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Isosteric exchange of the acylsulfonamide moiety in Abbott's Bcl-X_L protein interaction antagonist

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ABSTRACT

A multi-component reaction strategy was used for the fast and efficient synthesis of amide isosteres of known Bcl-2 inhibitors capable of disrupting protein–protein interactions. Ugi reaction and a subsequent nucleophilic aromatic substitution reaction provide a versatile path to libraries of compounds similar to Abbott's acylsulfonamides. Modeling arguments are used to explain the inferior activity of the amide as opposed to the sulfonamide series.

Human cancers overexpressing Bcl-2 and related survival proteins are often resistant to cytotoxic therapeutics.[1] These include breast, prostate, colorectal, gastric, and (non)-small-cell lung cancer, as well as neuroblastomas, B-cell lymphoma, and melanomas. Acquired resistance toward radiation or chemotherapy often leads to tumor relapse and aggressive formation of metastasis and finally to clinical failure of the current therapeutic regiment. Bcl-2 family members are important regulators of programmed cell death, belonging to the mitochondrial apoptosis pathway. Proapoptotic Bcl-2 family members such as Bcl-2, Bcl-X_L, and Bcl_w interact with antiapoptotic Bcl-2 family members, for example, Bax, Bak, and Bad and neutralize each other. Several experimental results indicate that Bcl-2 family members are promising new cancer targets. Thus synthetic cell permeable BH3 peptides[2] and newly discovered small molecules trigger apoptosis in cancer cells and show tumor regression in xenograft models.[3–6]

In a program to discover and develop Bcl2 family protein interaction antagonists Abbott scientists described a series of *N*-acylsulfonamide-based inhibitors, for example, **1**.[7,8] These compound series was developed using a NMR fragment-based approach, together with parallel chemistry (Fig. 1). Recently several potent in vitro and in vivo inhibitors together with structural information about their binding mode into Bcl-X_L have been reported and

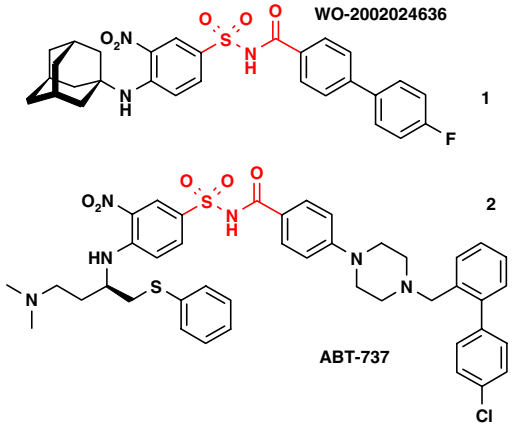

Figure 1. Examples of Abbott's acylsulfonamides.

two compounds currently undergo clinical trials. ABT-737, **2** a pan-Bcl family inhibitor targeting Bcl-2, Bcl-w, and Bcl-X_L has been shown to induce regression of lymphoma and small-cell lung cancer in corresponding xenograft models.

As a continuation of our interests to discover novel Bcl-2 family protein interaction antagonists we present here our attempts to prepare compounds similar to Abbott's Bcl-X_L inhibitors replacing the sulfonamide backbone against an Ugi backbone.[9]

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Our structural design of our novel inhibitors is based upon the analysis of the binding cleft of Bcl-2 and structural disclosure of the binding mode of a representative compound (Fig. 2). The Abbott Bcl-2 inhibitors adopt a V-shaped conformation with the *N*-acylsulfonamide moiety at the solvent rim of the deep and hydrophobic binding groove and the biphenyl and 2-amino nitrophenyl moieties pointing toward the floor of the groove. Due to the elongated binding groove which is not completely filled by the compound we reasoned that an elongation of the inhibitor by a 2-atom insertion fragment in the backbone would be accepted by the Bcl-2 protein. From the analysis of X-ray structures of Ugi backbones it appears that there is sufficient conformational freedom to readily adopt into a large binding groove. Therefore we reasoned that the quasi isosteric replacement of the *N*-acylsulfonamide against a α-acylaminocarbonamide would be tolerated by the Bcl-2 binding groove (Fig. 3). Although the sulfonamide consists of the three backbone atoms SNC and the Ugi backbone comprises the five backbone atoms NCCNC, modeling suggests a similar 3D shape and a reasonable overlap of the terminal residues. An isosteric replacement would have several advantages; the access to a novel class of potential Bcl-2 inhibitors by a convergent and comparatively short synthetic route; the circumvention of intellectual property issues and potentially improved pharmacokinetic properties.

