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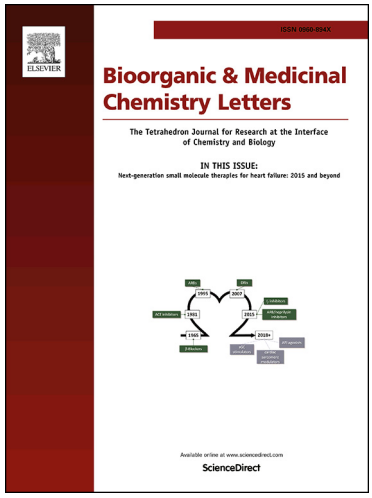

Discovery of novel biaryl sulfonamide based Mcl-1 inhibitors

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Graphical Abstract

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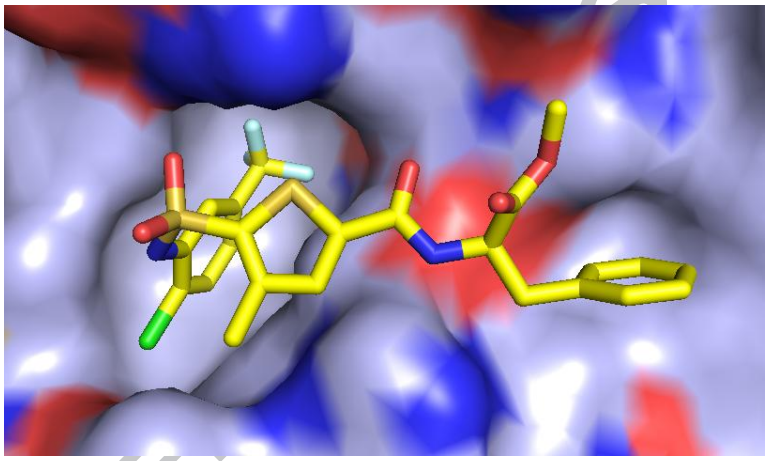

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Discovery of novel biaryl sulfonamide based Mcl-1 inhibitors

Bruce Follows,[*,a] Shawn Fessler,[a] Timm Baumeister,[a] Ann-Marie Campbell,[b] Mary Margaret Zablocki,[b] Hongbin Li,[b] Deepali Gotur,[a] Zhongguo Wang,[a] Xiaozhang Zheng,[a] Lisa Molz,[a] Cokey Nguyen,[a] Torsten Herbertz,[a] Liann Wang,[a] Kenneth Bair[a]

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ABSTRACT

Mcl-1 is an anti-apoptotic protein overexpressed in hematological malignancies and several human solid tumors. Small molecule inhibition of Mcl-1 would offer an effective therapy to Mcl-1 mediated resistance. Subsequently, it has been the target of extensive research in the pharmaceutical industry. The discovery of a novel class of Mcl-1 small molecule inhibitors is described beginning with a simple biaryl sulfonamide hit derived from a high through put screen. A medicinal chemistry effort aided by SBDD generated compounds capable of disrupting the Mcl-1/Bid protein-protein interaction *in vitro*. The crystal structure of the Mcl-1 bound ligand represents a unique binding mode to the BH3 binding pocket where binding affinity is achieved, in part, through a sulfonamide oxygen/Arg263 interaction. The work highlights the some of the key challenges in designing effective protein-protein inhibitors for the Bcl-2 class of proteins.

The Bcl-2 class of proteins, which is comprised of the anti-apoptotic (Mcl-1, Bcl-2, Bcl-xl, Bcl-w, Bfl-1/A1) and pro-apoptotic or BH3 proteins (Bim, Bid, Bad, Noxa) play critical roles in maintaining cellular homeostasis during normal cell differentiation during embryonic tissue development and remodeling. During this process, unwanted cells are removed through apoptosis as regulated by the intrinsic cell death pathway.[1] Mcl-1 functions as a negative regulator in this process, and like other anti-apoptotic proteins, sequesters the mediators or "apoptosis effectors" of the cell death response, BAX and BAK. When left unbound, BAK and BAX localize in the mitocondrial membrane to induce the release of caspases ultimately resulting in apoptosis.[2-4] Other pro-apoptotic BH3 proteins (Bid, Bim and Bad, Noxa, etc.) known as "apoptosis initiators" form tightly bound BH3-Mcl-1(Bcl-2,) complexes that effectively liberate BAK and BAX to tip the balance toward cell-death.[5]

Much evidence supports targeting Mcl-1 for the treatment of human cancer. A recent review reports that amplified levels of Mcl-1 occur in numerous haematological and solid tumor tissues including lung, breast, prostate, pancreatic, ovarian, cervical and melanoma.[6] Furthermore, many of these Mcl-1 overexpressing tissues exhibit tumor growth inhibition and cell death upon RNA silencing.[6] In pancreatic cancer cell lines, RNAi mediated tumor growth reduction was observed both *in vitro* and in mouse xenograft models further validating Mcl-1 as an oncology target.[7]

