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The Role of the Acidity of N-Heteroaryl Sulfonamides as Inhibitors of Bcl-2 Family Protein−Protein Interactions

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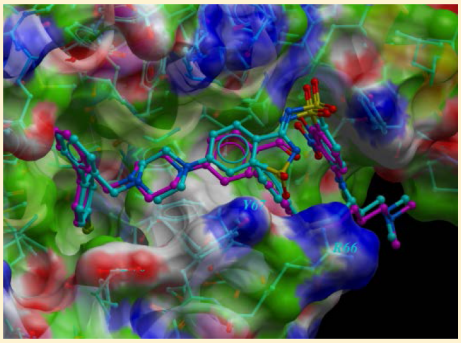
Supporting Information

ABSTRACT: Overexpression of the antiapoptotic members of the Bcl-2 family of proteins is commonly associated with cancer cell survival and resistance to chemotherapeutics. Here, we describe the structure-based optimization of a series of N-heteroaryl sulfonamides that demonstrate potent mechanism-based cell death. The role of the acidic nature of the sulfonamide moiety as it relates to potency, solubility, and clearance is examined. This has led to the discovery of novel heterocyclic replacements for the acylsulfonamide core of ABT-737 and ABT-263.



KEYWORDS: *B cell lymphoma, Bcl-2 family, protein−protein interaction, apoptosis, cancer*

Apoptosis, or programmed cell death, is the process by which the maintenance of tissue homeostasis is controlled. Evasion of cell death is a hallmark of cancer progression and development of resistance to chemotherapy.[1] Bcl-2 family proteins are key regulators of the mitochondrial apoptosis pathway. The Bcl-2 family is comprised of both proapoptotic (e.g., Bad, Bik, Bim, Bid, Noxa, and Puma) and antiapoptotic (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1) proteins, which regulate apoptosis through protein−protein interactions.[2,3] Protein−protein interactions are central to many biological processes and represent a large and important class of potential therapeutic targets. Disruption of protein−protein interactions with low molecular weight compounds remains a challenging endeavor. This is due in part to the relatively large surface area and lack of defined or specific pockets, which characterize most protein−protein interfaces.[4]

In an effort to discover and develop Bcl-2 family antagonists, Abbott scientists described a series of *N*-acyl sulfonamide-based inhibitors such as ABT-737 (Table 1, entry 1).[5−7] These compounds demonstrate potent mechanism-based killing of Bcl-2- and Bcl-xL-dependent cell lines by disrupting the protein−protein interaction of prosurvival Bcl-2 proteins with prodeath BH3-only proteins. One potential liability with this series of inhibitors is its limited solubility, which leads to dissolution-limited oral absorption.[8] Central to this series of inhibitors is an acylsulfonamide moiety, which acts as a linker for two pocket binding moieties. This acylsulfonamide linker presents a potential metabolic liability as exemplified by its use as a prodrug of a primary sulfonamide.[9,10] As a continuation of our interest in discovering novel Bcl-2 antagonists,[11] we present here our attempts to address these issues by replacing the acyl sufonamide moiety of **1** with heterocyclic rings.[12]

An examination of the cocrystal structure of 1 and Bcl-xL reveals the coplanarity of the aromatic ring and the carbonyl of the acyl sulfonamide group.[13] We rationalized that replacement of the carbonyl with an unsaturated ring would maintain the proper vectors for the two pocket binding moieties of the molecule (see Table 1). In addition, introduction of bicyclic ring systems with one saturated ring would decrease the planarity of the core ring system and result in an increase in aqueous solubility.[14]

Surface plasmon resonance (SPR) was used to characterize each Bcl-2 inhibitor. An IC_{50} was determined through a competition experiment with biotinylated Bak bound to the surface and subsequent injection of a mixture of inhibitor and Bcl-2 through the flowcell. For more potent compounds, binding constants (K_D) with Bcl-2 were determined by SPR to allow compound differentiation.[15] As shown in Table 1, initial replacement of the acyl sulfonamide with a naphthyl ring (entry **2**) led to a complete loss of binding affinity. While this result was disappointing, as a confirmation of the design hypothesis, we were encouraged by the quinoline **3**, which showed an IC_{50} of 68 nM. This highlights the importance of the acidity of the