To test this idea we had to synthesize a phenyl isocyanide capable to undergo subsequent nucleophilic aromatic substitution reactions.[10] Thus starting from 3-nitro-4-fluoroaniline **3** we assembled the corresponding isocyanide in the classical sequence formylation, dehydration according to Ugi in overall 60% yield (Scheme 1). This novel isocyanide allows for U-MCRs and even more subsequent diversification by nucleophilic aromatic substitution reactions.

Pursuing the idea of an isosteric replacement of the *N*-acylsulfonamide against an α-acylaminocarbonamide we prepared a larger amount of the Ugi intermediate **9** by the reaction of methylamine **8**, formaldehyde **7**, isocyanide **3**, and biphenyl carboxylic acid **6** (Scheme 2).[11] This intermediate was used as starting material for

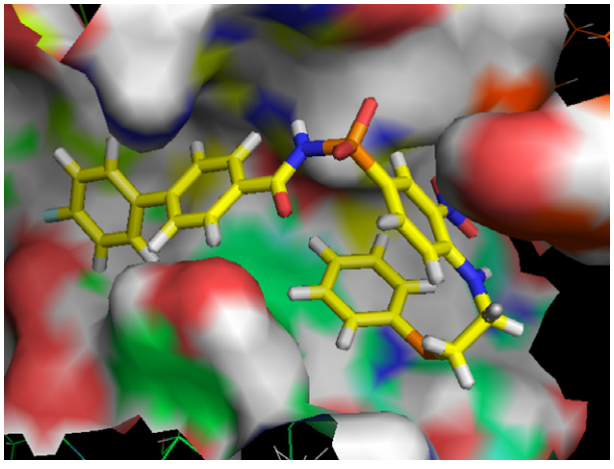


Figure 2. An Abbott sulfonamide-based inhibitor bound onto Bcl-X_L (PDB IP: 1YSI). Pictures generated using PyMol (www.pymol.com).

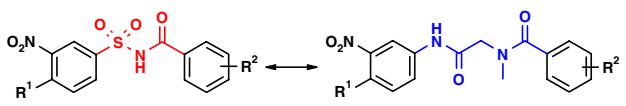
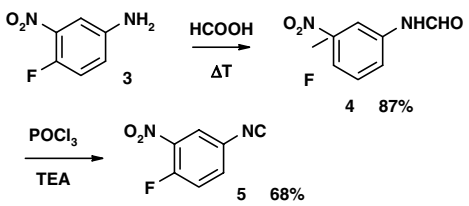
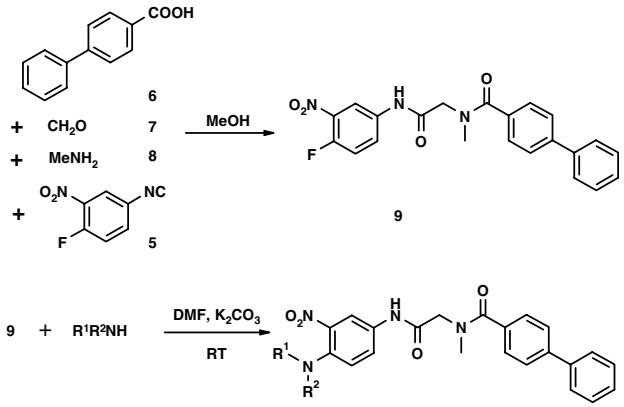


Figure 3. Structural comparison of the Abbott sulfonamide backbone with an Ugi backbone.



Scheme 1. Preparation of 4-fluoro-3-nitro-phenylisocyanide **5** useful for isocyanide-based multi-component reactions and subsequent nucleophilic aromatic substitution reactions.



Scheme 2. Synthesis of potential Bcl-2 protein interaction antagonists by an initial Ugi reaction and a second nucleophilic aromatic substitution reaction.

several nucleophilic aromatic substitutions under mild conditions for the preparation of a small library of potential Bcl-2 inhibitors (Fig. 4 and Table 1).[12]

To compare our compounds against authentic Abbott compounds we resynthesized several of those according to the patent procedures. Screening was performed as recently described using a FP assay.[13] The activities of the α-acylaminocarbonamides are summarized in Table 1.

