Published in final edited form as:
Pharmacol Ther. 2015 January ; 145: 76–84. doi:10.1016/j.pharmthera.2014.08.003.

Small molecule Mcl-1 inhibitors for the treatment of cancer

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Abstract

The Bcl-2 family of proteins serves as primary regulators of apoptosis. Myeloid cell leukemia 1 (Mcl-1), a pro-survival member of the Bcl-2 family of proteins, is overexpressed and the Mcl-1 gene is amplified in many tumor types. Moreover, the overexpression of Mcl-1 is the cause of resistance to several chemotherapeutic agents. Thus, Mcl-1 is a promising cancer target. This review highlights the current progress on the discovery of small molecule Mcl-1 inhibitors.

Keywords

Mcl-1; myeloid cell leukemia-1; inhibitors; small molecule; BH3-mimetic

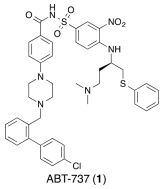
1. Introduction

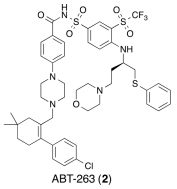
Apoptosis is a natural process for eliminating unwanted or damaged cells that represent a threat to the health of an organism. This process is highly regulated, and the B-cell lymphoma-2 (Bcl-2) family of proteins serve as the main regulators. Indeed, dysregulation and evasion of apoptosis is one of the hallmarks of cancer.(Hanahan & Weinberg, 2000, 2011)

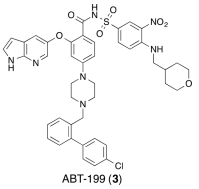
Members of the Bcl-2 family proteins share conserved sequences in regions known as Bcl-2 homology (BH) domains (BH1–BH4).(Korsmeyer, 1999; Pang, et al., 2012) Members within the same family can have opposite effects. The anti-apoptotic or pro-survival proteins, including Bcl-2, Bcl-X_L, Bcl-w, Bfl-1/A1 and Mcl-1,(Adams & Cory, 2007) keep cells alive; whereas, the pro-apoptotic proteins (e.g., Bim, tBid, Bad, Puma, Noxa, Bak, and Bax)(Youle & Strasser, 2008) promote cell death. The relative levels of the anti- and pro-apoptotic proteins govern whether a cell will live or die. Recently, much has been learned about how the Bcl-2 proteins regulate apoptosis.(Burlacu, 2003; M. F. van Delft & Huang,

Conflict of Interest Statement
The authors declare that there are no conflicts of interest.

2006; Volkmann, et al., 2014) Upon triggering a death signal, a subset of the pro-apoptotic proteins with homology only in the BH3 region cause Bak and Bax to homo-oligomerize and form pores in the mitochondrial membrane leading to cytochrome c release into the cytosol. This activates the caspase cascade and causes cell death. The anti-apoptotic proteins block cell death by binding and sequestering, with varying specificity the BH3-only proteins.(L. Chen, et al., 2005) The binding specificity and affinity exhibited by the anti-apoptotic proteins for the pro-apoptotic proteins is defined by hydrophobic and electrostatic interactions between the BH3 region of the pro-apoptotic proteins and the binding groove formed by the BH1, BH2 and BH3 regions of the anti-apoptotic proteins.(Dutta, et al., 2010; Moldoveanu, et al., 2014; Sattler, et al., 1997) Of the BH3-only proteins, Bim and Puma are the least selective, binding to all five anti-apoptotic proteins. Bad binds strongly to Bcl-2, Bcl-X_L and Bcl-w; whereas, Noxa binds exclusively to Mcl-1 and Bfl-1/A1. These observations suggest that apoptosis is regulated by the interactions between particular subsets of these proteins and that apoptosis can be initiated by the inhibition of the pro-survival members of the Bcl-2 family proteins. Indeed, this has been demonstrated by the BH3-mimetics ABT-737 (**1**)(Oltersdorf, et al., 2005) and its orally available derivative ABT-263 (**2**; navtioclax)(Tse, et al., 2008) which bind to Bcl-2, Bcl-X_L and Bcl-w. As expected, ABT-737 and ABT-263 induce apoptosis in tumor cells that are dependent on Bcl-2 and Bcl-X_L. More recently, a selective Bcl-2 inhibitor was discovered (ABT-199) that also demonstrates the utility of inhibitors of the Bcl-2 family.(Souers, et al., 2013) Indeed, Navitoclax and ABT-199 have shown efficacy in several clinical trials in patients with lymphoid malignancies that are believed to be Bcl-2 dependent.(Choo, et al., 2014; Roberts, et al., 2012; Tse, et al., 2008) However, there are some cancers that cannot be treated by these compounds alone. Several studies have shown that upregulation of Mcl-1 is a key factor in the development of resistance to ABT-737 and ABT-263 resistance in several tumor types.(Konopleva, et al., 2006; Tahir, et al., 2007; Mark F van Delft, et al., 2006)


ABT-737 (1)


ABT-263 (2)


ABT-199 (3)

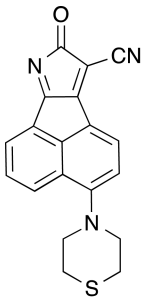
Mcl-1 has a number of functions and features that make it unique among the anti-apoptotic Bcl-2 family members. Mcl-1 is essential for early embryogenesis(Rinkenberger, et al., 2000) as well as the development and maintenance of lymphocytes(Dzhagalov, et al., 2008; Opferman, et al., 2003), neurons(Arbour, et al., 2008), synovial fibroblasts(Liu, et al., 2005) and hematopoietic stem cells(Opferman, et al., 2005). Mcl-1 is also unique in that it has a very short half-life of <1–4 h, depending on cellular conditions(Yang-Yen, 2006), and multiple pathways tightly regulate Mcl-1 transcription, translation, and degradation. (Thomas, et al., 2010) Structurally, Mcl-1's N-terminus also differs from that of the other anti-apoptotic Bcl-2 proteins in that it contains two PEST (proline/glutamic acid/serine/threonine–containing) regions.(Germain & Duronio, 2007) Indeed, the N-terminal region may serve as a regulatory domain for Mcl-1's rate of turnover, localization, and

phosphorylation, and may thus provide a mechanism to rapidly fine-tune the expression of Mcl-1 in response to environmental and cellular input.(Thomas, et al., 2010)

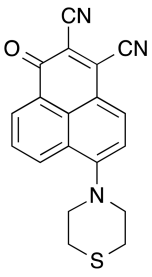
There is a lot of evidence to suggest that Mcl-1 is an important cancer target. For example, Mcl-1 overexpression is one of the most common genetic aberrations observed in human cancer,(Beroukhim, et al., 2010; G. Wei, et al., 2012) including lung(L. X. Song, et al., 2005), breast(Ding, et al., 2007), prostate(Krajewska, et al., 1996), pancreatic(Miyamoto, et al., 1999), ovarian and cervical cancers(Brotin, et al., 2010), as well as melanoma(Boisvert-Adamo, et al., 2009) and leukemia(Andersen, et al., 2005; Derenne, et al., 2002; Kang, et al., 2008). Furthermore, Mcl-1 overexpression induces resistance against the aforementioned Bcl-2-inhibitors, as well as a number of widely used anticancer therapies including paclitaxel,(Wertz, et al., 2011) vincristine(Wertz, et al., 2011) and gemcitabine(S.-H. Wei, et al., 2008). Moreover, RNA-mediated knockdown of Mcl-1 has shown tumor growth inhibition and cell death in Mcl-1 overexpressing lung, colon, ovarian and lymphoma cells. (Akgul, 2008; Boisvert-Adamo, et al., 2009; W. Chen, et al., 2010; Chetoui, et al., 2008; Hauck, et al., 2009; Keuling, et al., 2009; Konopleva, et al., 2006; Lucas, et al., 2012; Moulding, et al., 2000; Qin, et al., 2006; Thallinger, et al., 2003) Silencing of Mcl-1 also restores sensitivity in chemoresistant cells.(Lin, et al., 2007; Meng, et al., 2007; Taniai, et al., 2004) Given these data, Mcl-1 represents a very promising cancer target. An Mcl-1 inhibitor would be expected to be useful as a single agent against cancers that depend on Mcl-1 for survival and in combination with other drugs where Mcl-1 overexpression is the major resistance factor.

This Review focuses on the current state of Mcl-1 inhibitors. Although peptide-based inhibitors have been described, including stapled alpha-helix of Bcl-2 domains (SAHB) (Muppidi, et al., 2012; Stewart, et al., 2010), alpha-/beta-peptide foldamers(Smith, et al., 2013) and reverse BH3 (rBH3) peptides(Placzek, et al., 2011), we focus this review on small molecule inhibitors that have been reported to function as BH3-mimetics. As proposed by Lessene and coworkers true BH3-mimetics should exhibit Bak/Bax-dependant biological activity and high-affinity binding to at least one Bcl-2 family pro-survival protein, specifically Mcl-1 in the case of this review.(Lessene, et al., 2008) Therefore, Obatoclax (GX15-070)(Nguyen, et al., 2007), a putative pan-inhibitor that binds to all Bcl-2 family pro-survival proteins with low affinity(Nguyen, et al., 2007; Tse, et al., 2008) and kills wild-type and Bak/Bax-deficient cells with equal potency(Vogler, et al., 2009), as well as other chemical entities that exert their biological activities through possible off-target or unknown mechanisms of action are not included in this review.(Billard, 2013) In addition, this review does not cover molecular entities disclosed exclusively within patents. This information has been reviewed in detail elsewhere(Bajwa, et al., 2012).

2. S1



S1 (**4**)



Revised S1 (**5**)

Efforts aimed at designing novel DNA intercalating agents led to the discovery of S1 (**4**), a rigid, planar chromophore, which exhibited anti-tumor activity yet surprisingly lacked the ability to intercalate into DNA.(Zhang, et al., 2007) The structure of S1 was originally reported as possessing an 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carbonitrile (**4**) backbone. However, the structure was later revised by Song et al. to a 1-oxo-1*H*-phenalene-2,3-dicarbonitrile (**5**).(T. Song, Chen, et al., 2013) S1 has been touted as a pan-Bcl-2 family inhibitor as it has been reported to bind to Mcl-1 (K_d = 58 nM, Bid-BH3, FPA) and Bcl-2 (K_d = 310 nM, Bid-BH3, FPA), disrupt Bax/Bcl-2 and Bak/Mcl-1 complexes in a dose- and time-dependent manner, and induce Bax/Bak-dependent apoptosis.(Zhang, et al., 2010) However, Eastman and co-workers suggest that S1 does not function as a pan-Bcl-2 inhibitor in cells but rather it upregulates the BH3-only protein Noxa, which inhibits Mcl-1 and leads to its degradation and an increase in cellular sensitivity to apoptosis.(Albershardt, et al.) Furthermore, S1 has been shown to rapidly increase reactive oxygen species (ROS) which leads to the induction of endoplasmic reticulum (ER)-mediated stress.(Soderquist, et al., 2013) Finally, Zhong et al. have reported that S1-mediated cell death may be in part due to the induction of autophagy through (ER) stress and disruption of the interaction of Beclin 1 with Bcl-2.(Zhong, et al., 2012) Thus, S1-mediated cell death could be caused by several different mechanisms.

