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3-Substituted-*N*-(4-Hydroxynaphthalen-1-yl)arylsulfonamides as a Novel Class of Selective Mcl-1 Inhibitors: Structure-Based Design, Synthesis, SAR, and Biological Evaluation

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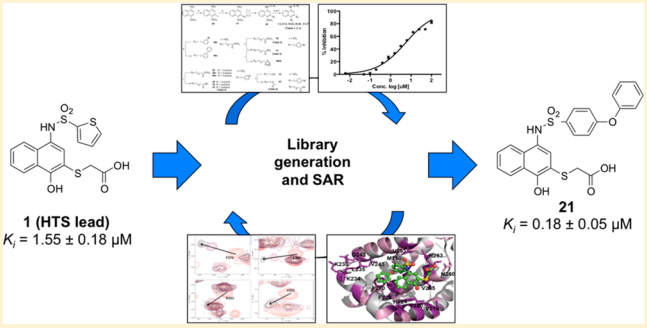
Supporting Information

ABSTRACT: Mcl-1, an antiapoptotic member of the Bcl-2 family of proteins, is a validated and attractive target for cancer therapy. Overexpression of Mcl-1 in many cancers results in disease progression and resistance to current chemotherapeutics. Utilizing high-throughput screening, compound **1** was identified as a selective Mcl-1 inhibitor and its binding to the BH3 binding groove of Mcl-1 was confirmed by several different, but complementary, biochemical and biophysical assays. Guided by structure-based drug design and supported by NMR experiments, comprehensive SAR studies were undertaken and a potent and selective inhibitor, compound **21**, was designed which binds to Mcl-1 with a K_i of 180 nM. Biological characterization of **21** showed that it disrupts the interaction of endogenous Mcl-1 and biotinylated Noxa-BH3 peptide, causes cell death through a Bak/Bax-dependent mechanism, and selectively sensitizes Eμ-myc lymphomas overexpressing Mcl-1, but not Eμ-myc lymphoma cells overexpressing Bcl-2. Treatment of human leukemic cell lines with compound **21** resulted in cell death through activation of caspase-3 and induction of apoptosis.



INTRODUCTION

Evasion of apoptosis or programmed cell death, a key regulator of physiological growth control and regulation of tissue homeostasis, is a hallmark of cancer and a contributor to the emergence of resistance to current therapies.[1−3] The B-cell lymphoma-2 (Bcl-2) family of proteins regulate the intrinsic (mitochondrial) pathway of apoptosis through a network of protein−protein interactions between pro- and antiapoptotic members.[4] Twenty-five known members of the family can be grouped functionally according to their pro- and antiapoptotic effects as well as structurally according to their Bcl-2 homology (BH) regions. The Bcl-2 antiapoptotic proteins, consisting of Bcl-2, Bcl-X_L, Bcl-b, Bcl-w, Mcl-1, and A1, share up to four BH domains which form the hydrophobic BH3-binding groove for binding their cognate partners. The pro-apoptotic proteins are divided into two groups: (a) multidomain proteins including Bax and Bak with BH1−BH4 domains and (b) BH3-only proteins including Bad, Bid, Bim, Noxa, and Puma, which share homology only in the BH3 α-helical domain. The BH3 domain possesses four conserved hydrophobic residues that are involved in the interaction with BH3-binding groove of the pro-survival Bcl-2 family members, which results in the sequestering and blocking of the function of pro-death members.

Overexpression of Bcl-2 survival members is observed in different types of human tumor samples and cancer cell lines, and much effort has been focused on developing therapeutics against this family of proteins for the treatment of cancers.[5−7] Selective and potent small-molecule inhibitors have been successfully developed against Bcl-2,[8] Bcl-X_L,[9] and Bcl-2/Bcl-X_L[10−13] with Navitoclax (ABT-263), currently in phase I/II clinical trials. The efficacy of ABT-263 as a single agent has been demonstrated in tumors with low levels of the pro-survival Mcl-1. Several studies[14−18] have shown that resistance to ABT-263 and its analogue ABT-737 is linked to high expression levels of Mcl-1, and in many instances this resistance can be overcome by treatment with agents that downregulate, destabilize, or inactivate Mcl-1. The biological significance of Mcl-1 protein expression in support of cell survival has been well documented in a number of cell systems, including human myeloblastic leukemia,[19] myeloma,[20−22] B-lymphoma,[23] non-small cell lung,[24] melanoma,[25] pancreatic,[26,27] and prostate.[28] Furthermore, Mcl-1 is overexpressed in many human tumor specimens[26,29−32] and metastatic tissue.[33] This overexpression contributes to chemoresistance and disease relapse.[34−36] It has

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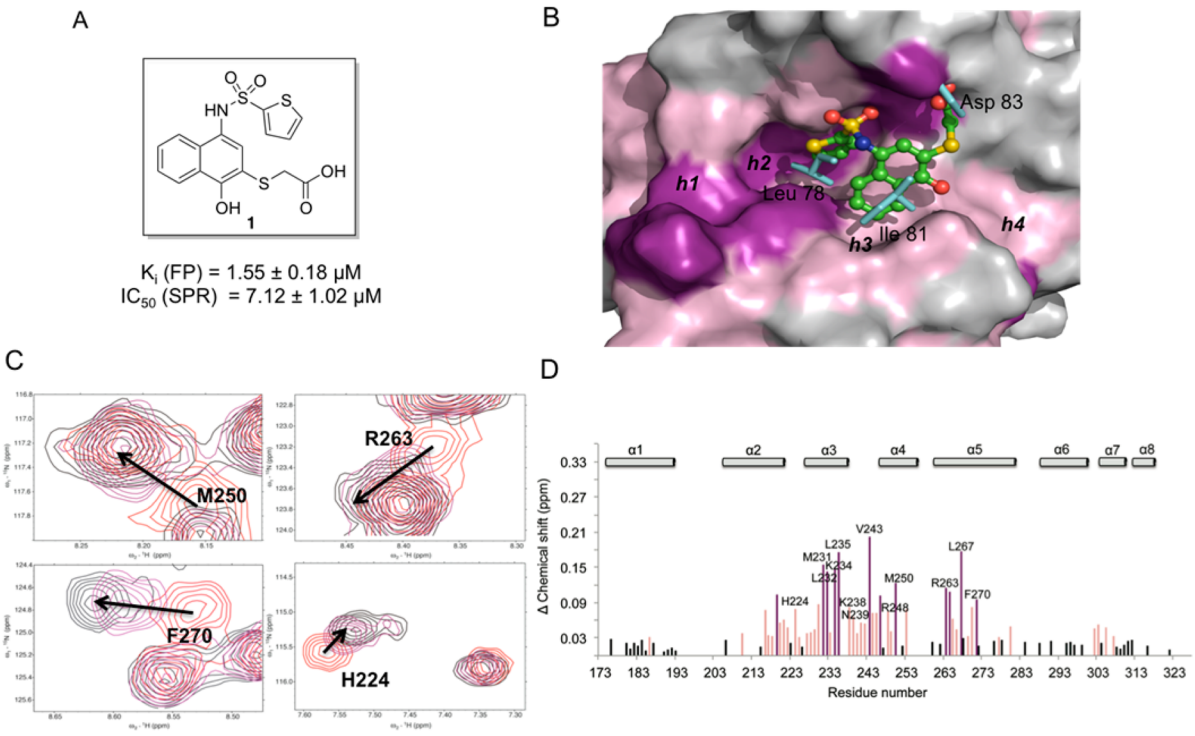

Figure 1. (A) Structure of the HTS lead compound **1**. (B) Putative binding mode of **1** to Mcl-1(PDB ID: 2NLA). Side chains residues of mNoxa peptide are shown in blue sticks. The surface of Mcl-1 protein is colored according to the chemical shift intensity. Significant shift (>0.09 ppm) is represented with purple, moderate shift (≥0.03 and ≤0.09 ppm) represented with pink. (C) Overlaid $^{15}N-^{1}H$ HSQC spectra of Mcl-1 (red) and in the presence of **1** (Mcl-1:**1** ratio of 1:2) (black), (Mcl-1:**1** ratio of 1:1) (purple). Arrows show the direction of chemical shift changes upon binding of **1**. (D) Plot of chemical shift changes of Mcl-1 amide upon addition of **1** (Mcl-1:**1** ratio of 1:2) as a function of Mcl-1 residue numbers.

been shown that Mcl-1 down-regulation is important toward making multiple myeloma cells susceptible to BH3-only proteins and therefore to mitochondrial disruption.[37,38] Such down-regulation can increase the sensitivity to rituximab-mediated killing of chronic and acute lymphoid leukemia (CLL and ALL).[39] Antisense strategies targeting Mcl-1 in vitro and in vivo have provided promising results in sensitizing human melanoma and pancreatic cancer.[40−42] These data suggest that therapies specifically targeting Mcl-1, either as a single agent or in combination, can be effective in the treatment of different human cancers.

Recently, several groups including us have reported on small molecules[43−50] and stapled peptides[51,52] as Mcl-1 inhibitors. Herein we disclose a novel series of small-molecule Mcl-1 inhibitors discovered through high-throughput screening (HTS) followed by the utilization of structure-based design to develop structure−activity relationships (SAR) around lead compound **1** (Figure 1A). Docking studies and two-dimensional $^{1}H-$ ^{15}N heteronuclear single quantum coherence spectroscopy (HSQC) NMR studies were employed to provide information about its binding mode which was used for the design and synthesis of additional analogues. A SAR was developed which resulted in the identification of several selective small-molecule Mcl-1 inhibitors with improved binding. Selected analogues were then tested in a series of complementary biochemical, biophysical, functional, and cellular assays to evaluate their potency, specificity, and mechanism of action.

RESULTS AND DISCUSSION

Lead Discovery. HTS of a 53.3K small-molecule library was performed using a fluorescence polarization (FP) binding assay based on the interaction between recombinant human Mcl-1 and fluorescently labeled Bid BH3 peptide (Flu-Bid). Compound **1** (Figure 1A) was one of the validated hits, which was also previously identified as a proteasome inhibitor.[53] Compound **1** was resynthesized and its binding to Mcl-1 was confirmed with a K_i of 1.55 ± 0.18 μM. Compound **1** exhibits similar potency as MIM1 (IC_{50} = 4.72 μM), Mcl-1 small-molecule inhibitor with a thiazolyl substituted core, which was also discovered with high-throughput competitive FP screen approach.[47] It is known that the binding efficiency index (BEI) is important in identifying leads that exhibit good potency relative to their size.[54] The BEI of compound **1**, calculated as a ratio between pK_i and molecular weight, is 14.7, which encouraged us to further pursue with modifications of this scaffold. To explore the binding mode of **1** with Mcl-1, in silico induced fit docking (IFD)[55] studies were performed using the crystal structure of Mcl-1 in complex with mNOXA BH3 peptide (PDB ID: 2NLA).[56] The predicted binding model of **1** in complex with Mcl-1 revealed that the thiophene and the naphthalene rings of **1** occupy two hydrophobic pockets, h2 and h3, in the Mcl-1 protein, mimicking two conserved hydrophobic residues in the BH3 binding motif represented by Leu 78 and Ile 81, respectively, in mNoxaB (Figure 1B). The importance of the two conserved hydrophobic residues, Leu and Ile, in the BH3 binding motif for the high affinity interaction and selective binding to Mcl-1 has been demonstrated with structural studies of Bims2A,[57] a highly selective peptide derived from Bim BH3-only protein, as well as

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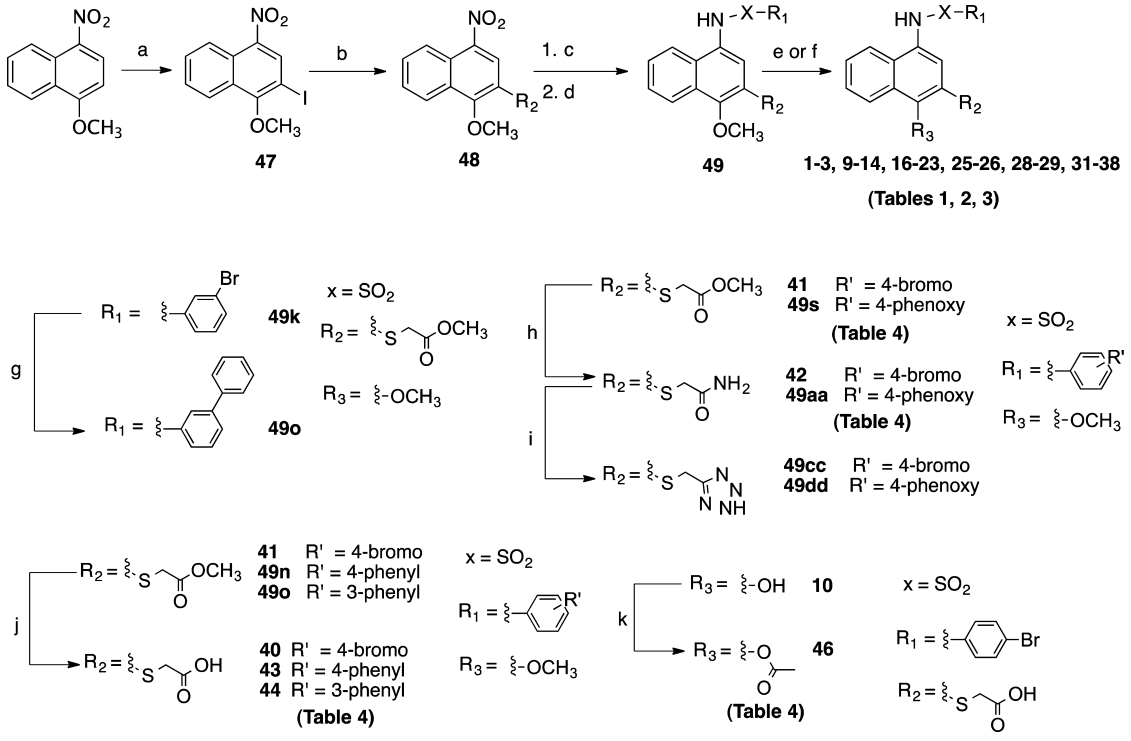
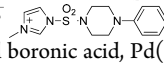
Scheme 1. Synthetic Route for 1 and Analogues[a]



[a]Reagents and conditions: (a) NIS, TFA, reflux, 24 h; (b) $HS(CH_2)_nCOOCH_3$ (n = 1, 2), $Pd(OAc)_2$, Xantphos, Cs_2CO_3, LiI, $ZnCl_2$, THF, 60 °C, overnight, or $HS(CH_2)_3CH_3$, $Pd_2(dba)_3$, Dppf, Et_3N, NMP, 80 °C, 2 h, or $HCC(CH_2)_nOH$ (n = 1, 2), $Pd(PPh_3)_2Cl_2$, CuI, Et_3N/THF, 60 °C, 2 h (4:1), 60 °C, 2 h; (c) Fe, AcOH, 70 °C, 1 h, or Pd/C, H_2 30 psi, EtOH/EtOAc (6:1), rt, overnight; (d) RSO_2Cl, pyridine, CH_2Cl_2, rt, overnight, or RCOCl, Et_3N, CH_2Cl_2, rt, overnight, or $^{TfO^-}$  , CH_3CN, 80 °C, 15 h; (e) BBr_3, CH_2Cl_2, 0 °C to rt, 1 h, or BBr_3, CH_2Cl_2, 0 °C to rt, 1 h, quench with MeOH at 0 °C; (g) phenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, THF/H_2O, 60 °C, 2 h; (h) NH_4OH, rt, 1 h; (i) NaN_3, $SiCl_4$, CH_3CN, 80 °C, 15 h; (j) LiOH, THF, rt, 1 h; (k) H_3CCOCl, Et_3N, 0 °C, rt, 30 min.

from recently reported selective small-molecule inhibitor in complex with Mcl-1.[45] The carboxylic acid group of **1** forms a network of hydrogen bonds with Arg 263 and Asn 260 of Mcl-1, mimicking the conserved Asp in BH3 peptides (Asp 83 in mNoxa). The predicted binding model also suggests that the phenolic group forms a hydrogen bond with His 224, which is one of the residues composing the h3 pocket of Mcl-1. A recent report[58] on the conformational flexibility of Mcl-1 and its binding hotspots identified His 224 as an acidic hotspot in the h3 site of Mcl-1, supporting the predicted hydrogen bonding in this region of Mcl-1.

To validate the computationally predicted binding site and confirm the binding of **1** to the BH3 groove of Mcl-1 protein, HSQC NMR spectroscopy studies were performed. For this purpose, the assignment of the backbone amides of apo human Mcl-1 was based on the work by Liu et al.,[59] and a series of ^1H,^{15}N-HSQC studies were carried out. The HSQC spectra were of good quality with well-dispersed peaks, and concentration-dependent perturbations of residues were observed (Figure 1C), indicating that **1** binds Mcl-1 specifically and causes dose-dependent perturbations of backbone amides. The chemical shift changes in the presence of a 2-fold excess of **1** were mapped and plotted against Mcl-1 residues (Figure 1D). Compound **1** caused moderate to significant chemical shift perturbations for residues of Met 231, Met 250, Leu 267, and Phe 270, which constitute the h2 and h3 pockets and which are predicted to be occupied by the thiophene and naphthalene rings of **1**, respectively. Moderate chemical shift perturbations of Arg 263 and His 224 were also observed, which are predicted to form hydrogen bonds with the thioacidic acid and phenolic moieties of **1**, respectively. Additionally, the residues in the vicinity of the predicted binding pose (Leu 232, Val 243, Arg 248) and the ones located on an unstructured loop connecting α-helix 3 to α-helix 4 (Lys 234, Leu 235, Lys 238, Asn 239) were also perturbed. Overall analysis of the chemical shift changes of the compound **1** in complex with Mcl-1 showed that **1** affects the residues forming the BH3-binding groove and provided conclusive evidence that **1** binds Mcl-1 protein at the same site that the conserved BH3 peptides interact with Mcl-1 protein. Therefore, on the basis of our modeling and NMR results, the substituted-*N*-(4-hydroxynaphthalen-1-yl)-arylsulfonamide represents a promising class for further optimization. A structure-based design approach was undertaken, and a focused library of analogues of **1** was designed and synthesized to improve the potency of this series.

Synthesis. Except for analogues **4**−**8**, **15**, and **39**, which were commercially available, all analogues were synthesized through a novel, modular route (Scheme 1), which differs from that recently reported by Ge et al.[53] Our route allows for facile access to a variety of analogues with variations at R_1, R_2, and R_3 and the linker region (X). It starts with an electrophilic aromatic substitution of 1-methoxy-4-nitronaphthalene with *N*-iodosuccinamide to provide aryl iodide (**47**).[60] This was subjected to Pd-catalyzed C−S or C−C cross-coupling using conditions previously reported,[61,62] or developed in our lab based on recent literature,[63−65] to provide several desired intermediates (**48**). The nitro function was reduced with iron[66] or via catalytic hydrogenation[67] to provide the corresponding

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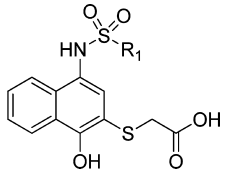
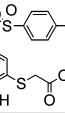
amines. Reaction of the amines with appropriate sulfonyl or acyl chlorides or 3-methyl-1-((4-phenylpiperazin-1-yl)-sulfonyl)-1*H*-imidazol-3-ium[68] provided the penultimate compounds (**49**), which were demethylated with BBr_3 followed by purification by trituration or reverse-phase HPLC to afford the target compounds (**1**−**3**, **9**−**14**, **16**−**23**, **25**−**26**, **28**−**29**, **31**−**38**) of >95% purity (Tables 1−3). In the case of analogues with an ester side chain, the BBr_3 step provided a convenient way to concomitantly hydrolyze the ester in a single pot. To preserve the methyl ester of analogue **32** (Table 3), analogue **41** (Table 4) was subjected to BBr_3 conditions followed by a quench with MeOH. For the synthesis of **49o**, intermediate **49k** underwent Suzuki−Miyaura coupling[69,70] with phenyl boronic acid to provide the desired compound which was subjected to BBr_3 to provide **17** (Table 1). Aminolysis of **41** and **49s** with NH_4OH[71] provided carboxamides **42** (Table 4) and **49aa**, respectively. **42** was then subjected to BBr_3 to give **33** (Table 3). The terminal amide of **42** and **49aa** were converted by a known procedure[72] to tetrazoles **49cc** and **49dd**, which after BBr_3 demethylation provided **36** and **37** (Table 3), respectively. The thioacidic acid analogues with a methoxy at R_3 (**40**, **43**−**44**) (Table 4) were obtained via LiOH hydrolysis of esters **41**, **49n**, and **49o**, respectively. Acetylation of **10** (Table 1) with acetyl chloride[73] provided **46** (Table 4). Analogue **24** (Table 1) with a phenyl scaffold in place of naphthalene was synthesized using conditions similar to those described for **10** starting from 2-iodoanisole (Supporting Information Scheme S1). The syntheses of compounds **27** (Table 2) and **30** (Table 3) were completed starting from 4-methoxy-1-naphthaldehyde and 1-nitronaphthalene, respectively (Supporting Information Schemes S2−S3).

