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Towards the next generation of dual Bcl-2/Bcl-x_L inhibitors

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ABSTRACT

Structural modifications of the left-hand side of compound **1** were identified which retained or improved potent binding to Bcl-2 and Bcl-x_L in in vitro biochemical assays and had strong activity in an RS4;11 apoptotic cellular assay. For example, sulfoxide diastereomer **13** maintained good binding affinity and comparable cellular potency to **1** while improving aqueous solubility. The corresponding diastereomer (**14**) was significantly less potent in the cell, and docking studies suggest that this is due to a stereochemical preference for the R_S versus S_S sulfoxide. Appending a dimethylaminoethoxy side chain (**27**) adjacent to the benzylic position of the biphenyl moiety of **1** improved cellular activity by approximately three-fold, and this activity was corroborated in cell lines overexpressing Bcl-2 and Bcl-x_L.

The B-cell lymphoma 2 (Bcl-2) family of proteins play a central role in regulating cell death through the outer mitochondrial pathway of apoptosis. Antiapoptotic members, such as Bcl-2, Bcl-x_L, and Mcl-1, are comprised of four Bcl homology (BH) domains. Three of these domains (BH1–BH3) are shared by the proapoptotic Bcl-2 effector proteins Bax and Bak, while the proapoptotic activator proteins (e.g., Bim, Bid) only contain BH3. In a normal healthy cell, antiapoptotic proteins sequester their apoptotic counterparts through BH3 domain binding. In response to cellular injury, Bax and Bak are activated, resulting in oligomerization. Oligomer insertion into the outer mitochondrial membrane forms an exit pore through which cytochrome *c* enters the cytosol. Subsequent initiation of the caspase cascade culminates in cell death.[1]

Pro-survival members of the Bcl-2 family of proteins are overexpressed in a variety of neoplastic malignancies, and this dysregulation has been identified as a critical component of tumor development and chemotherapy resistance.[2] As a result of overexpression, proapoptotic proteins are perpetually sequestered, and the intrinsic apoptotic pathway is blocked. A developing treatment to restore this process in cells that are 'primed for death' is the inhibition of antiapoptotic Bcl-2 proteins with BH3-mimetics.[3] Two such examples are ABT-737[4] and ABT-263[5] (Navitoclax; Fig. 1), which bind to Bcl-2, Bcl-x_L, and Bcl-w with subnanomolar affinity. Navitoclax, which is an orally bioavailable second generation Bcl-2 inhibitor, is in Phase I/II clinical trials for solid tumors and hematologic malignancies.[5,6]

Dose-limiting observations for Navitoclax include thrombocytopenia, which is a target-driven toxicity resulting from Bcl-x_L inhibition in platelets.[7a] Bcl-x_L inhibition also induces transient thrombocytopathy, leading to increased tail-bleeding time in mice.[7b] Thrombocytopenia has been managed in the clinic by starting with low lead-in dosing prior to dose escalation to avoid severe platelet nadirs,[6d] and blood platelet levels have been shown to revert to pre-dosing levels after cessation of Navitoclax administration.[6b] Therefore, a desirable outcome in developing second generation dual Bcl-2/Bcl-x_L inhibitors is an amelioration or at least attenuation of the severity and duration of thrombocytopenia in patients. An alternative approach is to develop a selective Bcl-2 inhibitor and thereby side-step thrombocytopenia as a dose-limiting toxicity.[8]

Herein we discuss structural modification of the left-hand side of known triflone **1**[9] (Fig. 1, green box). Modifications of the right-hand side of this compound will be discussed in a future publication. This tool compound was selected because its synthetic accessibility enabled the rapid profiling of scaffold changes. One goal of these studies was to improve aqueous solubility, with the aim of increasing the fraction absorbed and, ultimately, bioavailability. We were also keen to selectively improve Bcl-2 potency to determine if this might translate to an improved safety profile in vivo.

As a protein–protein interaction inhibitor that binds to a hydrophobic BH3 groove,[10] Navitoclax is not surprisingly very lipophilic ($c\log P = 12.4$). There are a number of functionalities, such as the

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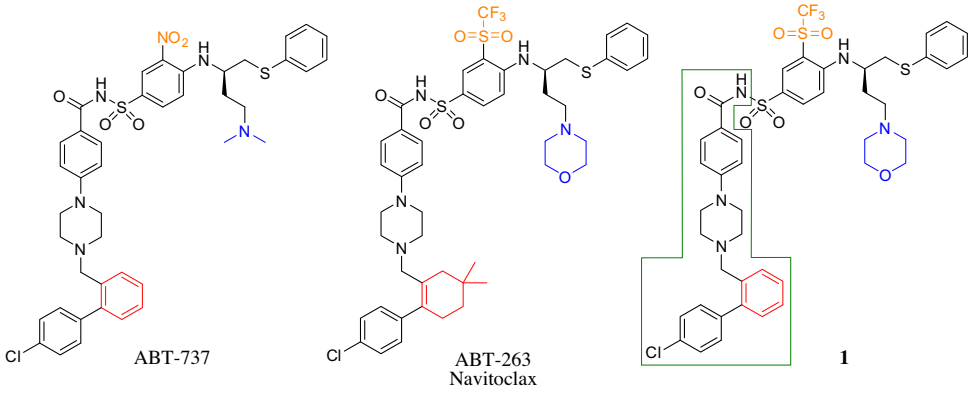