Initial docking studies using MOLOC indicate that our α-acylaminocarbonamides could indeed potentially bind deep into the hydrophobic Bcl-2 groove (Fig. 4).[14]

However, as by the FP assay performed the affinity of our first generation of compounds is at least 2–3 magnitudes less. This can be explained in part by the fact that the more active compounds of the Abbott series have a molecular weight which is still

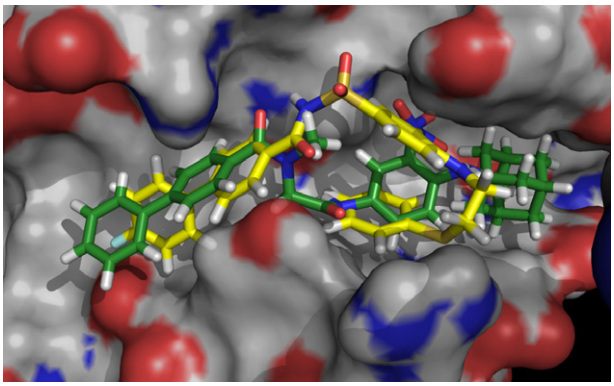
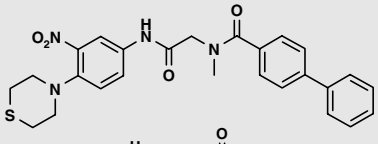
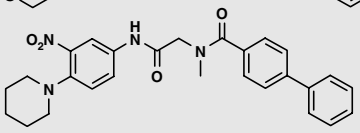
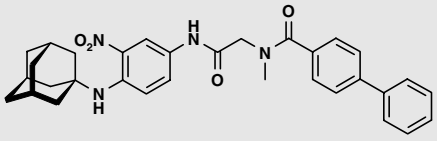


Figure 4. Compound **12** (green sticks) docked into the high resolution NMR structure of an Abbott compound (yellow sticks) in Bcl-X_L (PDB IP: 1YSI).

Table 1
In vitro FP data of selected Bcl-2 family protein–protein interrupters (Bcl-w)

Compound	Structure	K_i (μM)
10		12.95 ± 0.1
11		10.80 ± 0.7
12		9.43 ± 1.3

1/3 higher (cf. ABT-737 812 Da vs compound **10** 490 Da) as compared to our initial series of compounds. Analyzing the published molecular structure it appears that the interaction between the small molecule and the Bcl-2 protein is almost exclusively governed by van der Waals interactions and shape complementarity. Moreover the *N*-acylsulfonamides can undergo a favorable electrostatic interaction with the carboxyl group of the rim amino acid R139, whereas the present α-acylaminocarbonamides can apparently not undergo such a hydrogen bond interaction. However based on the promising initial low micromolar activity and the still rather low molecular weight we are confident that we will be able to design a second generation of more potent Bcl-2 inhibitors based on the Ugi backbone in the future.

In summary, we have prepared several potential Bcl-2 protein–protein interaction antagonists in two steps utilizing an IMCR and a subsequent aromatic substitution reaction. For this purpose we developed a versatile bifunctional *p*-fluorophenyl isocyanide useful for nucleophilic aromatic substitution reactions. The chemistry is high yielding and potentially useful to prepare arrays of compounds. The designed compounds are based upon the described acylsulfonamide inhibitors of Abbott. However, in contrast to the acylsulfonamides the best inhibitors of the newly described series are at least 2–3 orders of magnitude less active. Possible reasons for this drop of activity and possible routes towards more potent inhibitors are discussed. However, regarding the yet small molecular weight of the described compounds, the current backbone comprises a good starting point for further medicinal chemistry to yield higher active compounds.

