Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Scaffold hopping from indoles to indazoles yields dual MCL-1/BCL-2 inhibitors from MCL-1 selective leads

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Supporting Information

General Procedures

<u>*General Procedure 1: Synthesis of functionalized sulfonamides* (**13a–13r**):</u> Either 4-fluoro-3-nitrobenzenesulfonamide or 3-fluoro-4-nitrobenzenesulfonamide (1 eq.) was placed in a reaction flask and solubilized in DMF (0.1 M). Selected amines (1.1 eq.) and K_2CO_3 (2.0 eq.) were added to the reaction mixture. The reaction was stirred at RT for 16 h. Reaction completion was monitored via TLC (CH_2Cl_2/MeOH/NH_4OH, 92:7:1). Upon completion, the reaction was partitioned between EtOAc and brine. The organic layer was washed five times with brine to remove the DMF and excess amine. The organic layer was then collected, dried with Na_2SO_4, filtered and concentrated to produce the desired functionalized sulfonamides that needed no further purification.

<u>*General Procedure 2: Sulfonamide coupling of N-alkylated indazole carboxylic acids:*</u> An N-alkylated indazole (1 eq.) was dissolved in anhydrous DMF (0.1 M). DIPEA (1 eq.), DMAP (0.5 eq.) and EDCI (1.2 eq.) were then added. The reaction was stirred at RT for 10 min before the addition of the appropriate sulfonamide (**13a–13r**; 1.1 eq.). The reaction was stirred at RT for 16 h. Completion of the reaction was monitored via TLC (CH_2Cl_2/MeOH/NH_4OH, 92:7:1). The reaction was partitioned between EtOAc and brine, then the organic layer was washed 5 times with brine, collected, dried with Na_2SO_4, filtered and concentrated. The crude material was adsorbed onto silica gel, then purified by flash column chromatography eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1.

<u>*General Procedure 3: Esterfication of carboxylic acids:*</u>

The carboxylic acid (1 eq.) was dissolved in MeOH (0.1 M) at 0 ºC. $SOCl_2$ (3 eq.) was slowly added to the reaction, and then it was stirred at 65℃ for 16 h. Completion of the reaction was monitored via TLC (CH_2Cl_2/MeOH/CH_3COOH, 92:7:1). The reaction was concentrated, then partitioned between EtOAc and sat. $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, filtered and concentrated to yield the target methyl ester.

<u>*General Procedure 4: N-Alkylation of the heterocyclic cores:*</u>
A heterocyclic core (1 eq.) was added to a reaction flask, followed by the addition of DMF (0.1 M). The desired alkyl bromide (1.1 eq.) and K_2CO_3 (2 eq.) were then added to the reaction mixture. The reaction was stirred for at a given temperature for 16 h. Completion of the reaction was monitored via TLC (hexanes/EtOAc, 2:1). The reaction mixture was partitioned between

EtOAc and brine. The organic layer was washed 5 times with brine, collected, dried with Na_2SO_4, filtered and concentrated. The crude material was adsorbed onto silica gel and purified via flash column chromatography eluting with a gradient of EtOAc in hexanes.

General Procedure 5: Saponification of methyl esters:
A methyl ester (1 eq.) was dissolved in a 3:1 mixture of THF/MeOH (0.1 M). H_2O was then added (0.2 M) followed by the addition of NaOH (3.0 eq). The reaction was stirred at RT for 16h. Completion of the reaction was monitored via TLC (CH_2Cl_2/MeOH/CH_3COOH, 92:7:1). The reaction mixture was partitioned between EtOAc and 1 M HCl. The organic layer was collected, dried with Na_2SO_4, filtered and concentrated to yield the target acid that did not require further purification.

1-(3-(4-Chloro-3,5-dimethylphenoxy)propyl)-1H-benzo[d]imidazole-2-carboxylic acid (**2**). 1H-Benzo[*d*]imidazole-2-carboxylic acid was esterified on a 3.08 mmol scale according to General Procedure 3 to deliver methyl 1H-benzo[*d*]imidazole-2-carboxylate as a viscous oil (521 mg, 96%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 13.55 (s, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 3.97 (s, 3H). Subsequently, this ester (2.04 mmol scale) was treated with 5-(3-bromopropoxy)-2-chloro-1,3-dimethylbenzene according to General Procedure 4, heating the reaction at 45 ºC for 16 h. After flash column chromatography, methyl 1-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1H-benzo[d]imidazole-2-carboxylate (the less polar of the two products) was yielded as a viscous oil (530 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, J = 7.2 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.36-7.34 (m, 2H), 6.58 (s, 2H), 4.85 (t, J = 7.0 Hz, 4.00 (s, 3H), 3.91 (t, J = 5.6, 2H), 2.35-2.33 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 158.7, 154.6, 140.0, 138.7, 135.5, 134.6, 124.8, 124.1, 122.2, 120.3, 112.8, 109.0, 63.0, 51.2, 40.8, 28.4, 19.3. Methyl 1-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1H-benzo[d]imidazole-2-carboxylate was saponified on a 1.29 mmol scale according to General Procedure 5 to deliver 1-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-1H-benzo[d]imidazole-2-carboxylic acid (**3**) as an off-white solid (439 mg, 95%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 9.61 (s, 1H), 8.02 (m, 1H), 7.90 (m, 1H), 7.60 (m, 2H), 6.67 (s, 2H), 4.67 (t, J = 6.6 Hz, 2H), 4.05 (t, J = 5.6 Hz, 2H), 2.38 (t, J = 5.8 Hz, 2H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 161.3, 147.1, 141.6, 137.2, 136.6, 131.1, 130.8, 130.3, 120.5, 119.8 (2), 118.2, 70.1, 48.9, 33.4, 25.6.

5-Chloro-2-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-2H-indazole-3-carboxylic acid (**3**). A solution of methyl 5-chloroindazole-3-carboxylate (**10**; 400 mg, 2.27 mmol, 1 eq) in anhydrous THF (15 mL) was treated with 3-(4-chloro-3,5-dimethylphenoxy)propan-1-ol (487 mg, 2.27 mmol, 1 eq), PPh_3 (715 mg, 2.72 mmol, 1.2 eq) and DIAD (540 mL, 2.72 mmol, 1.2 eq). The reaction was stirred at RT for 16 h. Flash column chromatography was performed using a gradient hexanes/ethyl acetate to yield methyl 5-chloro-2-(3-(4-chloro-3,5-dimethylphenoxy)propyl)-2*H*-indazole-3-carboxylate as the major product: (570 mg, 67%): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95 (s, 1H), 7.66 (d, J = 9.2 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 6.51 (s, 2H), 5.06 (t, J = 7.2 Hz, 2H), 3.95 (m, 5H), 2.45-2.40 (m, 2H), 2.28 (s, 6H). Subsequently, the material was subjected to General Procedure 5 on a 1.35 mmol scale to deliver the title carboxylic acid (**3**) as a white solid (469 mg, 88%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 13.2 (s, 1H), 8.02 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 6.62 (s, 2H), 4.64 (t, J = 6.8 Hz, 2H), 3.92 (t, J = 5.6 Hz, 2H), 2.29-2.22 (m, 8H); ^{13}C NMR (400 MHz, DMSO) δ = 163.0, 156.2, 139.2, 136.4, 134.5, 127.6, 126.9, 125.1, 123.8, 120.2, 114.7, 112.6, 64.8, 46.2, 28.8, 20.4.

5-Chloro-2-(2-(4-chloro-3,5-dimethylphenoxy)ethyl)-2H-indazole-3-carboxylic acid (**4**): Methyl 5-chloroindazole-3-carboxylate (**10**; 2.37 mmol) and 5-(2-bromoethoxy)-2-chloro-1,3-dimethylbenzene were reacted together according to General Procedure 4 at RT. Flash column chromatography was performed using a gradient hexanes/ethyl acetate to yield methyl 5-chloro-2-(3-(4-chloro-3,5-dimethylphenoxy)ethyl)-2*H*-indazole-3-carboxylate (the less polar of the two products) as a viscous oil (367 mg, 39%): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.96 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 8.8 Hz, 1H), 6.55 (s, 2H), 5.26 (t, J = 5.8 Hz, 2H), 4.43 (t, J = 6.0 Hz, 2H), 4.03 (s, 3H), 2.26 (s, 6H). Subsequently, the material was subjected to General Procedure 5 on a 0.559 mmol scale to deliver the title carboxylic acid (**4**) as a white solid (188 mg, 89%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.99 (s, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.38 (d, J = 9.2 Hz, 1H), 6.73 (s, 2H), 5.25 (t, J = 5.2 Hz, 2H), 4.50 (t, J = 5.4 Hz, 2H), 2.24 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 165.8, 161.1, 150.3, 141.7 (2), 134.4, 132.4 (2), 130.5, 128.6, 125.3, 119.9, 71.8, 57.1, 25.5.