In the clinic, Mcl-1 overexpression has been observed as a primary resistance mechanism following treatment with vincristine[8], paclitaxel,[8] or gemcitabine.[7] Bcl-2 inhibitors in the clinic show efficacy in lymphoid tumors presumed to be Bcl-2 dependent where the mechanism of action is direct competition with anti-apoptotic proteins. The first in class intravenous drug ABT-737 and ABT-263, an orally available, dual Bcl-2/Bcl-XL inhibitor, were evaluated as therapies in small-cell lung cancer and B-cell malignancies.[9] The dual inhibitor caused dose limiting toxicity (thrombocytopenia) attributed to an on-target effect of Bcl-XL inhibition. A selective Bcl-2 inhibitor ABT-199[10] offered tolerability advantages over its predecessors and was approved by the US FDA for patients with chronic lymphocytic leukemia carrying a 17-p deletion. Limited efficacy has also been observed in certain instances of Mcl-1 overexpression.[2, 11]

While a number of small molecule Mcl-1 inhibitors[12-17] as well as peptide related BH3 protein mimetics[18-21] have been reported, three Mcl-1 specific inhibitors have entered clinical trials.[12, 14] A Phase 1 study with Novartis' MIK665 to treat patients with refractory or relapsed lymphoma or multiple myeloma will reach completion by April of 2020. Amgen initiated clinical studies with AMG-176, currently recruiting patients with relapsed or refractory disease states of multiple myeloma or acute myeloid leukemia. A newer compound, AMG-397, entered Phase I trials to treat patients with multiple myeloma, non-hodgkin's lymphoma or acute myeloid leukemia. These selective, reversible small molecules inhibitors represent first in class drugs capable of disrupting Mcl-1 from high affinity BH3 binding proteins *in vivo*. Alternative routes of inhibition have been reported including inactivation of Mcl-1 through a covalent bond with a noncatalytic lysine side chain.[22] Thus,

much effort is directed toward the development of Mcl-1 inhibitors as drugs to treat cancer.

Herein we describe the discovery of novel (double digit nanomolar), small molecule inhibitors of the Mcl-1/BH3 protein-protein interaction.

Hit identification was carried out using a high-throughput screening campaign which identified several chemotypes as starting points (Figure 1). Our testing paradigm incorporated validation activities run in parallel to expedite the early validation stage. While routine re-testing of purified material was conducted, exploratory libraries around each chemical series established preliminary SAR trends. Our high-throughput synthesis platform and screening capabilities were subsequently useful in differentiating initial leads. A fluorescence polarization assay was used to determine the IC_{50} values required to disrupt the recombinant human Mcl-1 and a FITSI labeled BID protein.[23] Several of the initial hit series, including the tetrahydroquinolines (**1**), pyrroles (**2**) and pyrazolidinones (**3**) were quickly eliminated during hit validation due to uninterpretable SAR upon re-synthesis of the actives and near neighbors. While the actual mechanism of inhibition is unknown, examples from the literature are often the result of compound aggregation, interference in the light detection format or compound reactivity.[24-25]

Among the original HTS hits, a pull-down experiment indicated that **4** disrupts the BH3/Mcl-1 binding interaction. Western blot analysis revealed a dose dependent reduction in the levels of the Mcl-1/tBid complex, purportedly at the Mcl-1/BH3 interface. The protein levels at 3 inhibitor concentration were quantified and normalized to the control as depicted in Figure 3. Taken together, the data suggested **4** was a competitive, reversible Mcl-1 inhibitor warranting further medicinal chemistry optimization.

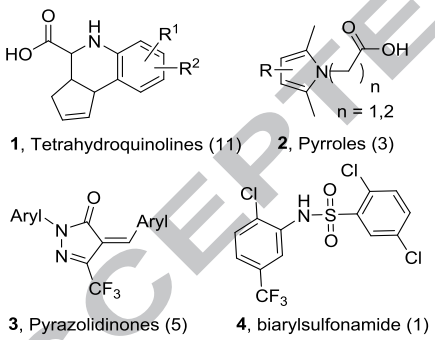


Figure 1. Actives from HTS screen

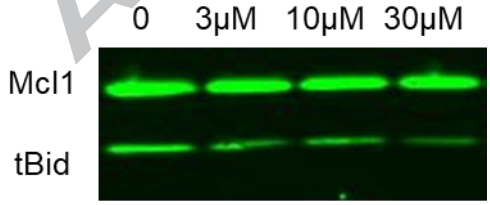


Figure 2. Western blot of Mcl-1/tBid complex after treatment with 0, 3, 10 and 30 μM concentration of **4**.

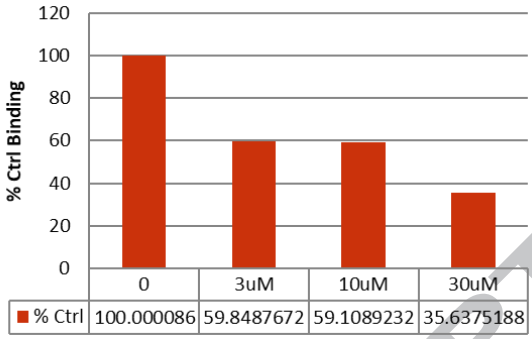


Figure 3. quantitative densitometry of Western blot normalized to the control

Several synthetic libraries based upon **4** were prepared to systematically interrogate SAR around the molecule (Figure 4). Left-hand modifications (Library A; Table 1) revealed that variations to the size, polarity or substitution pattern around the ring were detrimental to activity. Deleting either the 2-Cl (**8**) or the 5-CF_3 (**7**) group resulted in a complete loss of activity. Incorporating polarity by the introduction of a heterocycle (**9**) or appending polar groups on the phenyl ring system (**10**, **12**) also resulted in an abrogation of activity shown by representative examples in Table 1.