Structure−Activity Relationships. Structure-based design of analogues based on **1** yielded a focused library of compounds leading to clear SAR for this series. The binding affinities of our Mcl-1 inhibitors were determined by using competitive fluorescence polarization (FP) and surface plasmon resonance (SPR) binding assays, which test the ability of inhibitors to disrupt interaction between Mcl-1 and two different BH3 peptides, fluorescently labeled Bid and biotin-labeled Bim, respectively. Concurrently, HSQC NMR experiments were performed to provide structural insights of protein-bound ligand and experimental validation for the modeling studies.

The predicted binding model showed that the thiophene ring at R_1 of **1** projects into the h2 pocket (Figure 1B), which is the biggest and deepest pocket among the four hydrophobic pockets of Mcl-1.[56] To investigate the importance of hydrophobic interaction at this site and increase the binding affinity of **1**, a series of analogues with variation at R_1 was synthesized and evaluated (Table 1). When R_1 is changed to a methyl group in **2**, the binding affinity is significantly reduced, confirmed by SPR (IC_{50} > 100 μM) and NMR experiments which showed lack of chemical shift perturbation of backbone residues in the Mcl-1 BH3 binding site after adding **2** (Supporting Information Figure S1). As was expected, isosteric replacement of the thiophene in **1** to a phenyl in **3** maintained binding affinity with K_i of 3.56 ± 0.45 μM. To probe the hydrophobic interactions in the h2 pocket, analogues with alkyl and halogen substituents of various sizes at the *para*-position of the phenyl ring were prepared. From this set of compounds, a trend emerged showing improved binding with increasing size and hydrophobicity of substituents. *tert*-Butyl phenyl (**7**) exhibited 4.5-fold enhancement in FP assay (K_i = 0.81 ± 0.04 μM) and 1.5-fold in SPR assay (IC_{50} = 8.73 ± 0.95 μM), and bromo phenyl

Table 1. Binding Affinities of Sulfonamide Analogues with Variations at R_1



Cpd	R_1	FP K_i ± SD (μM)	SPR IC_{50} ± SD (μM)
1		1.55 ± 0.18	7.12 ± 1.02
2		74.92 ± 6.33	>100
3		3.56 ± 0.45	12.58 ± 3.60
4		3.13 ± 0.22	11.57 ± 7.86
5		1.55 ± 0.37	8.68 ± 1.63
6		1.06 ± 0.17	4.00 ± 1.06
7		0.81 ± 0.04	8.73 ± 0.95
8		4.23 ± 0.93	5.01 ± 1.12
9		0.82 ± 0.10	2.70 ± 0.60
10		0.49 ± 0.06	2.40 ± 0.17
11		9.31 ± 3.10	7.17 ± 2.81
12		2.35 ± 0.19	9.68 ± 0.88
13		4.42 ± 0.86	6.18 ± 1.24
14		1.54 ± 0.32	7.5 ± 2.98
15		0.73 ± 0.05	2.76 ± 1.72
16		0.37 ± 0.10	2.03 ± 1.10
17		0.38 ± 0.03	2.31 ± 0.76
18		2.03 ± 0.41	9.90 ± 3.50
19		0.17 ± 0.04	0.88 ± 0.15
20		0.24 ± 0.06	0.92 ± 0.20
21		0.18 ± 0.05	1.45 ± 0.29
22		0.29 ± 0.04	0.90 ± 0.42
23		2.22 ± 0.36	16.20 ± 5.80
24		283.36 ± 81.50	>100

(**10**) 7-fold in FP assay (K_i = 0.49 ± 0.06 μM) and 5-fold in SPR assay (IC_{50} = 2.40 ± 0.17 μM) improved binding over **3**. Introduction of a more polar methoxy group in **11** was accommodated but resulted in a 3-fold decreased binding (K_i = 9.31 ± 3.1 μM) compared to **3**. Bromine substitution at the *meta*-(**12**) and *ortho*-positions (**13**) of the phenyl ring was also explored, and the obtained binding results from both assays, FP

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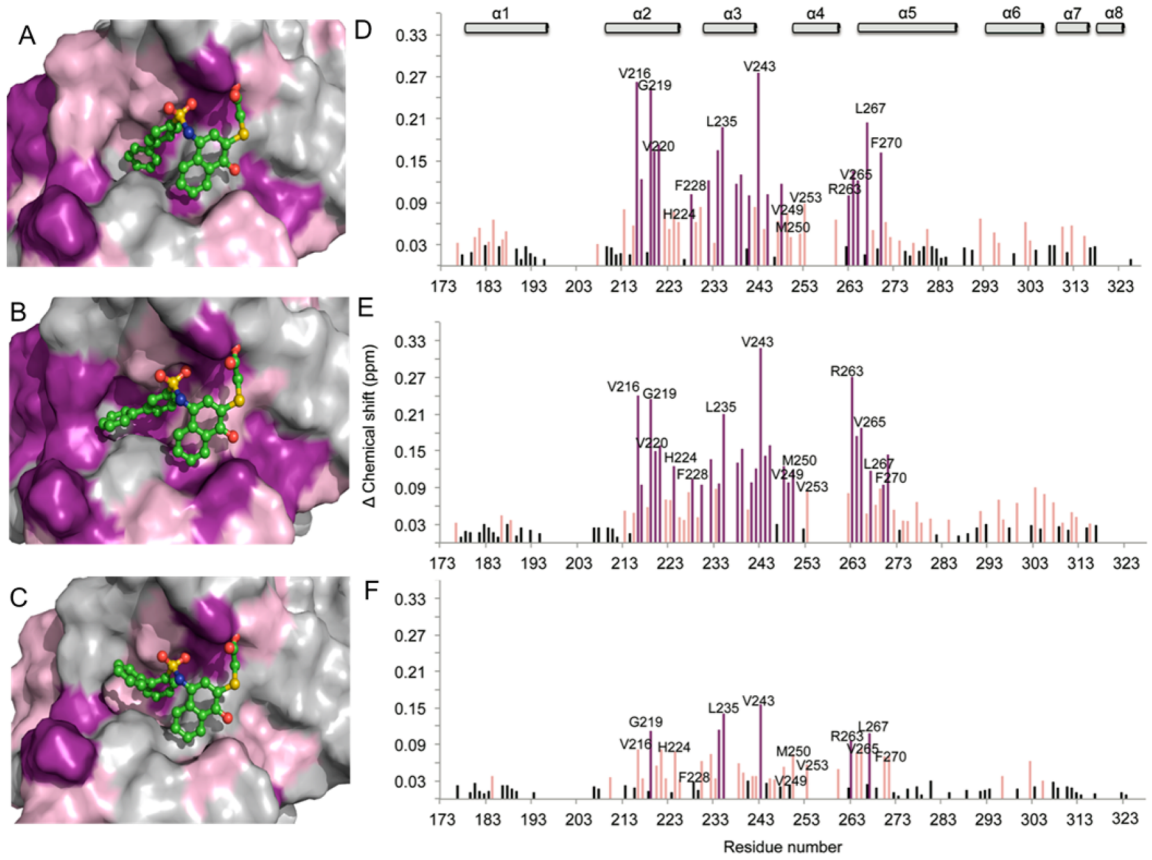

Figure 2. Putative binding modes of (A) **16**, (B) **17**, (C) **18** to Mcl-1 (PDB ID: 2NLA). Surface of Mcl-1 protein is colored according to the chemical shift intensity. Significant shift (>0.09 ppm) is represented with purple, moderate shift (≥0.03 and ≤0.09 ppm) represented with pink. Plots of chemical shift changes of Mcl-1 amide upon addition of (D) **16** (Mcl-1:**16** ratio of 1:2), (E) **17** (Mcl-1:**17** ratio of 1:2), (F), and **18** (Mcl-1:**18** ratio of 1:2) as a function of Mcl-1 residue numbers.

(K_i of 2.35 ± 0.19 μM and 4.42 ± 0.86 μM, respectively) and SPR (IC_{50} = 9.68 ± 0.88 μM and IC_{50} = 6.18 ± 1.24 μM, respectively), indicated that *para*-bromo substitution in **10** is the best, exhibiting the highest binding affinity to Mcl-1 among these three isomers. Because of peak overlap and possibility of ambiguous assignment of the peaks in the presence of a ligand and to improve the quality and accuracy of ligand-induced Mcl-1 chemical shift perturbation, resonance assignments were also determined for **10** in complex with Mcl-1 using ^{13}C,^{15}N double-labeled Mcl-1 protein in the presence of a 2-fold excess of **10**. The HSQC spectrum of **10** showed a similar pattern of chemical shift perturbation as with **1**, which we attribute to the structural similarity of the two analogues. Consistent with the 7-fold improved binding of **10** in comparison with **1**, the chemical shift perturbations were larger for **10** (Supporting Information Figure S2A). In particular, significant perturbation of residues Ser 247, Arg 248, and Val 253 on α-helix 4, which forms the upper rim of the h2 pocket, and residues Val 216, Val 220, and Gln 221 located toward the C-terminus of α-helix 2, which are at the border between the h3 and h4 pockets, were observed for **10**, suggesting that the Mcl-1 conformational flexibility accommodates larger R_1 substituent into the h2 pocket. Chemical shift perturbation plots derived from ^{1}H,^{15}N-HSQC experiments of the other two bromophenyl isomers showed a similar perturbation pattern as **10** with chemical shift changes that correlate well with the binding data in which the strongest perturbation is observed for **10**, followed by **12** and **13** (Supporting Information Figure S2A–C).

To further extend into the h2 pocket and gain additional interaction, analogues with biphenyl substituents were synthesized because biphenyl is considered a privileged moiety in targeting protein–protein interactions.[74] Analogues **16** and **17**, with *para*- and *meta*-biphenyl substituents, respectively, showed a similar 10-fold improvement in FP-based binding assay compared to **3** with K_i values of 0.37 ± 0.10 and 0.38 ± 0.03 μM, respectively, further confirmed with SPR assay showing 6-fold improvement. On the other hand, the *ortho*-biphenyl analogue **18** showed almost the same binding affinity as **3** with K_i of 2.03 ± 0.41 μM and IC_{50} = 9.90 ± 3.50 μM, in FP and SPR assays, respectively. The predicted binding models of these compounds showed that the distal phenyl ring of **16** and **17** inserts deeper into the h2 pocket (parts A and B of Figure 2, respectively). For **18**, this phenyl ring is partially solvent exposed (Figure 2C), which might explain its lower affinity compared to **16** and **17**. In agreement with their binding affinities, chemical shift perturbation plots of **16** and **17** show stronger perturbation of residues involved in the binding site compared to **18** (Figure 2D–F).

Analysis of the HSQC spectra of analogues **16**–**18** resulted in an important finding that strongly supports their predicted binding mode. One long-standing question for us was the possibility of a flipped binding mode for this class of analogues where R_1 would occupy the h4 pocket instead of h2. The possibility of a flipped conformation has been previously documented for a different class of dual Mcl-1 and Bcl-xL inhibitors.[49] The differential chemical shift mapping meth-

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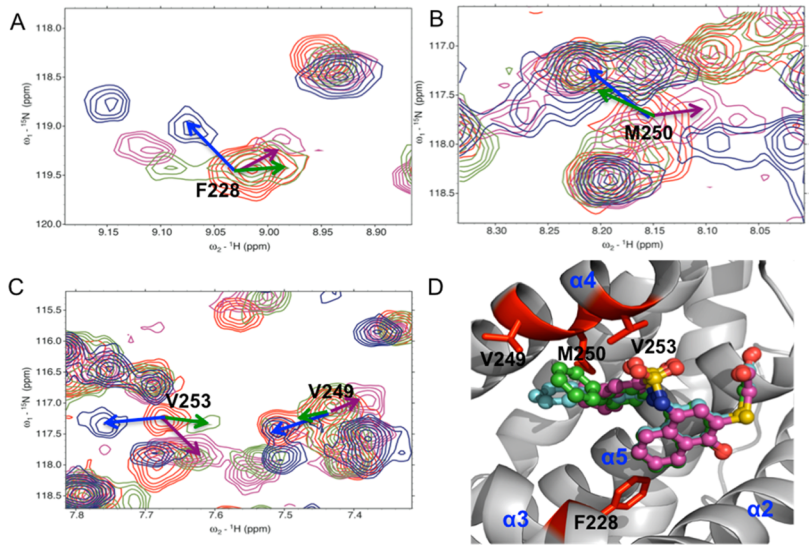

Figure 3. Overlaid $^{15}N-^{1}H$ HSQC spectra of Mcl-1 (red) and in the presence of **16** (Mcl-1:**16** ratio of 1:2) (purple), **17** (Mcl-1:**17** ratio of 1:2) (blue), **18** (Mcl-1:**18** ratio of 1:2) (green) for (A) Phe 228, (B) Met 250, and (C) Val 249 and Val 253. Arrows show the direction of chemical shift changes upon binding of compounds. (D) Overlay of putative binding modes of **16** (purple), **17** (blue), and **18** (green) to Mcl-1 (PDB ID: 2NLA) highlighting in red Val 249, Met 250, and Val 253 on helix 4, Phe 228 on helix 3 of Mcl-1.

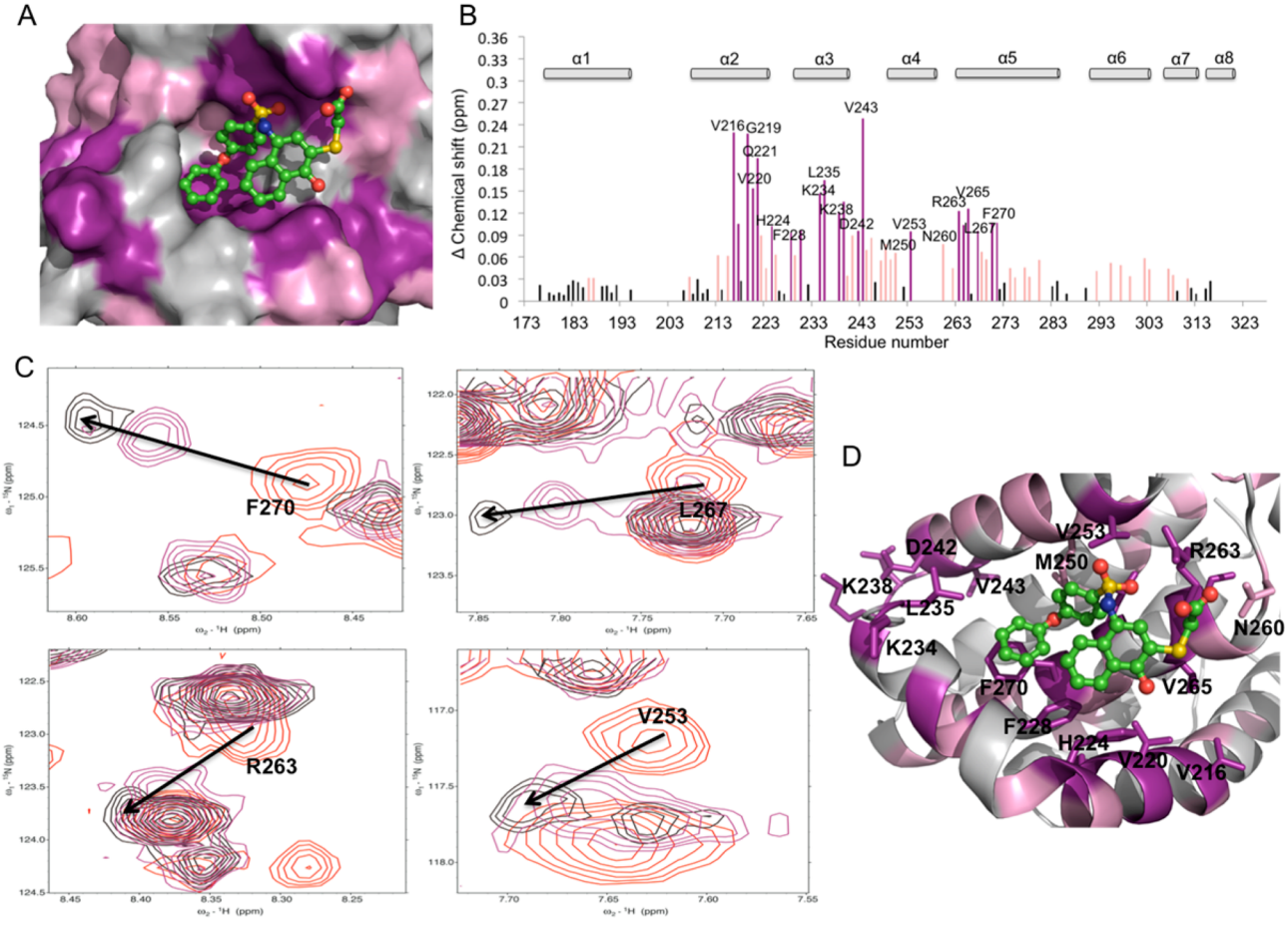

Figure 4. Putative binding mode of **21**. (A) Surface of the Mcl-1 protein (PDB ID: 2NLA) is colored according to the chemical shift intensity. Significant shift (>0.09 ppm) is represented with purple, moderate shift (≥0.03 and ≤0.09 ppm) represented with pink. (B) Plot of chemical shift changes of Mcl-1 amide upon addition of **21** (Mcl-1:**21** ratio of 1:2) as a function of Mcl-1 residue numbers. (C) Overlaid $^{15}N-^{1}H$ HSQC spectra of Mcl-1 (red) and in the presence of **21** (Mcl-1:**21** ratio of 1:2) (black), (Mcl-1:**21** ratio of 1:1) (purple). Arrows show the direction of chemical shift changes upon binding of **21**. (D) Mcl-1 residues shown to be perturbed in HSQC NMR in the presence of **21** (Mcl-1:**21** ratio of 1:2) (green).

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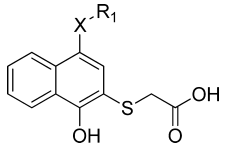
od[75,76] was applied, using the three biphenyl analogues (**16**, **17**, and **18**) differing only in the orientation of distal phenyl ring. Comparison of the spectra of the Mcl-1 bound to these three analogues shows distinct changes in chemical shifts exclusively for the resonances of the residues that are in direct contact with the modified structure of the inhibitors, facilitating binding site mapping. Careful analysis of the HSQC spectra of these analogues reveals a different direction of the chemical shift perturbations of the residues forming the h2 pocket (Met 250) or in its vicinity (Phe 228) as well as residues located on α-helix 4 (Val 249, Val 253) (Figures 3A–C). On the other hand, residues which are not part of the h2 pocket, such as Leu267, Val265, His224, and Arg263, show very similar chemical shift perturbations in the presence of the biphenyl analogues (Supporting Information Figure S6). This finding provides conclusive evidence for biphenyl occupation of the h2 pocket and further supports that α-helix 4 of Mcl-1 is flexible.[58]

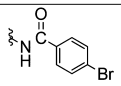
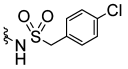
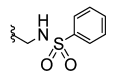
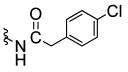
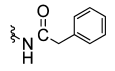
Because of encouraging binding data with **16**, analogues **19** and **20** with halogenated biphenyl rings at R_1 were synthesized to further increase potency. Satisfyingly, **19** with *para*-chlorobiphenyl showed a 21-fold improved binding compared to **3**, becoming the most potent analogue in our series, with a K_i = 0.17 ± 0.04 μM determined by the FP binding assay using fluorescent labeled Bid BH3 peptide and IC_{50} of 0.88 ± 0.15 μM in displacement of biotin labeled Bim BH3 peptide in the SPR-based assay (14-fold improvement). The chemical shift perturbation plot of **19** (Supporting Information Figure S3) showed a similar pattern of perturbations to **16**, suggesting a similar binding conformation but with a lower perturbation magnitude, likely due to the lower solubility of **19** in our NMR studies. To improve the physicochemical properties of the biphenyl analogues, we synthesized and evaluated analogues **21** with *para*-phenoxyphenyl at R_1 and its fluorine substituted congener, **22**. Analogue **21** showed similar binding affinity as **19** (K_i = 0.18 ± 0.05 μM in FP, and IC_{50} = 1.45 ± 0.29 μM in SPR) but stronger chemical shift perturbation (Figure 4A–C), probably due to its improved solubility. Computational docking of **21** predicted a π–π stacking of the distal phenyl of the *para*-phenoxyphenyl moiety with the phenyl of Phe270 of Mc-1 (Figure 4D), which could account for its improved affinity. Compounds **19** and **21** became the most potent analogues of our series and exhibited BIEs of 13.5 and 14.0, respectively, maintaining **1**'s BIE. In comparison with compound **53** (K_i of 0.055 μM), recently reported Mcl-1 selective inhbitor with an indole core structure, discovered and optimized by fragment-based screening strategy,[45] **19** and **21** have binding affinity in a similar nanomolar range, being 3-fold less potent than **53**. To further increase the aqueous solubility of this class of analogues, a *para*-phenylpiperazine group at R_1 was introduced in **23** which led to a 6-fold decrease in binding based on FP assay (K_i = 2.22 ± 0.36 μM) and 8-fold decrease based on SPR assay (IC_{50} = 16.20 ± 5.80 μM) in comparison with the biphenyl substituent in **16**. This can be attributed to a relatively polar substitutent projecting into the hydrophobic h2 pocket and to a less optimal conformational preference of phenylpiperazine versus biphenyl for the h2 pocket.