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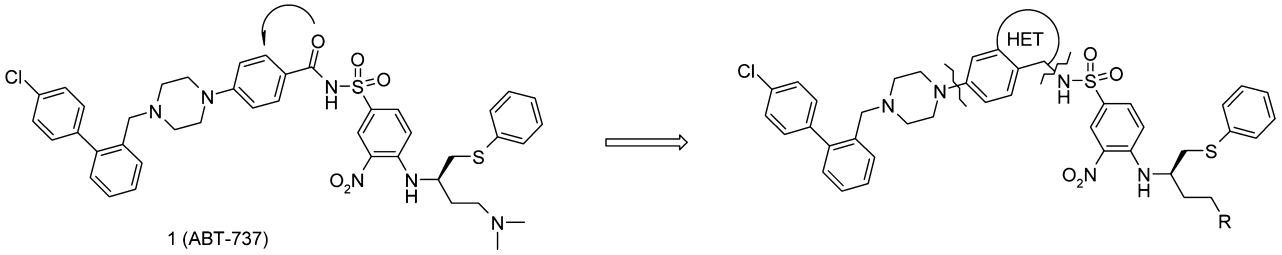
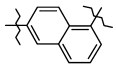
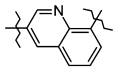
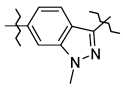
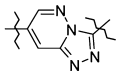
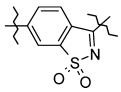
Table 1. Activity of N-Heteroaryl Sulfonamides



cmpd	HET	R	Bcl-2/BAK IC_{50}[a] (nM)	Bcl-2 K_D (nM)	Toledo LD_{50}[a] (μM)	pKa[b]	HT-eq Sol FaSSIF (μM)
1	ABT-737		20	0.6	0.06	4.02	7
2			>4000	nd	>30	8.17	<5
3		NMe_2	68	nd	3.95	7.26	44
4		NMe_2	8500	nd	>30	5.59	nd
5		NMe_2	25	8	4.8	5.77	31
6		NMe_2	17	14	>30	3.29	<5

[a]Average of at least two measurements (duplicate IC_{50} or LD_{50} values were generated in each assay). [b]Calculated pK_a for compounds in table using Moka.

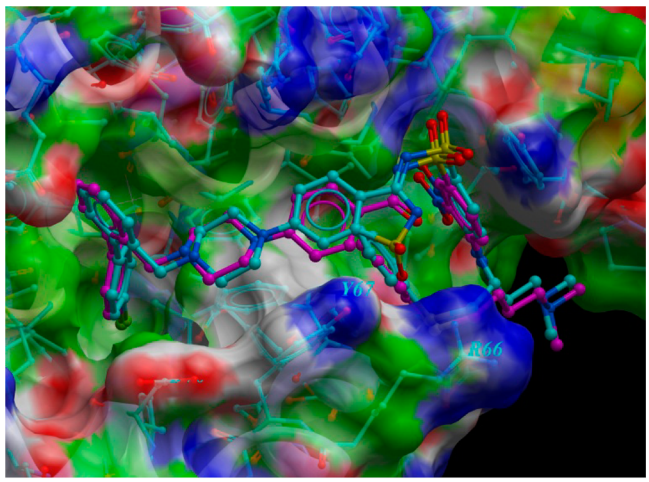


Figure 1. Crystal structure of **6** (blue) complexed with Bcl-2 at 2.1 Å (4IEH) overlaid with the crystal structure of ABT-737, **1** (purple), complexed with Bcl-2.

sulfonamide NH. While the naphthyl NH has a predicted pK_a of 8, the quinoline NH is expected to be significantly more acidic due to the combined inductive effect and the potential for intramolecular H-bonding. This observation led to the design of analogues, which would contain more acidic sulfonamide moieties to more closely match the acidity of the acyl sulfonamide moiety of ABT-737.

It was hypothesized that the synthesis of electron-withdrawing heterocyclic cores would result in increased acidity of the appended sulfonamide NH as compared to the naphthyl analogue **2**. The pyrazolo pyrimidine **4** was synthesized and shown to have an IC_{50} of 8500 nM. Conversely, the triazole **5** had a potency of 25 nM and induced apoptosis in the Bcl-2-dependent Toledo cell line with an LD_{50} of 4.8 μM. The saccharin analogue **6** was a similarly potent Bcl-2 inhibitor with an IC_{50} of 17 nM, although the compound had poor cell activity. The lack of cell activity of **6** is rationalized by the poor permeability demonstrated in in vitro assays (Table 3) perhaps due to its high PSA (160).

Examination of the cocrystal structure of the saccharin analogue with the Bcl-2 protein gave some insight into its

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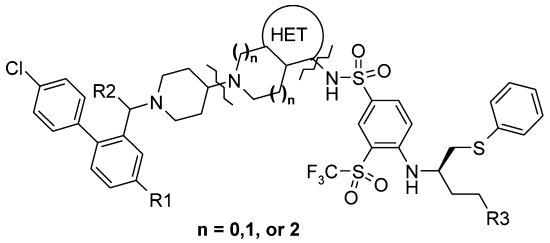
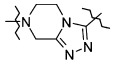
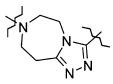
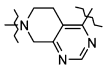
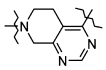
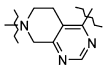
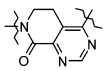
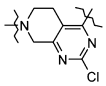
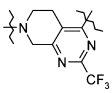
Table 2. Activity of Saturated Ring Systems



