

Synthesis and evaluation of substituted hexahydronaphthalenes as novel inhibitors of the Mcl-1/BimBH3 interaction

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ABSTRACT

Mcl-1, an anti-apoptotic member of the Bcl-2 protein family, is overexpressed in a broad range of human cancers and plays a critical role in conferring resistance to chemotherapy. In the course of screening a natural product-like library of sesquiterpenoid analogs, we identified substituted hexahydronaphthalenes that showed activity against the Mcl-1/BimBH3 interaction in vitro. Here, we describe the synthesis of a small library of analogs and their biological evaluation. The most potent inhibitor in the series (**19**) exhibits an IC₅₀ of 8.3 μM by ELISA and disrupts the interaction between endogenously expressed Mcl-1 and Bim in cultured MDA-MB-468 breast cancer cells.

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Bcl-2 family proteins regulate apoptosis through their influence on mitochondrial outer membrane (MOM) permeability and the release of cell death factors such as cytochrome c in response to cellular stress.^{1–3} Bax and Bak are pro-apoptotic multidomain (BH1–BH4) family members necessary for apoptosis and are directly involved in binding to the MOM.⁴ Other pro-apoptotic Bcl-2 proteins such as Bim, Bid, Bad, Bik, Puma, and Noxa contain only a single BH3 domain and indirectly modulate MOM permeability upstream of Bax and Bak.⁵ The anti-apoptotic family members Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and Bfl-1 exert their influence by heterodimerizing with these pro-apoptotic substrates.^{6–8}

Mcl-1 is over-expressed in greater than 50% of hepatocellular carcinomas,⁹ pancreatic adenocarcinomas,¹⁰ cervical cancers,¹¹ non-Hodgkin's lymphomas,¹² and non-small cell lung cancers.¹³ Mcl-1 is known to compensate for the loss of Bcl-2 or Bcl-xL activity induced by selective antagonists^{14,15} and is especially well suited to provide protection from apoptosis due to its relatively short half-life (between 0.5 and 3 h).¹⁶ Mcl-1 is thus a critical survival factor in a variety of human tumors and has emerged as a promising target for small molecule inhibitors.

In our search for new antagonists of anti-apoptotic Bcl-2 proteins, we screened a small in-house library of natural product-like

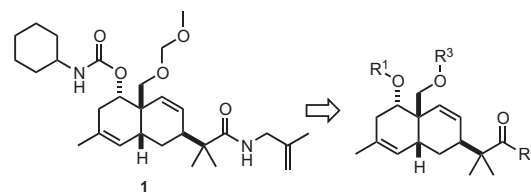
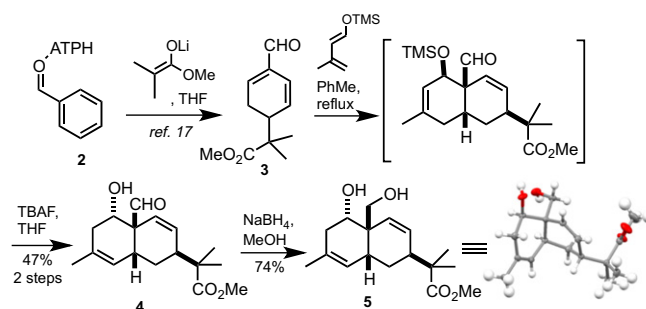