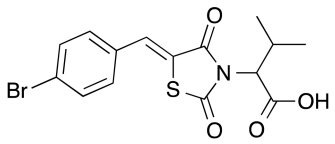
3. S1 Derivatives

Zhang and co-workers have disclosed a number of S1 derivatives using scaffold hopping approaches. Based on their NMR-derived model for the binding of S1 to Mcl-1, Song et al. selected the C3 and C6 positions of S1 (**5**, Fig. 1) for synthetic elaboration in order to extend further along the hydrophobic BH3-binding groove.(T. Song, Li, et al., 2013; T. Song, et al., 2014) Structure–activity studies at the C3 and C6 positions led to the identification of **6**, which binds Mcl-1 (IC_{50} = 10 nM, Bim-BH3, ELISA) and Bcl-2 (IC_{50} = 20 nM, Bim-BH3, ELISA) with increased affinity relative to S1 (Mcl-1, IC_{50} = 95 nM; Bcl-2, IC_{50} = 715 nM, Bim-BH3, ELISA). Preliminary cell studies indicate that compound **6** exhibits increased apoptotic activity compared to S1.

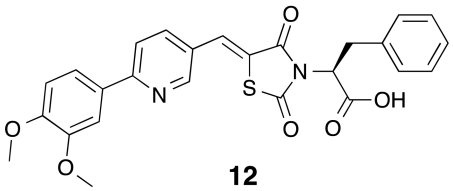
Additionally, Zhang and co-workers employed a fragment-based strategy to identify two novel Mcl-1 inhibitors. As shown in Figure 2, screening of several fragments derived from the dissection of the unrevised S1 structure using a fluorescence polarization assay led to the

identification of two novel hits: a 2-cyanoacetamide (**7**)(Zhang, Song, et al., 2013), and a 2-hydroxynicotinonitrile (**9**)(Zhang, Liu, et al., 2013). Synthetic elaboration of these molecules yielded compounds **8**, which is 6-fold more potent than S1, and **10**, which is equipotent to S1.

4. A*STAR Compounds



BH3I-1 (**11**)



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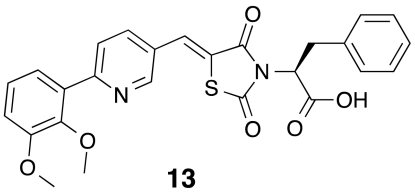


13

By synthesizing and screening a small, focused library of pyridine-based rhodanine derivatives of BH3I-1 (**11**)(Lugovskoy, et al., 2002), that binds to Bcl-2 at the BH3 site, Bernardo et al. identified two constitutional isomers, structurally-differentiated by the relative position of two methoxy groups. Compound **12** binds exclusively to Mcl-1 (K_d = 10 μM, ITC), and compound **13** binds Mcl-1 (K_d = 0.25 μM, ITC) with greater affinity and also binds to Bcl-X_L (K_d = 3.4 μM, ITC). (Bernardo, et al., 2010) While NMR-guided docking studies suggest that compounds **12** and **13** bind to the BH3-binding groove, the interaction(s) responsible for the difference in selectivity observed as a result of such a subtle structural change have not yet been identified. Although Bernardo and coworkers did not provide data validating the biological activity of the these compounds, compounds **12** and **13** were evaluated alongside other putative Mcl-1-inhibitors by Varadarjan et al. (Varadarajan, et al., 2013) This follow-up study determined that neither compound killed cells, even at high concentrations (<30 nM), as a single agent or in combination with ABT-737.

5. Compounds from Takeda Pharmaceutical Company

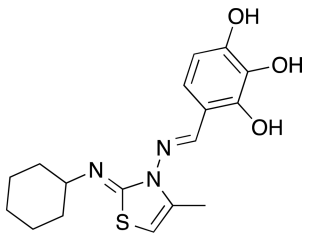
The desire to overcome Mcl-1 over-expression in cancer cells treated with Bcl-2 family inhibitors has prompted interest in the discovery of dual inhibitors, such as an Mcl-1/Bcl-X_L dual inhibitor. To this end, Tanaka et al. analyzed known inhibitors of Mcl-1 such as compound **14**(Elmore, et al., 2008) (Fig. 3) and the reported Bcl-X_L inhibitor, ABT-737, for two complimentary binding motifs. Merging of the two scaffold fragments, **15** and the

arylsulfonamide portion of ABT-737, resulted in compound **17**, which binds to both Mcl-1 (IC_{50} = 88 nM, Bid-BH3, TR-FRET) and Bcl- X_L (IC_{50} = 3.7 nM, Bid-BH3, TR-FRET). (Tanaka, et al., 2013) Compound **16**, the des-methyl analog of **17**, was cocrystallized with Mcl-1 (PDB ID 3WIY) and Bcl- X_L (PDB ID 3WIZ) and, for the most part, confirmed the contribution of each half of the molecule for binding to the respective anti-apoptotic proteins (Fig. 4). The portion of the molecule derived from ABT-737 does not appear to contribute much towards the binding of Mcl-1.

6. University of Michigan Compounds

Abulwerdi et al. identified *N*-(4-hydroxynaphthalen-1-yl)arylsulfonamide **18** (Fig.5) as part of a high-throughput screen (HTS) of 53,000 synthetic small molecules using a fluorescence polarization assay.(Abulwerdi, et al., 2014) Aided by NMR-based docking models and SAR studies, elaboration of the initial hit led to compound **19**, which exhibits selective affinity for Mcl-1 (K_d = 180 nM, Bid-BH3, FPA) over other pro-survival Bcl-2 family members (9- to 59-fold). Experiments in leukemia cell lines show that compound **19** inhibited cell growth and activated caspase-3 in a dose-dependent manner. Further, compound **19** shows moderate cytotoxicity against Bak/Bax-deficient cells, suggesting that apoptosis is Bak/Bax-dependent.

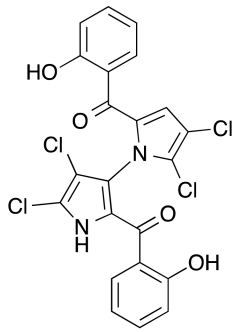
7. MIM1



MIM1 (**20**)

Cohen and coworkers screened a library of over 70,000 small molecules in a high-throughput competition fluorescence polarization-based assay for their ability to displace a fluorescently labeled Mcl-1 SAHBA(Stewart, et al., 2010) from Mcl-1.(Cohen, et al., 2012) In addition, the library was counter-screened for activity against Bcl- X_L, and the resulting hits were excluded from the hit pool to select compounds that selectively bind to Mcl-1. Following a series of increasingly stringent confirmatory assays, 28 compounds were progressed into liposome- and cell-based assays. Mcl-1 Inhibitor Molecule 1 (**20**; MIM1) was ultimately selected based on a combination of biological and physicochemical properties. MIM1 was shown to bind selectively to Mcl-1 with modest affinity (IC_{50} = 4.78 μM, Bid-BH3, FPA), trigger Bax/Bak-dependent apoptosis in leukemia cells, and to act in concert with ABT-737 by down-regulation of Mcl-1. However, a recent evaluation of putative Mcl-1 inhibitors disclosed that MIM1 induced Bak-dependent apoptosis only at high concentrations (>10 μM) and that it failed to induce apoptosis in Mcl-1-, Bcl-2- and Bcl-X_L-dependent cell lines.(Varadarajan, et al., 2013) Taken together, these data suggest that MIM1's inhibitory effects may be cell-line dependent.

8. Marinopyrrole A (Maritoclax)



marinopyrrole A (**21**)

Marinopyrrole A (**21**), a natural product isolated from an obligate marine *Streptomyces*, has received considerable attention due to its promising antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA).(Haste, et al., 2011; Hughes, et al., 2009; Nicolaou, et al., 2011) Marinopyrrole A, which was named Maritoclax, was found to also selectively bind to Mcl-1 (IC_{50} = 10.1 μM, Bim-BH3, ELISA), decrease Mcl-1 protein levels via proteasomal degradation and induce apoptosis in Mcl-1-dependent, but not Bcl-2- and Bcl-X_L-dependent, leukemia(Doi, et al., 2012) and melanoma cells(Pandey, et al., 2013). However, Eichhorn and coworkers disclosed that marinopyrrole A was equally effective against Bcl-2-dependent leukemia cells compared to Mcl-1-dependent cells, and that treatment with marinopyrrole A had no effect upon Mcl-1 expression levels.(Eichhorn, et al., 2013) Furthermore, the follow-up report indicates that marinopyrrole A does not lead to the degradation of Mcl-1 as no affect on Mcl-1 expression levels was observed upon treatment with this compound.

9. Compounds from Eutropics Pharmaceuticals

Richard et al. screened a library of 315,000 compounds in a high-throughput fluorescence polarization-based assay for the ability of compounds to inhibit Mcl-1.(Richard, et al., 2013) A subsequent FP assay was used as a counter-screen to the primary assay to identify compounds that displayed selectivity for Mcl-1 over Bcl-X_L. Evaluation of the hits identified in the HTS campaign for their synthetic tractability and quality gave the team their lead compound, the 7-hydroxyquinoline **22** (Fig. 6). Analysis of compound **22** identified a number of perceived liabilities, namely, the carboxylic acid and the 4-chloro groups, which were subsequently modified or eliminated. Synthetic modification and further SAR studies resulted in compound **23**, which yielded IC_{50}s of 310 nM for Mcl-1 and 40 μM for Bcl-X_L (Bim-BH3, FPA). Compound **23** was found to induce dose-dependent cytochrome c release and antiproliferative activity against several Mcl-1 dependent cell lines. Furthermore, the authors demonstrate that the cellular activity and selectivity of cell lines correlates with the degree of mitochondrial priming as determined by BH3 profiling(Certo, et al., 2006).

10. AbbVie Compounds

An NMR-based fragment screen against Mcl-1 of a 17,000 fragment library conducted by a team at AbbVie revealed a number of hits. Two of these hits were selected for additional studies based on the criteria of binding efficiency and synthetic tractability: (1) aryl sulfonamide **24** and (2) salicylic acid **26** (Fig. 7).(Petros, et al., 2014) In the absence of high resolution crystal structures, the binding modes for the respective fragments were determined by alternate means. The binding mode for the aryl sulfonamide fragment **24** was determined with the aid of nuclear Overhauser effect (NOE) restraint-driven docking and, in the case of the salicylic acid fragment **26**, the binding mode was elucidated by simply docking the fragment into the BH3-binding groove guided by a single electrostatic-contact restraint. The aryl sulfonamide fragment was elaborated into compound **25**, which exhibited an IC_{50} of 30 nM (Noxa-BH3, FPA) against Mcl-1, and the salicylic acid fragment was elaborated into compound **27**, which yielded an IC_{50} of 570 nM (Noxa-BH3, FPA). Cocrystal structures of aryl sulfonamide **28** (PDB ID 4OQ5) and salicylate **29** (PDB ID 4OQ6) were subsequently obtained (Fig. 8). Notably, the acid moieties of both **28** and **29** are fixed in the same region, and the hydrophobic naphthyl moiety of the more potent aryl sulfonamide **28** is located deep within the hydrophobic pocket of Mcl-1.