5-Chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-2H-indazole-3-carboxylic acid (**5**): Methyl 5-chloroindazole-3-carboxylate (2.09 g, 7.95 mmol, 1 eq) in anhydrous THF (40 mL) was treated with 4-(4-chloro-3,5-dimethylphenoxy)phenyl)methanol (1.40 g, 7.95 mmol, 1 eq), PPh_3 (2.50 g, 9.55 mmol, 1.2 eq) and DIAD (1.88 mL, 9.55 mmol, 1.2 eq). The reaction was stirred at RT for 16 h. Flash column chromatography was performed using a gradient hexanes/ethyl acetate to yield methyl 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-2*H*-indazole-3-carboxylate (**11**) as a viscous oil (2.18 g, 65%): R_f 0.59 (Hex/EtOAc, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (s, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.36 (d, J = 8.8 Hz, 2H), 7.30-7.26 (m, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.70 (s, 2H), 6.05 (s, 2H), 4.03 (s, 3H), 2.31 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 160.3, 157.3, 154.4, 145.9, 137.7, 131.2, 130.7, 129.7, 127.9, 124.0, 120.4, 119.9, 119.0, 118.5 (2), 56.1, 52.2, 21.7, 20.8. Isomer **12** (methyl 5-chloro-1-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-1*H*-indazole-3-carboxylate) was also isolated during this purification: Rf 0.43 (Hex/EtOAc, 2:1): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.24 (s, 1H), 7.34 (d, J = 2.8 Hz, 2H), 7.20 (d, J = 8.4, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.72 (s, 1H), 5.67 (s, 2H), 4.07 (s, 3H), 2.33 (s, 6H);^{13}C NMR (400 MHz, $CDCl_3$) δ = 162.6, 157.5, 154.2, 138.9, 137.8, 134.5, 129.8, 129.4, 128.9 (2), 127.9, 124.9, 121.6, 119.1, 118.7, 111.2, 53.8, 52.3, 20.9. Subsequently, ester **11** was subjected to General Procedure 5 to deliver the title carboxylic acid (**5**) as a white solid (yield = 92%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.96 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.83 (s, 2H), 6.02 (s, 2H), 2.24 (s, 6H);^{13}C NMR (400 MHz, $CDCl_3$) δ = 165.7, 161.6, 159.4, 150.5, 142.6, 136.7, 134.8 (2), 133.5, 132.5, 129.5, 128.7, 125.4, 124.2, 123.5 (2), 60.3, 25.5. Finally, ester **12** was likewise saponified to deliver 5-chloro-1-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-1*H*-indazole-3-carboxylic acid (**6**) as a white solid: ^{1}H NMR (400 MHz, DMSO) δ = 13.3 (s, 1H), 8.08 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 9.6 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 7.6 Hz, 2H), 6.87 (s, 2H), 5.78 (s, 2H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 168.2, 161.6, 159.4, 144.2, 142.6, 140.0, 136.5, 134.7, 133.5, 133.0, 132.4, 129.2, 125.6, 124.2, 123.7, 118.0, 57.3, 25.5; HRMS-ESI: *m/z* found 439.0609 $[M-H]^-$, $C_{23}H_{17}Cl_2N_2O_3$ requires 439.0622.

6-Chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-2H-indazole-3-carboxylic acid (**7**): 6-Chloro-1*H*-indazole-3-carboxylic acid (1.02 mmol) underwent General Procedure 3, followed by General Procedure 4 (0.266 mmol scale) with 5-(4-(bromomethyl)phenoxy)-2-chloro-1,3-dimethylbenzene at RT, and then the less polar product was subjected to General Procedure 5 (0.197 mmol scale) to yield the title compound (**7**) as a white solid (77 mg, 92%): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 8.07 (d, J = 9.2 Hz, 1H), 7.91 (s, 1H), 7.34 (d, J = 8.8 Hz, 3H), 6.98 (d, J = 8.4 Hz, 2H), 6.88 (s, 2H), 6.09 (s, 2H), 2.30 (6H); ^{13}C NMR (400 MHz, DMSO) δ = 161.1, 156.8, 154.7, 147.4, 137.9, 132.2, 131.5, 130.0, 128.7, 126.2, 125.7, 124.1, 121.9, 119.4, 118.8, 117.2, 55.4, 20.7.

Methyl 5-chloroindazole-3-carboxylate (**10**): 5-chloroisatin (**8**; 1.0 mmol) was added to a reaction flask, followed by the addition of H_2O (0.1 M) and NaOH (1.1 mmol). The reaction was heated to

45℃ and stirred for 30 minutes or until all the starting material was solubilized. The reaction mixture was then cooled to 0 ºC. $NaNO_2$ (1.0 mmol) was dissolved in H_2O and slowly added to the reaction. The reaction stirred for 10 minutes and then H_2SO_4 (2.5 mmol) was added to the reaction. After 30 minutes of stirring, $SnCl_2$ was dissolved in concentrated HCl and slowly added to the reaction mixture. The reaction was stirred at 0 ºC for 16 h. The resulting precipitate was filtered from the reaction mixture to deliver crude 5-chloroindazole-3-carboxylic acid (**9**), which was carried forwarded to the next reaction. Subsequently, this crude material was dissolved in MeOH (0.1 M), and $SOCl_2$ (3.0 mmol) was slowly added to the reaction. The reaction was stirred at 65℃ for 16 h. Completion of the reaction was monitored via TLC. The reaction was concentrated down and then partitioned between brine and ethyl acetate. The organic layer was collected, dried with Na_2SO_4, filtered and concentrated. The crude material was redistributed in CH_2Cl_2 and adsorbed to silica gel. Flash column chromatography was then performed to purify the desired product using a gradient of hexanes/ethyl acetate to yield methyl 5-chloroindazole-3-carboxylate (**10**): ^{1}H NMR (400 MHz, d_6-DMSO) δ = 8.02 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 9.2 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 162.8, 139.9, 135.1, 128.0, 127.6, 123.4, 120.3, 113.5, 52.2.

Synthesis of 4-chlorobenzenesulfonamide (**13a**): 4-chlorobenzenesufonyl chloride (1.0 mmol) was dissolved in dioxane (0.1 M), and cooled to 0 °C. NH_4OH (5.0 mmol) was added dropwise to the reaction. The reaction was stirred at 0 °C for 30 minutes. Completion of the reaction was monitored via TLC (hexanes/EtOAc). The reaction was partitioned between ethyl acetate and brine. The organic layer was collected, dried with Na_2SO_4, filtered, concentrated to deliver the title compound as a white solid (yield = 88%). ^{1}H and ^{13}C spectra were consistent with the literature.[25]

Synthesis of 4-morpholino-3-nitrobenzenesulfonamide (**13b**): Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and morpholine (yield = 77%): yellow solid; ^{1}H (400 MHz, d_6-DMSO) δ 8.22 (s, 1H), 7.92 (d, *J* = 7.4Hz, 1H), 7.46 (d, J = 8Hz, 2H), 3.72 (t, *J* = 6.8Hz, 4H), 3.14 (t, *J* = 6.8Hz, 4H), 2.00 (s, 1H); ^{13}C NMR (400 MHz, d_6-DMSO) δ= 152.3, 144.1, 140.2, 136.1, 129.5, 126.2, 71.0, 55.8, 51.0.

Synthesis of 4-((2-morpholinoethyl)amino)-3-nitrobenzenesulfonamide (**13c**): Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and 2-morpholinoethan-1-amine (yield = 65%): yellow solid; ^{1}H (400 MHz, d_6-DMSO) δ 8.78 (t, *J* = 7Hz, 1H), 8.48 (s, 1H), 7.85 (d, *J* = 7.4Hz, 1H), 7.35 (s, 2H), 7.23 (d, *J* = 7.4Hz, 1H), 3.61 (m, 4H), 3.50 (q, *J* = 6.8Hz, 2H), 2.64 (t, *J* = 6.8Hz, 2H), 2.46 (s, 4H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 151.6, 138.0, 135.3, 134.6, 129.8, 121.1, 71.5, 60.5, 58.0, 45.3.

Synthesis of 4-((furan-2-ylmethyl)amino)-3-nitrobenzenesulfonamide (**13d**): Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and furan-2-ylmethanamine (yield = 73%): yellow solid; ^{1}H (400 MHz, d_6-DMSO) δ 8.89 (t, *J* = 7.4 Hz, 1H), 8.49 (s, 1H), 7.83 (d, *J* = 7 Hz, 1H), 7.62 (s, 1H), 7.31-7.33 (m, 2H), 6.41 (s, 2H), 4.71 (d, *J* = 6.8 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 156.1, 151.3, 147.9, 137.8, 135.8, 129.8, 121.0, 115.7, 113.1, 45.1.

