 2 -fold better than the parent compound **1**. We acknowledge there are subtle differences between **5**, **7a** and **7b** and the parent **1**, but our previous work indicates that the salicylic hydroxyl of **1** contributes positively to binding, and inhibitors with $R^1 = iBu$ or Bn that are otherwise identical result in comparable binding affinities (see Tables 1 and 2 in ref. 11). Thus, the introduction of tetrazole and acylsulfonamides as carboxylic acid surrogates into this class of dual MCL-1/BCL-xL inhibitors is tolerated for MCL-1 inhibition.

The inhibition data for the acylsulfonamides were particularly encouraging, and so we decided to investigate them further, both by expanding the series as well as by evaluating their activities against BCL-xL. As illustrated in Table 2, acylsulfonamide **7a** inhibited BCL-xL about 4-fold weaker than parent compound **17cd**, although this is likely due to the different R^4 groups, as also observed in Table 2 in ref. 11. Nevertheless, switching the methyl sulfonamide of **7a** to the phenyl sulfonamide in **7b** elicited a 3-fold improvement in BCL-xL inhibitory activity, such that **7b** is on a par with “**17cd**”.¹¹ In addition, this trend was also observed in the MCL-1 inhibition data. Elaboration of the sulfonamide portion with that present in the BCL-2 inhibitor venetoclax yielded acyl sulfonamide **7c**, which afforded even greater improvements in inhibition of both proteins, although at the same time, these improvements were minimal, indicating limited interactions of these sulfonamide moieties with the protein surfaces. In order to create a better comparison, we synthesized **7d**, the congener of **7c** in which the R^4 group was the same as in **17cd**. We were delighted to observe further increases in activity, with **7d** equipotent to **26** at inhibiting both MCL-1 and BCL-xL. Switching the hydroxyl and acylsulfonamide functionalities as in **29** was unremarkable with similar binding affinities to isomer **7d** (and also to parent salicylic acids **27** and “**6e-OH-5**”¹¹). In summary, whilst the acylsulfonamide is clearly tolerated as a surrogate for the carboxylic acid in this class of dual MCL-1/BCL-xL inhibitors, it is surprising that the increase in size of the sulfonamide group from **7a** to **7b** to **7c** yielded minimal improvements in inhibition of MCL-1 and BCL-xL.

Lastly, we evaluated the cell killing activities of a small batch of our dual inhibitors with the human leukemia HL60 cell line that is known to be sensitive to MCL-1 and BCL-xL inhibitors,²⁴ and the data is shown in Table 3. Carboxylic acids **1**, **26** and **28** were just a few-fold worse than their cell-free K_i values with 0.5% FBS. Ester prodrugs **2** and **3** were not as effective at killing cells as the parent acid from which they were derived, which may suggest incomplete ester hydrolysis under the conditions of the experiment – we intend to investigate this in future work. Pleasingly, tetrazole **5** and acyl sulfonamide **7d** were somewhat

Table 3 Cell killing of select compounds in HeLa cells, in the presence of varying amounts of FBS

Compound	HL60 IC ₅₀ (μM)	
	0.15% FBS	10% FBS
1	18.5	>120
2	68.1	76.4
3	46.4	>120
5	15.7	>120
7d	7.45	>120
26	5.79	53.2
28	9.82	>120

comparable, or better, than the parent acid **1**. In all but one instance, the cell killing activities were significantly reduced in the presence of a greater concentration of FBS, which was anticipated since lipophilic molecules with acidic groups, such as ours, bind albumin.¹⁵ The similar activities for neutral methyl ester **2** in 0.5% FBS and 10% FBS suggests **2** likely does not bind albumin. Although the cell activity of ester congener **3** was impacted by a greater concentration of FBS, this may be explained by greater ester hydrolysis outside of the cell since the acetoxymethyl ester is far more prone to hydrolysis than is the methyl ester.¹⁷