11. Vanderbilt University Compounds

An NMR-based screen of a large fragment library (>13,800 compounds) by Friberg and coworkers led to the identification of several chemically distinct classes of fragment hits. Two of these hits, 5,6-ring-fused heterocyclic carboxylic acids, and a group of hydrophobic aromatics linked to a polar headpiece.(Friberg, et al., 2013) NMR-guided docking of the fragments revealed that the fragments bound in a mutually exclusive fashion in two closely situated binding sites within a large hydrophobic pocket. Based on this structural information, two fragments were merged together to produce compounds with markedly improved binding affinities (e.g., **30** and **31**, Fig. 9). Further analoging led to the discovery of the indole-2-carboxylic acid **33**, which was a potent inhibitor of Mcl-1 (K_d = 55 nM, Bak-BH3, FPA) that displayed a 16-fold selectivity over Bcl-2 and 270-fold over Bcl-X_L (870 nM and >15 μM, respectively, Bak-BH3, FPA). Cocrystal structures of compounds **32** (PDB ID 4HW3) and **33** (PDB ID 4HW2) bound to Mcl-1 confirmed that the merged compounds occupy both pockets identified by the initial fragments and that the carboxylic acid moiety interacts with R263 (Fig. 10). Further analysis of the X-ray structure aided in rationalizing the SAR observed in the merged series and illuminated opportunities to improve the potency by accessing additional binding sites.

12. Conclusion

Significant effort has been directed towards the discovery of Bcl-2 and Bcl-X_L inhibitors which has culminated in a number of very potent Bcl-2 family inhibitors such as ABT-737(Oltersdorf, et al., 2005), navitoclax (ABT-263)(Tse, et al., 2008), and ABT-199(Souers, et al., 2013). The remarkable in vitro and in vivo biological activities observed preclinically and in the clinic clearly demonstrates the feasibility of targeting the Bcl-2 family proteins with small molecule inhibitors. In contrast to the advances made in

drugging Bcl-2 and Bcl-X_L, the discovery of Mcl-1 inhibitors has lagged behind. This is unfortunate, since Mcl-1 appears to also be a promising cancer target. Encouragingly, the last half-decade has witnessed a rapid surge of interest towards the discovery of Mcl-1 inhibitors. This has resulted in a significant number of small molecule BH3-mimetics comprising a range of structurally diverse chemotypes. There remain, however, questions regarding the chemical liabilities, i.e., the inclusion of possible “bad actors”(Baell, 2010), and the non-drug-like physicochemical properties of some of the reported inhibitors. Also, the lack of in vivo data for the majority of proposed Mcl-1 inhibitors is striking.

The moderate potency of Mcl-1 inhibitors reported to date (Table 1) is likely responsible for the lack of convincing in vivo activity. It is tempting to speculate that the experiences observed in the discovery of Bcl-2 and Bcl-X_L inhibitors(Oltersdorf, et al., 2005; Souers, et al., 2013; Tse, et al., 2008) will be reflected in the Mcl-1 inhibitors. By comparison, a clinically useful Mcl-1 inhibitor may need to exhibit in vitro affinities approaching the low picomolar range. At present, there are no reported Mcl-1 inhibitors, peptide or small molecule-based, exhibiting this level of affinity for Mcl-1.

The discovery of potent Mcl-1 inhibitors is a unique challenge due to key structural differences between the BH3-binding grooves of Mcl-1 and Bcl-2 proteins. Unlike Bcl-2, the P2 pocket of Mcl-1 (Fig. 11), as confirmed by cocrystal structures of small molecule inhibitors bound to Mcl-1(Friberg, et al., 2013; Petros, et al., 2014; Tanaka, et al., 2013), has a high degree of plasticity. Indeed, the P2 pocket of Mcl-1 expands to form a large, hydrophobic cavity in the presence of ligands. The P4 pocket of Mcl-1, however, is less well defined and more solvent exposed.(Czabotar, et al., 2007) As such, this pocket is shallower and less hydrophobic than that found in Bcl-X_L. The importance of the P2 pocket in Mcl-1 has been confirmed experimentally by the results of two NMR-based fragment screening campaigns against Mcl-1 conducted by Friberg et al. and Petros et al.(Friberg, et al., 2013; Petros, et al., 2014). These two studies disclosed that fragments, bind exclusively in P2. In contrast, the NMR-based screen leading up to the discovery of ABT-737 resulted in the discovery of two fragments bound to two distinct areas of Bcl-X_L, P2 and P4.(Oltersdorf, et al., 2005) Future efforts to develop potent and specific ligands for Mcl-1 will need to fully exploit the binding opportunities available within the proximity of the P2 pocket as opportunities to gain significant contributions towards affinity from interactions outside of this region appear, at present, to be limited.

Significant advancements have been made over the past few years towards the discovery of Mcl-1 inhibitors. However, the Mcl-1 inhibitors described to date are still at a very early stage. Given the concerns described above, significant challenges still remain. With the recent increase in interest it is likely that these challenges will be overcome, and these efforts will lead to novel Mcl-1 inhibitors for the treatment of cancer.

Abbreviations

Bad	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist/killer

Bax	Bcl-2 associated X
Bcl-2	B-cell lymphoma-2
BH	Bcl-2 homology
BH3	Bcl-2 homology domain 3
Bcl-X_L	B-cell lymphoma-extra large
Bim	Bcl-2 interacting mediator
Bfl-1/A1	Bcl-2-related protein A1
Mcl-1	myeloid cell leukemia-1
Noxa	phorbol-12-myristate-13-acetate-induced protein 1
Puma	p53 upregulated modulator of apoptosis
SAHB	stapled alpha-helix of Bcl-2 domains
SAR	structure activity relationships
SPR	surface plasmon resonance
tBid	truncated BH3-interacting domain death agonist

References

Abulwerdi FA, Liao C, Mady A, Gavin J, Shen C, Cierpicki T, Stuckey JA, Showalter HDH, Nikolovska-Coleska Z. 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. J Med Chem. 2014; 57:4111–4133. [PubMed: 24749893]

Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007; 26:1324–1337. [PubMed: 17322918]

Akgul C. Mcl-1 is a potential therapeutic target in multiple types of cancer. Cell Mol Life Sci. 2008; 66:1326–1336. [PubMed: 19099185]

Albershardt TC, Salerni BL, Soderquist RS, Bates DJ, Pletnev AA, Kisselev AF, Eastman A. Multiple BH3 mimetics antagonize antiapoptotic MCL1 protein by inducing the endoplasmic reticulum stress response and up-regulating BH3-only protein NOXA. J Biol Chem. 2011; 286:24882–24895. [PubMed: 21628457]

Andersen MH, Becker JC, Straten PT. The antiapoptotic member of the Bcl-2 family Mcl-1 is a CTL target in cancer patients. Leukemia. 2005; 19:484–485. [PubMed: 15618955]

Arbour N, Vanderluit JL, Le Grand JN, Jahani-Asl A, Ruzhynsky VA, Cheung EC, Kelly MA, MacKenzie AE, Park DS, Opferman JT, Slack RS. Mcl-1 is a key regulator of apoptosis during CNS development and after DNA damage. J Neurosci. 2008; 28:6068–6078. [PubMed: 18550749]

Baell JB. Observations on screening-based research and some concerning trends in the literature. Future Medicinal Chemistry. 2010; 2:1529–1546. [PubMed: 21426147]

Bajwa N, Liao C, Nikolovska-Coleska Z. Inhibitors of the anti-apoptotic Bcl-2 proteins: a patent review. Expert Opin Ther Patents. 2012; 22:37–55.

Bernardo PH, Sivaraman T, Wan KF, Xu J, Krishnamoorthy J, Song CM, Tian LM, Chin JSF, Lim DSW, Mok HYK, Yu VC, Tong JC, Chai CLL. Structural Insights into the Design of Small Molecule Inhibitors That Selectively Antagonize Mcl-1. J Med Chem. 2010; 53:2314–2318. [PubMed: 20158203]

Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, Cho YJ, Haery L, Greulich H,

Reich M, Winckler W, Lawrence MS, Weir BA, Tanaka KE, Chiang DY, Bass AJ, Loo A, Hoffman C, Prensner J, Liefeld T, Gao Q, Yecies D, Signoretti S, Maher E, Kaye FJ, Sasaki H, Tepper JE, Fletcher JA, Tabernero J, Baselga J, Tsao MS, Demichelis F, Rubin MA, Janne PA, Daly MJ, Nucera C, Levine RL, Ebert BL, Gabriel S, Rustgi AK, Antonescu CR, Ladanyi M, Letai A, Garraway LA, Loda M, Beer DG, True LD, Okamoto A, Pomeroy SL, Singer S, Golub TR, Lander ES, Getz G, Sellers WR, Meyerson M. The landscape of somatic copy-number alteration across human cancers. Nature. 2010; 463:899–905. [PubMed: 20164920]

Billard C. BH3 Mimetics: Status of the Field and New Developments. Mol Cancer Ther. 2013; 12:1691–1700. [PubMed: 23974697]

Boisvert-Adamo K, Longmate W, Abel EV, Aplin AE. Mcl-1 Is Required for Melanoma Cell Resistance to Anoikis. Mol Cancer Res. 2009; 7:549–556. [PubMed: 19372583]

Brotin E, Meryet-Figuiere M, Simonin K, Duval RE, Villedieu M, Leroy-Dudal J, Saison-Behmoaras E, Gauduchon P, Denoyelle C, Poulain L. Bcl-x(L) and MCL-1 constitute pertinent targets in ovarian carcinoma and their concomitant inhibition is sufficient to induce apoptosis. Int J Cancer. 2010; 126:885–895. [PubMed: 19634140]

Burlacu A. Regulation of apoptosis by Bcl-2 family proteins. J Cell Mol Med. 2003; 7:249–257. [PubMed: 14594549]

Certo M, Del Gaizo Moore V, Nishino M, Wei G, Korsmeyer S, Armstrong SA, Letai A. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell. 2006; 9:351–365. [PubMed: 16697956]

Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DCS. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell. 2005; 17:393–403. [PubMed: 15694340]

Chen W, Bai L, Wang X, Xu S, Belinsky SA, Lin Y. Acquired activation of the Akt/cyclooxygenase-2/Mcl-1 pathway renders lung cancer cells resistant to apoptosis. Mol Pharmacol. 2010; 77:416–423. [PubMed: 19933775]

Chetoui N, Sylla K, Gagnon-Houde JV, Alcaide-Loridan C, Charron D, Al-Daccak R, Aoudjit F. Down-regulation of mcl-1 by small interfering RNA sensitizes resistant melanoma cells to fas-mediated apoptosis. Mol Cancer Res. 2008; 6:42–52. [PubMed: 18234961]

Choo EF, Boggs J, Zhu CQ, Lubach JW, Catron ND, Jenkins G, Souers AJ, Voorman R. The Role of Lymphatic Transport on the Systemic Bioavailability of the Bcl-2 Protein Family Inhibitors Navitoclax (ABT-263) and ABT-199. Drug Metab Dispos. 2014; 42:207–212. [PubMed: 24212376]

Cohen NA, Stewart ML, Gavathiotis E, Tepper JL, Bruekner SR, Koss B, Opferman JT, Walensky LD. A Competitive Stapled Peptide Screen Identifies a Selective Small Molecule that Overcomes MCL-1-Dependent Leukemia Cell Survival. Chemistry & Biology. 2012; 19:1175–1186. [PubMed: 22999885]

Czabotar PE, Lee EF, van Delft MF, Day CL, Smith BJ, Huang DCS, Fairlie WD, Hinds MG, Colman PM. Structural insights into the degradation of Mcl-1 induced by BH3 domains. PNAS. 2007; 104:6217–6222. [PubMed: 17389404]