Synthesis of 4-((2-(furan-2-yl)ethyl)amino)-3-nitrobenzenesulfonamide (**13e**): Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and 2-(furan-2-yl)ethan-1-amine (yield = 74%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.58 (t, *J* = 5.4 Hz, 1H), 8.50 (d, *J* = 2.4 Hz, 2H), 7.86 (d, *J* = 9.6 Hz, 1H), 7.59 (s, 1H), 7.38 (s, 2H), 7.25 (d, *J* = 9.2 Hz, 1H), 6.41 (s, 1H), 6.28 (d, *J* = 2.8 Hz, 1H), 3.72 (q, *J* = 6.4 Hz, 2H), 3.03 (t, J = 7.0 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 157.5, 151.5, 147.2, 138.0, 135.5, 134.7, 129.9, 120.6, 115.8, 111.9, 46.5, 32.1.

<u>*Synthesis of 4-(((1-methyl-1H-pyrazol-3-yl)methyl)amino)-3-nitrobenzenesulfonamide* (**13f**):</u> Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and (1-methyl-1*H*-pyrazol-3-yl)methanamine (yield = 63%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ= 8.83 (t, J = 5.6 Hz, 1H), 8.51 (s, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.71 (s, 1H), 7.48 (s, 1H), 7.36 (s, 2H), 7.28 (d, J = 9.6 Hz, 1H), 4.53 (d, J = 6 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 149.6, 141.5, 136.2, 133.7, 133.1, 128.2, 120.9, 119.3 (2), 40.6 (2).

<u>*Synthesis of 3-nitro-4-((pyridin-3-ylmethyl)amino)benzenesulfonamide* (**13g**):</u> Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and pyridin-3-ylmethanamine (yield = 69%): yellow solid; ^{1}H (400M Hz, d_6-DMSO) δ= 9.11 (t, *J* = 7.8 Hz, 1H), 8.64 (s, 1H), 8.47-8.50 (m, 2H), 7.77 (m, 2H), 7.35-7.38 (m, 1H), 7.31 (s, 2H), 7.11 (d, *J* = 8.4 Hz, 1H), 4.75 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 149.0, 148.8, 146.5, 135.3, 134.1, 133.2, 131.0, 130.6, 125.2, 124.1, 116.1, 43.8.

<u>*Synthesis of 3-nitro-4-((2-(pyridin-3-yl)ethyl)amino)benzenesulfonamide* (**13h**):</u> Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and 2-(pyridin-3-yl)ethan-1-amine (yield = 64%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ = 8.56 (d, *J* = 7.2 Hz, 2H), 8.50 (s, 1H), 8.47 (d, *J* = 4.0 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 12.0 Hz, 4H), 3.74 (q, *J* = 6.4 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 150.4, 148.1, 146.7, 136.9, 134.6, 133.3, 130.7, 129.9, 125.2, 123.9, 116.0, 43.9, 31.8.

<u>*Synthesis of 3-nitro-4-((pyridin-4-ylmethyl)amino)benzenesulfonamide* (**13i**):</u> Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and pyridin-4-ylmethanamine (yield = 58%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ = 9.13 (t, *J* = 6.2 Hz, 1H), 8.52 (d, *J* = 2.4 Hz, 3H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.35-7.33 (m, 4H), 6.97 (d, *J* = 8.4 Hz, 1H), 4.77 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 155.0, 154.9, 152.2, 151.7, 148.2, 135.8, 130.0, 129.9, 127.1, 127.0, 120.8, 49.9.

<u>*Synthesis of 3-nitro-4-((2-(pyridin-4-yl)ethyl)amino)benzenesulfonamide* (**13j**):</u> Follow *General Procedure 1* using 4-fluoro-3-nitrobenzenesulfonamide and 2-(pyridin-4-yl)ethan-1-amine (yield = 55%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.54 (bs, 2H), 8.26 (d, *J* = 8.4 Hz, 2H), 7.67 (s, 2H), 7.51 (s, 1H), 7.37 (bs, 2H), 7.08 (d, *J* = 8.8 Hz, 1H), 3.69 (t, *J* = 5.8 Hz, 2H), 3.03 (t, *J* = 6.6 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 155.7, 154.8, 153.0, 149.6, 137.5, 133.1, 129.5, 116.9, 116.7, 48.0, 38.5.

<u>*Synthesis of 3-morpholino-4-nitrobenzenesulfonamide* (**13k**):</u> Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and morpholine (yield = 75%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ= 8.02 (d, *J* = 8.8 Hz, 1H), 7.68 (s, 1H), 7.62 (s, 2H), 7.51 (d, *J* = 8.8 Hz), 3.72 (t, *J* = 4.4 Hz, 4H), 3.05 (t, *J* = 4.4 Hz, 4H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 148.7, 145.5, 144.0, 127.1, 118.9, 118.6, 66.4, 51.5.

<u>*Synthesis of 3-((furan-2-ylmethyl)amino)-4-nitrobenzenesulfonamide* (**13l**):</u> Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and furan-2-ylmethanamine (yield = 70%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.64 (t, *J* = 5.8 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 3H), 7.54 (s, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.41 (d, *J* = 16.4 Hz, 2H), 4.66 (d, J = 6.4 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ = 151.4, 150.7, 144.7, 143.2, 133.0, 128.2, 112.4, 112.3, 111.1, 108.3.

<u>*Synthesis of 3-((2-(furan-2-yl)ethyl)amino)-4-nitrobenzenesulfonamide* (**13m**):</u> Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and 2-(furan-2-yl)ethan-1-amine (yield = 78%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.30 (t, *J* = 5.4 Hz, 1H), 8.26 (d, *J* = 8.4 Hz,

1H), 7.65 (s, 2H), 7.59 (s, 1H), 7.47 (s, 1H), 7.08 (d, 9.6 Hz, 1H), 6.41 (t, *J* = 2.4 Hz, 1H), 6.28 (d, *J* = 2.8 Hz, 1H), 3.68 (q, *J* = 6.4 Hz, 2H), 3.05 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 157.5, 155.7, 149.6, 147.2, 137.4, 133.0, 116.8, 116.7, 115.7, 111.8, 46.4, 32.0.

Synthesis of 3-(((1-methyl-1H-pyrazol-3-yl)methyl)amino)-4-nitrobenzenesulfonamide (**13n**): Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and (1-methyl-1*H*-pyrazol-3-yl)methanamine (yield = 68%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.54 (t, *J* = 5.4 Hz, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 7.71 (s, 1H), 7.68 (s, 2H), 7.52 (s, 1H), 7.47 (s, 1H), 7.07 (d, *J* = 9.2 Hz, 1H), 4.48 (d, *J* = 5.6 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 153.8, 147.9, 141.5, 135.8, 133.2, 120.9, 115.5, 114.9, 43.7, 40.6.

Synthesis of 4-nitro-3-((pyridin-3-ylmethyl)amino)benzenesulfonamide (**13o**): Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and pyridin-3-ylmethanamine (yield = 60%): yellow solid; ^{1}H NMR (400 MHz, DMSO) δ 8.84 (t, J = 6.2 Hz, 1H), 8.63 (s, 1H), 8.49 (d, *J* = 3.6 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.61 (s, 2H), 7.40-7.37 (m, 1H), 7.33 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 4.70 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 150.7, 149.0, 144.7, 135.3, 134.1, 133.1, 128.3, 124.2, 112.21, 112.16, 44.0.

Synthesis of 4-nitro-3-((2-(pyridin-3-yl)ethyl)amino)benzenesulfonamide (**13p**): Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and 2-(pyridin-3-yl)ethan-1-amine (yield = 64%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.56 (s, 1H), 8.48 (s, 1H), 8.27 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.67 (s, 2H), 7.51 (s, 1H), 7.40 (t, *J* = 6.2 Hz, 1H), 7.08 (d, *J* = 9.6 Hz, 1H), 3.68 (q, *J* = 6.8 Hz, 2H), 3.03 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 150.9, 150.2, 148.1, 144.8, 137.0, 134.7, 132.7, 128.3, 124.1, 112.2, 111.9, 44.0, 31.6.

Synthesis of 4-nitro-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (**13q**): Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and pyridin-4-ylmethanamine (yield = 55%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.89 (t, *J* = 5.8 Hz, 1H), 8.55 (d, *J* = 5.6 Hz, 2H), 8.31 (d, *J* = 9.2 Hz, 1H), 7.60 (s, 1H), 7.39 (d, *J* = 4.4 Hz, 2H), 7.22 (s, 1H), 7.09 (d, *J* = 9.6 Hz, 1H), 4.73 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 155.5, 155.0, 152.5, 149.5, 137.9, 133.1, 127.1, 125.7, 117.0, 116.9, 50.1.