The most active compounds with single digit micromolar activity had either methyl or chloro groups in the ortho position. Modifications on the right-hand side of the molecule (Library B; Table 2) revealed several prominent SAR trends: Expanding the size of the ortho group or removing it led to potency losses. Within the set of ortho -Cl and -Me compounds, additional para/meta substitution neither helped nor hindered biochemical activity given that compounds bearing different functionality at the meta/ortho positions were equally potent as evident by thiophene (**20**, IC50 = 1.9 μM) and cyclic lactam (**26**, IC50 = 1.8 μM).

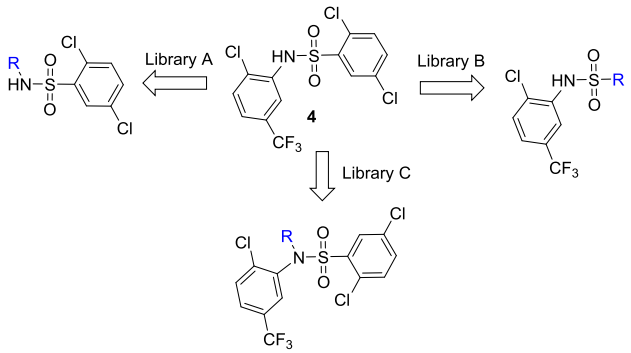
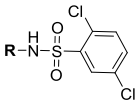


Figure 4. Exploratory library design from initial HTS hit. Library A = iterative changes on the left-hand side; Library B = iterative changes on the right-hand side; Library C = addition of functionality on the sulfonamide nitrogen.

Table 1. SAR summary of Library A



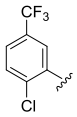
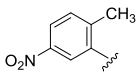
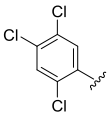
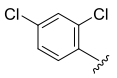
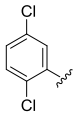
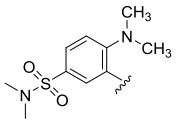
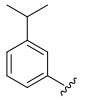
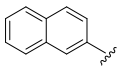
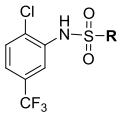
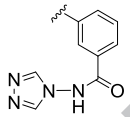
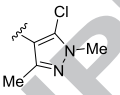
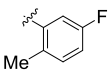
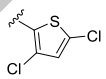
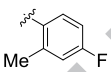
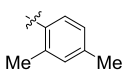
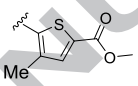
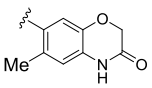
ID	R	FP IC_{50} μM	ID	R	FP IC_{50} μM
4		3.1	**10**		20
5		8.6	**11**		21
6		10.6	**12**		18
7		> 25	**13**		> 25
8		> 25	**14**		> 25
9		> 25			

Table 2. SAR Summary of Library B



ID	R	FP IC_{50} μM	ID	R	FP IC_{50} μM
15		7.3	**21**		19.2
16		13	**22**		23
17		11	**23**		17
18		5.5	**24**		8.6
19		10	**25**		12
20		1.9	**26**		1.8

To probe the central linker portion of **2**, a small set of derivatives was prepared including the reverse sulfonamide and 2 atom linkers comprised of N-C, C-O and S-C combinations (Figure 5). Since all analogs prepared had IC50s >25μM, we speculated that the sulfonamide linker may be optimal by virtue of its scaffolding effect or due to specific contacts made between the O- or N-atom with Mcl-1 protein residues.

The SAR trends from exploratory libraries allowed us to propose a tentative binding hypothesis. We speculated that the hydrophobic 2-Cl-5-CF_3-phenyl ring on the left-hand side of the molecule occupied a sterically confined hydrophobic pocket in the BH3 binding region of Mcl-1. The lipophilic pockets in Mcl-1, designated as P1-P4 (Figure 7a) may be suitable places for such a group since small hydrophobic residues from the Bim peptide alpha helix: ILE 86, LEU 90, VAL 93 and MET 97 each occupy the respective P1-P4 pockets based on Mcl-1/Bim co-crystals.[26-29] Analogous pockets (P2 and P4) in Bcl-2 have been targeted as "hot spots" in small molecule drug design using an SAR by NMR fragment based screening approach.[30] The 2,5-dichlorophenyl ring on the opposite side of the molecule may project away from the lipophilic pocket, toward another region of the BH3 binding helical domain. The observation that substitution on the edge of the right-hand side leads to flat SAR suggested that extending functional groups from the meta or para positions of the RHS ring may enable productive interactions with other residues in the BH3 binding domain. The thiophene scaffold present in **20** was therefore selected as a core template to test our hypothesis.