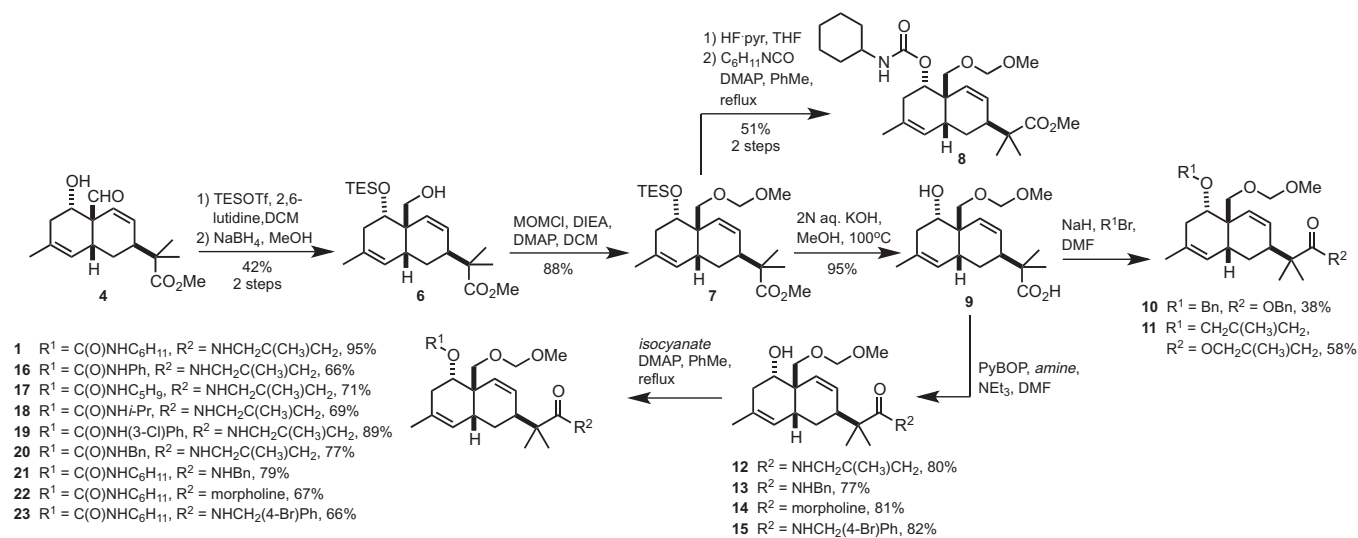
Figure 1. Structure of hexahydronaphthalene **1** and analogs.



Scheme 1. Synthesis of hexahydronaphthalene scaffold **5**.

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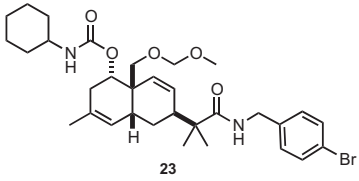
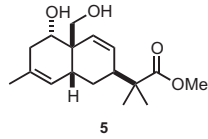
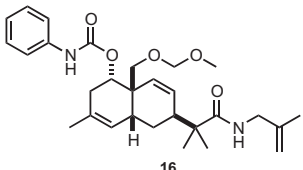
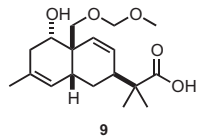
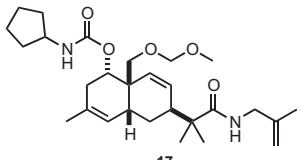
Scheme 2. Analog synthesis.

Table 1

Inhibition of the GST-Mcl-1/BimBH3 interaction by ELISA at 25 μ M (mean given with standard deviation, $n = 3$)

Compound	% Inhibition	Compound	% Inhibition
	53.8 \pm 5.1		31.2 \pm 7.1
	34.8 \pm 8.4		74.3 \pm 6.0
	17.1 \pm 1.8		40.3 \pm 8.4
	53.9 \pm 3.7		64.9 \pm 4.7
	26.5 \pm 5.9		31.2 \pm 1.0

Table 1 (continued)

Compound	% Inhibition	Compound	% Inhibition
	64.4 ± 5.9		25.0 ± 2.6
	61.8 ± 10.6		18.2 ± 6.0
	19.7 ± 4.0		

scaffolds for activity against the Mcl-1/BimBH3 interaction. This resulted in the identification of hexahydronaphthalene **1** (Fig. 1) as hit compound for further investigation. Here, we describe the synthesis of analogs of **1** and their biological evaluation as novel Mcl-1 antagonists.

The hexahydronaphthalene core structure was prepared via Diels-Alder reaction between dihydrobenzaldehyde derivative **3**¹⁷ and (*E*)-trimethyl((3-methylbuta-1,3-dien-1-yl)oxy)silane under thermal conditions (Scheme 1).¹⁸ Treatment of the intermediate cycloaddition adduct with TBAF in THF promoted silyl ether cleavage and concomitant alkene migration to give core structure **4** as a single diastereomer. Extensive 2D NMR experiments established the structural connectivity of the product while relative stereochemistry was confirmed by X-ray diffraction of reduced derivative **9**.¹⁸ Presumably, treatment of the intermediate Diels-Alder adduct with TBAF promotes retro-aldol ring opening and equilibration to the more stable α -OH isomer. While the precise mechanism of the concomitant alkene migration is unknown, this process seems to occur during the retro-aldol reaction and ultimately gives rise to a lower energy dihydrodecalin. The relative stereochemistry of the hexahydronaphthalene scaffold was confirmed by X-ray diffraction of diol derivative **5**.