Synthesis of 4-nitro-3-((2-(pyridin-4-yl)ethyl)amino)benzenesulfonamide (**13r**): Follow *General Procedure 1* using 3-fluoro-4-nitrobenzenesulfonamide and 2-(pyridin-4-yl)ethan-1-amine (yield = 58%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.57-8.50 (m, 4H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.39-7.36 (m, 5H), 3.78-3.73 (m, 2H), 3.00 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 154.8, 152.9, 151.4, 138.1, 135.5, 134.7, 129.9, 129.6, 120.8, 47.9, 38.6.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-chlorophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14a**): Follow *General Procedure 2* with **5** and **13a**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 55%); white solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.21 (s, 1H), 7.89 (d, *J* = 8.4, 2H), 7.69 (d, *J* = 9.6, 1H), 7.51 (d, *J* = 8.4, 2H), 7.27 (dd, *J* = 9.6, 1.6, 1H), 7.19 (d, *J* = 8.8, 2H), 6.86-6.82 (m, 4H), 6.03 (s, 2H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 156.7, 154.7, 145.5, 137.9, 136.2, 132.7, 130.3 (2), 129.6 (2), 128.5 (2), 127.7, 127.1, 122.9, 122.1, 119.8, 119.5, 118.4, 109.5, 54.7, 20.8; HRMS-ESI: *m/z* found 612.0293 [M-H]$^-$, $C_{29}H_{21}Cl_3N_3O_4S$ requires 612.0324.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-morpholino-3-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14b**): Follow *General Procedure 2* with **5** and **13b**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 42%): yellow solid; ^{1}H NMR (400 MHz, DMSO) δ 8.27 (d,

J = 8.0, 2H), 8.00 (d, J = 8.8, 1H), 7.67 (d, J = 9.2, 1H), 7.31 (d, J = 8.8, 1H), 7.26-7.23 (m, 3H), 6.85-6.84 (m, 4H), 6.08 (s, 2H), 3.66 (s, 4H), 3.03 (s, 4H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, DMSO) δ 156.5, 154.8, 146.8, 145.5, 139.5, 139.8, 137.8, 132.9 (2), 130.4, 128.6, 127.5, 126.9, 125.8, 123.0, 122.4, 120.4, 119.7, 119.3 (2), 118.6 (2), 66.3 (2), 51.3, 20.7; HRMS-ESI: *m/z* found 708.1098 [M-H]$^-$, $C_{33}H_{28}Cl_2N_5O_7S$ requires 708.1092.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-((morpholinoethyl)amino)-3-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14c**): Follow *General Procedure 2* with **5** and **13c**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 44%): yellow solid; ^{1}H NMR (400 MHz, DMSO) δ 8.64 (s, br, 1H), 8.58 (s, 1H), 8.29 (s, 1H), 8.97 (d, J = 8.0, 1H), 7.64 (d, J = 9.6, 1H), 7.27-7.22 (m, 3H), 7.07 (d, J = 7.6, 1H), 6.85-6.83 (m, 4H), 6.11 (s, 2H), 3.58 (s, br, 4H), 3.42 (s, br, 2H), 2.60 (s, br, 2H), 2.42 (s, br, 4H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, DMSO) δ = 163.7, 156.4, 154.8, 146.2, 145.5, 137.8, 135.3, 133.1, 132.7, 132.0, 130.4 (2), 128.6, 127.3, 126.9, 126.1, 123.0 (2), 122.6, 119.6, 119.2, 118.6, 114.6, 66.5, 55.6, 54.6, 53.0, 20.7; HRMS-ESI: *m/z* found 751.1571 [M-H]$^-$, $C_{35}H_{33}Cl_2N_6O_7S$ requires 751.1514.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-((furan-2-ylmethyl)amino)-3-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14d**): Follow *General Procedure 2* with **5** and **13d**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 56%): yellow solid; ^{1}H NMR (400 MHz, DMSO) δ 8.73 (m, 1H), 8.58 (s, 1H), 8.25 (s, 1H), 7.94 (d, J = 8.4, 1H), 7.66 (d, J = 8.4, 1H), 7.58 (s, 1H), 7.26-7.24 (m, 3H), 7.19 (d, J = 9.6, 1H), 6.84-6.83 (m, 4H), 6.38 (s, 2H), 6.08 (s, 2H), 4.65 (d, J = 6.4, 2H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, DMSO) δ 163.3, 156.5, 154.8, 151.5, 146.0, 145.5, 143.1, 137.8, 135.0, 133.0, 132.5, 130.3, 128.6, 127.5, 127.0, 126.2, 124.8, 123.7, 123.0, 122.4, 119.7, 119.3, 118.6, 118.2, 114.7, 110.9, 108.2, 54.6, 20.7; HRMS-ESI: *m/z* found 718.0912 [M-H]$^-$, $C_{34}H_{26}Cl_2N_5O_7S$ requires 718.0935.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-((2-(furan-2-yl)ethyl)amino)-3-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14e**): Follow *General Procedure 2* with **5** and **13e**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 54%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.64 (s, 1H), 8.61 (br, 1H), 8.03 (s, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.36 (d, J = 9.6 Hz, 1H), 7.21 (d, J = 9.2 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.0 Hz, 5H), 6.37 (s, 1H), 6.24 (d, J = 3.2 Hz, 1H), 5.93 (s, 2H), 3.68 (q, J = 6.4 Hz, 2H), 2.99 (t, J = 7.2 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 161.2, 156.6, 154.7, 152.7, 146.9, 145.5, 142.4 (2), 137.8, 135.0, 133.5, 132.4, 130.2, 127.4, 127.2, 122.7, 121.3, 120.1, 119.2, 118.6, 114.8, 112.0, 111.0 (2), 107.1 (2), 55.0, 41.7, 27.3, 20.7; HRMS-ESI: *m/z* found 732.1071 [M-H]$^-$, $C_{35}H_{28}Cl_2N_5O_7S$ requires 732.1092.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-(((1-methyl-1H-pyrazol-3-yl)methyl)amino)-3-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14f**): Follow *General Procedure 2* with **5** and **13f**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 44%): yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.69 (t, J = 5.6 Hz, 1H), 8.61 (s, 1H), 8.21 (s, 1H), 7.97 (d, J = 9.6 Hz, 1H), 7.71 (s, 1H), 7.69 (s, 1H), 7.45 (s, 1H), 7.31 (s, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 4.0 Hz, 2H), 7.05 (s, 1H), 6.84 (d, J = 7.2 Hz, 4H), 6.06 (s, 2H), 4.46 (d, J = 5.6 Hz, 2H), 3.78 (s, 3H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 156.5, 154.8, 146.2, 145.5, 138.4, 137.8, 135.1, 132.8, 130.3 (2), 130.0, 128.6, 127.7, 127.1, 126.5, 122.9, 122.1, 119.8, 119.3, 118.6, 117.8, 114.8, 54.7, 46.2, 41.8, 40.6, 38.9, 37.4, 20.7; HRMS-ESI: *m/z* found 732.1189 [M-H]$^-$, $C_{34}H_{28}Cl_2N_7O_6S$ requires 732.1204.

<u>Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-nitro-4-((pyridin-3-ylmethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide</u> <u>(**14g**):</u> Follow *General Procedure 2* with **5** and **13g**. The product was purified via flash column chromatography, eluting with $CH_2Cl_2/MeOH/NH_4OH$, 92:7:1 (yield = 39%): yellow solid; ^1H NMR (400 MHz, DMSO) δ 8.94 (t, *J* = 6.2, 1H), 8.64 (s, br, 1H), 8.59 (s, 1H), 8.45 (s, br, 1H), 8.23 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 9.6 Hz, 1H), 7.34 (m, 1H), 7.26-7.22 (m, 3H), 7.00 (d, *J* = 9.6 Hz, 1H), 6.85-6.83 (m, 4H), 6.09 (s, 2H), 4.69 (d, *J* = 6.4 Hz, 2H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ = 163.7, 156.5, 154.8, 148.8, 148.5, 145.9, 145.5, 137.8, 135.6, 135.1 (2), 133.1, 133.0, 131.9, 130.4, 128.6, 127.3, 126.9, 126.1, 123.0, 122.6, 119.6, 119.3, 118.6 (2), 114.6 (2), 54.5, 43.8, 20.7; HRMS-ESI: *m/z* found 731.1294 $[M+H]^+$, $C_{35}H_{29}Cl_2N_6O_6S$ requires 731.1241.

<u>Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-nitro-4-((2-(pyridin-3-yl)ethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide</u> <u>(**14h**):</u> Follow *General Procedure 2* with **5** and **13h**. The product was purified via flash column chromatography, eluting with $CH_2Cl_2/MeOH/NH_4OH$, 50:7:1 (yield = 35%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.63 (s, 1H), 8.60 (s, 1H), 8.55 (d, *J* = 4.0 Hz, 1H), 8.44 (t, *J* = 5.8 Hz, 1H), 8.29 (s, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.52 (t, *J* = 6.2 Hz, 1H), 7.37 (1H), 7.29-7.23 (m, 4H), 7.12 (s, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.82 (s, 2H), 6.11 (s, 2H), 3.69 (q, *J* = 6.4 Hz, 2H), 3.01 (t, *J* = 7.4 Hz, 2H), 2.27 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.1, 161.2, 159.6, 153.0, 150.9, 150.8, 150.2, 144.2, 142.5 (2), 140.7, 140.1, 137.8, 137.2, 136.5, 135.1 (2), 132.1, 131.7, 131.1, 129.5, 127.7, 127.2, 124.4, 123.9, 123.4, 119.2, 59.3, 48.4, 36.5, 25.5; HRMS-ESI: *m/z* found 745.14.14 $[M+H]^+$, $C_{36}H_{31}Cl_2N_6O_6S$ requires 745.1397.