4 Conclusions

In conclusion, we prepared ester prodrugs and carboxylic acid bioisosteres of one of our lead dual MCL-1/BCL-xL inhibitors. As predicted, the ester prodrugs **2** and **3** were inactive in the cell-free MCL-1 binding assay, which we presume was due to the lack of a carboxylic acid, and hence the loss of a crucial salt bridge most probably with Arg263, but unfortunately they were also less active than the parent carboxylic acid **1** in HL60 cells, likely due to incomplete ester hydrolysis. The introduction of a tetrazole as a carboxylic acid surrogate was tolerated, yielding the compound **5** that inhibited MCL-1 with a similar activity to the parent carboxylic acid. We also prepared a small library of acylsulfonamide bioisosteres (**7a-d**, **29**) and evaluated their inhibitory activities against both MCL-1 and BCL-xL. Pleasingly, this carboxylic acid surrogate was also tolerated across both proteins. At the same time, we were surprised that the series of acylsulfonamides exhibited rather similar inhibitory activities to one another, and so before we pursue the further optimization of our dual inhibitors, it is paramount that we obtain an X-ray co-crystal structure of one of our lead compounds with either MCL-1 or BCL-xL to facilitate ligand design. Lastly, we evaluated some of our best compounds in HL60 cells, and discovered that the tetrazole **5** and acylsulfonamide **7d** were as potent as, or slightly better than, the parent acids **1** and “**17cd**”.

Conflicts of interest

There are no conflicts to declare.