To briefly explore the importance of the naphthalene core to the binding potency, which occupies the h3 hydrophobic pocket, analogue **24** with a phenyl core was synthesized. Binding studies showed a significant drop in potency (K_i = 283.36 ± 81.50 μM in FP and IC_{50} > 100 μM in SPR) compared to the corresponding compound **10** with the naphthalene core, supporting the importance of hydrophobic interactions at h3 to the overall Mcl-1 binding.

The next focus of our SAR studies was the sulfonamide linker for which docking results suggested lack of any specific interactions with Mcl-1. To investigate the importance of the sulfonamide linker, several analogues were synthesized (Table 2). Replacement of the sulfonamide with a carboxamide in **25** led to significant 83-fold decrease of the binding affinity with K_i value of 40.8 ± 8.50 μM compared to **10**, possibly due to the unfavorable orientation of R_1 by the carboxamide linker. The decreased binding potency was confirmed by SPR and NMR (Supporting Information Figure S4) binding studies. To explore the impact of the flexibility of the linker, a methylene linker distal (**26**) or proximal (**27**) to the naphthalene core was inserted and both analogues showed decreased binding affinity with K_i = 5.23 ± 0.36 μM and K_i = 64.75 ± 12.61 μM, relative to **9** (6-fold) and **3** (18-fold), respectively. As expected, change of the sulfonamide linker in **26** to a carboxamide in **28** decreased the potency, but only by 2-fold (K_i = 12.53 ± 1.24 μM), suggesting that the insertion of the methylene linker between the carboxamide and the pendant aryl ring provids a degree of freedom to better orient R_1 into h2 pocket of Mcl-1.

Table 2. Binding Affinities of Analogues with Linker (X) Variations



Cpd	X-R_1	FP K_i ± SD (μM)	SPR IC_{50} ± SD (μM)
25		40.81± 8.52	45.08 ± 3.86
26		5.23 ± 0.36	9.59 ± 5.06
27		64.75 ± 12.61	30.20 ± 3.99
28		12.53 ± 1.24	18.27±6.65
29		59.35± 15.47	63.77 ± 10.76

Modeling studies showed that the thioacetic acid moiety at R_2 mimics the conserved Asp of BH3-only peptides and that the carboxylate is involved in electrostatic interaction with Asn 260 and Arg 263 of Mcl-1. Therefore, to further explore this site, analogues with different substituents at R_2 were synthesized (Table 3). Removal of the acid side chain (**30**) or its replacement with thiobutyl (**31**) did not show binding up to 100 μM in both FP and SPR assays. Introducing a methyl ester (**32**) or a primary carboxamide (**33**) resulted in decreased binding by 10-and 3-fold in FP assay respectively compared to **10** (6- and 2-fold decrease in SPR assay respectively), consistent with each of these functional groups, forming a weaker interaction with Arg 263 relative to the carboxylic acid moiety. When the carboxylic acid was changed to an alcohol

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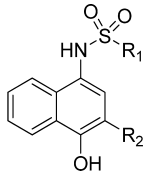
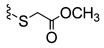
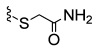
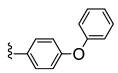
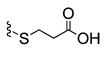
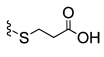
Table 3. Binding Affinities of Sulfonamide Analogues with Variations at R_1 and R_2[a]



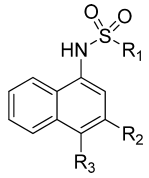
Cpd	R_1	R_2	FP $K_i \pm$ SD (μM)	SPR $IC_{50} \pm$ SD (μM)
30		-H	>27*	>100
31			>27*	>100
32			5.08 ± 1.35	14.88 ± 3.26
33			1.71 ± 0.35	3.87 ± 0.55
34			3.79 ± 0.62	10.95 ± 0.53
35			2.18 ± 0.91	19.68 ± 4.69
36			1.01 ± 0.22	1.50 ± 0.30
37			0.21 ± 0.03	ND[#]
38			1.13 ± 0.34	5.03 ± 1.88
39			>27*	64.9 ± 17.2

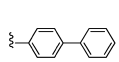
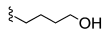
[a][#]ND: The IC_{50} was not determined because the compound showed nonspecific binding to the control surface; *Compounds were tested up to 100 μM.

and the sulfur to methylene (**34**) or ethylene (**35**), both analogues showed decreased binding compared to the parent congener **10** with K_i values of 3.79 ± 0.62 and 2.18 ± 0.91 μM, respectively. As was expected, bioisosteric replacement of the carboxylic acid group with a tetrazole in analogues **36** and **37** maintained the binding affinity in comparison with their parent congeners, **10** and **21**. Homologation of the thioacetic acid in **38** had no detrimental effect on binding and showed a similar K_i of 1.13 ± 0.34 μM in FP assay and IC_{50} = 5.03 ± 1.88 μM in SPR assay as **15**, which can be explained with the flexibility of both the thiopropanoic acid moiety and Arg 263 side chain. However, changing the point of fusion of the naphthalene ring in **38** substantially affected the binding and **39** with 1-naphthyl substituent showed a significant reduction in binding to Mcl-1. Our docking studies suggest that this might be attributed to a clash with the residues in the h2 pocket of Mcl-1.

Modeling showed that the phenolic group of the core naphthalene scaffold forms a hydrogen bond with His 224 of Mcl-1, consistent with the reported acidic hotspot in the h3 site of Mcl-1 close to His 224.[58] Several analogues were synthesized to probe the contribution of the phenolic group to binding to Mcl-1 (Table 4). When the hydroxyl group is changed to a methoxy in analogue **40**, the potency decreased by 170 fold (K_i = 86.98 ± 15.35 μM) compared to the phenolic congener **10**. The analogue **41**, where R_2 is methyl thioacetate and R_3 is methoxy, did not show binding up to 100 μM, and the loss of the binding was confirmed with SPR and HSQC experiment

Table 4. Binding Affinities of Sulfonamide Analogues with Variations at R_1, R_2, and R_3[a]



Cpd	R1	R2	R3	FP $K_i \pm$ SD (μM)	SPR $IC_{50} \pm$ SD (μM)
40			-OCH_3	86.98 ± 15.35	>100
41			-OCH_3	>27*	>100
42			-OCH_3	>27*	>100
43			-OCH_3	20.00 ± 0.79	>100
44			-OCH_3	24.2± 1.9	58.43 ±9.49
45			-OCH_3	>27*	>100
46				33.97 ± 15.96	26.84 ± 7.06

[a]*Compounds were tested up to 100 μM.

(Supporting Information Figure S5). Similar significant loss of binding is also apparent in compounds **42** to **45** compared to their corresponding phenolic analogues, clearly indicating the importance of the phenolic group to the overall binding to Mcl-1. Compound **46**, where the hydroxyl group was acetylated exhibited a K_i of 33.97 ± 15.96 μM, which is a 69-fold decrease in binding compared to **10**. However, **46** showed improved binding relative to **40**, which might be attributed to the formation of a hydrogen bond between the carbonyl of the acetyl group and the protonated His 224, supported by computational prediction.

We determined the selectivity of this class of compounds against four other Bcl-2 antiapoptotic proteins (Bcl-2, Bcl-xL, Bcl-w, Bfl-1/A1). The most potent analogues were tested in competitive FP-based assays that were optimized for each protein, and K_i values were calculated using equations developed previously[77] (Table 5). In general, all the analogues inhibit Mcl-1 most potently with the following order of selectively: Bfl-1/A1 > Bcl-w > Bcl-2 > Bcl-xL. As the BH3 domain binding profile of Bfl-1/A1, as well as its BH3 binding groove, is most similar to that of Mcl-1,[78] it is not surprising that the tested compounds showed less selective inhibition of A1. The most potent analogues in this series, **19** and **21**, show a profile for selectively inhibiting Mcl-1 with 7-and 19-fold versus Bfl-1/A1, 8-and 9-fold versus Bcl-w, 36- and 42-fold versus Bcl-2, and 56- and 59-fold versus Bcl-x_L, respectively. Other reported selective Mcl-1 inhibitors, **MIM1** and **53**, also show high selectivity against Bcl-xL (IC_{50} > 50 μM and K_i > 15 μM, respectively), while **19** and **21** show better selectivity against Bcl-2 in comparison with **53** which has 16-fold selectivity with K_i value of 0.87 μM.

Biological Characterization of Mcl-1 Inhibitors. To verify the specific binding of novel inhibitors to Mcl-1, we employed a pull-down assay using biotin-labeled Noxa (BL-Noxa) and whole cell lysate from the human breast cancer cell

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Table 5. Selectivity of Selected Analogues against Bcl-2 Antiapoptotic Proteins

compound	Mcl-1 K_i ± SD (μM)	A1/Bfl-1 K_i ± SD (μM)	Bcl-2 K_i ± SD (μM)	Bcl-w K_i ± SD (μM)	Bcl-X_L K_i ± SD (μM)
1	1.55 ± 0.18	6.14 ± 1.0	54.65 ± 9.56	37.53 ± 7.96	99.00 ± 22.63
10	0.49 ± 0.06	5.33 ± 1.01	23.83 ± 1.81	8.19 ± 1.91	32.99 ± 4.33
16	0.37 ± 0.10	2.34 ± 0.37	8.82 ± 0.65	1.92 ± 0.37	8.05 ± 0.50
17	0.38 ± 0.03	3.18 ± 0.41	7.85 ± 0.65	2.19 ± 0.45	15.14 ± 1.11
18	2.03 ± 0.41	17.26 ± 0.56	23.84 ± 3.16	4.41 ± 0.39	48.15 ± 3.28
19	0.17 ± 0.04	1.11 ± 0.19	6.11 ± 0.65	1.36 ± 0.51	9.59 ± 1.28
21	0.18 ± 0.05	3.36 ± 0.56	7.56 ± 1.08	1.58 ± 0.35	10.58 ± 1.53

line 2LMP. As shown in Figure 5, Mcl-1 was pulled down by BL-Noxa and, as was expected, the Bim BH3 peptide disrupted

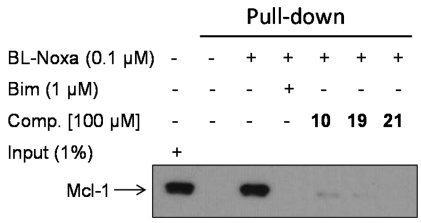

Figure 5. Interaction of Mcl-1 inhibitors with endogenous Mcl-1 protein and Noxa. Biotin-labeled Noxa (BL-Noxa, 0.1 μM) was incubated with whole cell lysates of 2LMP cells with or without tested Mcl-1 inhibitors and Bim BH3 peptide as a positive control, followed by incubation with precleared streptavidin agarose beads. Eluted beads were subjected to Western blot analysis with anti-Mcl-1 antibody.

the interaction between BL-Noxa and Mcl-1. Preincubation with several Mcl-1 inhibitors, **10**, **19**, and **21**, completely blocked the binding of BL-Noxa to Mcl-1, similar to Bim peptide, demonstrating that these inhibitors can recognize and specifically bind to the BH3 binding groove of endogenous Mcl-1 protein.

It is well established that Bax and Bak are required for the initiation of intrinsic, mitochondrial, apoptotic cell death, and they are maintained in an inactive state through interaction with the antiapoptotic proteins.[14,79] Therefore, cells subjected to inhibitors of antiapoptotic proteins are expected to undergo cell death in a Bax/Bak-dependent manner.[80] To determine the contribution of Bak and Bax in the cell death induced by our Mcl-1 inhibitors, we employed murine embryonic fibroblasts (MEFs) wild-type (wt) and deficient in both Bax and Bak (double knock out, DKO). Exposure of the wt MEFs to **21** resulted in a concentration-dependent cell death (assessed by PI staining), while Bax/Bak deletion significantly rescued cells from **21** induced cell death (Figure 6). Although **21** initiates cell death also in DKO MEFs (23% positive PI at 16 μM), it is clear that **21** is more potent in wt MEFs (61% positive PI at 16 μM), indicating that Bax and/or Bak are involved in cell death induction. Taken together, our data suggest that **21** induces cell death in a Bax/Bak-dependent manner due to its Mcl-1 inhibitory function.

To further confirm the specificity of our novel Mcl-1 inhibitors and to determine whether different prosurvival Bcl-2 proteins could suppress the apoptotic activities of novel Mcl-1 inhibitors, we used reported cell lines developed by retroviral transduction of lymphoma cells isolated from Eμ-myc transgenic mice which differ only in their expression of prosurvival Bcl-2 family proteins.[81] Lymphoma cells overexpressing Mcl-1 and Bcl-2 were treated with varying concentrations of tested compounds for 15−18 h, and then cell viability was determined by flow cytometry using a fluorescent reactive dye (LIVE/

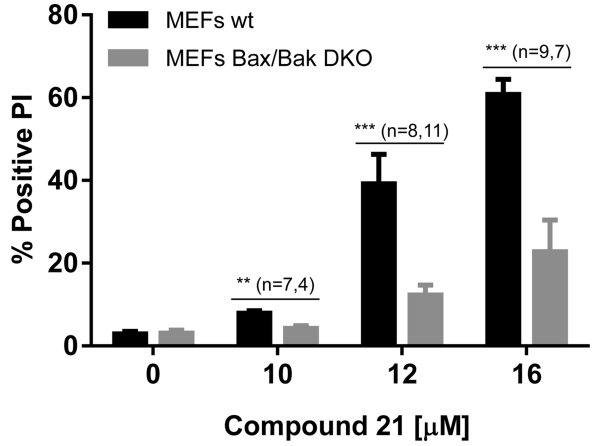

Figure 6. Cell death induced by Mcl-1 inhibitor **21** is Bax/Bak-dependent. MEFs deficient in Bax and Bak (gray bars) along with their wild-type counterpart (black bars) were exposed for 15 h to different concentrations of **21**, and the cell viability was assessed with PI staining. Error bars represent the mean ± SEM. The significance was calculated using unpaired t test, and the number of data is shown for each tested concentration with corresponding significance: (**) $p < 0.01$ and (***) $p < 0.001$.

DEAD fixable violet stain kit). ABT-263 (navitoclax), a selective inhibitor of Bcl-2, Bcl-xL, and Bcl-w, was used as a positive control. As predicted, lymphoma cells overexpressing Mcl-1 were significantly sensitive to **19** and **21** as assessed by an increased percentage of cell death in a concentration-dependent manner. In contrast, **19** and **21** were ineffective against Eμ-myc/Bcl-2 lymphomas (Figure 7). Importantly, **41** did not show any activity against both cell lines overexpressing Mcl-1 or Bcl-2, consistent with our binding studies which showed that **41** does not bind to Mcl-1. As expected, lymphoma cells overexpressing Bcl-2 were sensitive to cell death induced by ABT-263, while cells overexpressing Mcl-1 were insensitive to ABT-263, consistent with its binding specificity. Collectively, these results, demonstrate that **19** and **21** specifically bind and inhibit Mcl-1 and have no effect on Bcl-2, which is consistent with our biochemical data for their selectivity profiles. Furthermore, this supports the concept that tumor cells "addicted" to Mcl-1 protein will be the most sensitive target cell population for selective small-molecule Mcl-1 inhibitors.

We next evaluated our most potent compounds for their ability to inhibit cell growth in the leukemia cell lines HL-60, MV4,11, and K-562 (Figure 8). It has been shown that AML-derived cell lines, HL-60 and MV4,11, are sensitive to inhibition of the antiapoptotic protein Mcl-1, while CML-derived K-562 cell line is less sensitive to Mcl-1 inhibition.[82] Tested compounds from our series showed inhibition of the cell growth in a dose-dependent manner with similar potencies, IC_{50} values ranging from 2.06 to 11.72 μM against the HL-60

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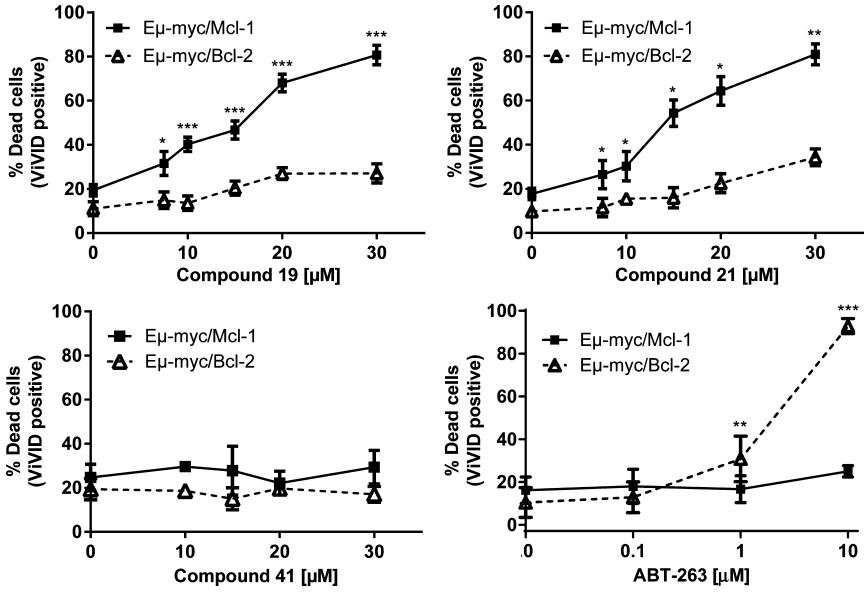

Figure 7. Sensitivity of Eμ-myc lymphoma cells overexpressing Mcl-1 and Bcl-2 antiapoptotic proteins to inhibitor-induced cell death. Eμ-myc/Mcl-1 and Eμ-myc/Bcl-2 lymphomas were treated for 15−18 h with increasing concentrations of **19**, **21**, **41**, and ABT-263. Dead cells were assessed by LIVE/DEAD fixable dead cell stain kit (ViVID). The data shown represents means ± SEM from 3−7 independent experiments. The significance was calculated using unpaired *t* test, and the number of data is shown for each tested concentration with corresponding significance: (*) is $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

and MV4,11 cell lines (Figure 8A). Interestingly, these compounds showed decreased ability to inhibit the cell growth of K-562, with IC_{50} values of 11.76 to 29.89 μM. Compound **41**, which does not bind to Mcl-1, did not show inhibition up to 50 μM. It is important to be pointed out that **36** and **37**, where the carboxylic acid was replaced with a bioisostere tetrazole group, show similar cellular activity in all tested cell lines in comparison with their parent compounds, **10** and **21**, respectively. These results demonstrate that the tetrazole group can effectively replace the acid group, achieving not only the same binding affinity to Mcl-1 but also comparable cellular activity. Furthermore, compound **32**, in which the acid group has been replaced with methyl ester and showed 10-fold less binding to Mcl-1 as compared with **10**, has also similar IC_{50} values in tested cell lines as **10**. This is probably due to enzymatic hydrolysis of the methyl ester group in the cells and releasing the corresponding compound with free acid group, thus functioning as a pro-drug. These data also demonstrate that the compounds with free acidic group in this series are cell permeable, although the mechanism of their cell permeability need to be further studied. Compounds **21** and **37** were evaluated for their ability to induce apoptosis in the HL-60 cell line using annexin-V and propidium iodide (PI) double staining by flow cytometry (Figure 8B). Both compounds effectively induced apoptosis in a dose-dependent manner. Treatment of the HL-60 cells by 2.5, 5.0, and 10 μM of **21** and **37** for 20 h results in 28.9% and 23.5%, 37.8% and 51.6%, and 78.4% and 74.2% of apoptotic cells (early + late), respectively, as compared to 7.6% and 7.3% of apoptotic cells in the DMSO controls. These results further confirmed that these two analogues have similar cellular activity, and tetrazole can effectively replace the acid group. To determine if the induction of apoptosis by compound **37** in the HL-60 cell line depends upon caspases, we treated the cells with compound **37** alone or in the presence of Z-VED-FMK, a pan-caspase inhibitor (Figure 8B and Supporting Information Figure S7). The obtained results showed that the induction of apoptosis was significantly inhibited in the presence of Z-VAD-FMK, clearly indicating that the apoptotic activity of compound **37** is mediated by caspases. Of note, Z-VAD-FMK alone has no effect on cells (Supporting Information Figure S7). Compound **41** at 40 μM has no effect on apoptosis induction just like untreated control, which is consistent with its lack of binding to Mcl-1 and inhibition of cell growth. We further tested compounds **19** and **21** in HL-60 cell line for their ability to induce caspase-3 activity, one of the important biochemical markers of apoptosis (Figure 8C). Both compounds induce activation of caspase-3 activity in a dose-dependent manner, with **21** effectively inducing activation over a 24 h period starting at 2.5 μM. Importantly, these results correlate with the ability of **21** to induce apoposis and inhibit HL-60 cell growth.