Figure 1. Structures of ABT-737, ABT-263, and **1**.

biphenyl and aryl acylsulfonamide, which likely contribute to its poor aqueous solubility. Additionally, the high acidity of the acylsulfonamide (pK_a 3.4) is suspected of reducing cellular permeability.[5] If this moiety could be replaced, a number of properties might therefore be improved.

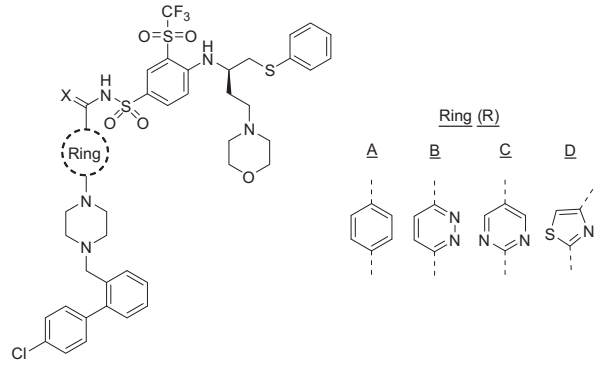
Conversion of the acylsulfonamide to the corresponding benzamidine analog **2**[11] (Table 1) resulted in a complete loss of cellular activity and poor solubility. Similarly, sulfonamide **3** was designed based on the ability of oxetane to act as a carbonyl isostere[15] but was inactive in both biochemical and cellular assays. A correlation between cellular activity and acyl sulfonamide acidity has been previously reported.[5b] Therefore, lack of cell potency for **2** (no calculated acidic centers; ACD pK_a v10, with correction library) and **3** (pK_a 9.0) was attributed to reduced acidity. However, other less quantifiable factors such as functional group binding incompatability (e.g., oxetane) were not ruled out.

Heterocyclic replacement of the phenyl ring of the aryl acylsulfonamide was also attempted as a means of reducing lipophilicity, improving solubility, and retaining or enhancing cellular activity. Pyridazine **4** (*c*log*P* = 8.7; pK_a ~3.6) was modestly active and offered an improvement in solubility, while pyrimidine **5** (*c*log*P* = 8.8; pK_a 3.5) demonstrated comparable binding to Bcl-2 and Bcl-x_L relative to **1**. Thiazole **6** (*c*log*P* = 9.8) was less active than analog **1**, despite an increase in acidity (pK_a 2.6).

As shown in Table 2 and similar to changes to the acylsulfonamide, heterocyclic replacements of the benzylpiperazine were well tolerated with respect to binding, but retaining cellular activity was a challenge (Table 2). For example, we designed piperazine analogs **8** and **9** with the goal of leveraging the polar character of phosphine oxides to enhance aqueous solubility. Analog **9** did indeed have improved solubility, but this was obviated by a lack of cell potency. Other point modifications such as thio ether **12** and sulfone **15** were equally tantalizing. For 4-substituted piperidines **16**–**19**, only the hydroxymethyl analog had appreciable activity in cells but was still 10-fold less active than **1**.[16]

Of the compounds in Table 2, only sulfoxide **13** met our criteria for in vitro potency and solubility, while the corresponding sulfoxide diastereomer (**14**) was inactive in the cell. The data suggest that the latter may be due to poor target inhibition (Bcl-2 FP IC_{50} 0.454 μM). Docking studies (Fig. 2) were used to evaluate potential differences stemming from changes in stereochemistry and build support for stereochemical assignment of the sulfoxide itself.

Table 1
Fluorescence polarization, cellular, and solubility data for **1**–**6**



Compd	R	X	Bcl-2 FP IC_{50}[a] (μM)	Bcl-x_L FP IC_{50}[a] (μM)	RS4;11 EC_{50}[b] (μM)	Sol[c] (μM)
1[9]	A	O	0.011	0.016	0.011	14
2[11]	A	NH	0.019	0.063	>3.19	<1
3	A	Oxetan-3,3-yl	>1	>1	>1.1	<1
4	B	O	0.270	0.378	0.563	38
5	C	O	0.018	0.039	0.177	3
6	D	O	0.119	0.098	0.385	2

[a] Fluorescence polarization assay.[12]
[b] Caspase-Glo® cellular assay.[13]
[c] Equilibrium solubility (pH 7.4).[14]