Structure-Based Design of Inhibitors of the Mcl-1 Oncoprotein

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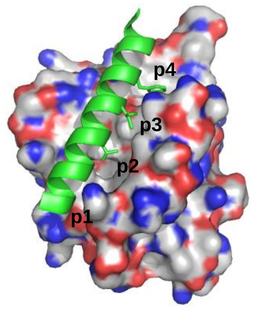
The Bcl-2 Proteins and Cancer

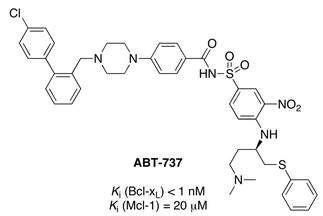
- The anti-apoptotic Bcl-2 proteins Bcl-x_L and Mcl-1 are frequently over-expressed in human cancers, including lung, pancreatic and colon cancers, as well as cancers of the blood, such as acute myeloid leukemia (AML).[1]
- Furthermore, the over-expression of Bcl-x_L and Mcl-1 contributes to resistance to conventional anti-cancer drugs, such as Taxol.[1]
- Expansion of targeted, small-molecule chemical artillery to battle human cancers is urgently required.
- Bcl-x_L and Mcl-1 exert their anti-apoptotic functions through "neutralizing" their pro-apoptotic Bcl-2 counterparts, such as Bak and Bim, by sequestering their helical BH3 "death" domains.[2]
- This protein–protein interface is an attractive target for structure-based drug design, and the BH3 helix of the pro-apoptotic Bcl-2 proteins provides a rational starting point.



➢ *Crystal structure of the Mcl-1–Bim-BH3 complex (PDB ID: 2PQK) highlighting the protein–protein interface: Bim-BH3 helix in green; Mcl-1 coloured by electrostatic surface potential (greys = hydrophobic, neutral hydrophilic; red = acidic; blue = basic). Hydrophobic residues insert into the p1–p4 sub-pockets.*

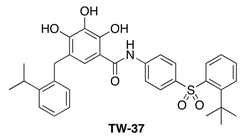
Current Inhibitors of the Bcl-2 Family

- There are many chemically diverse and selective inhibitors of Bcl-x_L.
- Arguably the most potent and selective inhibitor of Bcl-x_L is ABT-737, boasting a sub-nanomolar K_i.[3]
- ABT-263, the orally bioavailable congener of ABT-737, has caused thrombocytopenia in some patients during clinical trials.[4]
- Dual Bcl-x_L and Mcl-1 inhibitors, such as TW-37, are presently undergoing clinical trials.[4]
- Selective Mcl-1 inhibitors are beginning to be discovered, although none have progressed to clinical studies.[5]
- The pharmacologic inhibition of Mcl-1 represents a medical unmet need.



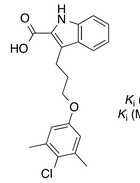
ABT-737

K_i (Bcl-x_L) < 1 nM
K_i (Mcl-1) = 20 μM



TW-37

K_i (Bcl-x_L) = 1.11 μM
K_i (Mcl-1) = 0.26 μM

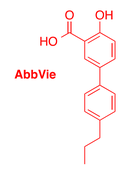


Fesik-53[6]

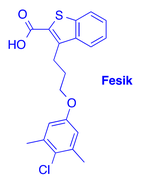
K_i (Bcl-x_L) > 15 μM
K_i (Mcl-1) = 0.055 μM

Structure-Based Rational Design of 4-Sulfamoyl-1-hydroxy-2-naphthoates as Specific Mcl-1 Inhibitors

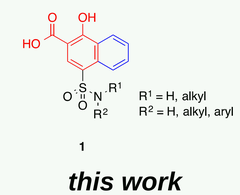
- Guided by NMR-based fragment screening, Abbvie[6] and Fesik[7] have discovered potent inhibitors of Mcl-1.
- The carboxylic acid of the Abbvie and Fesik inhibitors below bind Arg263 on the surface of Mcl-1, while the hydrophobic propyl and 4-chloro-3,5-dimethylphenyl groups bind the p1 and p2 sub-pockets, respectively.
- We hypothesized that merging the salicylate moiety of Abbvie's inhibitors with the benzene ring of Fesik's benzothiophenes will deliver a novel 1-hydroxy-2-naphthoate scaffold from which Mcl-1 inhibitors could be accessed in just two synthetic steps.
- Sulfamoyl groups may be readily introduced at the 4-position to allow inhibitors to probe deeply into the p2 pocket on the surface of Mcl-1.