CONCLUSIONS

Applying a HTS approach we have identified a novel class of small molecules as selective Mcl-1 inhibitors. Employing structure-based design supported by NMR studies, we synthesized a focused library of analogues and established a SAR for binding to Mcl-1. Careful HSQC analysis of analogues **16**−**18** provided strong evidence for the predicted binding model of these compounds and further confirmation that R_1 substituent of this class of inhibitors binds to one well-defined pocket of Mcl-1 known as h2 pocket. Analogues **19** and **21**, with K_i values of 170 and 180 nM, respectively, were developed as the most potent compounds in this series with an overall 9-fold increase in binding compared to **1**. Binding studies showed that **19** and **21** maintained the selectivity profile of **1**. Using wild-type and Bax/Bak double knockout MEFs cells, the contribution of these two multidomain pro-apoptotic proteins in **21**-induced cell death was determined and demonstrated that **21** primarily causes cell death in wild-type MEFs in a Bax/Bak-dependent manner. Furthermore, **19** and **21** led to sensitization of Eμ-myc lymphomas overexpressing Mcl-1 but did not show effect on cells overexpressing Bcl-2 antiapoptotic protein,

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A)

Cpd	HL-60 IC_{50} ± SD [μM]	MV4,11 IC_{50} ± SD [μM]	K-562 IC_{50} ± SD [μM]
10	3.13 ± 0.81	10.87±0.95	15.11 ± 2.45
16	5.09 ± 0.68	5.53 ± 0.96	12.53 ± 4.73
17	5.48 ± 1.29	4.65 ± 1.68	29.89 ± 9.18
19	7.56 ± 1.53	4.31 ± 1.99	14.91 ± 5.62
21	4.78 ± 0.92	4.24 ± 2.60	14.08 ± 3.63
32	2.06 ± 0.27	6.04 ± 0.19	11.87 ± 1.04
36	2.34 ± 0.44	6.58 ± 0.24	11.76 ± 2.12
37	4.47 ± 0.33	11.72 ± 0.94	15.29 ± 0.90
41	> 50	> 50	> 50

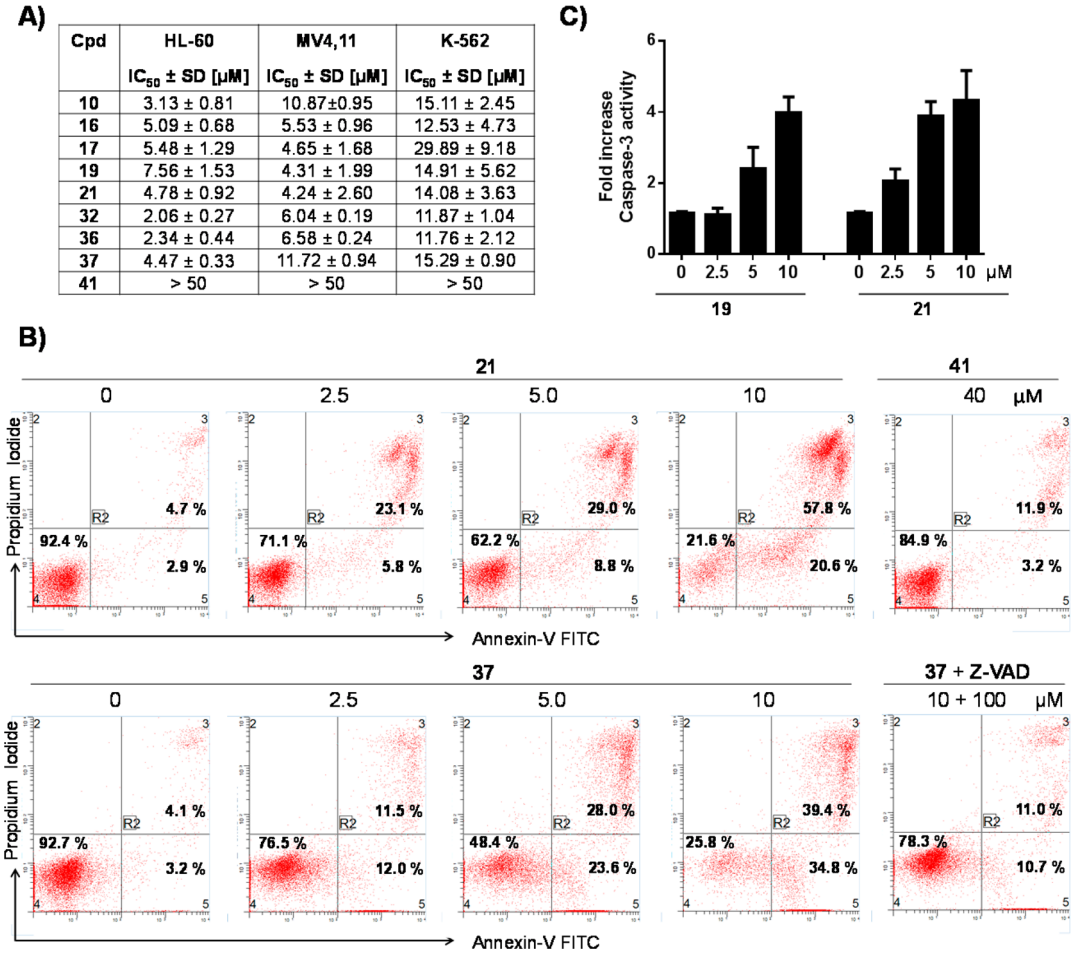

Figure 8. Cell death and apoptosis induction by Mcl-1 inhibitors in human leukemic cell lines. (A) Inhibition of cell growth by designed Mcl-1 inhibitors in the HL-60, MV4,11, and K-562 leukemia cell lines. Cells were treated for 3 days, and cell growth was determined using CellTiter Glo luminescent cell viability assay. (B) Analysis of apoptosis induced by **21** and **37** in the HL-60 leukemia cell line. Cells were treated with **21**, **37**, and **41** for 20 h using indicated concentrations, and apoptosis was analyzed with annexin-V and propidium iodide (PI) double staining by flow cytometry. Early apoptotic cells were defined as annexin-V positive/PI-negative, and late apoptotic cells as annexin-V/PI-double positive. Induction of the apoptosis by **37** was tested also in the presence of Z-VAD-FMK. (C) Induction of caspase-3 by **19** and **21** in the HL-60 cell line. Cells were treated for 20 h, and caspase-3 was detected with fluorometric-based assay. Results shown are the mean and SEM from at least three separate experiments.

confirming their selective targeting of Mcl-1. Most potent developed 3-substituted-*N*-(4-Hydroxynaphthalen-1-yl)-arylsulfonamide Mcl-1 inhibitors inhibited the cell growth of AML-derived cell lines, HL-60 and MV4,11. Compounds **19** and **21** induced activation of caspase-3 in HL-60 cell line and **21** effectively induced apoptosis starting at 2.5 μM. Future studies will be directed toward optimization of this class of compounds and in vivo evaluation.

EXPERIMENTAL SECTION

Chemistry Materials and Methods. All reactions were performed under anhydrous conditions. Reagents were used as supplied without further purification. Reactions were monitored by TLC using precoated silica gel 60 F254 plates. Silica gel chromatography was performed with silica gel (220−240 mesh) obtained from Silicycle. Purities of final compounds were assessed by analytical reverse-phase HPLC performed with one of the two methods. Method A: Agilent 1100 series with an Agilent Zorbax Eclipse Plus C18 (4.6 mm × 75 mm, 3.5 μm particle size) column with the gradient 10% ACN/water (1 min), 10−90% ACN/water (6 min), and 90% ACN/water (2 min) flow = 1 mL/min. Method B: Shimadzu system with a Restek Ultra C18 (4.6 mm × 150 mm, 5 μm particle size) column with the gradient 50% ACN/water (5 min), 50−70% ACN/water (2 min), and 70%−90% ACN/water (8 min) flow = 1 mL/min. Semipreparative reverse-phase HPLC was performed on a Shimadzu system with a Restek Ultra C18 (21.2 mm × 150 mm, 5 μm particle size) column. All NMR spectra were obtained in DMSO-d_6 or $CDCl_3$, and results were recorded at 400 or 500 MHz on a Varian 400 or 500 instrument. Mass spectra were recorded on a Micromass LCT time-of-flight instrument utilizing electrospray ionization operating in positive-ion (ESI+) or negative-ion (ESI) modes where indicated. High resolution mass spectrometry (HRMS) analysis was performed on an Agilent Q-TOF system. All final compounds were purified to >95% purity. Analogues **4**−**8**, **15**, and **39** were purchased from commercial vendors.

2-Iodo-1-methoxy-4-nitronaphthalene (47). Synthesized using reported procedure with modification.[60] A mixture of 1-methoxy-4-nitronaphthalene (2.1 g, 10.4 mmol) and *N*-iodosuccinimide (2.7 g, 12 mmol) in TFA (40 mL) was heated to reflux and stirred for 20 h under nitrogen. The reaction mixture was diluted with EtOAc (40 mL), washed with saturated aqueous $Na_2S_2O_3$ solution (30 mL), saturated aqueous $NaHCO_3$ (30 mL × 2), and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and silica was added to filtrate and the solvent was removed under reduced pressure. The adsorbed crude residue was purified by flash column chromatography (100% hexane) on silica gel to give **47** (2.4 g, 70%) as a light-yellow solid. Note: R_f of starting material and product were very close and a good separation was achieved with a relatively long silica gel column and 100% hexane gradient. >95% pure product was needed for pd-catalyzed coupling

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step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.58 (s, 1H), 8.21 (d, J = 8.42 Hz, 1H), 7.74 (t, J = 7.53 Hz, 1H), 7.65 (t, J = 7.53 Hz, 1H), 4.03 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.69, 142.85, 134.07, 129.99, 128.55, 128.08, 126.55, 123.92, 123.02, 83.35, 62.20. ESI MS: m/z 330.0 $(M + H)^+$.

Methyl 2-((1-Methoxy-4-nitronaphthalen-2-yl)thio)acetate (48a). Synthesized using reported procedures with modification.[63−65] To a solution of Cs_2CO_3 (1.5 g, 4.5 mmol) in dry THF (7 mL) under nitrogen was added methylthioglycolate (277 μL, 2.9 mmol). The mixture was stirred at room temperature for 10 min. At this time, a solution of $ZnCl_2$ (288 mg, 2.1 mmol) in dry THF (3 mL) was added and the mixture was stirred at room temperature for an additional 10 min. Meanwhile, in a separate flask, $Pd(OAc)_2$ (36 mg, 0.16 mmol) and xantphos (90 mg, 0.15 mmol) were premixed in dry THF (5 mL) under nitrogen and stirred at room temperature for about 20 min. To the solution of thiol, Cs_2CO_3, and $ZnCl_2$ was added **47** (1.0 g, 3.1 mmol), LiI (200 mg, 1.5 mmol), and premixed solution of the catalyst and ligand. The mixture was stirred at 60 °C under nitrogen for 20 h. The reaction mixture was filtered to remove Cs_2CO_3 and silica was added to the mixture and the solvent was removed under reduced pressure. The adsorbed crude residue was purified by column chromatography (hexane/EtOAc 4:1) on silica gel to give **48a** (606 mg, 66%) as a yellow oil which solidified. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.50 Hz, 1H), 8.37 (s, 1H), 8.19 (d, J = 8.50 Hz, 1H), 7.70 (t, J = 7.57 Hz, 1H), 7.64 (t, J = 7.57 Hz, 1H), 4.07 (s, 3H), 3.77 (s, 2H), 3.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.48, 159.95, 142.50, 129.73, 128.88, 127.85, 127.22, 125.94, 123.71, 122.93, 122.68, 61.92, 52.72, 35.10. ESI MS: m/z 308.1 $(M + H)^+$.

Methyl 3-((1-Methoxy-4-nitronaphthalen-2-yl)thio)-propanoate (48b). Synthesized using a similar procedure used to prepare **48a** except using methyl 3-mercaptopropionate. The mixture was stirred at 60 °C under nitrogen for 5 h. Crude was purified using flash column chromatography (hexane/EtOAc 4:1) on silica gel with dry loading to give **48b** (194 mg, 66%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 8.48 Hz, 1H), 8.27 (s, 1H), 8.16 (d, J = 8.48 Hz, 1H), 7.70−7.64 (m, 1H), 7.64−7.58 (m, 1H), 4.03 (s, 3H), 3.65 (s, 3H), 3.28 (t, J = 7.24 Hz, 2H), 2.65 (t, J = 7.24 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.64, 159.79, 142.48, 129.51, 128.93, 127.81, 126.94, 125.60, 123.62, 123.45, 122.57, 61.59, 51.90, 34.11, 28.11. ESI MS: m/z 322.0 $(M + H)^+$, 343.9 $(M + Na)^+$.

Butyl(1-methoxy-4-nitronaphthalen-2-yl)sulfane (48c). Synthesized using a reported procedure.[61] A stirred mixture of **47** (300 mg, 0.91 mmol), $Pd_2(dba)_3$ (42 mg, 0.05 mmol), Dppf (104 mg, 0.18 mmol), and Et_3N (0.2 mL) in dry NMP (7 mL) was flushed with nitrogen for 15 min at room temperature. Butanethiol (83 μL, 0.77 mmol) was then added, and the reaction mixture was heated to 80 °C and stirred for 2 h. The mixture was diluted with EtOAc (10 mL) and washed with H_2O (10 mL × 4) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and silica added to the filtrate, and the solvent was removed under reduced pressure. The adsorbed crude residue was purified by flash column chromatography (hexane to hexane/EtOAc 99:1) on silica gel to give **48c** (189 mg, 71%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (ddd, J = 0.72, 1.50, 8.36 Hz, 1H), 8.26 (s, 1H), 8.16 (ddd, J = 0.72, 1.50, 8.36 Hz, 1H), 7.68−7.59 (m, 2H), 4.03 (s, 3H), 3.03 (t, J = 7.36 Hz, 2H), 1.67 (p, J = 7.36 Hz, 2H), 1.48 (h, J = 7.36 Hz, 2H), 0.93 (t, J = 7.36 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.48, 142.53, 129.05, 128.81, 127.69, 125.69, 125.57, 125.03, 123.62, 122.34, 61.31, 32.23, 31.10, 21.92, 13.60. ESI MS: m/z 292.0 $(M + H)^+$.

3-(1-Methoxy-4-nitronaphthalen-2-yl)prop-2-yn-1-ol (48d). Synthesized using a reported procedure.[62] A mixture of **47** (453 mg, 1.4 mmol), $Pd(PPh_3)_2Cl_2$ (48 mg, 0.07 mmol), and CuI (28 mg, 0.15 mmol) in Et_3N (8 mL) and dry THF (3 mL) was added dropwise to a solution of 2-propyn-1-ol (0.15 mL, 2.6 mmol) in Et_3N (3 mL) under nitrogen at room temperature. Reaction mixture was heated to 60 °C and stirred for 2 h then diluted with EtOAc (10 mL) and washed with saturated aqueous NH_4Cl (15 mL × 2) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/EtOAc 3:2) on silica gel to give **48d** (342 mg, 97%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.66 Hz, 1H), 8.29 (s, 1H), 8.26 (d, J = 8.66 Hz, 1H), 7.74−7.66 (m, 1H), 7.64−7.55 (m, 1H), 4.58 (s, 2H), 4.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.40, 141.04, 130.49, 129.78, 128.26, 127.56, 126.29, 123.44, 123.24, 107.40, 93.96, 80.83, 61.95, 51.67. ESI MS: m/z 258.1 $(M + H)^+$.

4-(1-Methoxy-4-nitronaphthalen-2-yl)but-3-yn-1-ol (48e). Synthesized using a similar procedure used to prepare **48d** except using 3-butyn-1-ol as the alkyne. The crude was purified by flash column chromatography (hexane/EtOAc 3:2) on silica gel to give **48e** (338 mg, 83%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, J = 8.56 Hz, 1H), 8.23 (s, 1H), 8.19 (d, J = 8.56 Hz, 1H), 7.63 (t, J = 7.59 Hz, 1H), 7.53 (t, J = 7.59 Hz, 1H), 4.23 (s, 3H), 3.87 (t, J = 6.14 Hz, 2H), 2.76 (t, J = 6.14 Hz, 2H), 2.45 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.29, 141.11, 130.19, 129.91, 128.36, 127.48, 126.03, 123.43, 123.10, 109.99, 108.73, 93.90, 61.77, 60.96, 24.07. ESI MS: m/z 272.1 $(M + H)^+$.

A Representative Procedure for Iron Reduction of Nitro to Amine. *Methyl 2-((4-Amino-1-methoxynaphthalen-2-yl)thio)-acetate (49a).* Synthesized using a reported procedure.[66] To a suspension of iron powder (538 mg, 9.6 mmol) in glacial acetic acid (5 mL) was added **48a** (195 mg, 0.63 mmol) dissolved in glacial acetic acid (5 mL). The mixture was stirred at 70 °C under nitrogen for 1 h, when the mixture turned milky. The mixture was then diluted with EtOAc (15 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL × 2) and brine (20 mL). Organic layer was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to give **49a** as a crude as a purple oil. The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.29 Hz, 1H), 7.76 (d, J = 8.29 Hz, 1H), 7.50 (t, J = 7.58 Hz, 1H), 7.43 (t, J = 7.58 Hz, 1H), 6.78 (s, 1H), 3.91 (s, 3H), 3.73 (s, 2H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.48, 147.81, 138.68, 128.54, 126.54, 125.33, 124.24, 123.36, 122.45, 121.35, 111.08, 61.41, 52.53, 35.37. ESI MS: m/z 278.1 $(M + H)^+$, 300.1 $(M + Na)^+$.

Methyl 3-((4-Amino-1-methoxynaphthalen-2-yl)thio)propanoate (49b). Synthesized using the procedure for **49a** except using **48b** as the starting material. Crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.36 Hz, 1H), 7.75 (d, J = 8.36 Hz, 1H), 7.49 (t, J = 7.56 Hz, 1H), 7.42 (t, J = 7.56 Hz, 1H), 6.72 (s, 1H), 6.26 (s, 2H), 3.89 (s, 3H), 3.65 (s, 3H), 3.22 (t, J = 7.46 Hz, 2H), 2.64 (t, J = 7.46 Hz, 2H);. ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.38, 147.73, 138.77, 128.63, 126.51, 125.12, 123.92, 123.56, 122.36, 121.35, 110.85, 61.21, 51.78, 34.48, 27.90. ESI MS: m/z 292.0 $(M + H)^+$, 314.0 $(M + Na)^+$.