cmpd	HET	R1	R2	R3	Bcl-2/BAK IC_{50}[a] (nM)	Bcl-xL/BAK IC_{50}[a] (nM)	Bcl-2 K_D (nM)	Toledo LD_{50}[a] (μM)	HT-eq Sol FaSSIF (μM)
7		H	H		317	nd	nd	15.9	9
8		H	H		740	nd	nd	10.7	70
9		H	H	NMe_2	25	nd	5	3.47	87
10		H	H	NMe_2	19	24	7	0.298	64
18		F	H	NMe_2	33	74	3	0.197	25
19		H	Me	NMe_2	35	115	2	0.113	31
20		F	H	NMe_2	142	22	104	>30	63
21		H	H	NMe_2	32	20	17	5.37	22
22		H	H	NMe_2	46	62	10	3.9	36

[a]Average of at least two measurements (duplicate IC_{50} or LD_{50} values were generated in each assay).

increase in binding affinity. As shown in Figure 1, compound **6** has a high degree of overlap with the binding mode of ABT-737 cocrystallized with Bcl-2. This highlights the importance of the vector created by the saccharin core scaffold for the pocket-binding moieties. In addition, **6** benefits from an electrostatic interaction between Y67 and the oxygen of the sulfonyl group. The difference in potency of entry **4** versus **5** and **6** could be a result of the methyl substituent on the pyrazole ring of **4** interfering with the electrostatic interaction with Y67. Compound **5** also shows a 4-fold increase in solubility over compound **1**. The solubility benefit of **5** is lost in compound **6** as the ionic character of the sulfonamide increases due its increased acidity as a result of the electron-withdrawing saccharin core.

Our focus then turned to ring systems with one saturated ring to decrease the planarity of the core ring system in an effort to increase solubility and permeability of our compounds and thereby improve cellular potency. On the basis of the crystal structure of **6**, compounds were designed to maintain a hydrogen bond acceptor in a suitable position to interact with Y67. This led to the series shown in Table 2. The saturated six member ring compound **7** had an IC_{50} of 317 nM. The alternative six/five ring system **8**, where the five member ring was saturated, afforded a 2-fold loss in potency to 740 nM. The five/seven ring system **9** was synthesized to vary the vector in which the two pocket binding groups were and was found to have an IC_{50} of 25 nM. However, this compound had a cell potency of 3.47 μM. The six/six ring system **10** was found to

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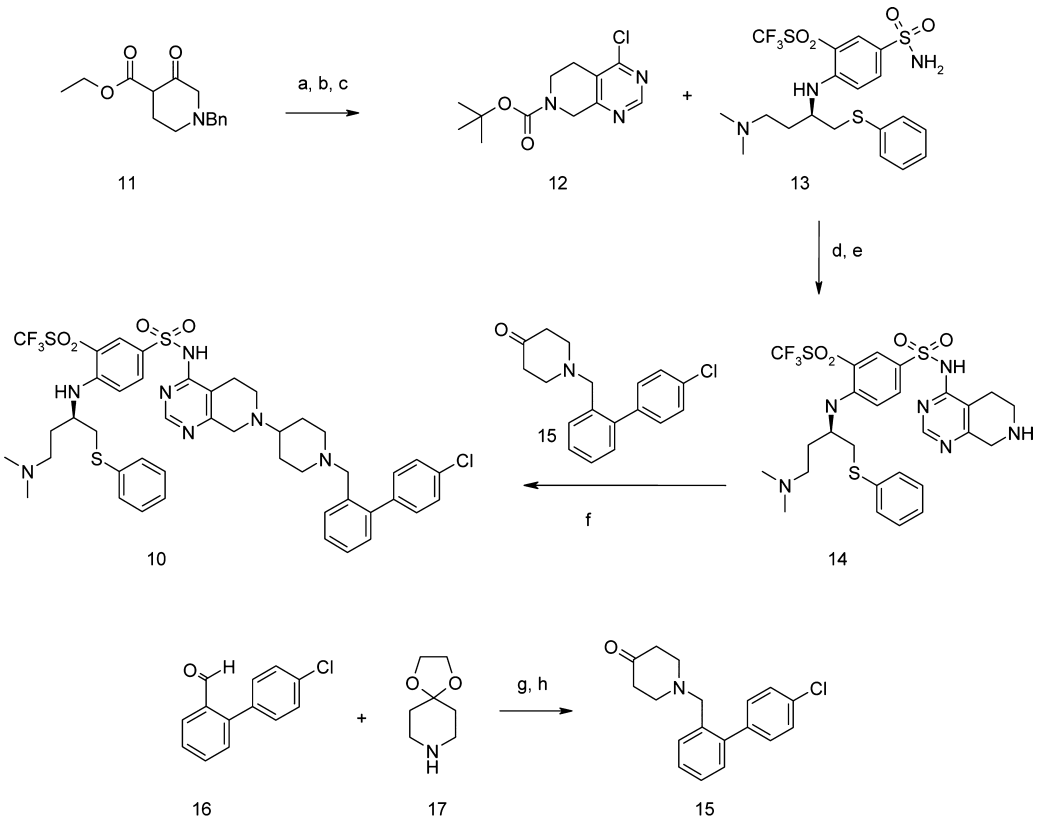
Scheme 1. Synthesis of Piperdyl Pyrimidine 10[a]



