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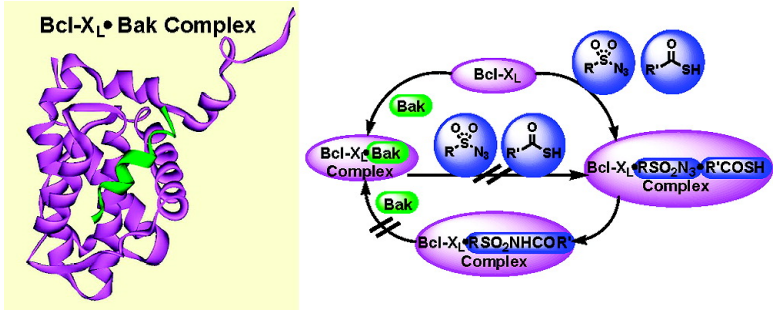

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Bcl-X_L-Templated Assembly of Its Own Protein−Protein Interaction Modulator from Fragments Decorated with Thio Acids and Sulfonyl Azides

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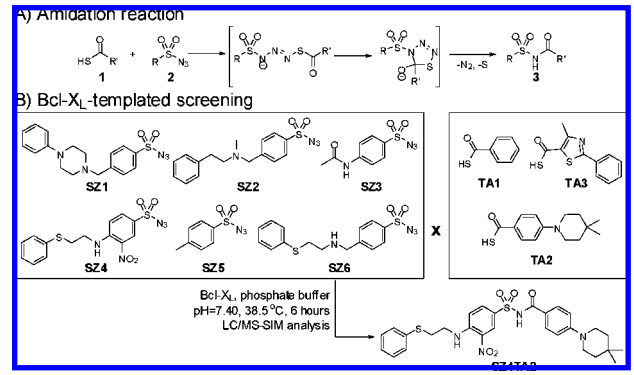
Protein−protein interactions are central to many biological processes and hence represent a large and important class of potential targets for human therapeutics.[1] Recent discovery of a variety of low-molecular-weight compounds interfering with protein−protein complexes launches and validates viable routes for a large number of new therapies.[2] However, disrupting or modulating protein−protein interactions with low-molecular-weight compounds remains challenging. Most protein−protein interfaces lack deep pockets that might provide binding sites for small molecules, and they are composed of two relatively large protein surfaces that are complementary with respect to shape and electrostatics.[3] Moreover, the adaptive and flexible nature of amino acid residues on protein surfaces creates additional challenges for lead compound design and discovery.[4]

The Bcl-2 family consists of both anti- and proapoptotic members, which are central regulators of programmed cell death.[5] The antiapoptotic proteins like Bcl-2, Bcl-X_L, and Mcl-1 heterodimerize with the proapoptotic constituents, which include the multidomain molecules Bax and Bak, and BH3-only proteins such as Bim, Bad, Bid, Noxa, or Puma, through the conserved BH3 domain.[5] The relative ratios of pro- and antiapoptotic Bcl-2 family proteins determine the ultimate sensitivity or resistance of cells to a wide variety of apoptotic signals.[6] Meanwhile, small molecules have been reported to modulate the extent of heterodimerization between anti- and proapoptotic Bcl-2 family members inducing apoptosis in cancer cells.[5] Using a combination of SAR by NMR screening, parallel synthesis, and structure-guided lead design, Abbott laboratories developed ABT-737 and a large series of analogues displaying inhibition constants in the nanomolar or subnanomolar range.[7]

Herein, we report our progress towards the development of lead synthesis and discovery methods that generate only biologically active compounds targeting protein−protein interactions. In the past decade, various fragment-based lead discovery approaches have been reported in which the biological target is actively engaged in the assembly of its own multidentate inhibitor from a pool of smaller reactive fragments. These approaches can roughly be divided into three different categories: (1) dynamic combinatorial chemistry (DCC),[8] (2) catalyst/reagent-accelerated target-supported assembly,[9] and (3) kinetic target-guided synthesis (TGS).[10] While DCC utilizes reversible reactions to create a library of all possible heterodimeric compounds, kinetic TGS approaches employ irreversible reactions generating only the biologically active bidentate members of the entire virtual library. In situ click chemistry,[10c] in particular, has been shown to produce potent triazole inhibitors of the enzymes acetylcholine esterase,[11] carbonic anhydrase,[12] and HIV protease.[13]So far, kinetic TGS approaches have been applied only for the discovery of enzyme inhibitors but not for protein−protein interaction modulators (PPIMs). Based on studies by Abbott laboratories, we investigated whether a kinetic TGS approach is a viable route for the discovery of PPIMs targeting the antiapoptotic protein Bcl-X_L.

Scheme 1. Bcl-X_L-Templated Assembly of Acylsulfonamide **SZ4TA2** from Fragments Decorated with Thio Acid or Sulfonyl Azide Functionalities



The reaction combining two fragments in the presence of a biological template into a larger molecule plays a crucial role for kinetic TGS. For example, the slow nature and the bio-orthogonality of the Huisgen cycloaddition, the compatibility of the reactants with water, and the stability of the triazole products are distinct characteristics of in situ click chemistry.[10c] Recently, Williams and co-workers developed an amidation reaction displaying a reactivity profile suitable for kinetic TGS applications.[14] This particular amidation reaction between thio acids **1** and sulfonyl azides **2** to give corresponding acylsulfonamides **3** is effective at room temperature in both organic and aqueous solvents (Scheme 1A).