Derenne S, Monia B, Dean NM, Taylor JK, Rapp MJ, Harousseau JL, Bataille R, Amiot M. Antisense strategy shows that Mcl-1 rather than Bcl-2 or BCI-x(L) is an essential survival protein of human myeloma cells. Blood. 2002; 100:194–199. [PubMed: 12070027]

Ding Q, He X, Xia W, Hsu JM, Chen CT, Li LY, Lee DF, Yang JY, Xie X, Liu JC, Hung MC. Myeloid cell leukemia-1 inversely correlates with glycogen synthase kinase-3beta activity and associates with poor prognosis in human breast cancer. Cancer Res. 2007; 67:4564–4571. [PubMed: 17495324]

Doi K, Li R, Sung SS, Wu H, Liu Y, Manieri W, Krishnegowda G, Awwad A, Dewey A, Liu X, Amin S, Cheng C, Qin Y, Schonbrunn E, Daughdrill G, Loughran TP, Sebti S, Wang HG. Discovery of Marinopyrrole A (Maritoclax) as a Selective Mcl-1 Antagonist that Overcomes ABT-737 Resistance by Binding to and Targeting Mcl-1 for Proteasomal Degradation. J Biol Chem. 2012; 287:10224–10235. [PubMed: 22311987]

Dutta S, Gullá S, Chen TS, Fire E, Grant RA, Keating AE. Determinants of BH3 Binding Specificity for Mcl-1 versus Bcl-xL. J Mol Biol. 2010; 398:747–762. [PubMed: 20363230]

Dzhagalov I, Dunkle A, He YW. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. J Immunol. 2008; 181:521–528. [PubMed: 18566418]

Eichhorn JM, Alford SE, Hughes CC, Fenical W, Chambers TC. Purported Mcl-1 inhibitor marinopyrrole A fails to show selective cytotoxicity for Mcl-1-dependent cell lines. Cell Death & Disease. 2013; 4:e880. [PubMed: 24157874]

Elmore SW, Souers AJ, Bruncko M, Song X, Wang X, Hasvold LA, Wang L, Kunzer AR, Park CM, Wendt MD. 7-substituted indole mcl-1 inhibitors. WO 2008/131000 A2. 2008

Friberg A, Vigil D, Zhao B, Daniels RN, Burke JP, Garcia-Barrantes PM, Camper D, Chauder BA, Lee T, Olejniczak ET, Fesik SW. Discovery of Potent Myeloid Cell Leukemia 1 (Mcl-1) Inhibitors Using Fragment-Based Methods and Structure-Based Design. J Med Chem. 2013; 56:15–30. [PubMed: 23244564]

Germain M, Duronio V. The N terminus of the anti-apoptotic BCL-2 homologue MCL-1 regulates its localization and function. J Biol Chem. 2007; 282:32233–32242. [PubMed: 17823113]

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]

Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144:646–674. [PubMed: 21376230]

Haste NM, Hughes CC, Tran DN, Fenical W, Jensen PR, Nizet V, Hensler ME. Pharmacological Properties of the Marine Natural Product Marinopyrrole A against Methicillin-Resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2011; 55:3305–3312. [PubMed: 21502631]

Hauck P, Chao BH, Litz J, Krystal GW. Alterations in the Noxa/Mcl-1 axis determine sensitivity of small cell lung cancer to the BH3 mimetic ABT-737. Mol Cancer Ther. 2009; 8:883–892. [PubMed: 19372561]

Hughes CC, Yang Y-L, Liu W-T, Dorrestein PC, La Clair JJ, Fenical W. Marinopyrrole A target elucidation by acyl dye transfer. J Am Chem Soc. 2009; 131:12094–12096. [PubMed: 19673475]

Kang MH, Wan Z, Kang YH, Sposto R, Reynolds CP. Mechanism of synergy of N-(4-hydroxyphenyl)retinamide and ABT-737 in acute lymphoblastic leukemia cell lines: Mcl-1 inactivation. J Natl Cancer Inst. 2008; 100:580–595. [PubMed: 18398104]

Keuling AM, Felton KE, Parker AA, Akbari M, Andrew SE, Tron VA. RNA silencing of Mcl-1 enhances ABT-737-mediated apoptosis in melanoma: role for a caspase-8-dependent pathway. PLoS ONE. 2009; 4:e6651. [PubMed: 19684859]

Konopleva M, Contractor R, Tsao T, Samudio I, Ruvalo PP, Kitada S, Deng XM, Zhai DY, Shi YX, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling XY, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC, Andreeff M. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell. 2006; 10:375–388. [PubMed: 17097560]

Korsmeyer SJ. BCL-2 gene family and the regulation of programmed cell death. Cancer Res. 1999; 59:1693–1700.

Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, Kitada S, Reed JC. Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol. 1996; 148:1567–1576. [PubMed: 8623925]

Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. Nat Rev Drug Discov. 2008; 7:989–1000. [PubMed: 19043450]

Lin X, Morgan-Lappe S, Huang X, Li L, Zakula DM, Vernetti LA, Fesik SW, Shen Y. 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. Oncogene. 2007; 26:3972–3979. [PubMed: 17173063]

Liu H, Eksarko P, Temkin V, Haines GK 3rd, Perlman H, Koch AE, Thimmapaya B, Pope RM. Mcl-1 is essential for the survival of synovial fibroblasts in rheumatoid arthritis. J Immunol. 2005; 175:8337–8345. [PubMed: 16339575]

Lucas KM, Mohana-Kumaran N, Lau D, Zhang XD, Hersey P, Huang DC, Weninger W, Haass NK, Allen JD. Modulation of NOXA and MCL-1 as a strategy for sensitizing melanoma cells to the BH3-mimetic ABT-737. Clin Cancer Res. 2012; 18:783–795. [PubMed: 22173547]

Lugovskoy AA, Degterev AI, Fahmy AF, Zhou P, Gross JD, Yuan J, Wagner G. A novel approach for characterizing protein ligand complexes: molecular basis for specificity of small-molecule Bcl-2 inhibitors. J Am Chem Soc. 2002; 124:1234–1240. [PubMed: 11841292]

Meng XW, Lee SH, Dai HM, Loegering D, Yu C, Flatten K, Schneider P, Dai NT, Kumar SK, Smith BD, Karp JE, Adjei AA, Kaufmann SH. MCL-1 as a buffer for proapoptotic BCL-2 family members during TRAIL-induced apoptosis - A mechanistic basis for sorafenib (bay 43-9006)-induced trail sensitization. J Biol Chem. 2007; 282:29831–29846. [PubMed: 17698840]

Miyamoto Y, Hosotani R, Wada M, Lee JU, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Doi R, Kato M, Shimada Y, Imamura M. Immunohistochemical analysis of Bcl-2, Bax, Bcl-X, and Mcl-1 expression in pancreatic cancers. Oncology. 1999; 56:73–82. [PubMed: 9885381]

Moldoveanu T, Follis AV, Kriwacki RW, Green DR. Many players in BCL-2 family affairs. Trends Biochem Sci. 2014; 39:101–111. [PubMed: 24503222]

Moulding DA, Giles RV, Spiller DG, White MR, Tidd DM, Edwards SW. Apoptosis is rapidly triggered by antisense depletion of MCL-1 in differentiating U937 cells. Blood. 2000; 96:1756–1763. [PubMed: 10961874]

Muppidi A, Doi K, Edwardraja S, Drake EJ, Gulick AM, Wang H-G, Lin Q. Rational Design of Proteolytically Stable, Cell-Permeable Peptide-Based Selective Mcl-1 Inhibitors. J Am Chem Soc. 2012; 134:14734–14737. [PubMed: 22920569]

Nguyen M, Marcellus RC, Roulston A, Watson M, Serfass L, Murthy Madiraju SR, Goulet D, Viallet J, Belec L, Billot X, Acoca S, Purisima E, Wiegmans A, Cluse L, Johnstone RW, Beauparlant P, Shore GC. Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. PNAS. 2007; 104:19512–19517. [PubMed: 18040043]

Nicolaou KC, Simmons NL, Chen JS, Haste NM, Nizet V. Total synthesis and biological evaluation of marinopyrrole A and analogs. Tetrahedron Lett. 2011; 52:2041–2043. [PubMed: 21499535]

Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005; 435:677–681. [PubMed: 15902208]

Opferman JT, Iwasaki H, Ong CC, Suh H, Mizuno S, Akashi K, Korsmeyer SJ. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science. 2005; 307:1101–1104. [PubMed: 15718471]

Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature. 2003; 426:671–676. [PubMed: 14668867]

Pandey MK, Gowda K, Doi K, Sharma AK, Wang H-G, Amin S. Proteasomal Degradation of Mcl-1 by Maritoclax Induces Apoptosis and Enhances the Efficacy of ABT-737 in Melanoma Cells. PLoS ONE. 2013; 8:e78570. [PubMed: 24223823]

Pang YP, Dai HM, Smith A, Meng XW, Schneider PA, Kaufmann SH. Bak Conformational Changes Induced by Ligand Binding: Insight into BH3 Domain Binding and Bak Homo-Oligomerization. Sci Rep-UK, 2. 2012

Petros AM, Swann SL, Song D, Swinger K, Park C, Zhang H, Wendt MD, Kunzer AR, Souers AJ, Sun C. Fragment-based discovery of potent inhibitors of the anti-apoptotic MCL-1 protein. Bioorg Med Chem Lett. 2014; 24:1484–1488. [PubMed: 24582986]

Placzek WJ, Sturlese M, Wu B, Cellitti JF, Wei J, Pellecchia M. Identification of a novel Mcl-1 protein binding motif. J Biol Chem. 2011; 286:39829–39835. [PubMed: 21953453]

Qin JZ, Xin H, Sitailo LA, Denning MF, Nickoloff BJ. Enhanced killing of melanoma cells by simultaneously targeting Mcl-1 and NOXA. Cancer Res. 2006; 66:9636–9645. [PubMed: 17018621]

Richard DJ, Lena R, Bannister T, Blake N, Pierceall WE, Carlson NE, Keller CE, Koenig M, He Y, Minond D, Mishra J, Cameron M, Spicer T, Hodder P, Cardone MH. Hydroxyquinoline-derived compounds and analoguing of selective Mcl-1 inhibitors using a functional biomarker. Bioorg Med Chem. 2013; 21:6642–6649. [PubMed: 23993674]

Rinkenberger JL, Horning S, Klocke B, Roth K, Korsmeyer SJ. Mcl-1 deficiency results in peri-implantation embryonic lethality. Genes Dev. 2000; 14:23–27. [PubMed: 10640272]

Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney DA, He SZ, Huang DCS, Xiong H, Cui Y, Busman TA, McKeegan EM, Krivoshik AP, Enschede SH, Humerickhouse R. Substantial Susceptibility of Chronic Lymphocytic Leukemia to BCL2 Inhibition: Results of a Phase I Study of Navitoclax in Patients With Relapsed or Refractory Disease. J Clin Oncol. 2012; 30:488–496. [PubMed: 22184378]

Sattler M, Liang H, Nettesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB, Fesik SW. Structure of Bcl-x(L)-Bak peptide complex: Recognition between regulators of apoptosis. Science. 1997; 275:983–986. [PubMed: 9020082]

Smith BJ, Lee EF, Checco JW, Evangelista M, Gellman SH, Fairlie WD. Structure -Guided Rational Design of α/β - Peptide Foldamers with High Affinity for BCL - 2 Family Prosurvival Proteins. ChemBioChem. 2013; 14:1564–1572. [PubMed: 23929624]

Soderquist R, Pletnev AA, Danilov AV, Eastman A. The putative BH3 mimetic S1 sensitizes leukemia to ABT-737 by increasing reactive oxygen species, inducing endoplasmic reticulum stress, and upregulating the BH3-only protein NOXA. Apoptosis. 2013; 19:201–209. [PubMed: 24072590]

Song LX, Coppola D, Livingston S, Cress D, Haura EB. Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells. Cancer Biology & Therapy. 2005; 4:267–276. [PubMed: 15753661]

Song T, Chen Q, Li X, Chai G, Zhang ZC. Correction to 3-Thiomorpholin-8-oxo-8 H-acenaphtho[1,2-b]pyrrole-9-carbonitrile (S1) Based Molecules as Potent, Dual Inhibitors of B-Cell Lymphoma 2 (Bcl-2) and Myeloid Cell Leukemia Sequence 1 (Mcl-1): Structure-Based Design and Structure-Activity Relationship Studies. J Med Chem. 2013; 56:9366–9367.