Exploratory targets were prepared from commercially available starting materials using three general reaction conditions as shown in Scheme 1: 1) Sulfonylation from the sulfonyl chloride and corresponding aniline (**4-27**) conducted in pyridine and dichloromethane; 2) Reductive amination using sodium borohydride and acetic acid in dichloroethane (**30, 31**); 3) Alkylation reactions from an alkyl halide and phenol/thiophenol using potassium carbonate or cesium carbonate as the base in DMF (**29, 32, 33**). Sulfide **29** was oxidized with *m*CPBA to afford sulfoxide **28**. Thiophene analogues **34-39**, **43** were prepared in three steps starting with sulfonamide formation between 2-chloro-5-(trifluoromethyl)aniline and methyl 5-(chlorosulfonyl)-4-methylthiophene-2-carboxylate (Scheme 2). The product was hydrolyzed with NaOH in MeOH-THF-water and treated in a subsequent step with the corresponding amine under BOP coupling conditions to give the desired products. A modification of this sequence was used to prepare acids **40-42** and **44-57** in which an additional hydrolysis step was added after the peptide coupling with an amino ester. Amides **63** and **64** as well as acylsulfonamides **61** and **62** were derived from reactions with chiral carboxylic acid **41** as shown in Scheme 2.

Incorporating larger, hydrophobic groups on the right-hand side of the molecule generally resulted in equipotent compounds. Exceptions to this occurred with the phenethyl derivatives. Key examples from this subseries included the first sub-micromolar inhibitor **37**, and structurally related chiral phenethanol compounds **38** and **43**.

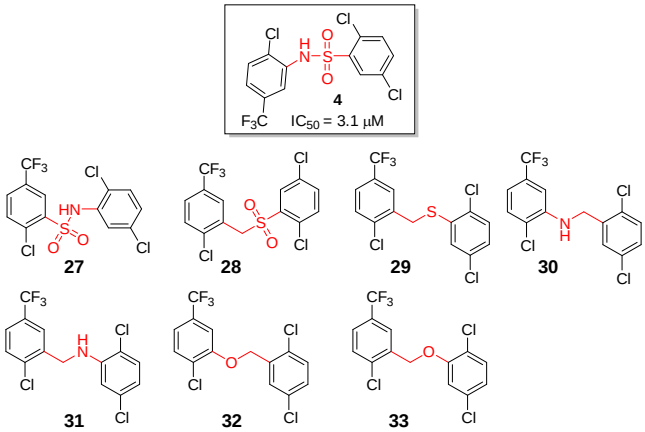
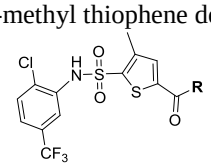


Figure 5. Modifications of the sulfonamide linker; deviations at the linker portion represented here lead to inactive compounds all showing IC50's > 25 μM

Previously reported structures of Mcl-1 show Arg residues such as Arg263 in close proximity to the hydrophobic pockets of the BH3 domain.[31] As part of our design strategy to introduce electrostatic interactions with the Arg residue, a carboxylic acid functionality was introduced on the right-hand side of the molecule. While many analogues from this design hypothesis failed to show improvements in potency, the phenylalanine derivative **40** displayed a 6-fold improvement in potency from the original hit **20**. Separation of the racemic mixture revealed that the R enantiomer preferentially binds to the target with an approximate 4-fold difference in IC_{50}. As a follow-up to **40**, further analogues were prepared to understand the role of the charged group and the phenyl ring. Simply removing the benzyl group (**41** vs. **56**) resulted in a ~3-fold loss in activity while extending the carboxylic acid chain by an atom length (**44**) resulted in an additional ~7-fold loss (**56** vs. **44**). Changes to the benzyl ring resulted in modest changes to the activity as shown in Table 4 with the following relative potencies: benzyl (**41**) > iPr (**48**) = CH_2CH_2Ph (**52**) > Me (**47**). Incorporating heteroatoms into the benzene ring also had a modest effect on reducing the activity shown by tryptophan derivative **53** and 3-pyridyl derivative **55**. More polar rings such as pyrrazole **50** were ~9-10 fold less potent.

Replacing the carboxylic acid with other ionizable groups resulted in comparable activities. Tetrazoles **59** and **60** had similar IC_{50s} vs. the corresponding carboxylic acid analogues. The acylsulfonamide derivatives **61** and **62** were slightly better and represented the most potent compounds in the series with IC_{50s} of 53 nM and 73 nM respectively. Expansion from this set however revealed that the negative charge was not essential for activity as shown by the ester and amide adducts **64** and **65** which were comparable in potency to their acid-containing counterparts.