Elaboration of **4** into the desired compounds was achieved through silylation of the secondary alcohol followed by aldehyde reduction and conversion to the methoxymethyl ether **7** (Scheme 2). To assess the impact of the amide substituent of **1** on biological activity, we prepared truncated analogue **8** via silyl ether cleavage and reaction of the resulting alcohol with cyclohexylisocyanate. Hydrolysis of **7** followed by dialkylation afforded analogs **10** and **11**. Condensation of **9** with various amines and subsequent carbamoylation gave a series of diversely substituted hexahydronaphthalenes for biological evaluation.

All newly synthesized compounds were tested for their ability to block the interaction between GST-Mcl-1 and the BimBH3 domain in an ELISA assay^{19,20} at a single concentration (25 μ M). A peptide corresponding to the BimBH3 helix was used as a reference inhibitor and average percent inhibition was calculated from three separate experiments. As shown in Table 1, initial lead compound **1** exhibited >50% inhibition of Mcl-1 at 25 μ M. Truncations at either end of the hexahydronaphthalene core as in **5**, **9**, and **12** resulted in diminished activity, as did the introduction of a more polar morpholine group in place of the isobutenyl amide as in **22**. Various

hydrophobic substitutions were well tolerated, with the exception of the smaller cyclopentyl and isopropyl carbamates in **17** and **18**. The 3-chlorophenyl carbamate derivative (**19**) exhibited slightly enhanced activity. Compound **10**, which is readily accessible from the core scaffold via dialkylation, also showed significant inhibition at 25 μ M.

We then selected the three most active compounds from single dose testing for determination of IC₅₀ values. Figure 2 depicts the ELISA dose response curves for compounds **10**, **19**, and **23** over a range of concentrations between 0.1 and 100 μ M. Each compound exhibited dose-dependant inhibition of Mcl-1 in the micromolar range. The most potent compound, **19**, showed an IC₅₀ of 8.3 μ M in vitro.

We next determined whether the most potent inhibitor in vitro (**19**) is able to enter human cancer cells, reach its target and disrupt the Mcl-1/Bim interaction. To this end, we treated MDA-MB-468 human breast cancer cells that ectopically express Bcl-xL and Bim with either vehicle V (0.1% DMSO) or **19** at 25 or 50 μ M, lysed the cells, immunoprecipitated Mcl-1 from the lysates and immunoblotted with Bim as described by us previously.²¹ As a positive control we used BH3M6, a substituted terphenyl derivative that has previously been shown to disrupt the interaction of various anti-apoptotic Bcl-2 family proteins with Bim in whole cells.²¹ TPC, an unsubstituted analog of BH3M6 with no appreciable in vitro activity was also included as a negative control. Figure 3 shows that in vehicle-treated MDA-MB-468 cells Bim co-immunoprecipitated with Mcl-1. Similarly, cells treated with negative

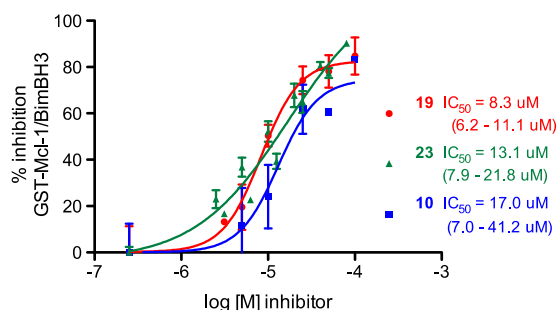


Figure 2. ELISA Mcl-1/BimBH3 dose-response curves for **10**, **19**, and **23** (95% confidence intervals in parentheses).

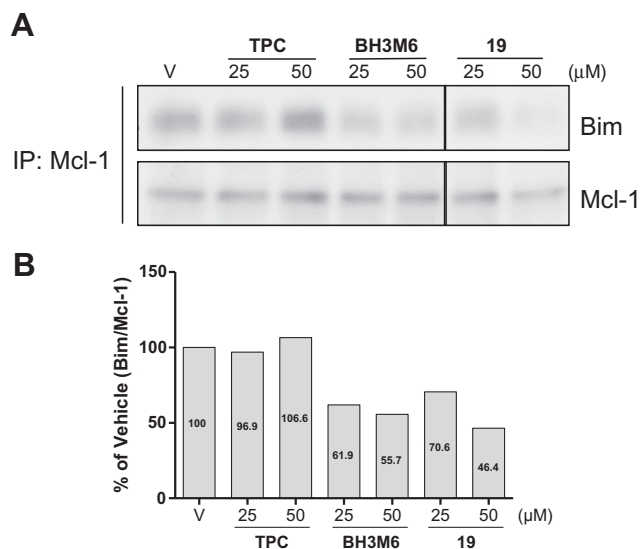


Figure 3. Co-immunoprecipitation of Mcl-1 (with Bim) from MDA-MB-468 cells. (A) Samples compared in the same gel for each protein and (B) densitometric analysis of inhibition.

control TPC also show complex formation between Mcl-1 and Bim. In contrast, **19** inhibited the interaction between Mcl-1 and Bim at both 25 and 50 μM. Compound **19** had similar potency to BH3M6 at both concentrations.