Song T, Li X, Chang X, Liang X, Zhao Y, Wu GY, Xie SH, Su PC, Wu Z, Feng YG, Zhang ZC. 3-Thiomorpholin-8-oxo-8H-acenaphtho [1,2-b] pyrrole-9-carbonitrile (S1) derivatives as pan-Bcl-2-inhibitors of Bcl-2, Bcl-xL and Mcl-1. Bioorg Med Chem. 2013; 21:11–20. [PubMed: 23206987]

Song T, Li XQ, Yang Y, Wu GY, Xie SH, Su PC, Feng YG, Zhang ZC. 3-Thiomorpholin-8-oxo-8H–acenaphtho [1,2-b] pyrrole-9-carbonitrile (S1) derivatives as pan-Bcl-2-inhibitors of Bcl-2, Bcl-x(L) and Mcl-1 (vol 21, pg 11, 2013). Bioorg Med Chem. 2014; 22:663–664.

Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, Huang DCS, Hymowitz SG, Jin S, Khaw SL, Kovar PJ, Lam LT, Lee J, Maecker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM, Oleksijew A, Park CH, Park C-M, Phillips DC, Roberts AW, Sampath D, Seymour JF, Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H, Humerickhouse RA, Rosenberg SH, Elmore SW. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013; 19:202–208. [PubMed: 23291630]

Stewart ML, Fire E, Keating AE, Walensky LD. The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. Nat Chem Biol. 2010; 6:595–601. [PubMed: 20562877]

Tahir SK, Yang XF, Anderson MG, Morgan-Lappe SE, Sarthy AV, Chen J, Warner RB, Ng SC, Fesik SW, Elmore SW, Rosenberg SH, Tse C. Influence of Bcl-2 family members on the cellular response of small-cell lung cancer cell lines to ABT-737. Cancer Res. 2007; 67:1176–1183. [PubMed: 17283153]

Tanaka Y, Aikawa K, Nishida G, Homma M, Sogabe S, Igaki S, Hayano Y, Sameshima T, Miyahisa I, Kawamoto T, Tawada M, Imai Y, Inazuka M, Cho N, Imaeda Y, Ishikawa T. Discovery of Potent Mcl-1/Bcl-xL Dual Inhibitors by Using a Hybridization Strategy Based on Structural Analysis of Target Proteins. J Med Chem. 2013; 56:9635–9645. [PubMed: 24215352]

Taniai M, Grambihler A, Higuchi H, Werneburg N, Bronk SF, Farrugia DJ, Kaufmann SH, Gores GJ. Mcl-1 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in human cholangiocarcinoma cells. Cancer Res. 2004; 64:3517–3524. [PubMed: 15150106]

Thallinger C, Wolschek MF, Wacheck V, Maierhofer H, Gunsberg P, Polterauer P, Pehamberger H, Monia BP, Selzer E, Wolff K, Jansen B. Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. J Invest Dermatol. 2003; 120:1081–1086. [PubMed: 12787138]

Thomas LW, Lam C, Edwards SW. Mcl-1; the molecular regulation of protein function. FEBS Lett. 2010; 584:2981–2989. [PubMed: 20540941]

Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zhang H, Fesik S, Rosenberg SH, Elmore SW. ABT-263: A Potent and Orally Bioavailable Bcl-2 Family Inhibitor. Cancer Res. 2008; 68:3421–3428. [PubMed: 18451170]

van Delft MF, Huang DC. How the Bcl-2 family of proteins interact to regulate apoptosis. Cell Res. 2006; 16:203–213. [PubMed: 16474435]

van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, Huang DCS. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell. 2006; 10:389–399. [PubMed: 17097561]

Varadarajan S, Vogler M, Butterworth M, Dinsdale D, Walensky LD, Cohen GM. Evaluation and critical assessment of putative MCL-1 inhibitors. Cell Death Differ. 2013; 20:1475–1484. [PubMed: 23832116]

Vogler M, Weber K, Dinsdale D, Schmitz I, Schulze-Osthoff K, Dyer MJS, Cohen GM. Different forms of cell death induced by putative BCL2 inhibitors. Cell Death Differ. 2009; 16:1030–1039. [PubMed: 19390557]

Volkmann N, Marassi FM, Newmeyer DD, Hanein D. The rheostat in the membrane: BCL-2 family proteins and apoptosis. Cell Death Differ. 2014; 21:206–215. [PubMed: 24162659]

Wei G, Margolin AA, Haery L, Brown E, Cucolo L, Julian B, Shehata S, Kung AL, Beroukhim R, Golub TR. Chemical Genomics Identifies Small-Molecule MCL1 Repressors and BCL-xL as a Predictor of MCL1 Dependency. Cancer Cell. 2012; 21:547–562. [PubMed: 22516262]

Wei S-H, Dong K, Lin F, Wang X, Li B, Shen J-j, Zhang Q, Wang R, Zhang H-Z. Inducing apoptosis and enhancing chemosensitivity to Gemcitabine via RNA interference targeting Mcl-1 gene in pancreatic carcinoma cell. Cancer Chemother Pharmacol. 2008; 62:1055–1064. [PubMed: 18297287]

Wertz IE, Kusam S, Lam C, Okamoto T, Sandoval W, Anderson DJ, Helgason E, Ernst JA, Eby M, Liu J, Belmont LD, Kaminker JS, O'Rourke KM, Pujara K, Kohli PB, Johnson AR, Chiu ML, Lill JR, Jackson PK, Fairbrother WJ, Seshagiri S, Ludlam MJC, Leong KG, Dueber EC, Maecker H, Huang DCS, Dixit VM. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature. 2011; 471:110–114. [PubMed: 21368834]

Yang-Yen HF. Mcl-1: a highly regulated cell death and survival controller. J Biomed Sci. 2006; 13:201–204. [PubMed: 16456709]

Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008; 9:47–59. [PubMed: 18097445]

Zhang Z, Jin L, Qian X, Wei M, Wang Y, Wang J, Yang Y, Xu Q, Xu Y, Liu F. Novel Bcl-2 Inhibitors: Discovery and Mechanism Study of Small Organic Apoptosis-Inducing Agents. ChemBioChem. 2007; 8:113–121. [PubMed: 17139689]

Zhang Z, Liu C, Li X, Song T, Wu Z, Liang X, Zhao Y, Shen X, Chen H. Fragment-based design, synthesis, and biological evaluation of N-substituted-5-(4-isopropylthiophenol)-2-hydroxynicotinamide derivatives as novel Mcl-1 inhibitors. Eur J Med Chem. 2013; 60:410–420. [PubMed: 23314054]

Zhang Z, Song T, Li X, Wu Z, Feng Y, Xie F, Liu C, Qin J, Chen H. Novel soluble myeloid cell leukemia sequence 1 (Mcl-1) inhibitor (E,E)-2-(benzylaminocarbonyl)-3-styrylacrylonitrile (4g) developed using a fragment-based approach. Eur J Med Chem. 2013; 59:141–149. [PubMed: 23220642]

Zhang Z, Song T, Zhang T, Gao J, Wu G, An L, Du G. A novel BH3 mimetic S1 potently induces Bax/Bak-dependent apoptosis by targeting both Bcl-2 and Mcl-1. Int J Cancer. 2010; 128:1724–1735. [PubMed: 20503275]

Zhong J-T, Xu Y, Yi H-W, Su J, Yu H-M, Xiang X-Y, Li X-N, Zhang Z-C, Sun L-K. The BH3 mimetic S1 induces autophagy through ER stress and disruption of Bcl-2/Beclin 1 interaction in human glioma U251 cells. Cancer Lett. 2012; 323:180–187. [PubMed: 22579788]

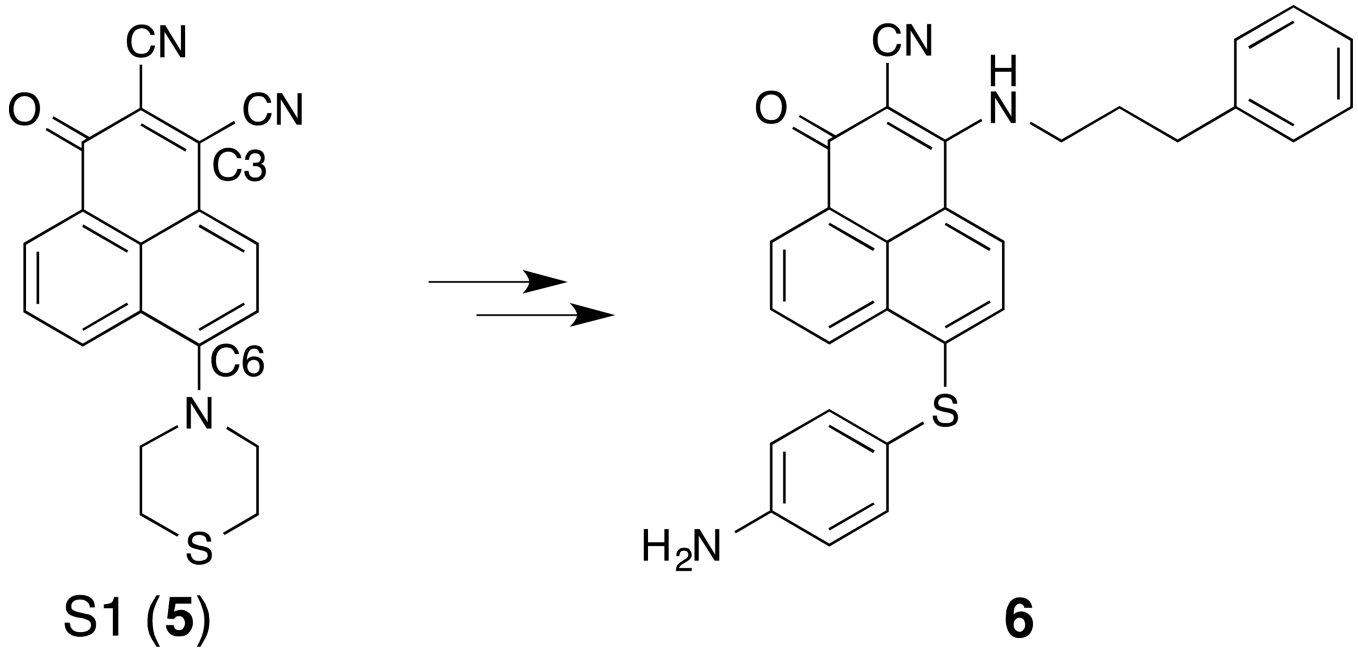


Figure 1.
SAR studies at C3 and C6 of S1 led to compound **6**.