Table 3. SAR of 4-methyl thiophene derivatives



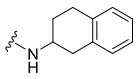
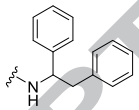
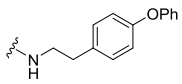
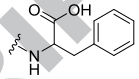
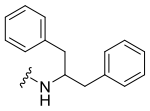
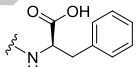
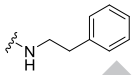
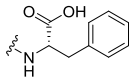
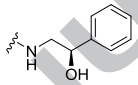
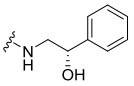
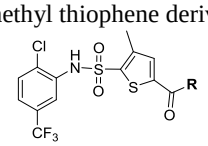
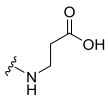
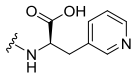
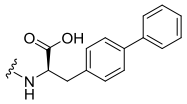
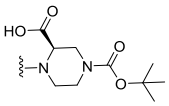
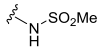
ID	R	FP IC_{50} μM	ID	R	FP IC_{50} μM
34		4.21	**39**		7.1
35		7.02	**40**		0.3
36		7.1	**41**		0.181
37		0.813	**42**		0.754
38		0.470	**43**		0.149

Table 4. SAR of 4-methyl thiophene derivatives



ID	R	FP IC_{50} μM	ID	R	FP IC_{50} μM
44		3.46	55		0.381
45		2.77	56		0.466
46		1.47	57		0.63
47		1.47	58		1.07
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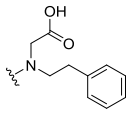
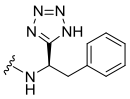
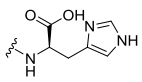
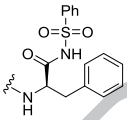
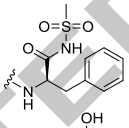
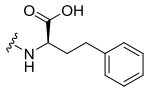
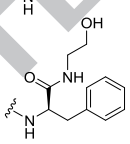
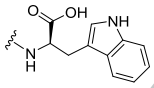
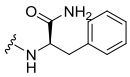
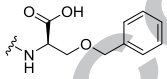
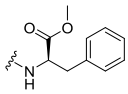
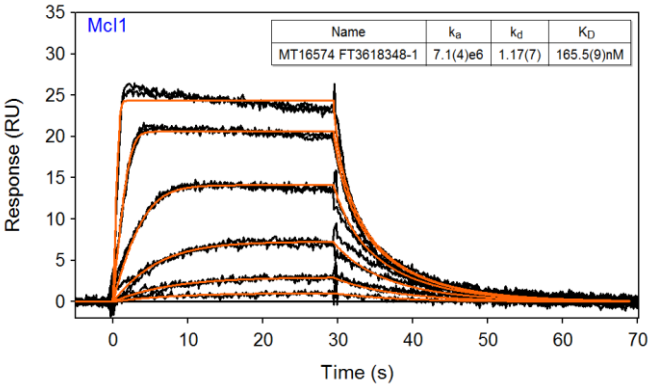
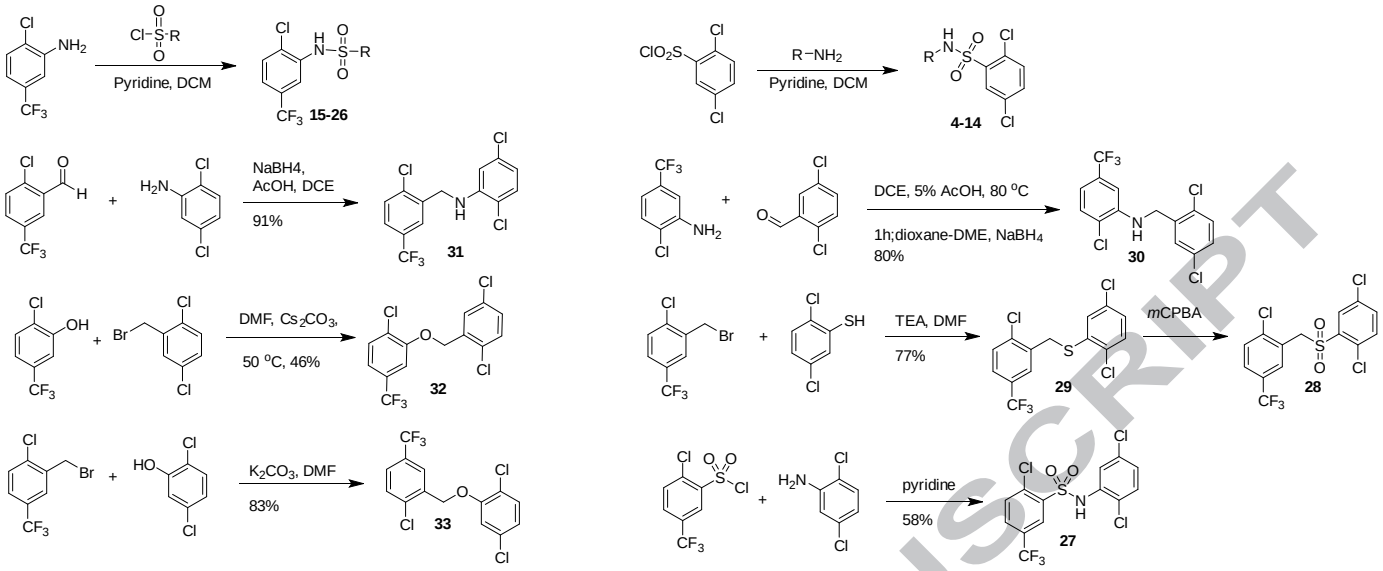
49		0.694	60		0.153
50		0.924	61		0.053
51		1.01	62		0.073
52		0.815	63		0.112
53		0.209	64		0.153
54		0.309	65		0.145