3-(Butylthio)-4-methoxynaphthalen-1-amine (49c). Synthesized using the procedure for **49a** except using **48c** as the starting material. Crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.30 Hz, 1H), 7.75 (d, J = 8.30 Hz, 1H), 7.49 (t, J = 7.42 Hz, 1H), 7.40 (t, J = 7.42 Hz, 1H), 6.72 (s, 1H), 6.30 (s, 2H), 3.92 (s, 3H), 2.96 (t, J = 7.33 Hz, 2H), 1.65 (p, J = 7.33 Hz, 2H), 1.47 (h, J = 7.33 Hz, 2H), 0.92 (t, J = 7.33 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.85, 138.46, 128.54, 126.42, 125.50, 124.73, 123.45, 122.20, 121.30, 110.24, 61.02, 32.29, 31.50, 22.02, 13.67. ESI MS: m/z 262.0 $(M + H)^+$.

A Representative Procedure for Hydrogenation Reaction. *3-(4-Amino-1-methoxynaphthalen-2-yl)propan-1-ol (49d).* Synthesized using a reported procedure.[67] A stirred solution of **48d** (325 mg, 1 mmol) in a mixture of EtOH (12 mL) and EtOAc (2 mL) was hydrogenated in the presence of 10% Pd/C (80 mg) at room temperature and under 30 psi of H_2 overnight. The suspension was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The crude was used in the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.35 Hz, 1H), 7.76 (d, J = 8.35 Hz, 1H), 7.51−7.43 (m, 1H), 7.43−7.35 (m, 1H), 6.54 (s, 1H), 3.85 (s, 3H), 3.54 (t, J = 6.08 Hz, 2H), 2.80 (t, J = 7.27 Hz, 2H), 1.85 (p, J = 6.69 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.20, 138.66, 129.80, 128.24, 126.01, 124.51, 123.79, 122.41, 121.33, 111.39, 62.18, 61.32, 33.18, 25.51. ESI MS: m/z 232.1 $(M + H)^+$.

*4-(4-Amino-1-methoxynaphthalen-2-yl)butan-1-ol (**49e**).* Synthesized using the procedure for **49d** except using **48e** as the starting material. Crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.39 Hz, 1H), 7.72 (d, J = 8.39 Hz, 1H), 7.43 (t, J = 7.33 Hz, 1H), 7.34 (t, J = 7.33 Hz, 1H), 6.52 (s, 1H), 3.79 (s, 3H), 3.56 (t, J = 6.43 Hz, 2H), 2.67 (t, J = 6.43 Hz, 2H), 1.65 (dt, J = 6.60, 14.36 Hz, 2H), 1.56 (dt, J = 6.60, 14.36 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.98, 138.36, 130.77, 128.39, 125.85, 124.33, 123.72, 122.34, 121.36, 111.52, 62.32, 61.97, 32.45, 29.21, 26.89.

A Representative Procedure for Sulfonamide/Amide Coupling Reaction of Aryl Amines with Sulfonyl/Acyl Chlorides. *Methyl 2-((1-Methoxy-4-(thiophene-2-sulfonamido)naphthalen-2-yl)thio)acetate (**49f**).* A solution of the crude amine **49a** dissolved in dry CH_2Cl_2 (4 mL) was added to 2-thiophenesulfonyl chloride (119 mg, 0.65 mmol). Addition of pyridine (0.08 mL, 0.99 mmol) was followed, and the mixture was stirred at room temperature under nitrogen overnight. The mixture was diluted with EtOAc (10 mL) and washed with H_2O (10 mL × 3) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure. Crude was purified by flash column chromatography (hexane/EtOAc 7:3) on silica gel to give **49f** (176 mg, 66% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.40 Hz, 1H), 7.82 (d, J = 8.40 Hz, 1H), 7.49−7.43 (m, 2H), 7.42−7.39 (m, 1H), 7.39−7.34 (m, 2H), 6.92−6.88 (m, 1H), 3.95 (s, 3H), 3.68 (s, 3H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.91, 154.12, 139.48, 133.09, 132.51, 130.06, 128.60, 127.74, 127.39, 126.96, 125.74, 123.39, 122.29, 61.49, 52.68, 35.12. ESI MS: m/z 423.9 $(M + H)^+$, 445.8 $(M + Na)^+$.

*Methyl 2-((1-Methoxy-4-(methylsulfonamido)naphthalen-2-yl)thio)acetate (**49g**).* Synthesized using the procedure for **49f** except using methanesulfonyl chloride, which afforded the title compound (69 mg, 39% over two steps) as a light-pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13−8.08 (m, 1H), 8.05−8.00 (m, 1H), 7.65 (s, 1H), 7.60−7.54 (m, 2H), 6.80 (s, 1H), 3.99 (s, 3H), 3.75 (s, 2H), 3.69 (s, 3H), 3.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.98, 154.06, 129.61, 128.85, 127.84, 127.36, 127.19, 125.15, 123.67, 122.67, 122.07, 61.50, 52.64, 39.91, 35.00. ESI MS: m/z 355.9 $(M + H)^+$, 377.9 $(M + Na)^+$.

*Methyl 2-((1-Methoxy-4-(phenylsulfonamido)naphthalen-2-yl)thio)acetate (**49h**).* Synthesized using the procedure for **49g** except using benzenesulfonyl chloride, which afforded the title compound (119 mg, 64% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.40 Hz, 1H), 7.74 (t, J = 9.06 Hz, 3H), 7.47 (q, J = 8.19, 8.80 Hz, 2H), 7.36 (t, J = 7.61 Hz, 3H), 7.29 (s, 1H), 6.93 (s, 1H), 3.95 (s, 3H), 3.68 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.80, 154.02, 139.11, 132.95, 129.95, 128.98, 128.63, 127.82, 127.33, 126.95, 126.90, 125.67, 123.36, 122.31, 122.28, 61.49, 52.63, 35.06. ESI MS: m/z 417.9 $(M + H)^+$, 439.9 $(M + Na)^+$.

*Methyl 2-((4-(4-Chlorophenylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49i**).* Synthesized using the procedure for **49f** except using 4-chlorobenzenesulfonyl chloride, which afforded the title compound (280 mg, 67% over two steps) as a light-pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.40 Hz, 1H), 7.74 (d, J = 8.40 Hz, 1H), 7.66 (t, J = 1.96 Hz, 1H), 7.65 (t, J = 1.96 Hz, 1H), 7.47 (ddd, J = 1.17, 6.86, 8.24 Hz, 1H), 7.38 (ddd, J = 1.17, 6.86, 8.24 Hz, 1H), 7.35−7.33 (m, 2H), 7.32 (t, J = 1.94 Hz, 1H), 7.07 (s, 1H), 3.95 (s, 3H), 3.69 (s, 3H), 3.63 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.84, 154.18, 139.50, 137.67, 129.86, 129.25, 128.78, 128.66, 127.49, 127.05, 125.81, 123.42, 122.41, 122.14, 61.50, 52.64, 35.02. ESI MS: m/z 451.9 $(M + H)^+$, 473.8 $(M + Na)^+$.

*Methyl 2-((4-(4-Bromophenylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**41**).* Synthesized using the procedure for **49f** except using 4-bromobenzenesulfonyl chloride, which afforded the title compound (185 mg, 61% over two steps) as a pink/purple solid. HPLC (method A, t_R = 7.66 min), purity 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 8.38 Hz, 1H), 7.72 (d, J = 8.38 Hz, 1H), 7.61−7.59 (m, 1H), 7.58 (t, J = 2.04 Hz, 1H), 7.54−7.47 (m, 3H), 7.44−7.38 (m, 1H), 7.34 (s, 1H), 6.76 (s, 1H), 3.97 (s, 3H), 3.71 (s, 3H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.83, 154.25, 138.21, 132.29, 129.91, 128.89, 128.71, 128.07, 127.41, 127.12, 125.92, 123.47, 122.48, 122.17, 122.09, 61.56, 52.70, 35.03. ESI HRMS: m/z 493.9724 $(M - H)^-$.

*Methyl 2-((1-Methoxy-4-(4-methoxyphenylsulfonamido)-naphthalen-2-yl)thio)acetate (**49j**).* Synthesized using the procedure for **49f** except using 4-methoxybenzenesulfonyl chloride, which afforded the title compound (190 mg, 77% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.42 Hz, 1H), 7.80 (d, J = 8.42 Hz, 1H), 7.69−7.63 (m, 2H), 7.48 (t, J = 7.64 Hz, 1H), 7.40 (t, J = 7.64 Hz, 1H), 7.29 (s, 1H), 6.86−6.80 (m, 3H), 3.95 (s, 3H), 3.79 (s, 3H), 3.70 (s, 3H), 3.63 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.82, 163.12, 153.79, 130.64, 129.85, 129.52, 128.61, 128.11, 126.95, 126.90, 125.20, 123.36, 122.31, 114.12, 61.50, 55.56, 52.65, 35.09. ESI MS: m/z 447.8 $(M + H)^+$, 469.8 $(M + Na)^+$.

*Methyl 2-((4-(3-Bromophenylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49k**).* Synthesized using the procedure for **49f** except using 3-bromobenzenesulfonyl chloride, which afforded the title compound (331 mg, 74%) as a purple/pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 8.40 Hz, 1H), 7.88 (s, 1H), 7.73 (d, J = 8.40 Hz, 1H), 7.62 (t, J = 9.41 Hz, 2H), 7.49 (t, J = 7.59 Hz, 1H), 7.40 (t, J = 7.59 Hz, 1H), 7.34 (s, 1H), 7.26−7.21 (m, 1H), 6.82 (s, 1H), 3.98 (s, 3H), 3.70 (s, 3H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.72, 154.55, 141.06, 135.89, 130.41, 130.24, 130.03, 128.75, 127.31, 127.04, 127.02, 126.33, 125.89, 123.45, 122.90, 122.48, 122.09, 61.48, 52.55, 35.16. ESI MS: m/z 495.8, 497.8 $(M + H)^+$, 517.8, 519.8 $(M + Na)^+$.

*Methyl 2-((4-(2-Bromophenylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49l**).* Synthesized using the procedure for **49f** except using 2-bromobenzenesulfonyl chloride, which afforded the title compound (314 mg, 74% over two steps) as a purple oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16−8.11 (m, 1H), 8.05−8.00 (m, 1H), 7.93 (d, J = 7.72 Hz, 1H), 7.78 (d, J = 7.72 Hz, 1H), 7.54−7.48 (m, 3H), 7.42−7.32 (m, 2H), 7.19 (s, 1H), 7.13 (s, 1H), 3.94 (s, 3H), 3.68 (s, 3H), 3.49 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.52, 154.35, 138.58, 135.17, 133.92, 132.04, 130.52, 128.75, 127.83, 127.55, 127.11, 127.07, 125.00, 123.16, 122.90, 122.26, 119.94, 61.40, 52.50, 35.13. ESI MS: m/z 495.8, 597.8 $(M + H)^+$, 517.8, 519.8 $(M + Na)^+$.

*Methyl 2-((4-(Benzo[b]thiophene-2-sulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49m**).* Synthesized using the procedure for **49f** except using 1-benzothiophene-2-sulfonyl chloride, which afforded the title compound (132 mg, 57% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.40 Hz, 1H), 7.90 (d, J = 8.40 Hz, 1H), 7.79 (d, J = 7.99 Hz, 1H), 7.74 (d, J = 7.99 Hz, 1H), 7.69 (s, 1H), 7.48−7.42 (m, 2H), 7.41 (s, 1H), 7.40−7.35 (m, 2H), 7.01 (s, 1H), 3.96 (s, 3H), 3.64 (s, 3H), 3.51 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.77, 154.23, 141.85, 139.53, 137.38, 130.61, 130.05, 128.66, 127.47, 127.37, 127.06, 125.72, 125.47, 125.44, 123.47, 122.62, 122.34, 122.25, 61.51, 52.58, 34.95. ESI MS: m/z 473.9 $(M + H)^+$, 495.8 $(M + Na)^+$.

*Methyl 2-((4-([1,1′-Biphenyl]-4-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49n**).* Synthesized using the procedure for **49f** except using 4-biphenylsulfonyl chloride, which afforded the title compound (152 mg, 71% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.34 Hz, 1H), 7.83−7.76 (m, 3H), 7.58 (d, J = 8.31 Hz, 2H), 7.51 (d, J = 7.14 Hz, 2H), 7.49−7.42 (m, 2H), 7.42−7.35 (m, 3H), 7.33 (s, 1H), 6.90 (s, 1H), 3.96 (s, 3H), 3.65 (s, 3H), 3.60 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.75, 153.99, 145.89, 139.09, 137.68, 129.97, 128.98, 128.66, 128.50, 127.90, 127.86, 127.56, 127.23, 126.98, 126.93, 125.58, 123.43, 122.35, 122.33, 61.49, 52.58, 35.02. ESI MS: m/z 494.1 $(M + H)^+$, 516.1 $(M + Na)^+$.

*Methyl 2-((4-([1,1′-Biphenyl]-3-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49o**).* Synthesized using reported procedures with modification.[69,70] A stirred mixture of **49k** (307 mg, 0.62 mmol), phenylboronic acid (113 mg, 0.91 mmol), 2 M aqueous Na_2CO_3 (0.93 mL), and $Pd(PPh_3)_4$ (72 mg, 0.06 mmol) in THF (6 mL) /H_2O (1 mL) was heated at 60 °C under nitrogen for 2 h. Mixture was diluted with EtOAc (10 mL) and washed with H_2O (15

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mL × 2) and brine (15 mL). Organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica to give **49o** (284 mg, 92%) as a light-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$/ couple of drops of D_2O) δ 8.00 (d, J = 8.39 Hz, 1H), 7.83 (s, 1H), 7.79−7.73 (m, 2H), 7.69 (dd, J = 7.58, 14.09 Hz, 2H), 7.47−7.40 (m, 2H), 7.37−7.31 (m, 6H), 3.92 (s, 3H), 3.62 (s, 3H), 3.58 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$/ couple of drops of D_2O) δ 169.86, 154.00, 142.22, 139.49, 138.99, 133.60, 131.46, 131.02, 130.00, 129.44, 128.89, 128.63, 128.14, 127.89, 127.03, 126.98, 126.88, 125.92, 125.87, 125.80, 123.46, 122.38, 122.27, 61.43, 52.60, 34.97. ESI MS: m/z 494.1 $(M + H)^+$.

*Methyl 2-((4-([1,1′-Biphenyl]-2-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49p**).* Synthesized using the procedure for **49f** except using 2-biphenylsulfonyl chloride which was synthesized as previously reported.[83] The title compound (57 mg, 58% over two steps) was obtained as a light-orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.20 Hz, 1H), 8.00 (d, J = 8.20 Hz, 1H), 7.62 (t, J = 7.54 Hz, 1H), 7.51 (q, J = 7.13, 7.54 Hz, 3H), 7.44−7.37 (m, 2H), 7.37−7.29 (m, 5H), 6.93 (s, 1H), 5.86 (s, 1H), 3.91 (s, 3H), 3.67 (s, 3H), 3.44 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.66, 152.79, 140.89, 138.72, 138.15, 132.77, 132.73, 129.66, 129.43, 128.59, 128.52, 128.39, 128.20, 128.11, 127.94, 127.07, 126.69, 123.16, 122.20, 122.04, 120.40, 61.44, 52.62, 34.96. ESI MS: m/z 494.0 $(M + H)^+$.

*Methyl 2-((4-(4′-Chloro-[1,1′-biphenyl]-4-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49q**).* Synthesized using the procedure for **49f** except using 4′-chlorobiphenyl-4-sulfonyl chloride, which afforded the title compound (284 mg, 80% over two steps) as a pink oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 7.14 Hz, 1H), 7.80 (d, J = 7.14 Hz, 1H), 7.78 (d, J = 7.54 Hz, 2H), 7.54 (d, J = 7.54 Hz, 2H), 7.49−7.44 (m, 2H), 7.44−7.40 (m, 3H), 7.40−7.35 (m, 1H), 7.33 (s, 1H), 6.97 (s, 1H), 3.96 (s, 3H), 3.65 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.73, 154.05, 144.56, 138.08, 137.53, 134.79, 129.97, 129.19, 128.67, 128.48, 128.01, 127.80, 127.40, 126.98, 126.93, 125.64, 123.40, 122.35, 122.33, 61.50, 52.60, 35.03. ESI MS: m/z 528.1 $(M + H)^+$, 550.1 $(M + Na)^+$.

*Methyl 2-((4-(2′,4′-Difluoro-[1,1′-biphenyl]-4-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49r**).* Synthesized using the procedure for **49f** except using 2′,4′-difluoro-biphenyl-4-sulfonyl chloride, which afforded the title compound (75 mg, 58% over two steps) as a purple oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.41 Hz, 1H), 7.83−7.76 (m, 3H), 7.50 (d, J = 8.16 Hz, 2H), 7.46 (d, J = 7.94 Hz, 1H), 7.40 (d, J = 7.94 Hz, 1H), 7.37−7.29 (m, 2H), 6.98 (s, 1H), 6.96−6.86 (m, 2H), 3.96 (s, 3H), 3.66 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.84, 154.01, 139.74, 138.18, 131.40 (dd, J = 4.57, 9.75 Hz), 130.03, 129.43 (d, J = 3.03 Hz), 128.65, 127.76, 127.61, 127.02, 126.96, 125.70, 123.44, 122.37, 122.32, 111.98 (dd, J = 3.71, 21.44 Hz), 105.88−103.01 (m), 61.51, 52.63, 34.95. ESI MS: m/z 530.1 $(M + H)^+$.

*Methyl 2-((1-Methoxy-4-(4-phenoxyphenylsulfonamido)-naphthalen-2-yl)thio)acetate (**49s**).* Synthesized using the procedure for **49f** except using 4-phenoxybenzenesulfonyl chloride, which afforded the title compound (244 mg, 72% over two steps) as a light-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.43 Hz, 1H), 7.80 (d, J = 8.43 Hz, 1H), 7.66 (d, J = 8.14 Hz, 2H), 7.52−7.46 (m, 1H), 7.42−7.32 (m, 4H), 7.23 (s, 1H), 7.17 (t, J = 7.38 Hz, 1H), 6.94 (d, J = 8.52 Hz, 2H), 6.87 (d, J = 8.52 Hz, 2H), 3.95 (s, 3H), 3.69 (s, 3H), 3.66 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.85, 161.65, 155.11, 153.85, 132.59, 130.09, 129.85, 129.59, 128.60, 128.03, 126.90, 126.85, 125.50, 124.81, 123.42, 122.38, 122.31, 120.02, 117.64, 61.50, 52.67, 35.05. ESI MS: m/z 509.8 $(M + H)^+$.

*Methyl 2-((4-(4-(4-Fluorophenoxy)phenylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49t**).* Synthesized using the procedure for **49f** except using 4-(4-fluoro-phenoxy)-benzenesulfonyl chloride, which afforded the title compound (93 mg, 72% over two steps) as a yellow oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.42 Hz, 1H), 7.80 (d, J = 8.42 Hz, 1H), 7.66 (d, J = 8.72 Hz, 2H), 7.51−7.45 (m, 1H), 7.42−7.36 (m, 1H), 7.34 (s, 1H), 7.07−7.00 (m, 2H), 6.94−6.88 (m, 2H), 6.84 (d, J = 8.72 Hz, 2H), 3.95 (s, 3H), 3.69 (s, 3H), 3.66 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.87, 161.82, 160.79, 158.36, 153.87, 150.83 (d, J = 2.72 Hz), 132.65, 129.83, 129.66, 128.61, 127.99, 126.88 (d, J = 4.95 Hz), 125.47, 123.39, 122.35 (d, J = 6.41 Hz), 121.67 (d, J = 8.41 Hz), 117.23, 116.86, 116.63, 61.50, 52.69, 35.06. ESI MS: m/z 528.1 $(M + H)^+$, 550.1 $(M + Na)^+$.