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10. *Experimental procedure for 4-fluoro-3-nitro-phenylisocyanide **5**:* One hundred millimoles (15.6 g) of 4-fluoro-3-nitro aniline is refluxed in 150 ml formic acid for 12 h. The residual formic acid is evaporated under reduced pressure to yield 18.4 g (100%) of crude formamide product **4**, which can be further purified by crystallization from ethanol. ^{1}H (400 MHz, d_6-DMSO; major and minor diastereomer; ratio 7.15:1) = 7.51 (m, 1H), 7.82 (m, 1H), 8.48 (d, 1H), 10.61 (br s, 1H); 7.61 (m, 1H), 7.92 (m, 1H), 8.83 (d, 1H), 10.40 (br d, 1H); ^{13}C (100 MHz, d_6-DMSO) = 113.8, 115.5, 115.6, 118, 8, 119.0, 119.2, 119.4, 124.4, 124.5, 126.3, 126.4, 134.8, 134.9, 135.4, 135.5, 136.2, 136.3, 149.2, 151.7, 160.1, 162.7. HPLC–MS 2.73 min; $[M+H]^+$ 185.
A solution of 40 mmol (7.36 g) formamide **4** and 100 mmol (10 g, 13.7 ml) triethylamine in 40 ml DCM is prepared and cooled to 0 °C. To this solution 40 mmol (3.7 ml) $POCl_3$ are added slowly to maintain the temperature at 0 °C. The mixture is maintained for another hour at 0 °C and then warmed-up to 20 °C and stirred for another 6 h. Thirty-two milliliters of a aqueous solution of 8 g Na_2CO_3 are carefully added under vigorous stirring for 30 min. The aqueous phase is extracted 3× with each 20 ml DCM. The combined organic phases are dried over K_2CO_3 and evaporated. The residue is crystallized from ether/DCM −20 °C and filtered to yield 5.45 g 3-nitro-4-fluorophenylisocyanide **5** (82%). ^{1}H (400 MHz, d_6-DMSO) = 7.76 (m, 1H), 8.08 (m, 1H), 8.49 (m, 1H); ^{13}C (100 MHz, d_6-DMSO) = 120.1, 120.3, 124.8, 134.2, 134.3, 153.2, 155.8, 166.2. HPLC–MS 2.79 min; $[M+H]^+$ 167.
11. *Experimental procedure for biphenyl-4-carboxylic acid [(4-fluoro-3-nitro-phenylcarbamoyl)-methyl]-methyl-amide **9**:* One millimole of each of the starting materials aldehyde, primary amine, carboxylic acid, and isocyanide are stirred in 1 ml of methanol for 48 h at 20 °C. The solvent is evaporated and the residue is purified using silica gel chromatography to yield 265 mg **9** (65% yield). ^{1}H (400 MHz, d_6-DMSO, mixture of two rotamers around the tertiary amide bond) = 3.04 (3H, br s, major), 3.07 (3H, br s, minor), 4.15 (2H, br s, minor), 4.32 (2H, br s, major); 7.38–7.91 (m, 11H), 8.51 (m, 1H, minor), 8.59 (m, 1H, major), 10.49 (br s, NH, minor), 10.63 (1H, br s, major). HPLC–MS 3.73 min; $[M+H]^+$ 408.
12. *Experimental procedure for biphenyl-4-carboxylic acid methyl-[(3-nitro-4-thiomorpholin-4-yl-phenyl-carbamoyl)-methyl]-amide **10**:* A solution of K_2CO_3 (0.246 mmol) and thiomorpholine (0.147 mmol) in DMF was mixed with 50 mg **9** (0.123 mmol). The solution was stirred at 20 °C for 3 days when the reaction was complete according to HPLC–MS analysis. The solvent was evaporated under reduced pressure and the residual material was purified using silicagel chromatography to yield 29 mg **10** (48% yield). HPLC–MS 3.92 min; $[M+H]^+$ 491.
13. A primary screen for Bcl project is based on fluorescence polarization (FP) technology and is well described in literature (Zhang, H.; Nimmer, P.; Rosenberg, S.H.; Ng, S.C.; Joseph, M. *Anal. Biochem.* **2002**, *307*, 70). We have established assays for Bcl-2 (SantaCruz Biotech), Bcl-X_L (R&D Systems), and Bcl-w (R&D Systems) using as the binding partner the 5-carboxyfluorescein-labeled 16-mer peptide tracer Flu-Bak-BH3 (sequence GQVGRQLAIIGDDINR is derived from the Bak BH3 domain). The assays were performed in a 384-well format, in 20 mM potassium phosphate buffer pH 7.4, containing 1 mM EDTA, 50 mM NaCl, and 0.05% pluronic F-68. The final concentration of DMSO in all assays was 10%. The reaction was carried in a 50-μL volume and the resulting polarization signal was measured at λ_{ex} = 485 nm/λ_{em} = 535 nm using an UltraReader (Tecan) after 2 h incubation of the reaction mixture at room temperature. Validation of the assays was performed using non-labeled Bak-BH3 peptide as a control inhibitor.
14. MOLOC: Paul Gerber, Gerber Molecular Design, http://www.moloc.ch.