Figure 6. SPR sensorgram

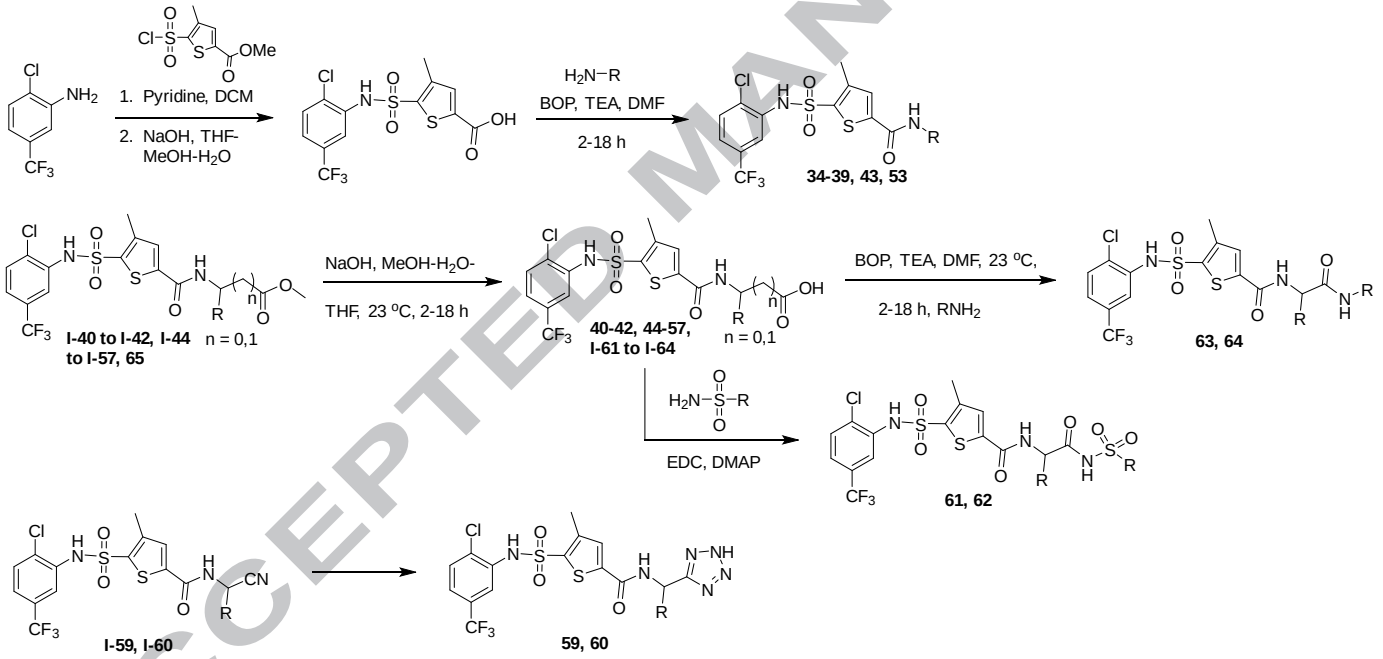
Biophysical characterization by surface plasmon resonance was performed with **43** at several concentrations with biotinylated Mcl-1. Carbonic anhydrase II was used as a control protein for measuring binding selectivity. The KD determined by SPR (165 nM) was consistent with the IC_{50} measured by fluorescence polarization (149 nM). Together, SPR data indicated that representative compound, **43**, showed selective and saturable binding consistent with a well behaved Mcl-1 inhibitor (Figure 6; additional material available in supplementary information).

A co-crystal structure of **65** with Mcl-1 revealed key insights into the critical ligand-protein interactions present in the series (Figure 8). The amide carbonyl oxygen lies within hydrogen bonding distance of His224 (3-3.5 Å). The benzyl ring on the right-hand side is loosely associated with the floor of the P4 pocket, which is shallow, hydrophobic and otherwise devoid of features that could be targeted in order to optimize interactions with small molecule inhibitors. The 2-Cl and 5-CF_3 groups project deep into hydrophobic regions of the P2 pocket, with an orthogonal orientation relative to the bound BH_3 peptide helix. The 2,5-disubstituted phenyl ring creates a unique pocket, absent in the BH3 bound Mcl-1 structure, highlighting the plasticity of the P2 pocket of Mcl-1. The sulfonamide oxygen lies 3.46 Å from the N-atom of Arg263. The –NH bond of the sulfonamide is extensively



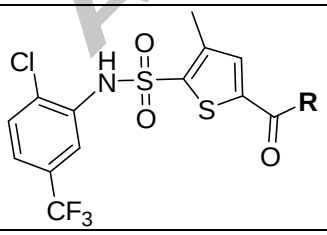
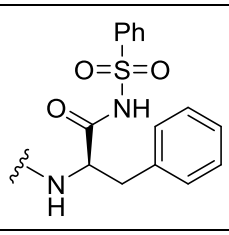
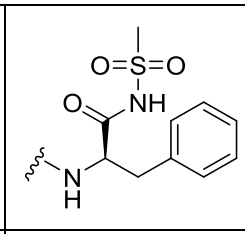
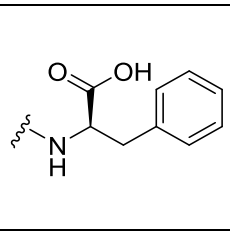
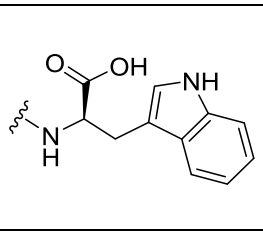


Scheme 1. Synthesis of exploratory chemistry targets



Scheme 2. Synthetic routes to targets spanning P2 to P4.