AbbVie



Fesik

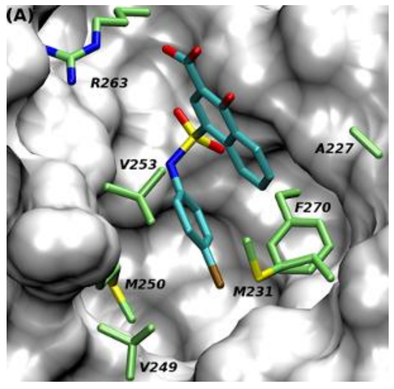


1

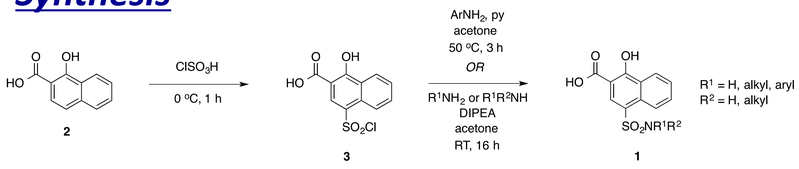
this work

Molecular Modelling

- Molecular modeling of compound **1a** (R^1 = 4-bromophenyl, R^2 = H) with Mcl-1 using the Site Identification by Ligand Competitive Saturation (SILCS)[8] methodology developed in the MacKerell laboratory at the University of Maryland is shown right.



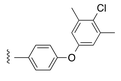
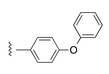
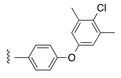
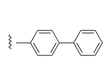
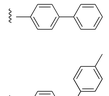
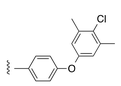
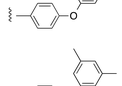
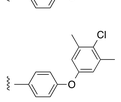
Synthesis



- The target molecules were prepared according to the expeditious synthetic sequence shown above. Starting from commercially available and cost effective 1-hydroxy-2-naphthoic acid (**2**), regioselective chlorosulfonylation was accomplished by stirring in chlorosulfonic acid for 1 h at 0 ° C to furnish **3**. Subsequently, reaction of **3** with various anilines and amines delivered the target molecules **1**.

Biological Evaluation of Naphthoates

- A library of 50 compounds was rapidly developed.
- An *in vitro* fluorescence polarization competition assay confirmed our novel 1-hydroxy-2-naphthoates are effective inhibitors of Mcl-1 (Table below).
- Biphenyl ethers wherein the distal phenyl ring is particularly hydrophobic resulted in potent inhibitors of Mcl-1.
- Double functionalization of the sulfonamide nitrogen afforded even greater inhibition of the oncoprotein.
- Up to 20-fold selectivity for Mcl-1 over Bcl-x_L was recorded.
- Limited cell activities were observed, likely due to the charged carboxylic acid impeding cell penetration.

Compound	R^1	R^2	K_i (μM)	Compound	R^1	R^2	K_i (μM)
1a			1.54 ± 0.47	1f			0.095 ± 0.005
1b			1.29 ± 0.11	1g			0.100 ± 0.006
1c			1.76 ± 0.18				
1d			0.361 ± 0.029	1h			0.047 ± 0.002
1e			0.361 ± 0.025	1i			0.045 ± 0.005

Conclusions and Future Directions

- 4-Sulfamoyl-1-hydroxy-2-naphthoates are potent inhibitors of Mcl-1.
- HSQC NMR experiments have provided orthogonal confirmation our compounds bind Mcl-1 in the hydrophobic crevice.
- In order to acquire cellular activity, carboxylic acid function will be converted into ester prodrugs, particularly acetoxymethyl ester, and bioisosteres, including tetrazole and hydroxyisoxazole functions.

References

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