*Methyl 2-((1-Methoxy-4-(4-phenylpiperazine-1-sulfonamido)-naphthalen-2-yl)thio)acetate (**49u**).* As reported previously,[68] to a solution of 1,1′-sulfonyldiimidazole (1.98 g, 10 mmol) in CH_2Cl_2 (40 mL) was added methyl trifluoromethanesulfonate (1.64 g, 10 mmol) at 0 °C. The solvent was removed after 3 h stirring. To the resulting residue in CH_3CN (40 mL) was added 1-phenylpiperazine (1.08 g, 6.67 mmol). After being stirred at room temperature overnight, the reaction mixture was concentrated under reduced pressure, and the crude was purified using flash column chromatography on silica to give 1-((1*H*-imidazol-1-yl)sulfonyl)-4-phenylpiperazine (1.17 g, 60% yield over 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.30−7.23 (m, 3H), 7.16 (s, 1H), 6.93 (t, J = 7.33 Hz, 1H), 6.89−6.84 (m, 2H), 3.37−3.30 (m, 4H), 3.26−3.20 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.21, 136.62, 130.80, 129.34, 121.43, 117.64, 117.20, 48.89, 46.42. ESI MS: m/z 293.0 $(M + H)^+$.

To a solution of 1-((1*H*-imidazol-1-yl)sulfonyl)-4-phenylpiperazine (85 mg, 0.29 mmol) in CH_2Cl_2 (3 mL) cooled at 0 °C was added methyl trifluoromethanesulfonate (0.035 mL, 0.32 mmol). After being stirred for 2 h at 0 °C, the reaction mixture was concentrated under reduced pressure to give 3-methyl-1-((4-phenylpiperazin-1-yl)-sulfonyl)-1*H*-imidazol-3-ium as a beige solid which was used in the next step without further purification.

A solution of 3-methyl-1-((4-phenylpiperazin-1-yl)sulfonyl)-1*H*-imidazol-3-ium (0.29 mmol) and aniline **49a** (0.29 mmol) in CH_3CN (3 mL) was stirred at 80 °C for 15 h. The reaction mixture was diluted with EtOAc (10 mL), washed with 1 N HCl, 1 N NaOH, H_2O, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, and the crude was purified using flash column chromatography on silica to give **49u** (36 mg, 25% over three steps) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12−8.06 (m, 1H), 8.06−8.00 (m, 1H), 7.71 (s, 1H), 7.59−7.52 (m, 2H), 7.22 (t, J = 7.94 Hz, 2H), 6.88−6.81 (m, 3H), 6.75 (s, 1H), 3.98 (s, 3H), 3.74 (s, 2H), 3.68 (s, 3H), 3.43−3.37 (m, 4H), 3.15−3.08 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.86, 153.45, 150.61, 129.16, 128.93, 128.69, 128.57, 127.15, 127.07, 123.77, 123.51, 122.71, 121.71, 120.67, 116.73, 61.50, 52.59, 49.15, 46.48, 35.20. ESI MS: m/z 502.1 $(M + H)^+$, 524.1 $(M + Na)^+$.

*Methyl 2-((4-(4-Bromobenzamido)-1-methoxynaphthalen-2-yl)-thio)acetate (**49v**).* Synthesized using the procedure for **49f** except using 4-bromobenzoyl chloride and Et_3N as the base, which afforded the title compound (245 mg, 66%) as a pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.09 (d, J = 8.38 Hz, 1H), 7.88 (s, 1H), 7.82−7.73 (m, 3H), 7.63−7.56 (m, 2H), 7.56−7.43 (m, 2H), 3.98 (s, 3H), 3.74 (s, 2H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.08, 165.42, 153.22, 133.17, 132.01, 131.74, 131.53, 128.82, 128.59, 128.43, 126.80, 126.77, 123.92, 123.50, 122.67, 121.57, 61.45, 52.57, 35.18. ESI MS: m/z 460.0, 462.0 $(M + H)^+$, 482.0, 484 $(M + Na)^+$.

*Methyl 2-((4-((4-Chlorophenyl)methylsulfonamido)-1-methoxy-naphthalen-2-yl)thio)acetate (**49w**).* Synthesized using the procedure for **49f** except using 4-chlorobenzylsulfonyl chloride, which afforded the title compound (110 mg, 41% over two steps) as a light-pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.31 Hz, 1H), 7.75 (d, J = 8.31 Hz, 1H), 7.65 (s, 1H), 7.57 (t, J = 7.54 Hz, 1H), 7.50 (t, J = 7.54 Hz, 1H), 7.22−7.16 (m, 4H), 6.73 (s, 1H), 4.37 (s, 2H), 4.00 (s, 3H), 3.75 (s, 2H), 3.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.00, 153.34, 135.04, 132.09, 128.91, 128.74, 128.31, 127.98, 127.18, 127.08, 126.88, 123.66, 122.67, 122.59, 121.65, 61.55, 57.41, 52.66, 34.96. ESI MS: m/z 465.8 $(M + H)^+$, 487.8 $(M + Na)^+$.

*Methyl 2-((4-(2-(4-Chlorophenyl)acetamido)-1-methoxynaphthalen-2-yl)thio)acetate (**49x**).* Synthesized using the procedure for **49f** except using 4-chlorophenylacetyl chloride and Et_3N as the base, which afforded the title compound (208 mg, 69% over two steps) as a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.34 Hz, 1H), 7.82 (s, 1H), 7.49 (m, 1H), 7.45−7.38 (m, 4H), 7.38−7.32 (m, 3H), 3.94 (s, 3H), 3.82 (s, 2H), 3.73 (s, 2H), 3.67 (s, 3H). ^{13}C NMR

(100 MHz, $CDCl_3$) δ 170.16, 169.79, 153.07, 133.95, 132.72, 130.93, 129.52, 128.47, 128.24, 127.90, 126.80, 126.72, 123.43, 123.32, 122.67, 120.80, 61.41, 52.59, 43.65, 35.14. ESI MS: *m/z* 429.9 $(M + H)^+$, 451.8 $(M + Na)^+$.

*Methyl 2-((1-Methoxy-4-(2-phenylacetamido)naphthalen-2-yl)-thio)acetate (**49y**).* Synthesized using the procedure for **49f** except using phenylacetyl chloride. Crude was triturated with cold methylene chloride to yield the title compound (154 mg, 49% over two steps) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.38 Hz, 1H), 7.94 (s, 1H), 7.53−7.44 (m, 4H), 7.40 (q, *J* = 7.27 Hz, 2H), 7.33 (s, 1H), 7.29−7.24 (m, 2H), 3.96 (s, 3H), 3.89 (s, 2H), 3.78 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.10, 169.71, 152.55, 134.54, 129.69, 129.46, 128.70, 128.40, 127.92, 127.64, 126.59, 123.48, 122.57, 122.48, 120.78, 61.40, 52.61, 44.64, 35.16. ESI MS: *m/z* 396.1 $(M + H)^+$, 418.1 $(M + Na)^+$.

*4-Bromo-N-(3-(butylthio)-4-methoxynaphthalen-1-yl)-benzenesulfonamide (**49z**).* Synthesized using the procedure for **49f** except using **49c** and 4-bromobenzenesulfonyl chloride, which afforded the title compound (55 mg, 57% over two steps) as a light-brown oil which solidified. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.44 Hz, 1H), 7.77 (d, *J* = 8.44 Hz, 1H), 7.59 (d, *J* = 8.36 Hz, 2H), 7.49 (d, *J* = 8.36 Hz, 2H), 7.46 (d, *J* = 7.85 Hz, 1H), 7.35 (t, *J* = 7.85 Hz, 1H), 7.21 (s, 1H), 7.16 (s, 1H), 3.94 (s, 3H), 2.81 (t, *J* = 7.24 Hz, 2H), 1.58 (p, *J* = 7.24 Hz, 2H), 1.45 (h, *J* = 7.24 Hz, 2H), 0.92 (t, *J* = 7.24 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.71, 138.26, 132.23, 128.92, 128.88, 128.65, 127.99, 127.25, 126.93, 126.37, 125.79, 124.49, 122.19, 122.04, 61.01, 31.92, 31.20, 21.95, 13.67. ESI MS: *m/z* 479.8, 481.8 $(M + H)^+$.

*2-((4-(4-Bromophenylsulfonamido)-1-methoxynaphthalen-2-yl)-thio)acetamide (**42**).* Synthesized using a reported procedure.[71] Compound **41** (82 mg, 0.16 mmol) was added to aqueous NH_4OH (29%, 3 mL). The mixture was stirred at room temperature for 1 h. Workup included diluting the mixture with EtOAc (10 mL) and washing with H_2O (10 mL × 2) and brine (10 mL). Organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **42** (69 mg, 90%) as a pink solid. Crude was used in the next step without further purification. HPLC (method A, t_R = 6.50 min), purity 98%. ^1H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.95 (d, *J* = 8.50 Hz, 1H), 7.85 (d, *J* = 8.50 Hz, 1H), 7.72 (d, *J* = 8.20 Hz, 2H), 7.60 (d, *J* = 8.20 Hz, 2H), 7.56−7.50 (m, 2H), 7.43−7.37 (m, 1H), 7.21 (s, 1H), 7.15 (s, 1H), 3.88 (s, 3H), 3.53 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.76, 151.88, 139.67, 132.70, 129.69, 129.18, 129.05, 128.23, 127.46, 127.03, 126.41, 125.24, 124.89, 123.95, 121.82, 61.27, 35.91. ESI MS: *m/z* 480.7, 482.7 $(M + H)^+$, 502.7, 504.7 $(M + Na)^+$. ESI HRMS: *m/z* 478.9742 $(M - H)^-$.

*2-((1-Methoxy-4-((4-phenoxyphenyl)sulfonamido)naphthalen-2-yl)thio)acetamide (**49aa**).* Synthesized using the procedure for **42** except using **49s** as the starting material, which afforded the title compound (179 mg, quantitative) as a light-purple solid. ^1H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.56−7.46 (m, 2H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.39−7.33 (m, 1H), 7.23−7.14 (m, 2H), 7.11 (s, 1H), 7.04−6.94 (m, 4H), 3.82 (s, 3H), 3.50 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.84, 155.74, 130.72, 129.77, 129.55, 128.23, 127.07, 125.57, 125.09, 124.94, 124.55, 121.45, 120.02, 118.17, 109.99, 61.17, 36.13. ESI MS: *m/z* 495.0 $(M + H)^+$, 517.0 $(M + Na)^+$.

*4-Bromo-N-(3-(3-hydroxypropyl)-4-methoxynaphthalen-1-yl)-benzenesulfonamide (**49bb**).* Synthesized using the procedure for **49f** except using **49d** and 4-bromobenzenesulfonyl chloride, which afforded the title compound (163 mg, 33% over two steps) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 7.94 (t, *J* = 7.53 Hz, 2H), 7.70 (d, *J* = 8.53 Hz, 2H), 7.53 (d, *J* = 8.53 Hz, 2H), 7.51−7.45 (m, 1H), 7.42−7.35 (m, 1H), 6.86 (s, 1H), 4.47 (s, 1H), 3.79 (s, 3H), 3.37 (t, *J* = 5.87 Hz, 2H), 2.69−2.60 (m, 2H), 1.59−1.49 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.33, 139.44, 132.62, 130.38, 130.34, 129.28, 128.55, 128.23, 127.00, 126.88, 126.78, 126.01, 124.09, 122.21, 62.33, 60.67, 33.79, 25.69. ESI MS: *m/z* 449.9, 451.9 $(M + H)^+$, 471.9, 473.9 $(M + Na)^+$.

*4-Bromo-N-(3-(4-hydroxybutyl)-4-methoxynaphthalen-1-yl)-benzenesulfonamide (**45**).* Synthesized using the procedure for **49f** except using **49e** and 4-bromobenzenesulfonyl chloride, which afforded the title compound (226 mg, 49% over two steps) as a light-pink solid. HPLC (method A, t_R = 6.94 min), purity 96%. ^1H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 7.99−7.91 (m, 2H), 7.70 (d, *J* = 8.43 Hz, 2H), 7.54 (d, *J* = 8.43 Hz, 2H), 7.49 (t, *J* = 7.34 Hz, 1H), 7.40 (t, *J* = 7.34 Hz, 1H), 6.81 (s, 1H), 4.33 (t, *J* = 5.42 Hz, 1H), 3.78 (s, 3H), 3.37 (q, *J* = 5.42 Hz, 2H), 2.59 (t, *J* = 7.01 Hz, 2H), 1.49−1.28 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.35, 139.45, 132.62, 130.46, 130.42, 129.33, 128.54, 128.24, 126.97, 126.82, 126.78, 126.02, 124.16, 122.21, 62.36, 60.92, 32.66, 28.85, 26.90. ESI MS: *m/z* 464.1, 466.1 $(M + H)^+$, 486.1, 488.1 $(M + Na)^+$. ESI HRMS: *m/z* 462.0378 $(M - H)^-$.

*N-(3-(((2H-Tetrazol-5-yl)methyl)thio)-4-methoxynaphthalen-1-yl)-4-bromobenzenesulfonamide (**49cc**).* Synthesized using a reported procdure.[72] To a suspension of **42** (127 mg, 0.26 mmol) and sodium azide (255 mg, 3.9 mmol) in dry CH_3CN (5 mL) in a glass tube was added $SiCl_4$ (0.15 mL, 1.3 mmol) via syringe. The tube was sealed, and the stirring reaction mixture was heated to 80 °C for 15 h. The reaction was quenched with 2 M Na_2CO_3. The solution was then washed with EtOAc (10 mL × 2). The aqueous phase was acidified with 1 N HCl, and the mixture was extracted with EtOAc (15 mL × 5). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **49cc** (66 mg, 50%) as a tan solid. The crude was used in the next step without further purification. ^1H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 7.93 (d, *J* = 8.40 Hz, 1H), 7.83 (d, *J* = 8.40 Hz, 1H), 7.65 (d, *J* = 8.04 Hz, 2H), 7.55−7.48 (m, 3H), 7.44−7.38 (m, 1H), 7.14 (s, 1H), 6.53 (s, 1H), 4.42 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.77, 139.46, 132.64, 130.46, 129.17, 129.07, 128.36, 127.58, 127.06, 126.23, 123.97, 122.53, 122.22, 61.68, 25.09. ESI MS: *m/z* 505.8, 507.8 $(M + H)^+$, 527.8, 529.8 $(M + Na)^+$.

*N-(3-(((2H-Tetrazol-5-yl)methyl)thio)-4-methoxynaphthalen-1-yl)-4-phenoxybenzenesulfonamide (**49dd**).* Synthesized using the procedure for **49cc** except using **49aa** as the starting material which afforded the title compound (128 mg, 95%) as a tan solid. ^1H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.48−7.43 (m, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.17 (s, 1H), 7.01−6.94 (m, 4H), 4.44 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.90, 155.45, 153.57, 134.18, 130.75, 130.55, 129.76, 129.50, 128.35, 127.49, 126.94, 126.14, 125.17, 124.15, 122.62, 122.16, 120.13, 118.27, 61.66, 25.13. ESI MS: *m/z* 517.9 $(M - H)^-$.

*Methyl 3-((1-Methoxy-4-(naphthalene-2-sulfonamido)-naphthalen-2-yl)thio)propanoate (**49ee**).* Synthesized using the procedure for **49f** except using **49b** and 2-naphthalenesulfonyl chloride, which afforded the title compound (180 mg, 59% over two steps) as a light-pink solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.98 (d, *J* = 8.39 Hz, 1H), 7.90−7.85 (m, 2H), 7.85−7.80 (m, 2H), 7.80−7.76 (m, 1H), 7.59 (t, *J* = 7.36 Hz, 1H), 7.53 (t, *J* = 7.36 Hz, 1H), 7.43 (t, *J* = 7.39 Hz, 1H), 7.34 (t, *J* = 7.39 Hz, 1H), 7.19 (s, 1H), 6.98 (s, 1H), 3.89 (s, 3H), 3.64 (s, 3H), 2.94 (t, *J* = 7.23 Hz, 2H), 2.40 (t, *J* = 7.23 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.84, 153.85, 135.99, 134.83, 131.94, 129.66, 129.43, 129.20, 129.02, 128.91, 128.75, 127.82, 127.78, 127.54, 126.95, 126.72, 125.21, 123.73, 122.39, 122.19, 109.99, 61.20, 51.79, 34.05, 27.65. ESI MS: *m/z* 481.9 $(M + H)^+$, 503.9 $(M + Na)^+$.

*2-((4-(4-Bromophenylsulfonamido)-1-methoxynaphthalen-2-yl)-thio)acetic Acid (**40**).* To a solution of **41** (425 mg, 0.85 mmol) in dry THF (2 mL) was added 1N aqueous LiOH (4 mL). The mixture was stirred at room temperature under nitrogen for 1 h. Reaction mixture was diluted with water (10 mL) and washed with EtOAc (10 mL × 2). Aqueous phase was acidified with 1 N HCl and extracted with EtOAc (10 mL × 3). Combined organic extracts were washed with brine, dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure. Crude was triturated with cold CH_2Cl_2 to give **40** (305 mg, 74%) as a white/tan solid. HPLC (method A, t_R = 6.85 min), purity >99%. ^1H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.92 (d, *J* = 8.38 Hz, 1H), 7.87 (d, *J* = 8.38 Hz, 1H), 7.68 (d, *J* = 8.08 Hz, 2H), 7.57 (d, *J* = 8.08 Hz, 2H), 7.51 (t, *J* = 7.56 Hz, 1H), 7.40 (t, *J* = 7.56

Hz, 1H), 7.09 (s, 1H), 3.84 (s, 3H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.59, 152.07, 139.60, 132.69, 129.83, 129.18, 129.13, 128.28, 127.54, 127.09, 126.57, 124.60, 124.56, 124.01, 121.86, 61.30, 34.63. ESI HRMS: m/z 479.9578 (M − H)$^-$.

*2-((4-([1,1′-Biphenyl]-4-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetic Acid (**43**).* Synthesized using the procedure for **40** except **49n** was used as the starting material. Crude was triturated with cold CH_2Cl_2 to give **43** (88 mg, 70%) as an white solid. HPLC (method A, t_R = 7.22 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 10.21 (s, 1H), 7.95 (d, J = 8.44 Hz, 1H), 7.91 (d, J = 8.44 Hz, 1H), 7.79−7.70 (m, 4H), 7.68−7.61 (m, 2H), 7.53−7.48 (m, 1H), 7.45 (t, J = 7.28 Hz, 2H), 7.39 (t, J = 7.28 Hz, 2H), 7.08 (s, 1H), 3.83 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.52, 151.84, 144.73, 139.09, 138.87, 129.90, 129.50, 128.93, 128.26, 127.89, 127.78, 127.49, 126.47, 124.56, 124.21, 124.18, 121.77, 61.26, 34.56. ESI HRMS: m/z 478.0787 (M − H)$^-$.

*2-((4-([1,1‴-Biphenyl]-3-ylsulfonamido)-1-methoxynaphthalen-2-yl)thio)acetic Acid (**44**).* Synthesized using the procedure for **40** except **49o** was used as the starting material. Crude was triturated with cold CH_2Cl_2 to give **44** (152 mg, 59%) as a white solid. HPLC (method A, t_R = 7.20 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 10.20 (s, 1H), 7.90 (d, J = 8.46 Hz, 1H), 7.86 (d, J = 8.46 Hz, 1H), 7.82 (d, J = 7.81 Hz, 1H), 7.72 (s, 1H), 7.63 (d, J = 7.81 Hz, 1H), 7.55 (t, J = 7.74 Hz, 1H), 7.50−7.44 (m, 1H), 7.43−7.38 (m, 4H), 7.38−7.30 (m, 2H), 7.11 (s, 1H), 3.82 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.46, 151.97, 141.49, 140.71, 138.92, 131.42, 130.33, 129.80, 129.49, 129.43, 128.66, 128.24, 127.47, 127.14, 126.43, 125.88, 125.21, 124.64, 124.59, 124.04, 121.77, 61.23, 34.56. ESI HRMS: m/z 478.0788 (M − H)$^-$.

A Representative Procedure for a Single-Pot Ester Hydrolysis and Demethylation with BBr_3. *2-((1-Hydroxy-4-(thiophene-2-sulfonamido)naphthalen-2-yl)thio)acetic Acid (**1**).* To a stirred solution of **49f** (48 mg, 0.12 mmol) suspended in dry CH_2Cl_2 (1.5 mL) was added BBr_3 (1 M in CH_2Cl_2, 0.5 mL, 0.5 mmol) dropwise at 0 °C under nitrogen. The mixture was allowed to warm up to room temperature. After 1 h of stirring, the starting material was entirely consumed and the product formed as determined by TLC and MS (ESI$^-$). The mixture was slowly added to a stirred solution of saturated aqueous NH_4Cl (20 mL) at 0 °C. The solution was extracted with EtOAc (15 mL × 2). The combined organic extracts were washed with brine (15 mL), dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure. The crude was purified using a C_{18} reverse phase semipreparative HPLC column with solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH_3CN) as eluents to give **1** (**59**) (28 mg, 59%) as a white/tan solid. HPLC (method A, t_R = 6.05 min), purity 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.17−8.14 (m, 1H), 7.89−7.84 (m, 2H), 7.52−7.40 (m, 2H), 7.36 (dd, J = 1.32, 3.74 Hz, 1H), 7.09 (s, 1H), 7.07 (dd, J = 3.74, 4.97 Hz, 1H), 3.56 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.54, 152.87, 140.58, 133.57, 132.80, 131.71, 129.80, 128.03, 127.20, 126.22, 125.46, 124.33, 123.61, 122.83, 113.36, 37.25. ESI HRMS: m/z 393.9870 (M − H)$^-$.