Table 5. Physico-chemical properties of select Mcl-1 inhibitors.

				
ID	**61**	**62**	**41**	**53**
MW	686	624	546	586
clog D pH 7.4	5.0	2.8	1.7	1.8
LiPE	2.3	4.3	5.1	5.1
MLM	81	91	95	94
HLM	74	99	66	98

PAMPA	0.24	0.14	--	0.12
Solubility (μM)	--	--	100	100

*PAMPA permeability values expressed as the mean log Papp in cm/sec; lipE = pIC_{50} – log P; MLM = mouse liver microsomes assay values expressed as a percentage of parent compound left after 30 min.

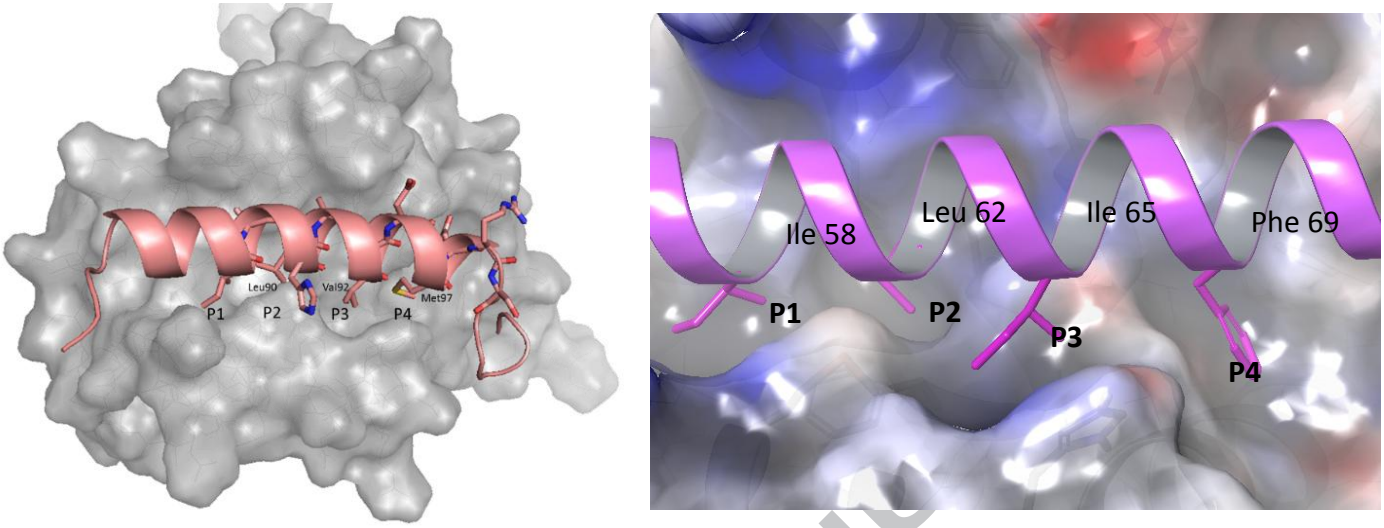


Figure 7. (a) Overview of BH3 alpha helix bound to Mcl-1 (b) Conserved residues of BH3 Bim protein interact with P1-P4 region of Mcl-1's binding domain (2NL9). Residues of the key hydrophobic protein-protein interactions between the BH3 alpha helix and corresponding P1- P4 pockets of Mcl-1.