<u>Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-nitro-4-((pyridin-4-ylmethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide</u> <u>(**14i**):</u> Follow *General Procedure 2* with **5** and **13i**. The product was purified via flash column chromatography, eluting with $CH_2Cl_2/MeOH/NH_4OH$, 75:14:2 (yield = 37%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 9.06 (bs, 1H), 8.70 (bs, 1H), 8.67 (d, *J* = 18.0 Hz, 2H), 8.22 (s, 1H), 7.89 (d, *J* = 9.2 Hz, 1H), 7.74 (bs, 2H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 3H), 6.86 (d, *J* = 3.2 Hz, 5H), 6.08 (s, 2H), 4.89 (d, *J* = 4.8 Hz, 2H), 2.30 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 163.0, 156.5, 155.9, 154.8, 145.9, 145.5, 144.9, 137.8, 135.1, 132.9, 132.5, 131.1, 130.9, 130.3, 128.6, 127.6, 127.0, 126.5, 124.3, 122.9, 122.2, 119.8, 119.3, 118.6, 114.6, 71.5, 45.6, 20.8; HRMS-ESI: *m/z* found 729.1091 $[M-H]^-$, $C_{35}H_{27}Cl_2N_6O_6S$ requires 729.1095.

<u>Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-nitro-4-((2-(pyridin-4-yl)ethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide</u> <u>(**14j**):</u> Follow *General Procedure 2* with **5** and **13j**. The product was purified via flash column chromatography, eluting with $CH_2Cl_2/MeOH/NH_4OH$, 50:7:1 (yield = 39%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.66 (d, *J* = 4.8 Hz, 2H), 8.30 (s, 1H), 8.24 (t, *J* = 6.0 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 5.6 Hz, 2H), 7.68 (d, *J* = 9.6 Hz, 2H), 7.31-7.23 (m, 4H), 7.11 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 3.8 Hz, 4H), 6.11 (s, 2H), 3.75 (q, *J* = 6.0 Hz, 2H), 3.20 (t, *J* = 7.0 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.6, 161.2, 160.4, 159.5, 157.3, 150.3, 149.7, 149.1, 142.6, 137.7, 136.8, 136.5, 134.9 (2), 132.2, 131.8, 131.7, 131.3, 127.8, 127.3, 124.5, 124.1, 123.3 (2), 118.5, 59.4, 47.7, 39.0, 25.5; HRMS-ESI: *m/z* found 743.1244 $[M-H]^-$, $C_{36}H_{29}Cl_2N_6O_6S$ requires 743.1252.

<u>Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-morpholino-4-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide</u> <u>(**14k**):</u> Follow *General Procedure 2* with **5** and **13k**. The product was purified via flash column chromatography, eluting with

CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 57%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.29 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.76 (s, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.57 (d, *J* = 9.2 Hz, 1H), 7.25 (d, *J* = 9.2 Hz, 3H), 6.87 (d, *J* = 11.2 Hz, 4H), 6.09 (s, 2H), 3.66 (bs, 4H), 2.98 (bs, 4H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.0, 163.9, 156.6, 154.7, 150.5, 145.6, 144.9, 143.7, 137.9, 133.0, 131.6, 130.3, 128.7, 127.5, 127.0, 125.9, 123.0, 122.5, 120.7 (2), 119.7, 119.4, 118.5, 66.4, 51.8, 20.7; HRMS-ESI: *m/z* found 708.1098 $[M-H]^-$, $C_{33}H_{28}Cl_2N_5O_7S$ requires 708.1092.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-((furan-2-ylmethyl)amino)-4-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14l**): Follow *General Procedure 2* with **5** and **13l**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 50%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.53 (t, *J* = 5.8 Hz, 1H), 8.31 (s, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.61 (s, 1H), 7.48 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 3H), 7.11 (d, *J* = 9.2 Hz, 1H), 6.86 (t, *J* = 8.0 Hz, 4H), 6.29 (d, *J* = 2.4 Hz, 1H), 6.19 (s, 1H), 6.10 (s, 2H), 4.60 (d, *J* = 5.6 Hz, 2H), 2.28 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 163.8, 156.5, 154.8, 152.9, 151.5, 145.5, 144.6, 142.9 (2), 137.8, 133.1, 132.2, 131.7, 130.2, 128.6, 127.4, 126.94, 126.9, 123.1, 122.6, 119.7, 119.3, 118.6, 114.1, 113.3, 110.8, 108.2, 40.6, 20.7; HRMS-ESI: *m/z* found 720.1048 $[M+H]^+$, $C_{34}H_{28}Cl_2N_5O_7S$ requires 720.1081.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-((2-(furan-2-yl)ethyl)amino)-4-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14m**): Follow *General Procedure 2* with **5** and **13m**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 52%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.27 (br, 1H), 8.24 (s, 1H), 8.14 (d, *J* = 9.6 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.62 (s, 1H), 7.51 (s, 1H), 7.30 (d, *J* = 10.0 Hz, 1H), 7.27 (s, 1H), 7.23 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 9.2 Hz, 1H), 6.84 (d, *J* = 6.0 Hz, 4H), 6.34 (s, 1H), 6.21 (d, *J* = 3.2 Hz, 1H), 6.05 (s, 2H), 3.66 (q, *J* = 4.4 Hz, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.23 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 187.3, 162.9, 156.6, 154.7, 152.8, 145.5, 144.6, 142.3 (2), 137.8, 132.7, 132.2, 130.2, 128.6, 127.9, 127.2, 127.1, 122.9, 122.1, 119.9, 119.3, 118.5, 113.6 (2), 110.9, 106.9, 54.8, 41.7, 27.2, 20.7; HRMS-ESI: *m/z* found 732.1071 $[M-H]^-$, $C_{35}H_{28}Cl_2N_5O_7S$ requires 732.1092.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((3-(((1-methyl-1H-pyrazol-3-yl)methyl)amino)-4-nitrophenyl)sulfonyl)-2H-indazole-3-carboxamide (**14n**): Follow *General Procedure 2* with **5** and **13n**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 47%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.42 (t, *J* = 5.6 Hz, 1H), 8.33 (s, 1H), 8.11 (d, *J* = 9.6 Hz, 1H), 7.67 (t, *J* = 11.8 Hz, 3H), 7.45 (s, 1H), 7.28 (t, *J* = 7.0 Hz, 3H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.85 (s, 2H), 6.14 (s, 2H), 4.44 (d, *J* = 4.4 Hz, 2H), 3.71 (s, 3H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 163.8, 156.5, 152.9, 145.5, 144.6, 138.5 (2), 137.8 (2), 133.1, 131.8, 130.2, 130.0, 128.6, 127.4, 126.9 (2), 123.1, 122.6, 119.7, 119.3, 118.6, 118.0, 113.7, 113.5, 54.6, 38.8, 37.4, 20.7; HRMS-ESI: *m/z* found 732.1189 $[M-H]^-$, $C_{34}H_{28}Cl_2N_7O_6S$ requires 732.1204.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-nitro-3-((pyridin-3-ylmethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide (**14o**): Follow *General Procedure 2* with **5** and **13o**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 92:7:1 (yield = 40%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.72 (s, 1H), 8.25 (s, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.74 (bs, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.45 (s, 1H), 7.25 (d, *J* = 7.6 Hz, 4H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 4H), 6.07 (s, 2H), 4.66 (s, 2H), 2.27 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 168.5, 161.2, 159.5, 157.7, 153.6, 153.2, 150.3, 149.3, 142.6, 140.3 (2), 139.2, 137.8, 137.1, 136.4, 135.0, 132.2, 131.7, 128.8, 128.4, 127.8, 127.4, 124.4, 124.1, 123.3, 118.7, 118.1, 59.4, 48.8, 25.5; HRMS-ESI: *m/z* found 731.1176 $[M+H]^+$, $C_{35}H_{29}Cl_2N_6O_6S$ requires 731.1241.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-nitro-3-((2-(pyridin-3-yl)ethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide (**14p**): Follow *General Procedure 2* with **5** and **13p**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 50:7:1 (yield = 38%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.31 (s, 1H), 8.23 (t, *J* = 5.6 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.46 (s, 1H), 7.33 (s, 1H), 7.25 (d, *J* = 10.8 Hz, 3H), 7.21 (s, 1H), 7.11 (d, *J* = 9.2 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 4H), 6.10 (s, 2H), 3.68 (q, *J* = 7.2 Hz, 2H), 3.07 (t, *J* = 7.0 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.6, 161.2, 159.5, 157.4, 153.0, 150.8, 150.3, 149.3, 143.9, 142.6, 137.7, 136.7, 136.5, 134.9, 133.4, 132.2, 131.8, 131.7, 129.6, 127.8, 127.3, 124.5, 124.1, 123.2, 118.5, 118.4, 76.1, 59.4, 48.5, 36.3, 25.5; HRMS-ESI: *m/z* found 745.1414 $[M+H]^+$, $C_{36}H_{31}Cl_2N_6O_6S$ requires 745.1397.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-nitro-3-((pyridin-4-ylmethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide (**14q**): Follow *General Procedure 2* with **5** and **13q**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 75:14:2 (yield = 35%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.83 (t, *J* = 6.2 Hz, 1H), 8.63 (d, *J* = 5.2 Hz, 2H), 8.24 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 5.6 Hz, 2H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.30-7.23 (m, 4H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.90-6.86 (m, 4H), 6.04 (s, 2H), 4.88 (d, *J* = 6.4 Hz, 2H), 2.30 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.4, 161.3, 159.5, 157. 6, 150.2, 149.8, 148.9, 142.6 (2), 137.8, 136.3, 134.9, 132.2, 131.9, 131.7, 128.9 (2), 127.7, 127.3, 124.5, 124.1, 123.6, 123.4, 119.1, 118.0, 59.3, 50.4, 25.5; HRMS-ESI: *m/z* found 729.1091 $[M-H]^-$, $C_{35}H_{27}Cl_2N_6O_6S$ requires 729.1095.