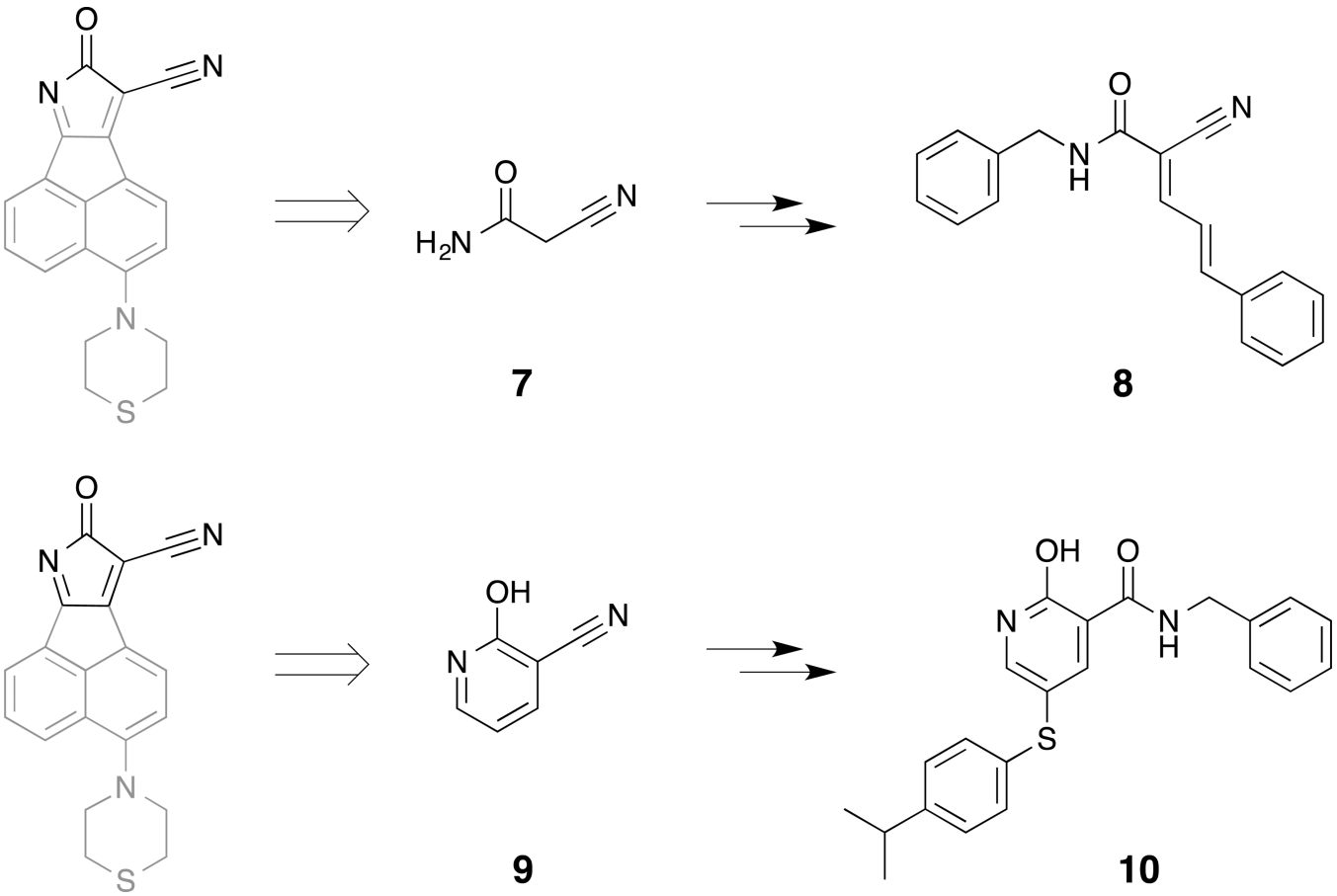


Figure 2.
Fragments derived from the **4** led to two novel classes of Mcl-1 inhibitors.

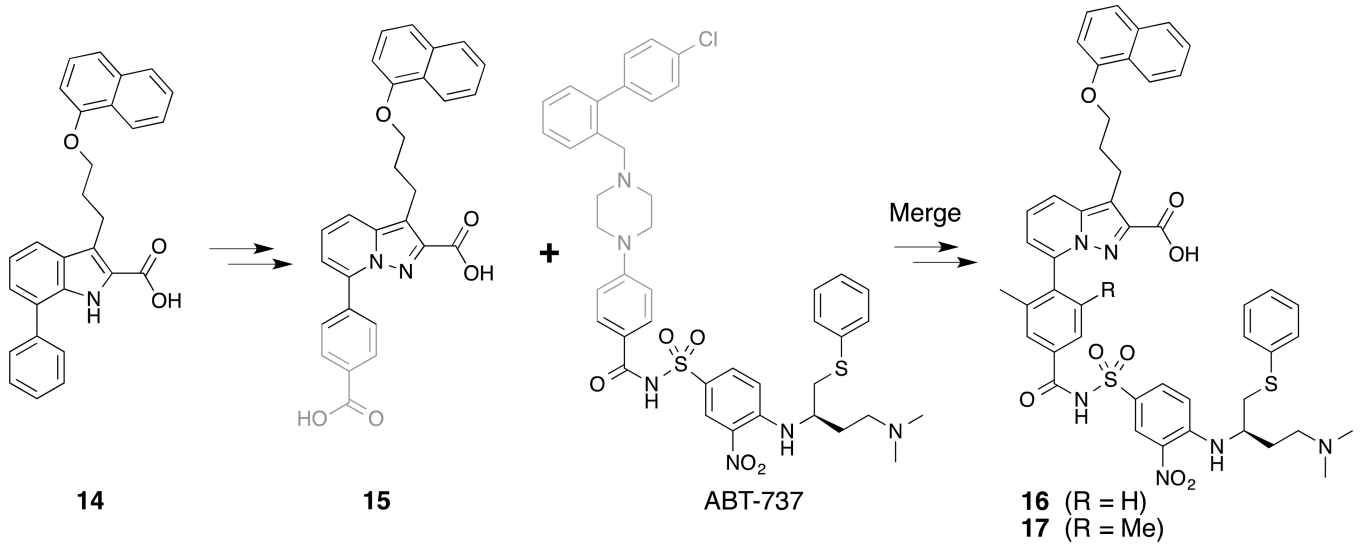


Figure 3.

Merging of fragments derived from two known inhibitors of Mcl-1 and Bcl-X_L resulted in novel dual-inhibitors.

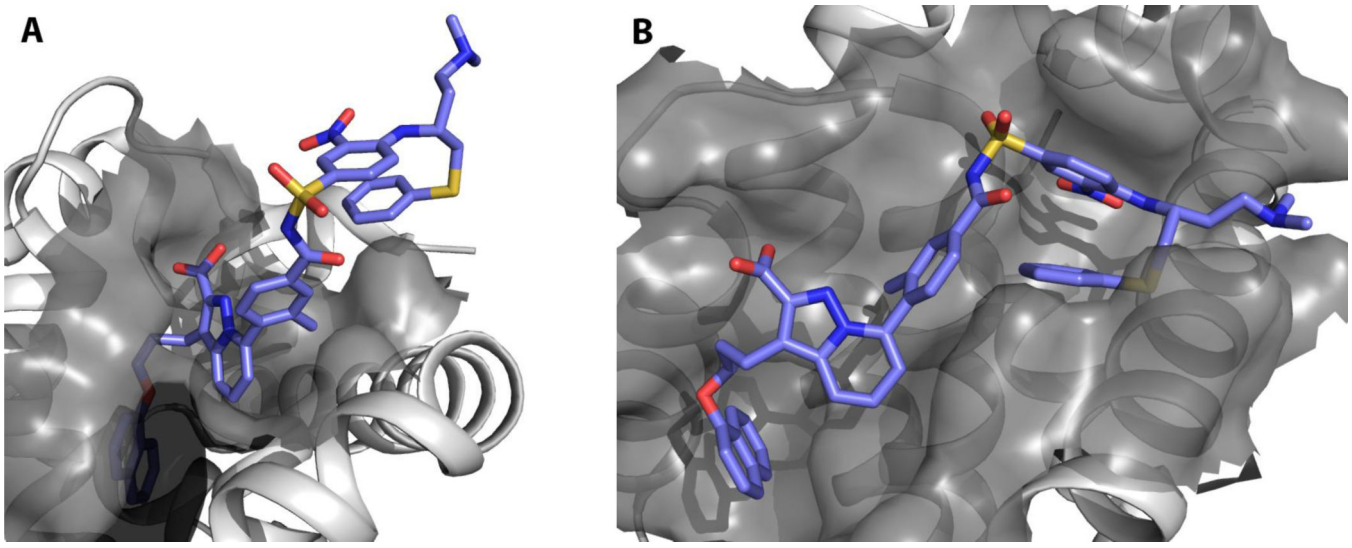


Figure 4.
Crystal structures of **15** bound to Mcl-1 (A) and to Bcl-X_L (B).