[a]Conditions: (a) H_2 Pd/C, Boc_2O, DIPEA. (b) Formamidine, EtONa. (c) CCl_4, PPh_3, EtOH. (d) $Pd_2(dba)_3$, Cs_2CO_3, DavePhos, dioxane. (e) TFA, DCM. (f) $Na(OAc)_3BH$, THF. (g) $Na(OAc)_3BH$, THF. (h) HCl, dioxane.

Table 3. In Vivo Pharmacokinetics in Sprague Dawley Rat[a]

parameters	10	6	19
Cl (mL/min/kg)	68	25	89
Vdss (L/kg)	8.6	0.09	5.28
IV AUC (nmol/h)	232	761	190
PAMPA log P_e (cm^2/s)	−4.6	<−6	−4.8
% *F*	<5	<5	ND

[a]Plasma Cl and AUC calculated following 1 mg/kg iv dose. Oral bioavailability (% *F*) calculated following a 10 mg/kg po dose. The compound was administered as a solution in (a) 10% PG (polyethylene glycol), 5% cremophor EL, and 85% pH citrate buffer (**10** and **6**) and (b) 10% PG, 50 (10%) Solutol, and 40% WFI (water for injection) (**19**).

have the best potency of the series with a Bcl-2 K_D of 7 nM and a cell potency of 0.298 μM. Compound **10** was also found to inhibit Bcl-xL with an IC_{50} of 24 nM. Compounds **8**, **9**, and **10** demonstrated an increase in water solubility as compared to compound **1**.

With the improved cell potency of **10**, we focused our efforts on this six/six ring system. The synthesis of **10** is shown in Scheme 1. Removal of the benzyl group of **11** and protection with a Boc group affords the ketoester. Treatment of the ketoester with formamidine forms the pyrimidine ring. Chlorination of the pyrimidine ring proved problematic due to incompatible reaction conditions with the needed BOC protecting group; however, treatment with PPh_3/CCl_4 afforded **12**. With **12** in hand, the stage was set for the challenging N-heteroarylation of a complex sulfonamide. While copper-based methods failed, a variant of the Pd coupling reported by Rivero et al. delivered the desired product in excellent yield.[16] Thus, Pd-mediated coupling with the known aryl sulfonamide **13**[8] forms **14**. Removal of the Boc group and reductive amination with ketone **15** (which is separately prepared from coupling of the aldehyde **16** and piperdine **17**) affords the final product **10**.

While the cell potency of **10** was encouraging, the pharmacokinetics in rat, as shown in Table 3, demonstrated high clearance with little oral bioavailability. We attempted to block potential metabolically labile positions to decrease clearance. Substitution on the biphenyl ring system with fluorine, **18**, afforded a 2-fold increase in potency in both binding and cell assays. Substitution at the benzylic position of the biphenyl with a methyl, **19**, gave a further increase in cell potency to 0.113 μM. However, the clearance of compound **19** remained high, affording low exposure in plasma (Table 3). It was observed that the clearance of compound **6** was significantly reduced as compared to **10**, affording a 4-fold increase in AUC. Potentially, this reduction in clearance is influenced by the greater acidity of the sulfonamide due to the electron-withdrawing saccharin core of **6**. Thus, in an attempt to reduce the clearance of the series, analogues were designed to increase the acidity of the sulfonamide by adding electron-withdrawing groups to the piperidyl pyrimidine ring system. This led to the synthesis of compounds **20**, **21**, and **22**. The piperidone analogue **20** had diminished binding and cell potency. The Cl and CF_3 substituted compounds **21** and **22** were potent binders of Bcl-2 with a K_D of 17 and 10 nM, respectively, although they had reduced potency in cell assays.

In summary, a series of Bcl-2 inhibitors with heterocyclic cores has been prepared, which led to the discovery of potent

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inhibitors with improved solubility over ABT-737. The acidic nature of the sulfonamide NH has been shown to impact the potency, solubility, and clearance rates of these compounds. Potent cell active compounds have been discovered, and strategies to modulate in vivo clearance rates have been identified within the series. Further efforts to improve this series are in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and characterization data (^{1}H NMR, HRMS, and HPLC analysis) for selected compounds and biochemical assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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