Synthesis of 5-chloro-2-(4-(4-chloro-3,5-dimethylphenoxy)benzyl)-N-((4-nitro-3-((2-(pyridin-4-yl)ethyl)amino)phenyl)sulfonyl)-2H-indazole-3-carboxamide (**14r**): Follow *General Procedure 2* with **5** and **13r**. The product was purified via flash column chromatography, eluting with CH_2Cl_2/MeOH/NH_4OH, 50:7:1 (yield = 36%): yellow solid; ^1H NMR (400 MHz, d_6-DMSO) δ 8.65 (d, *J* = 4.0 Hz, 2H), 8.61 (s, 1H), 8.43 (t, *J* = 5.4 Hz, 1H), 8.29 (s, 1H), 8.02 (d, *J* =9.6 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 4.8 Hz, 2H), 7.30-7.24 (m, 5H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.82 (s, 2H), 6.12 (s, 2H), 3.73 (q, *J* = 6.4 Hz, 2H), 3.09 (t, *J* = 7.2 Hz, 2H), 2.27 (s, 6H); ^{13}C NMR (400 MHz, d_6-DMSO) δ 168.1, 161.2, 159.6, 158.3, 151.1, 150.8, 150.2, 142.6 (2), 140.1, 137.8, 137.2, 136.4, 135.1, 134.8, 132.1, 131.7, 131.1, 130.9, 127.7, 127.3, 124.4, 123.9, 123.4, 119.2, 59.3, 47.6, 39.0, 25.5; HRMS-ESI: *m/z* found 745.1414 $[M+H]^+$, $C_{36}H_{31}Cl_2N_6O_6S$ requires 745.1397.

Fluorescence Polarization Competition Assay

The fluorescence polarization assays were performed in 96 well polypropylene F-bottom black microplates (Greiner Bio-One) with a final volume of 100 µL. During the competition assay, a fluorescently-labeled Bak-BH3 peptide (FITC-Ahx-GQVGRQLAIIGDDINR-$CONH_2$, hereafter “FITC-Bak”, where FITC = fluorescein isocyanate; Ahx = 6-aminohexanoyl linker) was competed off of either MCL-$1^{172-327}$, BCL-X_L^{1-212} or BCL-2^{1-211} with the synthesized inhibitors. The binding affinities of FITC-Bak to MCL-1, BCL-X_L and BCL-2 were determined via a fluorescence polarization assay where various concentrations of the selected proteins were titrated into solutions of 10nM FITC-Bak in 20 mM HEPES, pH 6.8, 50 mM NaCl, 3 mM DTT, 0.01% Triton X-100 and 5% DMSO at room temperature. The changes in the fluorescence polarization were then measured using a BMG PHERAstar FS multimode microplate reader equipped with two PMTs for simultaneous measurements of both the perpendicular and parallel fluorescence emission at a 485 nm excitation and 520 nm emission filter. Regression analysis was then performed on the polarization data using Origin (OriginLab, Northampton, MA) and the data was fitted to the Hill equation, thus producing binding curves for FITC-Bak with MCL-1, BCL-X_L and BCL-2. FITC-Bak's K_d's were then determined to be 42 nM for MCL-1, 6 nM for BCL-X_L and 33 nM for BCL-2.

The fluorescence polarization competition assays were setup using a Biomek FXP Automated Liquid Handling System. Protein concentrations of either 100nM of MCL-1, 15 nM of BCL-X_L or 75 nM of BCL-2 with 10 nM of FITC-Bak (in 20 mM HEPES, pH 6.8, 50 mM NaCl, 3 mM DTT, 0.01% Triton X-100 and 1% DMSO) were chosen and various concentrations of the inhibitors were titrated into the solutions. Wells possessing only the peptide, only the desired protein and both the peptide plus the desired protein without inhibitor were used as controls. Changes in fluorescence polarization were measured after 4 hours of incubation at room temperature using the BMG PHERAstar FS multimode plate reader previously mentioned and regression analysis was performed using Prism 8 (Graphpad) with the data fitted to a sigmoidal curve to determine inhibitor IC_{50} values. The IC_{50} values were then converted to K_i values using an equation derived by Nikolovska-Coleska *et al*. All inhibitors were tested in triplicate.

SILCS-Molecular Simulations

Methods

The Site-Identification by Ligand Competitive Saturation (SILCS) approach, as used in our previous MCL-1 study [1], was used to predict docking poses and contributions to the binding affinities of the synthesized compounds for MCL-1. The SILCS FragMaps were previously generated from SILCS GCMC-MD simulations [2] conducted using the crystal structure of MCL-1 (PDB entry 4HW3)[3]. SILCS simulations for BCL-2 were performed using the crystal structure PDB entry 6O0K. For MCL-1, the molecular structures of all compounds were prepared using MOE (Chemical Computing Group) and aligned with the crystal binding mode of compound **1** (PDB: 4HW2) for compounds **5** and **6** and with the crystal binding mode of compound **49** from Fesik's work on a series of **14** compounds (PDB: 5FDO)[4]. For BCL-2, molecular structures of all compounds were aligned with the crystal binding mode of compound Venetoclax (PDB:6O0K). Exhaustive SILCS-MC docking studies were performed from the aligned compound structures using SILCS FragMaps. The computational details about SILCS and SILCS-MC setups can be found in our previous work [2].

Results and discussion

Figure 1(A) shows the predicted binding mode of compound **5** aligned with the crystal binding mode of Fesik's compound **1**. The SILCS FragMaps capture important functional group contributions to the binding of compound 1 including the hydrophobic moieties as indicated by the apolar green meshes and the carboxylate group as shown by the negative orange meshes, as described previously [1]. Compound **5**, with the indole in **1** being replaced by indazole, is predicted to adopt a similar binding orientation as **1**, with the 4-Cl-3,5-diMe-phenyl being deeply inserted into the P2 pocket. The linker between the bi-cyclic ring and halogenated phenyl is changed from a propyl in **1** to a phenyl ring in compound 5, but still presents a very similar binding orientation. The carboxylate groups in both **1** and **5** occupy the P2 pocket entrance and form ionic interactions with near-by R263 residue. However, since the carboxylate group in compound **5** is at the 3 position of indazole while is at the 2 position of the indole in **1**, the binding orientation of the indazole ring in compound **5** is turned away from that of the compound 1 indole ring. This is suggested to explain why Fesik's compound **1** has higher affinity than our compound **5**.

The arrangement of the carboxylate (2 position) and (4-Cl-3,5-diMe-phenoxy)propyl (3 position) groups around the indole ring (Figure 1A) allows the indole ring to be fully accommodated by the hydrophobic environment at P2 pocket as indicated by the favorable apolar FragMaps (green meshes). However, for compound **5**, the arrangement of carboxylate (3 position) and the (4-Cl-3,5-diMe-phenoxy)-phenyl (2 position) on the indazole ring, though still allowing the carboxylate and (4-Cl-3,5-diMe-phenoxy)phenyl groups to fully occupy the desired regions, lead to the indazole ring being rotated 90˚ from the indole binding mode, and thus is less favorably accommodated by the pocket (off the favorable region as indicated by the apolar FragMaps.