To probe if this amidation reaction is suited for kinetic TGS applications targeting protein−protein interactions, building blocks, some of which are structurally related to fragments of ABT-737, were decorated with sulfonyl azide or thio acid groups (Scheme 1B). Thio acids **TA1-TA3** and sulfonyl azides **SZ1-SZ6** were incubated as binary mixtures in the presence of buffer solution containing Bcl-X_L for 6 h at 38 °C. As a control, 18 identical binary building block combinations were kept under the same incubation conditions in buffer without Bcl-X_L. All incubations were then analyzed by HPLC with product detection by electrospray ionization in the positive selected ion mode (LC/MS-SIM).[11b] From all the screened samples, only one incubation sample led to an increased amount of acylsulfonamide **SZ4TA2**[7e] in the Bcl-X_L-containing sample compared to the incubation without the protein. We synthesized this compound for hit validation.[15] Comparison of the LC/MS-SIM traces of the Bcl-X_L incubation mixture with the trace of the corresponding synthesized **SZ4TA2** clearly confirmed that

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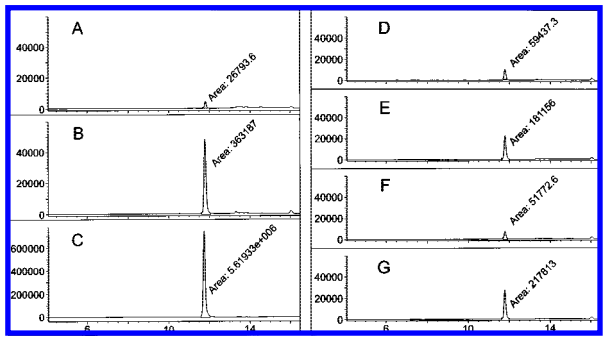

Figure 1. Hit identification of acylsulfonamide **SZ4TA2** by LC/MS-SIM. (A) Incubation of **SZ4** and **TA2** in buffer without Bcl-X_L. (B) Bcl-X_L-templated reaction after 6 h of incubation. (C) Synthesized **SZ4TA2** as reference. (D) Suppression of Bcl-X_L-templated reaction by Bak BH3 peptide. (E) Bcl-X_L-templated incubation in presence of mutant Bak BH3 peptide. (F) Suppression of Bcl-X_L-templated reaction by Bim BH3 peptide. (G) Bcl-X_L-templated incubation in presence of mutant Bim BH3 peptide.

Bcl-X_L templates the formation of the characterized hit compound (Figure 1A−C).

To assess whether the Bcl-X_L-templated reactions occur at the BH3 binding pocket or randomly elsewhere on the protein surface, control experiments were performed in which the reactive fragments **SZ4** and **TA2** were incubated with Bcl-X_L and various proapoptotic BH3-containing peptides.[15] Bak BH3 and Bim BH3 peptides bind to Bcl-X_L through their BH3 domain and theoretically compete with the reactive building blocks for binding during these incubations. In comparison, their mutants exhibit lower affinity toward Bcl-X_L and hence do not suppress the Bcl-X_L-templated acylsulfonamide formation to the same extent. Comparison of the LC/MS-SIM traces (Figure 1D−G) between the Bcl-X_L incubations with and without these peptides suggests that the generation of **SZ4TA2** occurs at the BH3 binding site on Bcl-X_L.

An advantage of kinetic TGS approaches is the increased throughput screening capability with incubations containing more than two reactive building blocks. Experiments were undertaken to test whether our amidation kinetic TGS screening can be performed as incubations containing more than two complimentary reacting building blocks. Best results were obtained with reactions containing one thio acid and six sulfonyl azides.[15] Samples of Bcl-X_L with all 9 reactive building blocks at the same time failed at giving clear results. Although the multicomponent screening of the entire library failed, these experiments prove that the amidation kinetic TGS approach can be tested in an enhanced screening throughput.

To investigate the ability of the hit compound to disrupt the interaction between Bcl-X_L and Bak, we performed the well-established fluorescence polarization competition assay using Bcl-X_L and fluorescein-labeled Bak BH3 peptide.[16] Abbott laboratories reported that **SZ4TA2** is a good Bcl-X_L PPIM with a K_i constant of 19 nM, as determined by a competitive fluorescence polarization assay using a fluorescein-labeled Bad-BH3 peptide.[7e] Consistently, compound **SZ4TA2** is validated again as a Bcl-X_L inhibitor with an IC_{50} constant of 78.8 nM by our assay.[15] To compare the activity of acylsulfonamides not assembled by Bcl-X_L with that of the hit compound **SZ4TA2**, compounds **SZ2TA1**, **SZ2TA2**, **SZ2TA3**, **SZ4TA1**, **SZ5TA1**, and **SZ5TA2** have been synthesized and tested for their binding to Bcl-X_L. The IC_{50} values of these compounds have been determined to be 5 μM or higher.[15] Finally, we also determined the IC_{50} constants for the corresponding reactive building blocks **SZ4** and **TA2** to be higher than 100 μM. Taken together, these results indicate that the hit compound **SZ4TA2** identified through the kinetic TGS screening is indeed a respectable ligand of the biological target, which underscores the utility of kinetic TGS as a valuable approach to PPIM discovery and optimization.

In a proof-of-concept study, we have shown for the first time that kinetic TGS can be applied not only for enzymatic targets but also for protein−protein interaction disruption by using the recently reported amidation reaction between sulfonyl azides and thio acids. In the future, we will study in great detail the kinetics and mechanism of the kinetic TGS approach. Additionally, we will investigate the scope and limitations of the herein reported lead discovery and optimization method targeting other protein−protein interactions related to various diseases.

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Supporting Information Available: Synthetic procedures, LC/MS-SIM traces, and determination of IC_{50} values. This material is available free of charge via the Internet at http://pubs.acs.org.

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