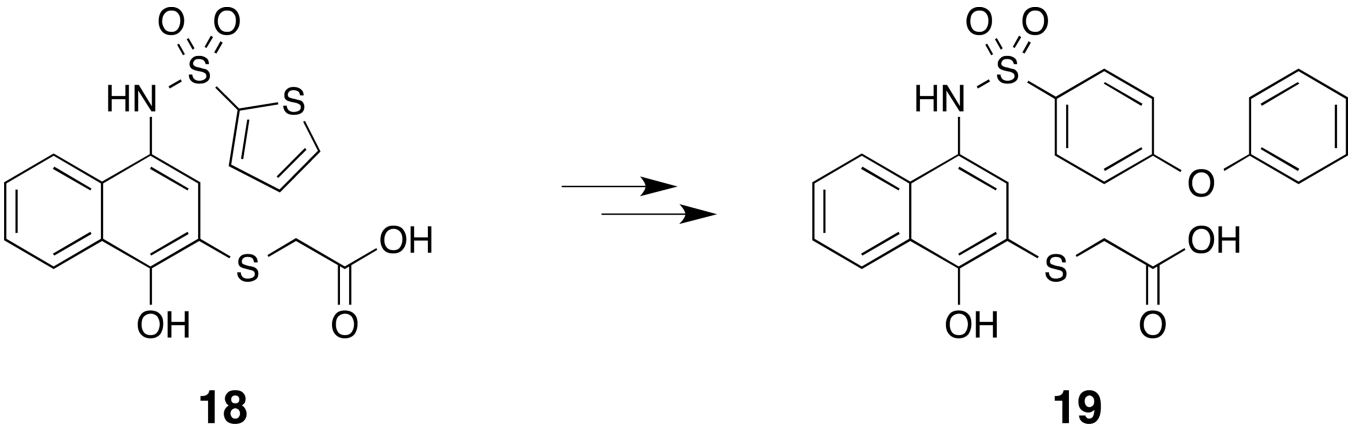
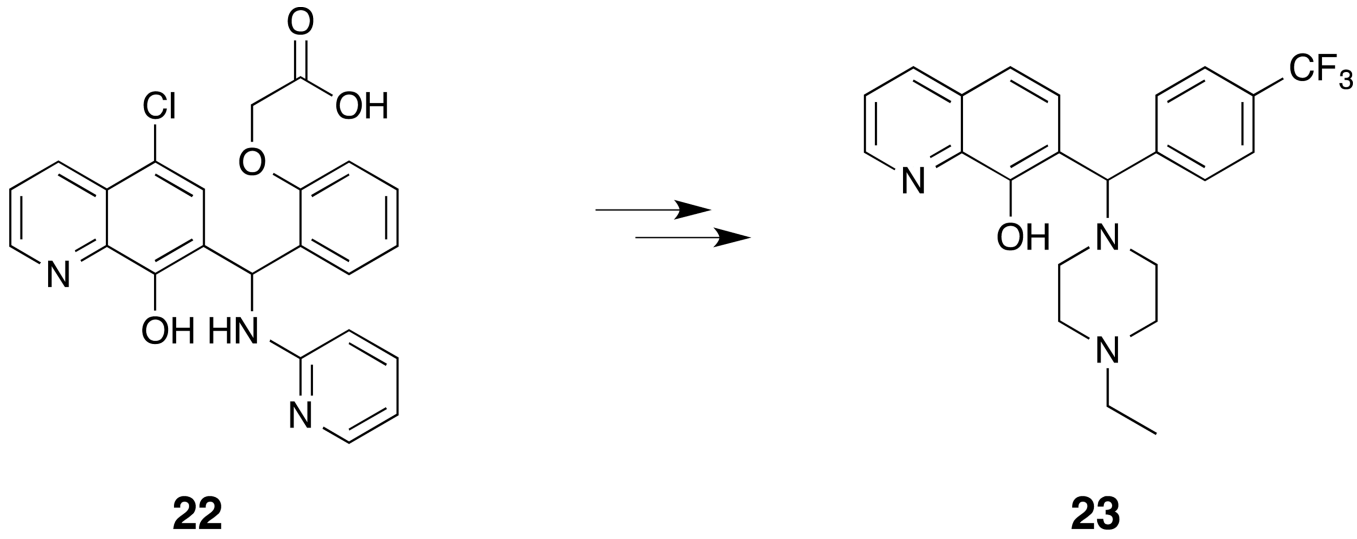


Figure 5.
Discovery of an *N*-(4-hydroxynaphthalen-1-yl)sulfonamide Mcl-1 inhibitor.



22 **23**

Figure 6.
Synthetic modification of 7-hydroxyquinoline **22** led to compound **23**.

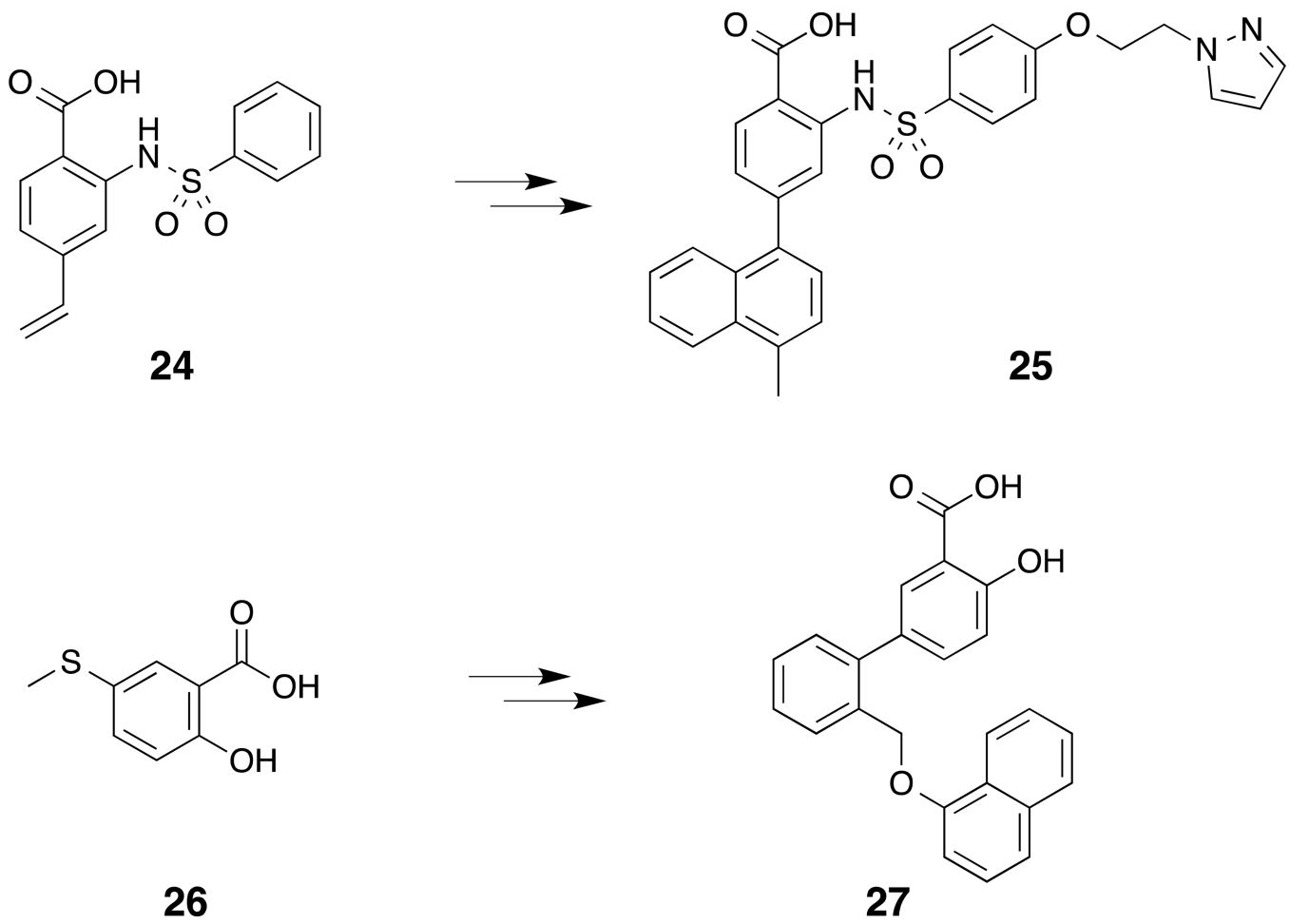


Figure 7.
Fragments **24** and **26** were elaborated to give compounds **25** and **27**.

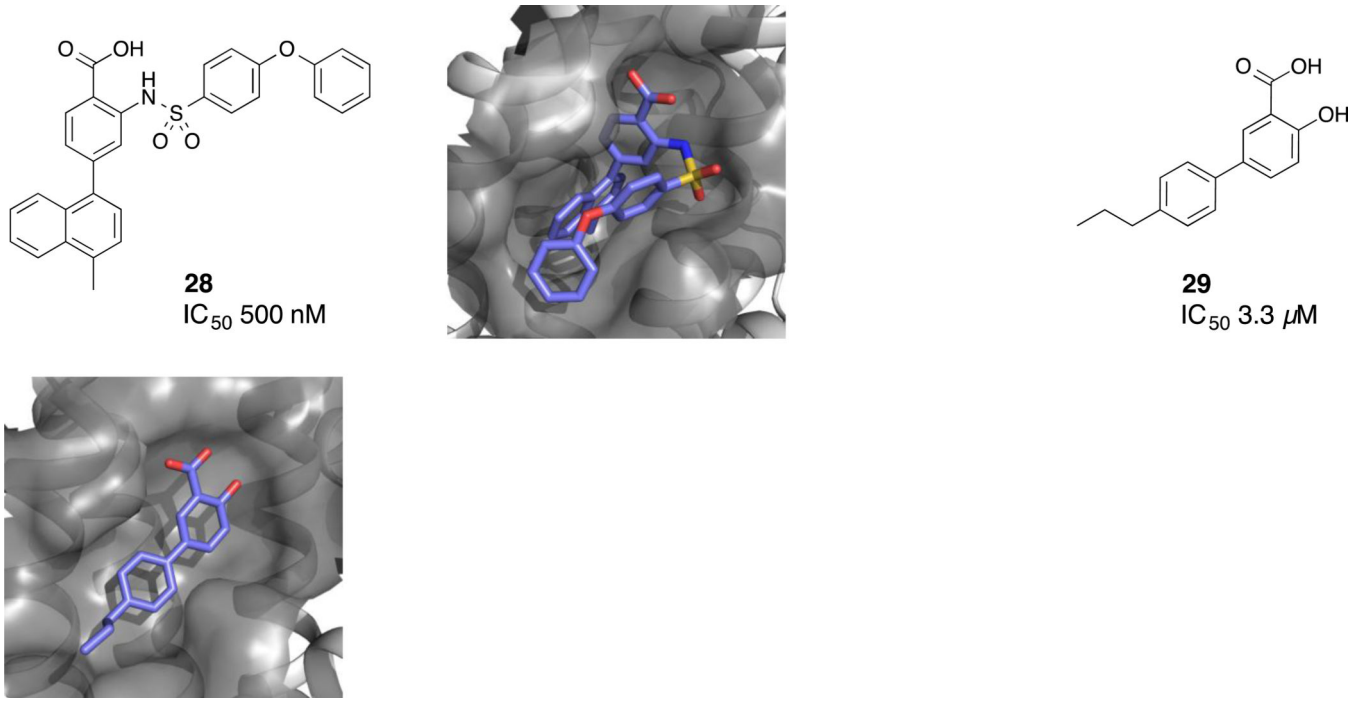


Figure 8.
Cocrystal structures of aryl sulfonamide **28** and salicylate **29** with Mcl-1.