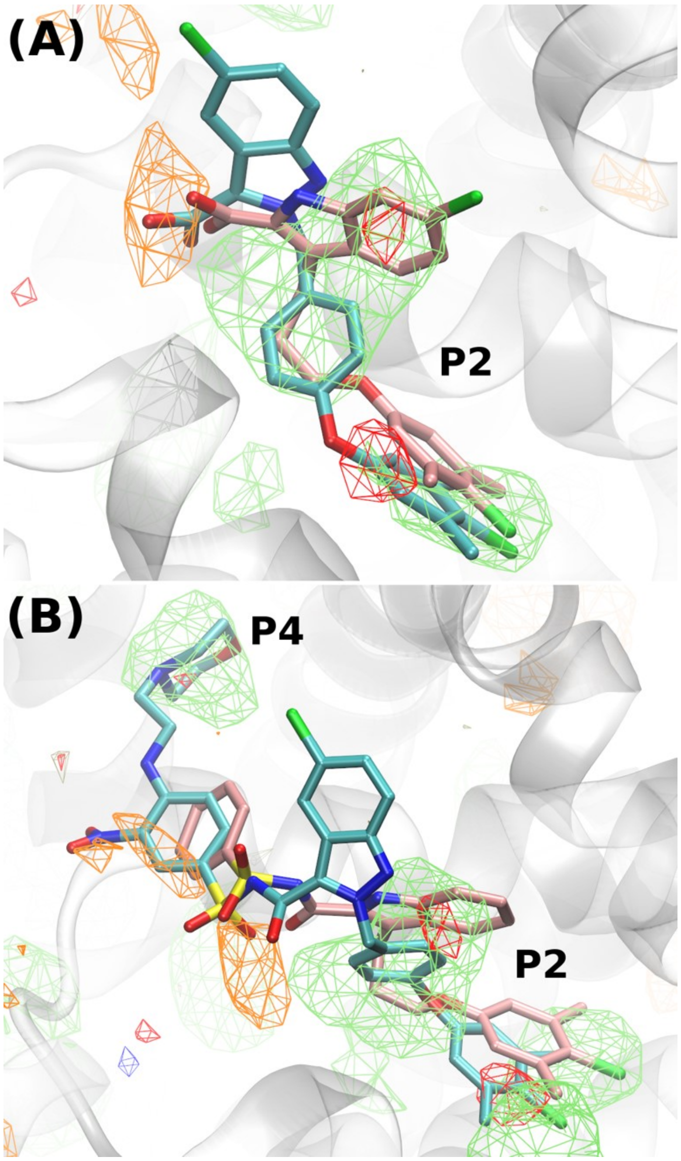
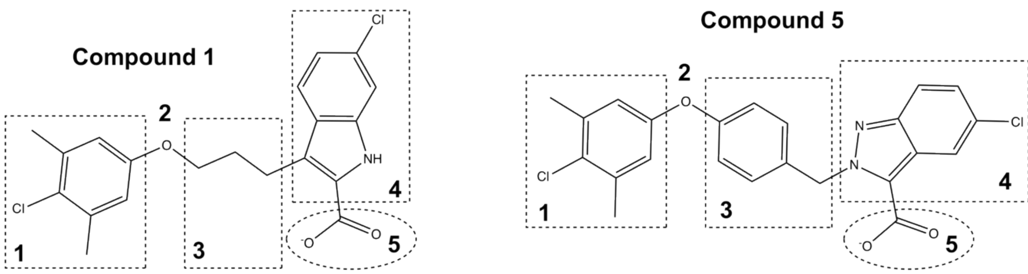


Figure 1 Predicted MCL-1 binding poses (carbons in cyan) of compound **5** (A) and **14c** (B) by SILCS-MC. Crystal orientation (carbons in pink) of compound **1** (PDB:4HW2) and compound **49** from Ref [3] (PDB:5FDO) are overlaid on **5** and **14c**, respectively. SILCS FragMaps for apolar, H-bond acceptor and negatively charged types are shown in green, red, and orange meshes, respectively, and rendered at a GFE level of -1.2 kcal/mol.

To quantitatively describe the differences between Fesik compound **1** and **5**, the LGFE scores and the GFE contributions for different functional groups to the total LGFE of the compounds are shown in Table 1. The predicted binding affinities based on the LGFE scores captures the more favorable binding of compound **1** (Ki = 0.25 μM) as compared to compound **5** (Ki = 2.36 μM). GFE contributions from the di-Me-4-Cl-phenyl, the ester oxygen, the linker and the carboxylate groups are very similar for both **1** and **5**. The difference in binding is due to the GFE contribution from the bi-cyclic ring with the indole ring in compound **1** making a more favorable contribution than the indazole ring in **5**. Such analyses will facilitate future design of MCL-1 compounds. For example, swapping the substituent positions of carboxylate and (4-Cl-3,5-diMe-phenoxy) phenyl groups on the indazole ring might allow the resulting benzimidazole ring to bind in a similar way as the indole ring in compound **1** and let the entire compound benefit from this orientation for binding.

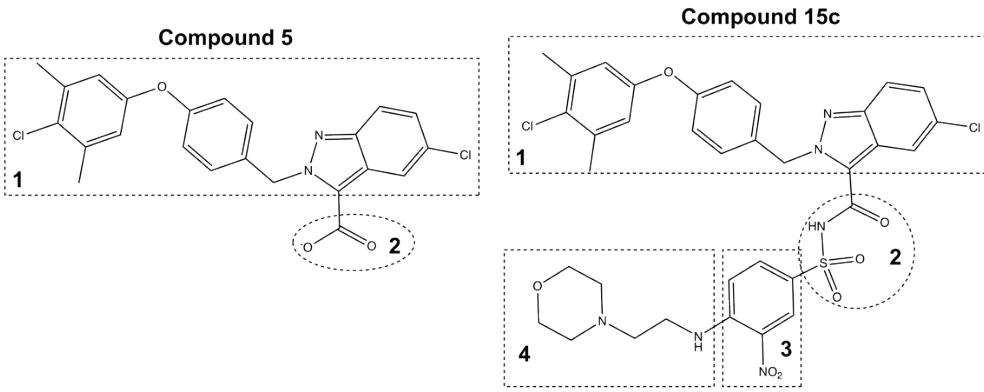
Table 1 LGFE scores and GFE contributions (kcal/mol) from different functional groups to the overall predicted binding affinities of compound 1 and 5 to MCL-1.



Compound	LGFE	GFE				
		1	2	3	4	5
		4-Cl-3,5-diMe-phenyl	Ester O	Propyl / phenyl	Indole / indazole	carboxylate
1	-7.8	-2.0	-0.1	-1.8	-1.8	-2.1
5	-7.4	-2.4	-0.4	-2.1	-0.4	-2.1

The predicted binding mode of one of our acylsulfonamides compound, **14c**, aligned with the crystal binding mode of one Fesik's acylsulfonamide compound **49** is shown in Figure 1(B) [4]. The overall similar binding orientations are evident. The core part of compound **14c** retains a similar binding orientation as with compound **5** with the acyl-sulfonamide group of **14c** occupying the position of the acid group in compound **5** as designed. The morpholine group occupies the MCL-1 P4 pocket as indicated by the apolar FragMaps. Such additional occupancy of P4 pocket helps to improve the binding of the **14** series of compounds over compound **5**. However, the binding improvement (Ki is 1.61 μM for compound **14c** compared to 2.32 μM for **5**) is not that promising given the number of additional functional groups being added.

Table 2 LGFE scores and GFE contributions (kcal/mol) from different functional groups to the overall predicted binding affinities of compound **5** and **14c** to MCL-1.



Compound	LGFE	GFE			
		1	2	3	4
		P2 pocket	carboxylate/ acyl-sulfonamide	nitrophenyl	P4 pocket
5	-7.4	-5.3	-2.1	-	-
14c	-8.5	-4.4	-1.5	-0.5	-2.1

To understand the relatively small change in the overall binding functional group GFE analysis was performed (Table 2). The overall predicted binding affinity based on LGFE captures the favored binding of compound **14c** compared to 5. The GFE contribution from the additional functional groups in compound **14c** targeting the P4 pocket do yield very favorable binding contributions (group 3 and 4 together). However, the acyl-sulfonamide group in compound **14c** which replaces the acid group in compound 5, contribute a slightly smaller binding benefit as compared to the acid group, and the P2 pocket core part (group 1) in compound **14c** also offsets the contribution from the additional functional groups designed to target the P4 pocket. Clearly, acyl-sulfonamide group designed to retain the acceptor role of the acid group in compound 5 while enabling linking to additional groups to access the P4 pocket binding, does not fully maximize the benefits from the additional groups to the binding. Similar data are observed for other **14** series compounds as listed in the Supplementary Information Table S1. This observationon our designed compounds is consistent with Fesik's acyl-sulfonamide designs. One acyl-sulfonamide design from their study [4], compound **50**, which has exactly the same P2 pocket part with the original acid compound 1, but with acyl-sulfonamide-phenyl group to replace the acid group, has a measured Ki of 91 nM, compared to the 55 nM measured Ki for compound **1**, which indicates the acyl-sulfonamide group does not retain the same level of affinity benefit as the acid group.