*2-((1-Hydroxy-4-(methylsulfonamido)naphthalen-2-yl)thio)-acetic Acid (**2**).* Synthesized using the procedure for **1** except **49t** was used as the starting material. The title compound (27 mg, 48%) as a white solid after HPLC purification. HPLC (method A, t_R = 5.14 min), purity 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.20 (d, J = 8.09 Hz, 1H), 8.14 (d, J = 8.09 Hz, 1H), 7.56 (p, J = 6.86 Hz, 2H), 7.47 (s, 1H), 3.69 (s, 2H), 2.99 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.73, 152.58, 131.89, 129.38, 127.31, 126.30, 125.65, 125.18, 124.32, 122.82, 113.55, 39.79, 37.03. ESI HRMS: m/z 326.0164 (M − H)$^-$.

*2-((1-Hydroxy-4-(phenylsulfonamido)naphthalen-2-yl)thio)acetic Acid (**3**).* Synthesized using the procedure for **1** except **49h** was used as the starting material. The title compound (21 mg, 22%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.15 min), purity 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 9.91 (s, 1H), 9.79 (s, 1H), 8.14 (d, J = 8.22 Hz, 1H), 7.88 (d, J = 8.22 Hz, 1H), 7.67−7.61 (m, 2H), 7.61−7.56 (m, 1H), 7.54−7.47 (m, 3H), 7.46−7.39 (m, 1H), 7.01 (s, 1H), 3.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.46, 152.56, 140.16, 133.03, 131.56, 129.53, 129.44, 127.25, 127.05, 126.17, 125.46, 124.56, 123.76, 122.75, 113.34, 37.16. ESI HRMS: m/z 388.0319 (M − H)$^-$.

*2-((4-(4-Chlorophenylsulfonamido)-1-hydroxynaphthalen-2-yl)-thio)acetic Acid (**9**).* Synthesized using the procedure for **1** except **49i** was used as the starting material. The title compound (48 mg, 20%) was obtained as a white solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method B, t_R = 6.72 min), purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 10.03 (s, 1H), 9.84 (s, 1H), 8.15 (d, J = 8.12 Hz, 1H), 7.85 (d, J = 8.12 Hz, 1H), 7.66−7.59 (m, 2H), 7.59−7.52 (m, 2H), 7.46 (dt, J = 7.28, 14.85 Hz, 2H), 7.05 (s, 1H), 3.54 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.48, 152.72, 139.06, 137.95, 131.47, 129.66, 129.60, 129.19, 127.15, 126.25, 125.48, 124.24, 123.63, 122.84, 113.38, 37.11. ESI HRMS: m/z 421.9930 (M − H)$^-$.

*2-((4-(4-Bromophenylsulfonamido)-1-hydroxynaphthalen-2-yl)-thio)acetic Acid (**10**).* Synthesized using the procedure for **1** except **41** was used as the starting material. The title compound (134 mg, 45%) was obtained as a white solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method A, t_R = 6.53 min), purity 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (s, 1H), 10.05 (s, 1H), 9.87 (s, 1H), 8.14 (d, J = 8.17 Hz, 1H), 7.84 (d, J = 8.17 Hz, 1H), 7.71 (d, J = 8.48 Hz, 2H), 7.54 (d, J = 8.48 Hz, 2H), 7.48 (t, J = 7.03 Hz, 1H), 7.43 (t, J = 7.03 Hz, 1H), 7.04 (s, 1H), 3.53 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.52, 152.74, 139.45, 132.56, 131.45, 129.66, 129.28, 127.18, 126.92, 126.27, 125.48, 124.21, 123.63, 122.86, 113.38, 37.10. ESI HRMS: m/z 465.9418 (M − H)$^-$.

*2-((1-Hydroxy-4-(4-methoxyphenylsulfonamido)naphthalen-2-yl)thio)acetic Acid (**11**).* Synthesized using the procedure for **1** except **49j** was used as the starting material. The title compound (25 mg, 29%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.20 min), purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.10 (d, J = 8.07 Hz, 1H), 7.89 (d, J = 7.78 Hz, 1H), 7.52 (d, J = 8.63 Hz, 2H), 7.49−7.36 (m, 2H), 7.00−6.93 (m, 3H), 3.75 (s, 3H), 3.47 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.47, 162.75, 152.44, 131.81, 131.63, 129.49, 129.37, 127.05, 126.18, 125.48, 124.92, 123.93, 122.75, 114.57, 113.35, 56.02, 37.24. ESI HRMS: m/z 418.0424 (M − H)$^-$.

*2-((4-(3-Bromophenylsulfonamido)-1-hydroxynaphthalen-2-yl)-thio)acetic Acid (**12**).* Synthesized using the procedure for **1** except **49k** was used as the starting material. The title compound (21 mg, 35%) was obtained as a white/tan solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method A, t_R = 6.69 min), purity 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.15 (d, J = 7.56 Hz, 1H), 7.87−7.78 (m, 2H), 7.75 (t, J = 1.78 Hz, 1H), 7.62−7.56 (m, 1H), 7.52−7.39 (m, 3H), 7.01 (s, 1H), 3.52 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.52, 152.92, 142.07, 135.93, 131.78, 131.49, 129.77, 129.59, 127.18, 126.34, 126.28, 125.53, 124.02, 123.58, 122.89, 122.35, 113.43, 37.24. ESI HRMS: m/z 465.9414 (M − H)$^-$.

*2-((4-(2-Bromophenylsulfonamido)-1-hydroxynaphthalen-2-yl)-thio)acetic Acid (**13**).* Synthesized using the procedure for **1** except **49l** was used as the starting material. The title compound (34 mg, 40%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.41 min), purity 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (s, 1H), 10.23 (s, 1H), 9.88 (s, 1H), 8.16−8.10 (m, 1H), 8.07−8.02 (m, 1H), 7.86 (dd, J = 1.52, 7.83 Hz, 1H), 7.78 (dd, J = 1.52, 7.83 Hz, 1H), 7.52−7.46 (m, 3H), 7.42 (td, J = 1.33, 7.60 Hz, 1H), 7.05 (s, 1H), 3.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.42, 152.60, 139.23, 135.82, 134.64, 131.92, 131.77, 129.49, 128.52, 127.18, 126.32, 125.44, 123.98, 123.78, 122.78, 119.92, 113.54, 37.03. ESI HRMS: m/z 465.9408 (M − H)$^-$.

*2-((4-(Benzo[b]thiophene-2-sulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**14**).* Synthesized using the procedure for **1** except **49m** was used as the starting material. The title compound (13 mg, 22%) was obtained as an orange solid after HPLC purification. HPLC (method B, t_R = 7.52 min), purity 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.11 (d, J = 8.14 Hz, 1H), 8.02 (d, J = 8.14

Hz, 1H), 7.89 (t, J = 8.09 Hz, 2H), 7.72 (s, 1H), 7.47 (t, J = 7.56 Hz, 1H), 7.41 (t, J = 7.35 Hz, 2H), 7.38−7.32 (m, 1H), 7.14 (s, 1H), 3.40 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.57, 153.29, 141.31, 141.04, 137.80, 131.73, 129.99, 129.94, 127.65, 127.22, 126.21, 125.86, 125.54, 123.96, 123.54, 123.41, 122.92, 113.33, 37.41. ESI HRMS: m/z 444.0041 (M − H)$^-$.

*2-((4-([1,1′-Biphenyl]-4-ylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**16**).* Synthesized using the procedure for **1** except **49n** was used as the starting material. The title compound (29 mg, 45%) was obtained as a white/tan solid after HPLC purification. HPLC (method B, t_R = 8.81 min), purity 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.11 (d, J = 7.93 Hz, 1H), 7.90 (d, J = 7.93 Hz, 1H), 7.76 (d, J = 7.47 Hz, 2H), 7.72−7.62 (m, 4H), 7.50−7.42 (m, 3H), 7.43−7.36 (m, 2H), 7.02 (s, 1H), 3.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.52, 152.74, 144.57, 139.00, 138.90, 131.65, 129.57, 129.52, 128.92, 128.00, 127.63, 127.46, 127.08, 126.18, 125.55, 124.53, 123.85, 122.81, 113.42, 37.27. ESI HRMS: m/z 464.0630 (M − H)$^-$.

*2-((4-([1,1′-Biphenyl]-3-ylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**17**).* Synthesized using the procedure for **1** except **49o** was used as the starting material. The title compound (48 mg, 34%) was obtained as a white/tan solid after HPLC purification. HPLC (method A, t_R = 6.99 min), purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 9.93 (s, 1H), 9.79 (s, 1H), 8.10 (d, J = 8.25 Hz, 1H), 7.88−7.81 (m, 2H), 7.79 (s, 1H), 7.60−7.50 (m, 2H), 7.50−7.41 (m, 4H), 7.41−7.32 (m, 3H), 7.04 (s, 1H), 3.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.44, 152.66, 141.46, 140.82, 139.01, 131.50, 131.30, 130.18, 129.60, 129.55, 128.66, 127.17, 127.07, 126.23, 126.07, 125.51, 125.26, 124.59, 123.73, 122.81, 113.44, 37.16. ESI HRMS: m/z 464.0632 (M − H)$^-$.

*2-((4-([1,1′-Biphenyl]-2-ylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**18**).* Synthesized using the procedure for **1** except **49p** was used as the starting material. The title compound (20 mg, 43%) was obtained as a white/tan solid after HPLC purification. HPLC (method B, t_R = 8.95 min), purity 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 9.76 (s, 1H), 9.70 (s, 1H), 8.12 (d, J = 8.03 Hz, 1H), 7.95 (d, J = 8.03 Hz, 1H), 7.74 (d, J = 8.30 Hz, 1H), 7.58 (t, J = 7.46 Hz, 1H), 7.52 (t, J = 7.46 Hz, 1H), 7.48−7.41 (m, 1H), 7.41−7.34 (m, 1H), 7.23 (q, J = 4.82, 5.81 Hz, 1H), 7.17 (t, J = 7.46 Hz, 3H), 7.01−6.95 (m, 1H), 6.93 (s, 1H), 6.91 (s, 1H), 3.43 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.57, 152.24, 141.36, 139.92, 138.75, 133.21, 132.68, 131.42, 129.62, 129.37, 128.85, 128.36, 127.55, 127.48, 126.97, 126.21, 125.49, 124.57, 123.79, 122.69, 113.65, 37.07. ESI HRMS: m/z 464.0636 (M − H)$^-$.

*2-((4-(4′-Chloro-[1,1′-biphenyl]-4-ylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**19**).* Synthesized using the procedure for **1** except **49q** was used as the starting material. The title compound (36 mg, 25%) was obtained as a white solid after HPLC purification. HPLC (method B, t_R = 10.23 min), purity >99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 9.96 (s, 1H), 9.81 (s, 1H), 8.10 (d, J = 8.09 Hz, 1H), 7.88 (d, J = 8.09 Hz, 1H), 7.77 (d, J = 8.24 Hz, 2H), 7.72−7.64 (m, 4H), 7.52 (d, J = 8.24 Hz, 2H), 7.47−7.36 (m, 2H), 6.99 (s, 1H), 3.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.45, 152.51, 143.18, 139.25, 137.67, 133.88, 131.56, 129.50, 129.39, 129.27, 128.05, 127.65, 127.09, 126.24, 125.50, 124.54, 123.84, 122.78, 113.44, 37.04. ESI HRMS: m/z 498.0247 (M − H)$^-$.

*2-((4-(2′,4′-Difluoro-[1,1′-biphenyl]-4-ylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**20**).* Synthesized using the procedure for **1** except **49r** was used as the starting material. The title compound (31 mg, 48%) as a white solid after HPLC purification. HPLC (method A, t_R = 7.12 min), purity 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 9.97 (s, 1H), 9.80 (s, 1H), 8.10 (d, J = 8.16 Hz, 1H), 7.86 (d, J = 8.16 Hz, 1H), 7.69 (d, J = 7.72 Hz, 2H), 7.62 (d, J = 7.72 Hz, 2H), 7.59−7.53 (m, 1H), 7.47−7.34 (m, 3H), 7.19 (t, J = 8.31 Hz, 1H), 7.00 (s, 1H), 3.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.46, 152.55, 139.44, 138.77, 132.51 (dd, J = 4.88, 9.80 Hz), 131.58, 129.82 (d, J = 2.86 Hz), 129.50, 127.66, 127.06, 126.23, 125.50, 124.50, 123.81, 122.78, 113.47, 112.73 (dd, J = 3.78, 21.26 Hz), 105.15 (t, J = 26.72 Hz), 37.04. ESI HRMS: m/z 500.0441 (M − H)$^-$.

*2-((1-Hydroxy-4-(4-phenoxyphenylsulfonamido)naphthalen-2-yl)thio)acetic Acid (**21**).* Synthesized using the procedure for **1** except **49s** was used as the starting material. The title compound (18 mg, 13%) was obtained as a white solid after HPLC purification. HPLC (method B, t_R = 10.24 min), purity 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 9.87 (s, 1H), 9.83 (s, 1H), 8.14 (d, J = 8.31 Hz, 1H), 7.86 (d, J = 8.31 Hz, 1H), 7.59 (d, J = 8.75 Hz, 2H), 7.50 (t, J = 7.09 Hz, 1H), 7.44 (t, J = 7.81 Hz, 3H), 7.23 (t, J = 7.09 Hz, 1H), 7.05−6.98 (m, 5H), 3.55 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.53, 160.76, 155.60, 152.58, 134.17, 131.57, 130.75, 129.84, 129.76, 127.06, 126.16, 125.48, 125.07, 124.60, 123.78, 122.80, 120.03, 118.33, 113.38, 37.15. ESI HRMS: m/z 480.0579 (M − H)$^-$.

*2-((4-(4-(4-Fluorophenoxy)phenylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**22**).* Synthesized using the procedure for **1** except **49t** was used as the starting material. The title compound (36 mg, 44%) as a white solid after HPLC purification. HPLC (method A, t_R = 7.17 min), purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 1H), 9.83 (s, 1H), 9.79 (s, 1H), 8.11 (d, J = 8.21 Hz, 1H), 7.83 (d, J = 8.21 Hz, 1H), 7.55 (d, J = 8.46 Hz, 2H), 7.44 (dt, J = 6.92, 21.86 Hz, 2H), 7.25 (t, J = 8.52 Hz, 2H), 7.11−7.02 (m, 2H), 6.99−6.96 (m, 2H), 6.95 (s, 1H), 3.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.53, 161.09, 152.57, 151.54 (d, J = 2.57 Hz), 134.14, 131.59, 129.86, 129.68, 127.08, 126.19, 125.48, 124.61, 123.80, 122.80, 122.17 (d, J = 8.70 Hz), 117.90, 117.45, 117.22, 113.38, 37.17. ESI HRMS: m/z 498.0485 (M − H)$^-$.

*2-((1-Hydroxy-4-(4-phenylpiperazine-1-sulfonamido)-naphthalen-2-yl)thio)acetic Acid (**23**).* Synthesized using the procedure for **1** except **49u** was used as the starting material. The title compound (14.5 mg, 48%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.55 min), purity 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.59 (s, 1H), 8.15 (t, J = 7.91 Hz, 2H), 7.59−7.53 (m, 1H), 7.53−7.48 (m, 2H), 7.20−7.14 (m, 2H), 6.89 (d, J = 7.91 Hz, 2H), 6.77 (t, J = 7.26 Hz, 1H), 3.64 (s, 2H), 3.24−3.16 (m, 4H), 3.14−3.04 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.57, 152.02, 150.84, 131.08, 129.40, 128.56, 127.32, 126.28, 125.52, 125.43, 123.76, 122.95, 120.04, 116.48, 113.63, 48.54, 46.36, 37.06. ESI HRMS: m/z 472.1002 (M − H)$^-$.

*2-((4-(4-Bromobenzamido)-1-hydroxynaphthalen-2-yl)thio)-acetic Acid (**25**).* Synthesized using the procedure for **1** except **49v** was used as the starting material. The title compound was obtained (8.5 mg, 30%) as a white solid after HPLC purification. HPLC (method A, t_R = 6.72 min), purity 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.23 (d, J = 5.36 Hz, 1H), 8.02 (d, J = 8.05 Hz, 2H), 7.83 (d, J = 5.36 Hz, 1H), 7.78 (d, J = 8.05 Hz, 2H), 7.57−7.50 (m, 3H), 3.72 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.74, 165.71, 151.94, 133.90, 131.89, 130.66, 130.30, 128.64, 127.11, 126.17, 126.13, 125.80, 125.47, 123.80, 122.97, 113.65, 37.15. ESI HRMS: m/z 429.9747 (M − H)$^-$.

*2-((4-((4-Chlorophenyl)methylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**26**).* Synthesized using the procedure for **1** except **49w** was used as the starting material. The title compound (15.5 mg, 20%) was obtained as a white solid after HPLC purification. HPLC (method B, t_R = 6.22 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H), 9.84 (s, 1H), 9.55 (s, 1H), 8.20 (d, J = 8.36 Hz, 1H), 8.07 (d, J = 8.36 Hz, 1H), 7.60−7.51 (m, 2H), 7.44 (s, 1H), 7.43−7.38 (m, 4H), 4.49 (s, 2H), 3.71 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.81, 152.35, 133.45, 133.18, 131.79, 129.37, 128.93, 128.72, 127.23, 126.30, 125.63, 124.99, 124.28, 122.79, 113.67, 57.16, 36.96. ESI HRMS: m/z 436.0086 (M − H)$^-$.

*2-((4-(2-(4-Chlorophenyl)acetamido)-1-hydroxynaphthalen-2-yl)thio)acetic Acid (**28**).* Synthesized using the procedure for **1** except **49x** was used as the starting material. The title compound (19 mg, 10%) was obtained as a white solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method B, t_R = 5.77 min), purity 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.23−8.17 (m, 1H), 7.89−7.83 (m, 1H), 7.57−7.50 (m, 3H), 7.47−7.39 (m, 4H), 3.78 (s, 2H), 3.66 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.72, 169.89, 151.36, 135.73, 131.66, 131.49, 129.70, 128.69, 127.15, 127.00, 126.11, 126.07,

125.40, 123.21, 123.02, 113.51, 42.24, 37.21. ESI HRMS: m/z 400.0417 $(M - H)^-$.

*2-((1-Hydroxy-4-(2-phenylacetamido)naphthalen-2-yl)thio)-acetic Acid (**29**).* Synthesized using the procedure for **1** except **49y** was used as the starting material. The title compound (50 mg, 61%) was obtained as a white solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method A, t_R = 6.07 min), purity 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 1H), 9.97 (s, 1H), 9.66 (s, 1H), 8.26−8.12 (m, 1H), 7.92−7.77 (m, 1H), 7.59−7.47 (m, 3H), 7.42 (d, J = 7.64 Hz, 2H), 7.37 (t, J = 7.40 Hz, 2H), 7.31−7.24 (m, 1H), 3.77 (s, 2H), 3.67 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.73, 170.25, 151.26, 136.74, 129.70, 129.58, 128.76, 127.10, 126.97, 126.92, 126.19, 126.06, 125.38, 123.22, 122.99, 113.49, 43.09, 37.13. ESI HRMS: m/z 366.0802 $(M - H)^-$.