Compound **19** also exhibited approximately 80% growth inhibition toward MDA-MB-468 cells above 100 μM by MTT assay (see Supplementary data). However, increased concentrations did not

result in greater cytotoxicity. Poor solubility in culture medium beyond 100 μM precluded an accurate determination of GI₅₀ values.

To gain structural insight into the possible binding interaction between **19** and Mcl-1, we performed computational docking experiments using the GLIDE protocol. Since **19** was produced as a racemic mixture, we docked both enantiomers to the X-ray crystal structure of human Mcl-1 (derived from PDB code 2NL9).²² Figure 4 depicts the top scoring docking poses for each antipode, both of which bind to the hydrophobic cleft occupied by the BH3 helical domains of pro-apoptotic partners. Notably, the two large hydrophobic groups of the 1R,4aS,6R,8aS enantiomer (isobutenyl and 3-chlorophenyl) bind to the sites on Mcl-1 that normally accommodate the Leu62 (*i*+4) and Phe69 (*i*+11) side chains in human Bim (Fig. 4A). While the 3-chlorophenyl substituent in the 1S,4aR,6S,8aR enantiomer also appears to mimic the Phe69 residue, the isobutenyl substituent resides well outside of the Leu62 pocket (Fig. 4B). In both docked structures, the methoxymethyl ether substituent of **19** makes extensive stabilizing contacts with Asn260 and Arg263 in Mcl-1. Furthermore, this functional group overlays well with the carboxy side chain of Asp67 (*i*+9) in human Bim.

In summary, we have described the synthesis and preliminary SAR for a series of hexahydronaphthalenes that disrupt the Mcl-1/BimBH3 interaction in vitro. The most potent inhibitor in the series (**19**) exhibits an IC₅₀ of 8.3 μM by ELISA. Compound **19** also disrupts the interaction between endogenously expressed Mcl-1 and Bim in intact MDA-MB-468 breast cancer cells. Computational docking suggests that **19** interacts with the BH3-binding hydrophobic cleft in human Mcl-1. Efforts toward the synthesis of enantiopure **19** and other analogs for evaluation against a wider panel of anti-apoptotic Bcl-2 family proteins are currently underway.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2012.07.050>.

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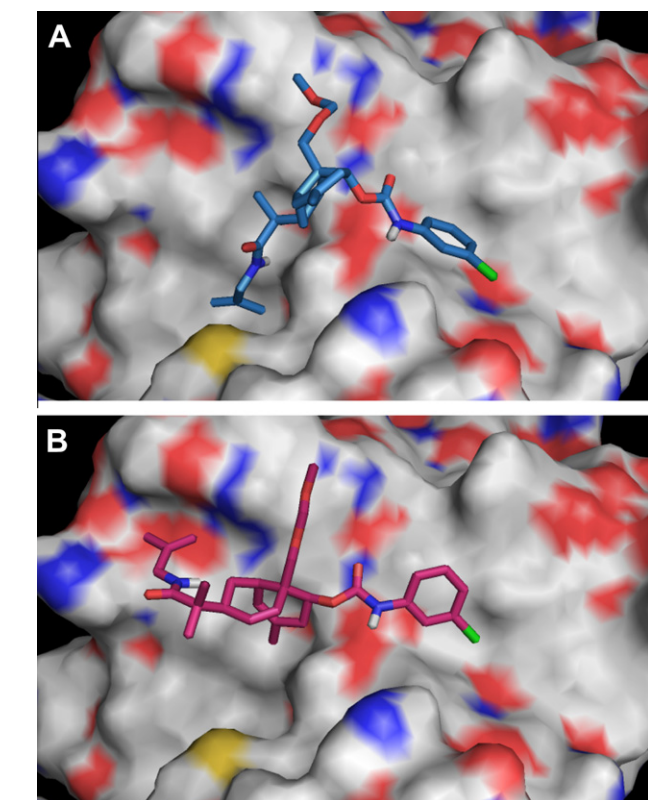


Figure 4. Top-scoring poses for (A) (1R,4aS,6R,8aS)-**19** and (B) (1S,4aR,6S,8aR)-**19** docked to human Mcl-1 (PDB code 2NL9) using GLIDE.

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