Thus, based on the SILCS GFE analyses, this suggests that to further efficiently extend the scaffold to utilize the P4 pocket, additional functional groups that extend from the acyl-sulfonamide group need to be carefully optimized to maintain the binding contribution of the P2 pocket core part as well as the acyl-sulfonamide group. An alternative is to keep the carboxylate group while extending the molecule from other positions in the current scaffold. This approach actually was explored in work from Souers et al [5]. In that work, compound 5 is similar to the Fesik's compound 1 with the di-Me-4-Cl-phenyl ring replaced by naphthalene and the 6-Cl being absent. And to further utilize the P4 pocket, instead of altering the carboxylate group, they introduced substituent at the 1 and 7 positions on the indole ring and extend the scaffold from there to design a series of compounds to utilize the P4 pocket. One compound **30b** (Ki = 0.43

nM) showed a thousand-fold binding affinity increase compared to the compound **5** (Ki = 0.34 μM).

Table S3 LGFE scores and GFE contributions (kcal/mol) from different functional groups to the overall predicted binding affinities of compound **14c** to MCL-1 and BCL-2.

Target	LGFE	GFE			
		1	2	3	4
		P2 pocket	carboxylate/ acyl-sulfonamide	nitrophenyl	P4 pocket
MCL-1	-8.5	-4.4	-1.5	-0.5	-2.1
BCL-2	-9.3	-4.3	-1.0	-1.0	-3.0

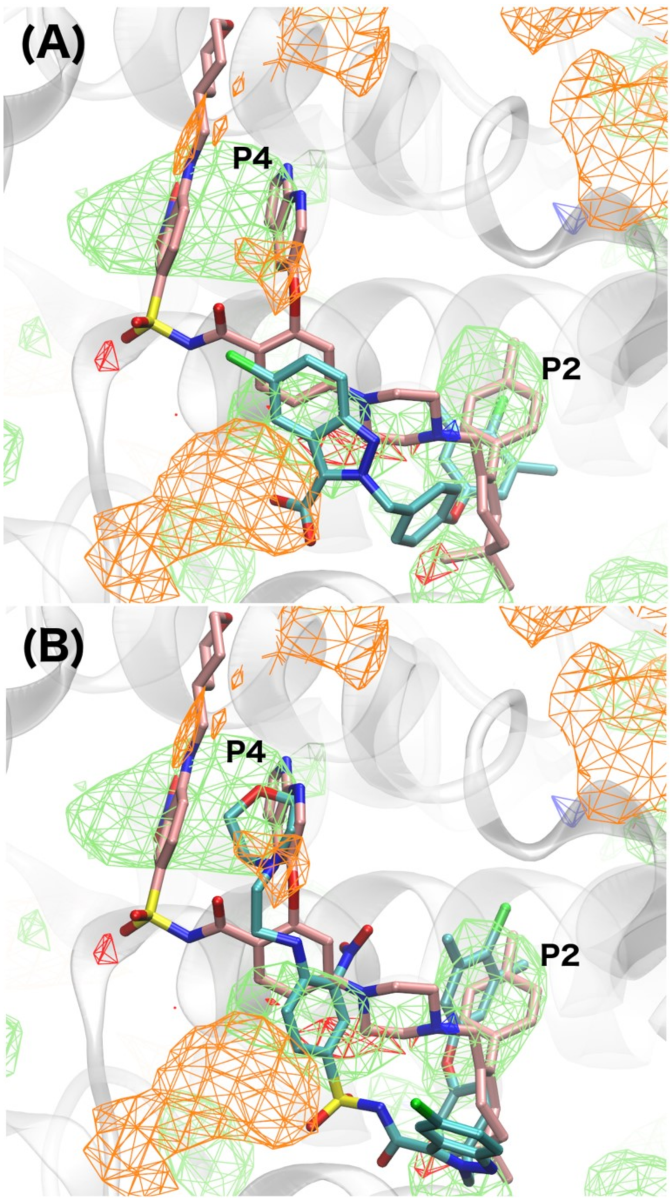


Figure S2 Predicted binding poses (carbons in cyan) of compound **5** (A) and **14c** (B) by SILCS-MC targeting BCL-2. Crystal orientation (carbons in pink) of compound venetoclax (PDB:6O0K) is overlaid on **5** and **14c**. SILCS FragMaps for apolar, H-bond acceptor and negatively charged types are shown in green, red, and orange meshes, respectively, and rendered at a GFE level of -1.2 kcal/mol.

The designed compounds were also found to be active toward BCL-2. Figure S2 shows the predicted binding modes of compound **5** and **14c** to BCL-2. The binding orientations of both compound **5** and **14c** are similar to the crystal binding mode of venetoclax with the di-Me-4-Cl-phenyl bound in the BCL-2 P2 pocket, carboxylate or acyl-sulfonamide bound near residue R105 and morpholine group in compound **14c** bound to the P4 pocket. Table 3 lists the LGFE and GFE contributions for compound **14c** targeting MCL-1 and BCL-2. Data for other synthesized compounds are listed in Tables S4 and S5. Predicted binding affinities in terms of LGFE capture the favorable binding to BCL-2 over MCL-1 for studied compounds. For compound **14c**, the GFE contributions indicate the more affinity gain on BCL-2 over MCL-1 was brought by the nitrophenyl and morphiline groups. And for other compounds as shown in Tables S4 and S5, binding of the nitrophenyl group is more favorable targeting BCL-2 than MCL-1 in general. This is consistent with the observed apolar FragMaps overlaid on the nitrophenyl group as shown in Figure S2 for BCL-2, while MCL-1 lack of such binding patterns at this position as shown in Figure S1.

As a conclusion, the predicted docking poses using the SILCS FragMaps along with the GFE analyses helps to explain the experimental binding observations on MCL-1 and BCL-2 and shed insights on how to further optimize our scaffold to maximize its binding to MCL-1.

Table S4 GFE contributions (kcal/mol) from different functional groups in **14** series of compounds to the predicted LGFE scores for MCL-1.

Compound	LGFE	GFE			
		1	2	3	4
14a	-7.0	-5.1	-1.3	-0.6	-
14b	-7.6	-3.4	-1.6	-0.5	-2.1
14c	-8.5	-4.4	-1.5	-0.5	-2.1
14d	-7.2	-4.0	-1.6	-0.7	-0.9
14e	-7.5	-4.2	-1.6	-0.5	-1.2
14f	-7.4	-4.3	-1.6	-0.6	-0.9
14g	-8.0	-4.4	-1.5	-0.5	-1.6
14h	-8.2	-4.5	-1.6	-0.5	-1.6
14i	-7.4	-3.9	-1.6	-0.8	-1.1
14j	-7.9	-4.1	-1.6	-0.6	-1.6
14k	-7.4	-4.0	-1.6	-0.6	-1.2
14l	-7.0	-4.0	-1.7	-0.6	-0.7
14m	-7.1	-3.8	-1.5	-0.7	-1.1
14n	-7.6	-4.0	-1.6	-0.7	-1.3

14o	-7.8	-4.0	-1.6	-0.7	-1.5
14p	-8.2	-3.8	-1.6	-0.7	-2.1
14q	-8.1	-4.6	-1.6	-0.5	-1.4
14r	-8.2	-3.9	-1.7	-0.4	-2.2

Table S5 GFE contributions (kcal/mol) from different functional groups in **14** series of compounds to the predicted LGFE scores for BCL-2.

Compound	LGFE	GFE			
		1	2	3	4
14a	-7.7	-4.7	-1.8	-1.2	-
14b	-8.3	-4.4	-1.2	-1.3	-1.4
14c	-9.3	-4.3	-1.0	-1.0	-3.0
14d	-8.2	-4.1	-1.6	-1.2	-1.3
14e	-8.9	-4.5	-0.8	-1.8	-1.8
14f	-9.0	-3.6	-1.4	-1.6	-2.4
14g	-8.8	-3.0	-1.4	-1.5	-2.9
14h	-9.0	-4.6	-0.8	-1.5	-2.1
14i	-8.5	-3.5	-1.0	-2.0	-2.0
14j	-9.1	-3.9	-1.6	-1.3	-2.3
14k	-8.9	-4.3	-2.1	-1.9	-0.6
14l	-8.2	-4.6	-2.1	-0.5	-1.1
14m	-8.3	-4.3	-1.9	-0.6	-1.5
14n	-8.4	-4.2	-1.8	-0.7	-1.7
14o	-8.3	-4.3	-1.8	-1.1	-1.1
14p	-8.7	-3.6	-2.3	-1.1	-1.7
14q	-8.1	-4.1	-2.1	-0.5	-1.4
14r	-8.7	-4.1	-2.3	-0.9	-1.4

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