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References

- J. Kale, E. J. Osterlund and D. W. Andrews, BCL-2 family proteins: changing partners in the dance towards death, *Cell Death Differ.*, 2018, **25**(1), 65–80, DOI: [10.1038/cdd.2017.186](https://doi.org/10.1038/cdd.2017.186).
- S. P. Glaser, E. F. Lee, E. Trounson, *et al.*, Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia, *Genes Dev.*, 2012, **26**(2), 120–125, DOI: [10.1101/gad.182980.111](https://doi.org/10.1101/gad.182980.111).
- S. Wuillème-Toumi, N. Robillard, P. Gomez, *et al.*, Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival, *Leukemia*, 2005, **19**(7), 1248–1252, DOI: [10.1038/sj.leu.2403784](https://doi.org/10.1038/sj.leu.2403784).
- J. M. Balko, J. M. Giltnane, K. Wang, *et al.*, Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets, *Cancer Discovery*, 2014, **4**(2), 232–245, DOI: [10.1158/2159-8290.CD-13-0286](https://doi.org/10.1158/2159-8290.CD-13-0286).
- H. Wang, M. Guo, H. Wei and Y. Chen, Targeting MCL-1 in cancer: current status and perspectives, *J. Hematol. Oncol.*, 2021, **14**(1), 67, DOI: [10.1186/s13045-021-01079-1](https://doi.org/10.1186/s13045-021-01079-1).
- S. Fletcher, MCL-1 inhibitors – where are we now (2019)?, *Expert Opin. Ther. Pat.*, 2019, **29**(11), 909–919, DOI: [10.1080/13543776.2019.1672661](https://doi.org/10.1080/13543776.2019.1672661).
- J. L. Yap, L. Chen, M. E. Lanning and S. Fletcher, Expanding the Cancer Arsenal with Targeted Therapies: Disarmament of the Antiapoptotic Bcl-2 Proteins by Small Molecules, *J. Med. Chem.*, 2017, **60**(3), 821–838, DOI: [10.1021/acs.jmedchem.5b01888](https://doi.org/10.1021/acs.jmedchem.5b01888).
- K. H. Lin, P. S. Winter, A. Xie, *et al.*, Targeting MCL-1/BCL-xL Forestalls the Acquisition of Resistance to ABT-199 in Acute Myeloid Leukemia, *Sci. Rep.*, 2016, **6**, 27696, DOI: [10.1038/srep27696](https://doi.org/10.1038/srep27696).
- D. A. Luedtke, X. Niu, Y. Pan, *et al.*, Inhibition of Mcl-1 enhances cell death induced by the Bcl-2-selective inhibitor ABT-199 in acute myeloid leukemia cells, *Signal Transduction Targeted Ther.*, 2017, **2**, 17012, DOI: [10.1038/sigtrans.2017.12](https://doi.org/10.1038/sigtrans.2017.12).
- E. Proschak, H. Stark and D. Merk, Polypharmacology by Design: A Medicinal Chemist's Perspective on Multitargeting Compounds, *J. Med. Chem.*, 2019, **62**(2), 420–444, DOI: [10.1021/acs.jmedchem.8b00760](https://doi.org/10.1021/acs.jmedchem.8b00760).
- L. Chen, J. Chauhan, J. L. Yap, C. C. Goodis, P. T. Wilder and S. Fletcher, Discovery of N-sulfonylated aminosalicylic acids as dual MCL-1/BCL-xL inhibitors, *RSC Med. Chem.*, 2023, **14**(1), 103–112, DOI: [10.1039/d2md00277a](https://doi.org/10.1039/d2md00277a).
- L. Chen, P. T. Wilder, B. Drennen, *et al.*, Structure-based design of 3-carboxy-substituted 1,2,3,4-tetrahydroquinolines as inhibitors of myeloid cell leukemia-1 (Mcl-1), *Org. Biomol. Chem.*, 2016, **14**(24), 5505–5510, DOI: [10.1039/c5ob02063h](https://doi.org/10.1039/c5ob02063h).
- M. E. Lanning, W. Yu, J. L. Yap, *et al.*, Structure-based design of N-substituted 1-hydroxy-4-sulfamoyl-2-naphthoates as selective inhibitors of the Mcl-1 oncoprotein, *Eur. J. Med. Chem.*, 2016, **113**, 273–292, DOI: [10.1016/j.ejmchem.2016.02.006](https://doi.org/10.1016/j.ejmchem.2016.02.006).
- A. Friberg, D. Vigil, B. Zhao, *et al.*, Discovery of potent myeloid cell leukemia 1 (Mcl 1) inhibitors using fragment based methods and structure based design, *J. Med. Chem.*, 2013, **56**(1), 15–30, DOI: [10.1021/jm301448p](https://doi.org/10.1021/jm301448p).
- B. Zhao, J. Sensintaffar, Z. Bian, *et al.*, Structure of a Myeloid cell leukemia-1 (Mcl-1) inhibitor bound to drug site 3 of human serum albumin, *Bioorg. Med. Chem.*, 2017, **25**(12), 3087–3092, DOI: [10.1016/j.bmc.2017.03.060](https://doi.org/10.1016/j.bmc.2017.03.060).
- Z. Nikolovska-Coleska, R. Wang, X. Fang, *et al.*, Development and optimization of a binding assay for the XIAP BIR3 domain using fluorescence polarization, *Anal. Biochem.*, 2004, **332**(2), 261–273, DOI: [10.1016/j.ab.2004.05.055](https://doi.org/10.1016/j.ab.2004.05.055).
- H. Wang, J. Chauhan, A. Hu, *et al.*, Disruption of Myc-Max Heterodimerization with Improved Cell-Penetrating Analogs of the Small Molecule 10074-G5, *Oncotarget*, 2013, **4**(6), 936–947, accessed October 10, 2021. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3757250/>.
- C. Ballatore, D. M. Huryn and A. B. Smith, Carboxylic Acid (Bio)Isosteres in Drug Design, *ChemMedChem*, 2013, **8**(3), 385–395, DOI: [10.1002/cmdc.201200585](https://doi.org/10.1002/cmdc.201200585).
- N. F. Pelz, Z. Bian, B. Zhao, *et al.*, Discovery of 2-Indoleacylsulfonamide Myeloid Cell Leukemia 1 (Mcl-1) Inhibitors Using Fragment-Based Methods, *J. Med. Chem.*, 2016, **59**(5), 2054–2066, DOI: [10.1021/acs.jmedchem.5b01660](https://doi.org/10.1021/acs.jmedchem.5b01660).
- P. Politzer and J. S. Murray, Some Intrinsic Features of Hydroxylamines, Oximes and Hydroxamic Acids: Integration of Theory and Experiment, in *The Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids*, John Wiley & Sons, Ltd, 2008, pp. 1–27, DOI: [10.1002/9780470741962.ch1](https://doi.org/10.1002/9780470741962.ch1).
- S. Fletcher and P. T. Gunning, Mild, efficient and rapid O-debenzylation of ortho-substituted phenols with trifluoroacetic acid, *Tetrahedron Lett.*, 2008, **49**(33), 4817–4819, DOI: [10.1016/j.tetlet.2008.06.022](https://doi.org/10.1016/j.tetlet.2008.06.022).
- R. Chidambaram, A robust palladium-catalyzed cyanation procedure: beneficial effect of zinc acetate, *Tetrahedron Lett.*, 2004, **45**(7), 1441–1444, DOI: [10.1016/j.tetlet.2003.12.040](https://doi.org/10.1016/j.tetlet.2003.12.040).
- T. Lee, P. P. Christov, S. Shaw, *et al.*, Discovery of Potent Myeloid Cell Leukemia-1 (Mcl-1) Inhibitors That Demonstrate *in Vivo* Activity in Mouse Xenograft Models of Human Cancer, *J. Med. Chem.*, 2019, **62**(8), 3971–3988, DOI: [10.1021/acs.jmedchem.8b01991](https://doi.org/10.1021/acs.jmedchem.8b01991).
- Q. Wang and S. Hao, A-1210477, a selective MCL-1 inhibitor, overcomes ABT-737 resistance in AML, *Oncol. Lett.*, 2019, **18**(5), 5481–5489, DOI: [10.3892/ol.2019.10891](https://doi.org/10.3892/ol.2019.10891).