*4-Bromo-N-(3-(butylthio)-4-hydroxynaphthalen-1-yl)-benzenesulfonamide (**31**).* Synthesized using the procedure for **1** except **49z** was used as the starting material. The title compound (10 mg, 8%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 8.44 min), purity 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 7.40 Hz, 1H), 7.79 (d, J = 7.40 Hz, 1H), 7.59−7.50 (m, 4H), 7.50−7.45 (m, 1H), 7.37 (s, 1H), 7.26 (s, 1H), 6.60 (s, 1H), 3.49 (s, 1H), 2.65 (t, J = 7.24 Hz, 2H), 1.52−1.45 (m, 2H), 1.45−1.35 (m, 2H), 0.90 (t, J = 7.24 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.04, 138.33, 132.18, 131.86, 131.23, 128.98, 128.09, 127.90, 126.29, 123.80, 123.47, 122.92, 122.16, 111.25, 36.61, 31.70, 21.74, 13.60. ESI HRMS: m/z 463.9998 $(M - H)^-$.

*Methyl 2-((4-(4-Bromophenylsulfonamido)-1-hydroxynaphthalen-2-yl)thio)acetate (**32**).* To a suspension of **41** (75 mg, 0.15 mmol) in dry CH_2Cl_2 (1.5 mL) was added BBr_3 (1 M in CH_2Cl_2, 0.4 mL, 0.4 mmol) dropwise at 0 °C. The mixture was allowed to warm up to room temperature and stirred under nitrogen. The starting material was entirely consumed as determined by TLC and analytical HPLC after 30 min. The mixture was again cooled down to 0 °C and MeOH (2 mL) was added. After addition, the mixture was allowed to warm up to room temperature and stirred for 1 h when a new spot formed as monitored by TLC. The mixture was slowly added to a stirring solution of saturated aqueous NH_4Cl (15 mL) at 0 °C. The solution was extracted with EtOAc (15 mL × 2). The combined organic extracts were washed with brine (15 mL), dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure. The crude was purified using a C_{18} reverse phase semipreparative HPLC column with solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH_3CN) as eluents to give **32** (31 mg, 43%) as a white solid. HPLC (method A, t_R = 7.45 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.90 (s, 1H), 8.15 (d, J = 8.22 Hz, 1H), 7.85 (d, J = 8.22 Hz, 1H), 7.73 (d, J = 8.58 Hz, 2H), 7.55 (d, J = 8.58 Hz, 2H), 7.52−7.41 (m, 2H), 6.99 (s, 1H), 3.60 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.21, 152.67, 139.44, 132.58, 131.45, 129.39, 129.23, 127.20, 126.93, 126.30, 125.51, 124.25, 123.67, 122.85, 113.07, 52.64, 36.18. ESI HRMS: m/z 479.9586 $(M - H)^-$.

*2-((4-(4-Bromophenylsulfonamido)-1-hydroxynaphthalen-2-yl)-thio)acetamide (**33**).* Synthesized using the procedure for **1** except **41** was used as the starting material. The title compound (24 mg, 42%) as a yellow solid after trituration with a mixture of CH_3CN:H_2O 1:1 and cold CH_2Cl_2 and without a need for HPLC purification. HPLC (method B, t_R = 9.37 min), purity 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.98 (s, 1H), 8.14 (d, J = 8.15 Hz, 1H), 7.87−7.78 (m, 2H), 7.69 (d, J = 8.16 Hz, 2H), 7.56−7.48 (m, 3H), 7.44 (p, J = 6.86 Hz, 2H), 6.98 (s, 1H), 3.47 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.00, 154.82, 139.41, 132.55, 132.15, 131.51, 129.34, 127.54, 126.92, 126.20, 125.68, 123.58, 123.23, 112.74. ESI HRMS: m/z 464.9591 $(M - H)^-$.

*4-Bromo-N-(4-hydroxy-3-(3-hydroxypropyl)naphthalen-1-yl)-benzenesulfonamide (**34**).* Synthesized using the procedure for **1** except **49aa** was used as the starting material. The title compound (16 mg, 32%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.48 min), purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.17 (s, 1H), 8.11 (d, J = 8.34 Hz, 1H), 7.82 (d, J = 8.34 Hz, 1H), 7.68 (d, J = 8.50 Hz, 2H), 7.49 (d, J = 8.50 Hz, 2H), 7.38 (t, J = 7.23 Hz, 1H), 7.31 (t, J = 7.23 Hz, 1H), 6.73 (s, 1H), 3.34 (t, J = 6.35 Hz, 2H), 2.61 (t, J = 7.48 Hz, 2H), 1.54 (p, J = 6.68 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.29, 139.59, 132.49, 130.41, 129.31, 128.21, 126.80, 126.16, 125.66, 125.40, 123.69, 123.54, 122.46, 122.39, 60.51, 33.15, 26.18. ESI HRMS: m/z 434.0063 $(M - H)^-$.

*4-Bromo-N-(4-hydroxy-3-(4-hydroxybutyl)naphthalen-1-yl)-benzenesulfonamide (**35**).* Synthesized using the procedure for **1** except **45** was used as the starting material. The title compound (49 mg, 34%) was obtained as a white solid after HPLC purification. HPLC (method B, t_R = 9.60 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 9.13 (s, 1H), 8.12 (d, J = 8.17 Hz, 1H), 7.87 (d, J = 8.17 Hz, 1H), 7.69 (d, J = 8.49 Hz, 2H), 7.51 (d, J = 8.49 Hz, 2H), 7.39 (t, J = 7.31 Hz, 1H), 7.33 (t, J = 7.31 Hz, 1H), 6.67 (s, 1H), 4.36 (s, 1H), 3.42−3.33 (m, 2H), 2.58 (t, J = 5.70 Hz, 2H), 1.45−1.26 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.19, 139.58, 132.50, 130.54, 129.39, 128.16, 126.78, 126.20, 125.66, 125.39, 123.64, 123.61, 122.58, 122.46, 61.16, 32.47, 29.39, 26.67. ESI HRMS: m/z 448.0223 $(M - H)^-$.

*N-(3-(((2H-Tetrazol-5-yl)methyl)thio)-4-hydroxynaphthalen-1-yl)-4-bromobenzenesulfonamide (**36**).* Synthesized using the procedure for **1** except **49cc** was used as the starting material. The title compound (20 mg, 42%) was obtained as a white solid after HPLC purification. HPLC (method A, t_R = 6.45 min), purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 8.13 (d, J = 8.04 Hz, 1H), 7.75 (d, J = 8.04 Hz, 1H), 7.64 (d, J = 8.62 Hz, 2H), 7.49−7.43 (m, 3H), 7.42−7.36 (m, 1H), 6.91 (s, 1H), 4.25 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.78, 139.47, 132.51, 131.79, 130.43, 129.21, 127.45, 126.87, 126.27, 125.59, 124.10, 123.57, 123.08, 26.54. ESI HRMS: m/z 489.9647 $(M - H)^-$.

*N-(3-(((2H-Tetrazol-5-yl)methyl)thio)-4-hydroxynaphthalen-1-yl)-4-phenoxybenzenesulfonamide (**37**).* Synthesized using the procedure for **1** except **49dd** was used as the starting material. The title compound (32 mg, 53%) as a white solid after HPLC purification. HPLC (method A, t_R = 6.88 min), purity 96%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.51−7.46 (m, 1H), 7.44−7.38 (m, 3H), 7.20 (t, J = 7.4 Hz, 1H), 6.99−6.94 (m, 5H), 4.28 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.70, 155.63, 153.61, 134.27, 131.88, 130.73, 130.54, 129.77, 127.33, 126.16, 125.60, 125.06, 124.51, 123.71, 123.04, 119.98, 118.35, 111.64, 26.59. ESI MS: m/z 503.9 $(M - H)^-$.

*3-((1-Hydroxy-4-(naphthalene-2-sulfonamido)naphthalen-2-yl)-thio)propanoic Acid (**38**).* Synthesized using the procedure for **1** except **49ee** was used as the starting material. The title compound (23 mg, 15%) was obtained as a white solid after HPLC purification. HPLC (method B, t_R = 7.08 min), purity >99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.01 (s, 1H), 9.60 (s, 1H), 8.23 (s, 1H), 8.14−8.06 (m, 2H), 8.06−7.97 (m, 3H), 7.80 (d, J = 8.52 Hz, 1H), 7.68 (t, J = 7.36 Hz, 1H), 7.60 (t, J = 7.36 Hz, 1H), 7.43 (p, J = 6.84 Hz, 2H), 6.87 (s, 1H), 2.62 (t, J = 6.83 Hz, 2H), 2.17 (t, J = 6.83 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.97, 152.44, 137.07, 134.56, 131.90, 131.48, 129.68, 129.51, 129.23, 129.20, 128.33, 128.20, 128.00, 126.94, 126.22, 125.51, 124.61, 123.96, 122.93, 122.70, 113.33, 34.12, 29.59. ESI HRMS: m/z 452.0630 $(M - H)^-$.

*2-((1-Acetoxy-4-(4-bromophenylsulfonamido)naphthalen-2-yl)-thio)acetic Acid (**46**).* Synthesized using a reported procedure.[73] A stirred solution of **10** (50 mg, 0.11 mmol) in dry THF (1.5 mL) was cooled to 0 °C. Et_3N (31 μL, 0.22 mmol) was added, and the mixture was stirred for 5 min before acetyl chloride (10 μL, 0.14 mmol) was added at 0 °C. The mixture was stirred under nitrogen for an additional 20 min then diluted with EtOAc (10 mL) and washed with H_2O (10 mL × 3). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude was subjected to flash column chromatography (hexane/EtOAc 4:1) on silica gel to afford **46** (34 mg, 61%) as a white solid. HPLC (method A, t_R = 7.87 min), purity 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 8.10 Hz, 1H), 7.96 (d, J = 8.48 Hz, 2H), 7.76 (d, J = 8.10 Hz, 1H), 7.71 (d, J = 8.48 Hz, 2H), 7.69−7.59 (m, 2H), 7.37 (s, 1H), 3.63 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.13, 161.35, 147.04, 137.50, 132.14, 131.33, 131.18, 129.73, 129.46, 128.87, 128.46, 126.94, 125.55,

122.76, 121.64, 114.58, 28.48, 24.29. ESI HRMS: m/z 507.9533 $(M - H)^-$.

Fluorescence Polarization-Based Binding Assays. Sensitive and quantitative FP-based binding assays were developed and optimized to determine the binding affinities of small-molecule inhibitors to the recombinant Mcl-1, A1/Bfl-1, Bcl-w, Bcl-2, and Bcl-xL proteins. Protocols for expression and purification of used antiapoptotic proteins and determination of K_d values of fluorescent probes to proteins are provided in the Supporting Information. On the basis of the K_d values, the concentrations of the proteins used in the competitive binding experiments were 90 nM for Mcl-1, 40 nM for Bcl-w, 50 nM for Bcl-xL, 60 nM for Bcl-2, and 4 nM for A1/Bfl-1. The fluorescent probes, Flu-BID and FAM-BID, were fixed at 2 nM for all assays except for A1/Bfl-1, where FAM-BID was used at 1 nM. Then 5 μL of the tested compound in DMSO and 120 μL of protein/probe complex in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 μg/mL bovine gamma globulin; 0.02% sodium azide, purchased from Invitrogen, Life Technologies, supplemented with 0.01% Triton X-100) were added to assay plates (Microfluor 2Black, Thermo Scientific), incubated at room temperature for 3 h, and the polarization values (mP) were measured at an excitation wavelength at 485 nm and an emission wavelength at 530 nm using the plate reader Synergy H1 Hybrid, BioTek. IC_{50} values were determined by nonlinear regression fitting of the competition curves (GraphPad Prism 6.0 Software). The K_i values were calculated as described previously.[77]

Solution Competitive Surface Plasmon Resonance Based Assay Using Immobilized Biotin-Labeled Bim BH3 Peptide. The solution competitive SPR-based assay was performed on Biacore 2000. N terminal biotin-labeled Bim BH3 peptide (141−166 amino acids) was immobilized on streptavidin (SA) chip giving density of 1400 RU (Response Units). The preincubated Mcl-1 protein (20 nM) with tested small-molecule inhibitors for at least 30 min was injected over the surfaces of the chip. Response units were measured at 15 s in the dissociation phase, and the specific binding was calculated by subtracting the control surface (Fc1) signal from the surfaces with immobilized biotin-labeled Bim BH3. IC_{50} values were determined by nonlinear least-squares analysis using GraphPad Prism 6.0 software.

Molecular Modeling. Crystal structure of Mcl-1 in complex with mNoxa BH3 peptide (PDB entry 2NLA) and in silico Schrödinger's IFD were used to model the binding poses of our designed compounds with Mcl-1. IFD is allowing incorporation of the protein and ligand flexibility in the docking protocol, which consists of the following steps: (i) constrained minimization of the protein with an RMSD cutoff of 0.18 Å, (ii) initial Glide docking of the ligand using a softened potential (van der Waals radii scaling), (iii) one round of Prime side chain prediction for each protein/ligand complex, on residues within defined distance of any ligand pose, (iv) prime minimization of the same set of residues and the ligand for each protein/ligand complex pose, (v) Glide redocking of each protein/ligand complex structure within a specified energy of the lowest energy structure, (vi) estimation of the binding energy (IFDScore) for each output pose. All docking calculations were run in the extra precision (XP) mode of Glide. The center of the grid box of the Mcl-1 was defined by the Val 249 (in h1), Phe 270 (in h2), Val 220 (in h3/h4), and Val 216 (in h4). The size of the grid box was set to 15 Å. Default values were used for all other parameters. Schrödinger's MC/SD dynamic simulation performs constant temperature calculations that take advantage of the strengths of Monte Carlo methods for quickly introducing large changes in a few degree of freedom and stochastic dynamics for its effective local sampling of collective motions. The MC/SD dynamic simulation time in our study was set to 100 ps by allowing movement of the docked ligand and the residues, which is less than 6 Å to the ligand. The force field used was set to OPLS_2001. Default values were used for all other parameters.

NMR Studies. ^{15}N-labeled or ^{15}N, ^{13}C-labeled Mcl-1 proteins for NMR studies were prepared and purified using the same protocol as for unlabeled protein with the exception that the bacteria were grown on M9 minimal media supported with 3 g/L of ^{13}C-glucose and/or 1 g/L of $(^{15}NH_4)_2SO_4$. Protein samples were prepared in a 20 mM sodium phosphate, 150 mM NaCl, and 1 mM DTT solution at pH 7 in 7% D_2O. The binding mode of the compounds has been characterized by recording $^1H,^{15}N$ -HSQC experiments with a 138 μL solution of uniformly ^{15}N-labeled Mcl-1 (75 μM) in the absence and presence of added compounds with the indicated molar ratio concentrations. All spectra were acquired at 30 °C on a Bruker 600 MHz NMR spectrometer equipped with a cryogenic probe, processed using Bruker TopSpin and rNMR,[84] and were analyzed with Sparky.[85] Plots of chemical shift changes were calculated as $((\Delta^1H\ ppm)^2 + (0.2(\Delta^{15}N\ ppm))^2)^{0.5}$ of Mcl-1 amide upon addition of compound. The absence of a bar in a chemical shift plot indicates no chemical shift difference or the presence of a proline or residue that is overlapped or not assigned.

Biotin−Streptavidin Pull-Down Experiment. Human breast cancer 2LMP cells, a subclone of the MDA-MB-231 cell line, were lysed in CHAPS buffer (10 mM HEPES (pH 7.4), 2.5 mM EDTA, 150 mM NaCl, 1.0% CHAP). Precleared cell lysates were incubated with different concentrations of compounds followed by incubation with biotinylated Noxa BH3 peptide (18−43) and streptavidin−agarose beads to pull-down Mcl-1 protein bound to Noxa peptide. Beads were washed with CHAPS buffer, and Mcl-1 protein was eluted by boiling in SDS-PAGE sample buffer and analyzed by Western blotting using Mcl-1 antibody (Santa Cruz).

Cell Culture and Cell Viability Assays. MEFs cells, wild-type and Bax/Bak double knockout, were gifts from Shaomeng Wang at the University of Michigan and were cultured in DMEM (Life Technologies), supplemented with 10% fetal bovine serum (FBS) (Thermo Scientific HyClone). The retroviral transduced lymphoma cells isolated from Eμ-myc transgenic mice were gifts from Ricky W. Johnstone at University of Melbourne, Melbourne, Australia, and cultured as previously described.[81] Human leukemia cell lines HL-60, K-562, and MV-4−11 were obtained from American Type Culture Collection (ATCC). The cells were cultured in RPMI 1640 medium (Life Technologies), all supplemented with 10% FBS. MEFs cells were seeded in 12-well plates at 0.5×10^6 cells/well, left to adhere, and then treated for 15 h with increasing concentrations of the compounds. The cells were harvested, washed with phosphate-buffered saline (PBS), and stained with 0.025 mg/mL propidium iodide (MP Biomedicals). The percentage of propodium iodide positive population was determined by flow cytometry and calculated using WinList 3.0. The Mcl-1 and Bcl-2 retroviral transduced lymphoma Eμ-myc cells were seeded in 24-well plates at 0.5×10^6 cells/well. They were treated with different concentrations of tested compounds for 15−18 h. The cells were harvested and stained with violet LIVE/DEAD fixable dead cell stain kit (Invitrogen) according to manufacturer's protocol. The percentage of fluorescent positive cells was determined by flow cytometry and calculated using WinList 3.0. The effect of the compounds on tested leukemia cells viability was evaluated by CellTiter Glo luminescent cell viability assay (Promega). Cells were plated in 12-well plates at 0.5×10^6 cells/well and treated with various concentrations of the compounds and incubated for 3 days. Cell viability was determined by measuring intracellular ATP levels with the CellTiter Glo reagent and reading the luminescence with the Synergy H1 Hybrid BioTek plate reader. Percent cell growth was calculated relative to DMSO treated cells, and IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism 6.0 Software.

Determination of Caspase Activity. HL-60 cells were seeded in 12-well plates at 0.5×10^6 cells/well. After 20 h treatment with different concentrations of the compounds, caspase 3 activity was determined using the fluorometric substrates DEVD-AFC following the protocol of the Caspase-3 fluorometric assay kit (BioVision). Caspase 3 activity is reported as the fold change relative to DMSO treated cells.

Analysis of Apoptosis. To determine the induction of apoptosis, HL-60 cells were plated in 12-well plates at 0.5×10^6 cells/well and treated with various concentrations of tested compounds. After 20 h, cells were harvested, washed with PBS, and treated with annexin-V FITC and propidium iodide using BD annexin V FITC assay kit (BD Biosciences Pharmingen). The percentage of cells undergoing apoptosis was assessed by flow cytometry within 1 h and analyzed using WinList 3.0. Apoptosis was also determined in the presence of

Z-VAD-FMK (Bachem), pan caspase-inhibitor. For this purpose sells were pretreated with 100 μM Z-VAD-FMK for about 1 h before adding tested compounds.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details for synthesis of analogues **24**, **27**, and **30** and intermediates, protein expression and purification, fluorescence polarization-based binding assay, and additional HSQC chemical shift perturbation plots and apoptosis studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Bcl-2, B-cell lymphoma-2; BH, Bcl-2 homology; DKO, double knockout; FP, fluorescence polarization; IFD, induced fit docking; MEF, murine embryonic fibroblast; Mcl-1, myeloid cell leukemia sequence 1; PI, propidium iodide; SPR, surface plasmon resonance. Solvent and reagent abbreviations used:; CH_3CN, acetonitrile; NH_4Cl, ammonium chloride; NH_4OH, ammonium hydroxide; $(NH_4)_2SO_4$, ammonium sulfate; Dppf, 1,1′-bis(diphenylphosphino)ferrocene; $Pd(PPh_3)_2Cl_2$, bis-(triphenylphosphine)palladium(II) dichloride; BBr_3, boron tribromide; Cs_2CO_3, cesium carbonate; CuI, copper(I) iodide; CH_2Cl_2, dichloromethane; DTT, dithiothreitol; EtOAc, ethyl acetate; EtOH, ethanol; HCl, hydrogen chloride; LiOH, lithium hydroxide; $MgSO_4$, magnesium sulfate; MeOH, methanol; NMP, *N*-methyl-2-pyrrolidone; $Pd(OAc)_2$, palladium acetate; $NaHCO_3$, sodium bicarbonate; Na_2CO_3, sodium carbonate; NaCl, sodium chloride; NaOH, sodium hydroxide; $Na_2S_2O_3$, sodium thiosulfate; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)-palladium(0); THF, tetrahydrofuran; Et_3N, triethylamine; TFA, trifluoroacetic acid; $Pd_2(dba)_3$, tris(dibenzylideneacetone)-dipalladium(0); $ZnCl_2$, zinc chloride; Z-VAD-FMK, Z-Val-Ala-DL-Asp-fluoromethylketone

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