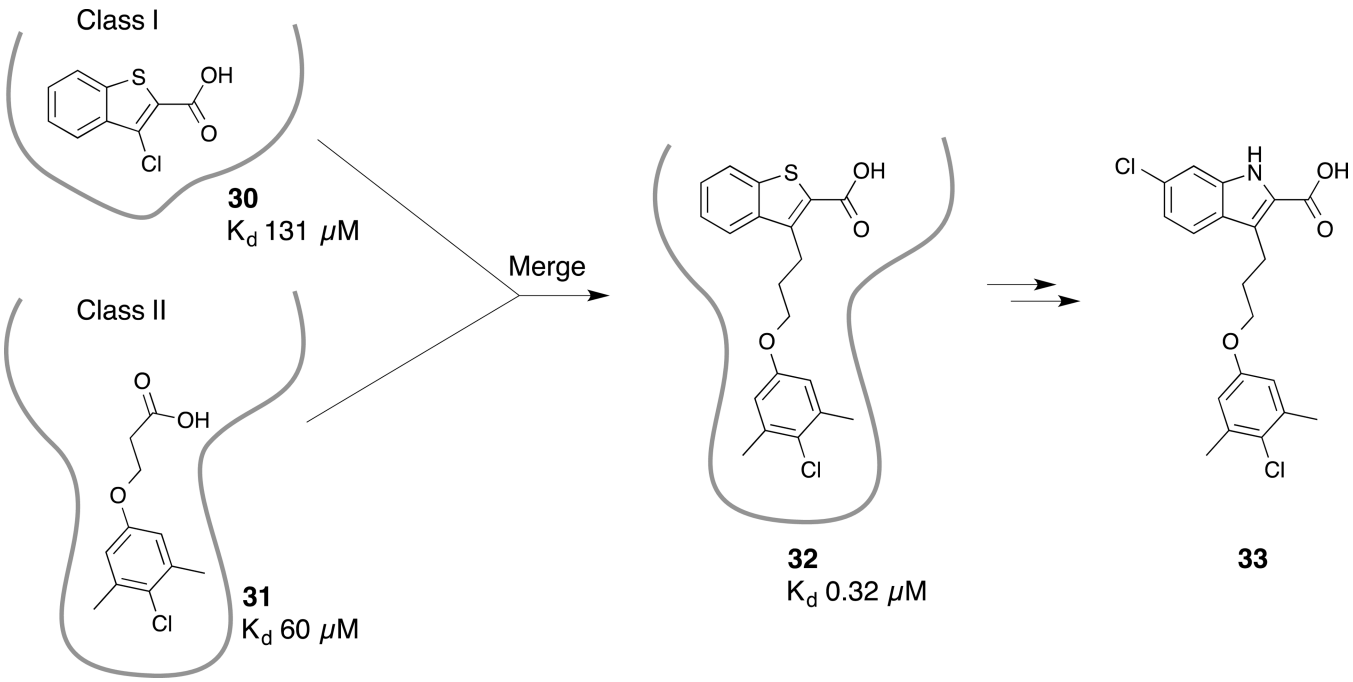


Figure 9.

Merging fragments from two distinct classes produced a compound with significantly improved binding affinity.

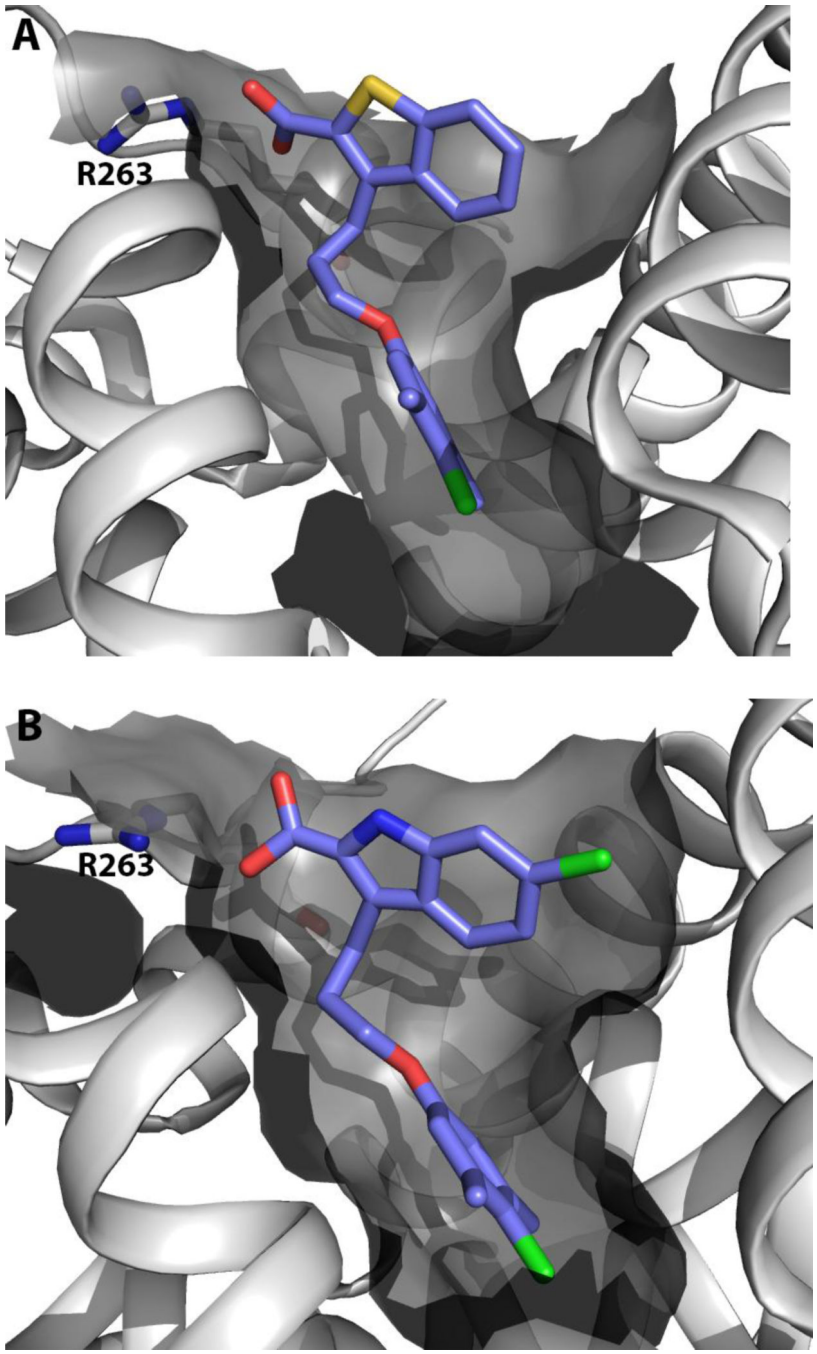


Figure 10.
Cocrystal structures of benzothiophene-2-carboxylic acid **32** (A) and indole-2-carboxylic acid **33** (B).

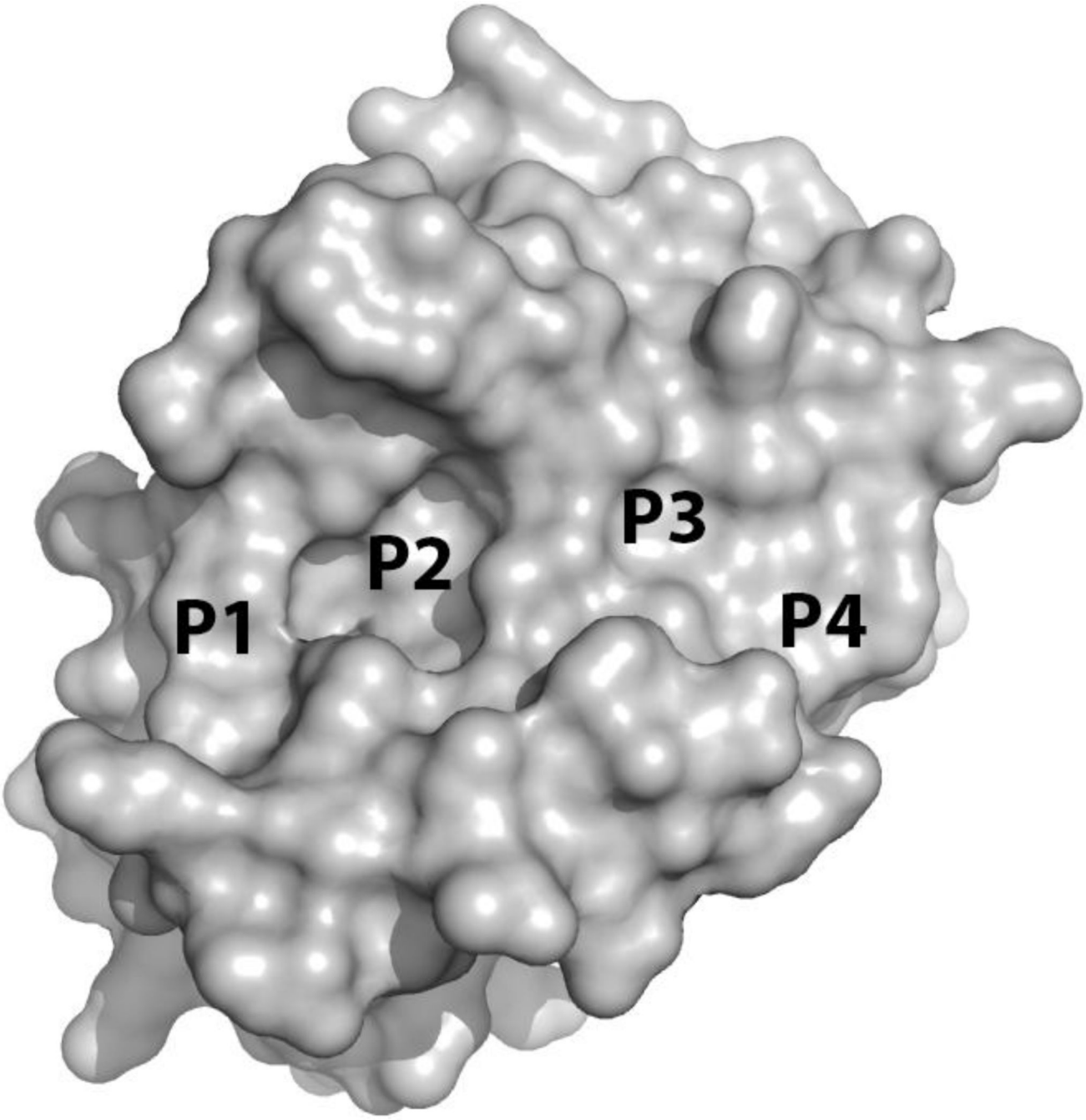


Figure 11.
X-ray structure of Mcl-1 (PDB ID 3MK8).

Table 1

Small molecule Mcl-1 inhibitors.

	Inhibition/Selectivity Data*			
Compound	**Mcl-1**	**Bcl-2**	**Bcl-X_L**	**Reference**
S1 (**5**)	K_d 58 nM	K_d 310 nM	NA	T. Song, Chen, et al. (2013)
6	IC_{50} 10 nM	IC_{50} 20 nM	NA	T. Song, et al. (2014)
8	K_d 160 nM	NA	NA	Zhang, Song, et al. (2013)
10	IC_{50} 54 nM	NA	NA	Zhang, Liu, et al. (2013)
12	K_d 10 μM	NA	K_d >750 μM	Bernardo, et al. (2010)
13	K_d 250 nM	NA	K_d 3.4 μM	Bernardo, et al. (2010)
16	IC_{50} 610 nM	NA	IC_{50} 4.4 nM	Tanaka, et al. (2013)
17	IC_{50} 88 nM	NA	IC_{50} 3.7 nM	Tanaka, et al. (2013)
19	K_d 180 nM	K_d 7.6 μM	K_d 10.6 μM	Abulwerdi, et al. (2014)
MIM1 (**20**)	IC_{50} 4.8 μM	NA	IC_{50} >50 μM	Cohen, et al. (2012)
marinopyrrole A (**21**)	IC_{50} 10 μM	NA	IC_{50} >80 μM	Doi, et al. (2012)
23	IC_{50} 310 nM	NA	IC_{50} 40 μM	Richard, et al. (2013)
25	IC_{50} 30 nM	NA	NA	Petros, et al. (2014)
27	IC_{50} 570 nM	NA	NA	Petros, et al. (2014)
33	K_d 55 nM	K_d 870 nM	K_d >15 μM	Friberg, et al. (2013)

Abbreviations: NA, not available at time of writing.

*Determined using varying competitive binding assays and ITC.