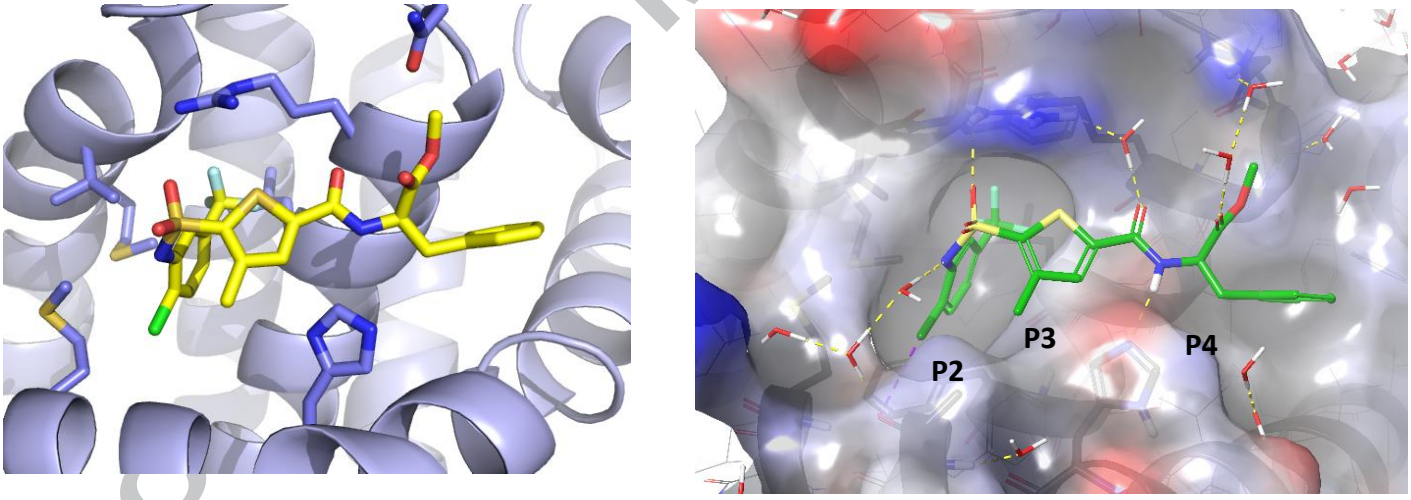


Figure 8. (a) X-Ray crystal structure of **65** and Mcl-1 showing the key H-bonding interactions; (b) bound structure (65) depicting the degree of plasticity in the P2 binding site (PDB code 6P3P).

ionized under the buffered conditions of the biochemical assay, which we speculated may enhance a potential electrostatic interaction with Arg263. Perhaps more important is the bond angle of the sulfonamide, where, unlike the simple 2-atom linkers in **27**-**33**, maintains an optimal 90° bend to allow the 2- chloro-5-CF_3 phenyl group to project into the deepest part of the P2 pocket while keeping the methyl thiophene ring close to the ridge between the P2 and P3 sites. The structural changes at P2 highlight the extent of flexibility inherent in this target. Reported small molecule Mcl-1 co-crystal structures[32-35] also show high degrees of plasticity in the region of the P2 pocket.

Table 5 shows the series possesses favorable solubility and microsomal stability in human and mouse microsomes. In general, the highly lipophilic components required for binding combined with the ionized, acidic residue provide a balance of physicochemical properties maintaining the clogD value within drug-like space. The most potent analogue, **61**, has a high clogD of 5 and therefore much lower lipE values when compared to **41** or **53** (lipEs of 2.3 vs. 5). Since the affinity of **41** and **53** is derived to a greater degree from specific interactions with the target, they were considered to provide a better starting point for lead optimization efforts.

The lack of prominent binding features in P4 created a challenge to attaining additional binding affinity.[36,6] Indeed, none of our medicinal chemistry design efforts succeeded in capturing additional interactions in P4. While the orientation of the thiophene core provided ample opportunities to probe the region,

derivatives of the phenethyl group including para and meta phenyl ring substituents, branching substitution at the carbon atoms alpha and beta to the amide nitrogen, as well as extending the length of the linker region all proved unsuccessful in gaining additional ligand/protein interactions to enhance biochemical potency. Little insight can be gained from X-ray structures in this regard as the aromatic rings lie perpendicular to the floor of the relatively flat and featureless P4 pocket. The benzyl ring of **65** lies in a strikingly similar position as the BH3 peptide residue Phe69 in the Bim bound Mcl-1 complex (Figure 7a-b). BH3 proteins Nova, Bax, and Bak all bind tightly to Mcl-1 in a binding groove spanning ~10 Å. An efficacious Mcl-1 inhibitor, therefore, will likely need extraordinary binding affinity to effectively compete with the BH3 binding proteins *in vivo*. The clinical candidates ABT199 and ABT263 engage multiple pockets of Bcl-2 spanning P2-P4 and similar design approaches may also be required to produce effective Mcl-1 candidates. Inhibitors reported by Abbvie occupy P2-P4 and maintain an ionic charge interaction between the ligand's carboxylic acid and Arg263. Amgen recently reported successfully designing potent and drug-like Mcl-1 inhibitors by restricting the conformational degrees of freedom using structure-based drug design. The macrocycle AMG-176 maintained a rigid, bioactive conformer with desirable potency profiles against Mcl-1 dependent cell lines.[14]

More extensive design approaches targeting the P4 pocket or addition of specific groups that target the Arg263 residue will inevitably be needed to drive potency in this series of inhibitors.

In summary, the discovery of Biaryl sulfonamide Mcl-1 inhibitors is described. Traditional medicinal chemistry efforts aided by X-Ray crystallography successfully advanced an HTS hit to a more lead-like, potent inhibitor of the Mcl-1/Bid complex. The lessons learned here underscore some of the key challenges in designing small molecule inhibitors of protein-protein interactions.

ASSOCIATED CONTENT

Supporting Information. Analytical methods, materials, instrumentation, general materials for the synthesis of compounds 41, 61, 62, and 63, protein production and structure determination, data collection and refinement statistics, Mcl-1-tBid pulldown assay, biophysical characterization with surface plasmon resonance. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS

Mcl-1, myeloid cell leukemia; Bcl-2, B-cell lymphoma 2.

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