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COMMUNICATIONS

Published on Web 09/12/2002

Development of a Potent Bcl-x_L Antagonist Based on α-Helix Mimicry

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Received May 10, 2002

The design of low-molecular weight ligands that disrupt protein−protein interactions is still a challenging goal in medicinal chemistry due in part to the large interfacial surface areas invovled.[1] Conventional methods for identifying inhibitors of protein−protein interactions from chemical libraries have had limited success. Therefore, new strategies for rational design of such inhibitors are necessary.[2]

Our approach to this problem involves the design of molecular scaffolds that mimic the surface functionality projected along one face of an α-helix. We have synthesized several designed *proteomimetics* based on a terphenyl scaffold, which in a staggered conformation closely reproduce the projection of functionality on the surface of an α-helix (Figure 1A).[3] To further extend this strategy we have tried to mimic parts of the α-helical, pro-apoptotic Bak- and Bad-proteins, which interact by heterodimerization with the anti-apoptotic protein Bcl-x_L.[4] Bcl-x_L is overexpressed in many types of cancer and protects transformed cells from cell death, leading to uncontrolled cell growth even if apoptotic signals generated by chemo- or radiotherapy are present.[5] A designed Bak/Bad-mimetic could interact with the anti-apoptotic Bcl-x_L protein and thus enable the apoptotic cascade leading to cell death. We herein report the identification of a potent inhibitor of the Bak/Bcl-x_L interaction based on α-helix mimicry of a part of the pro-apoptotic Bak protein.

We based our design on the crystal and solution structures of Bak/Bcl-x_L complex,[6,7] which show the helical Bak-peptide binding into a hydrophobic cleft formed by the BH1−BH3 domains of Bcl-x_L (Figure 2A). From alanine scans of the Bak-peptide it is clear that four hydrophobic residues (Val^{74}, Leu^{78}, Ile^{81}, Ile^{85}) along one edge of the helix are involved in binding.[8] In addition, Asp^{83} forms an ion pair with a lysine residue of Bcl-x_L. A related 26-mer peptide derived from the Bad-protein binds better to Bcl-x_L,[9] exploiting larger hydrophobic residues (Tyr, Phe) to induce a slight structural change in the binding region of Bcl-x_L. Furthermore, it has been shown that the α-helix propensity of these peptides is decisive for strong binding to Bcl-x_L.[9a]

On the basis of these structural requirements we designed a series of terphenyl molecules (**1**, **3**−**5**) containing alkyl or aryl substituents on the three ortho positions (to mimic the key hydrophobic substituents on the helical exterior of Bak or Bad) and carboxylic acid substituents on either end (to mimic the additional ion pair) (Figure 1B).

A modular synthetic route to terphenyl derivatives **1**−**5** was developed involving sequential Suzuki coupling of the corresponding methoxyphenylboronate and phenyltriflate derivatives.

The binding affinity of these molecules for Bcl-x_L was assessed by a fluorescence polarization assay using fluorescein-labeled 16-mer Bak-peptide.[8] Displacement of this probe through competitive binding of the terphenyl into the hydrophobic cleft of Bcl-x_L would lead to a decrease in its fluorescence polarization which in turn could be related to the known affinity of the 16-mer Bak/Bcl-x_L complex. This assay showed (Figure 3) that the terphenyl molecule with two carboxylic acids and the isobutyl, 1-naphthalenemethylene, isobutyl sequence (**4**) shows the strongest binding to Bcl-x_L with a K_D value of 114 nM. The less hydrophobic terphenyls (**1** and **3**) show lower affinity (K_D = 2.09 and 1.89 μM, respectively), emphasizing the importance of hydrophobic interactions for binding to the recognition cleft in Bcl-x_L.[10] Scrambling the position of the substituents, as in **5**, leads to a significant loss in binding affinity (K_D = 2.70 μM), suggesting an effective shape complementarity for **4**, as in the natural peptide. The importance of the hydrophobic groups is further confirmed by the weak binding of an analogue of **4** lacking the naphthyl and two isobutyl substituents (**7**[3b], K_D = 27.4 μM). Finally, the role of the carboxylate groups was probed by partial removal (as in **6**[3a], K_D = 6.8 μM) or conversion to positively charged groups (as in **2**,[3b] K_D = 13.7 μM), leading in both cases to significant loss of activity.

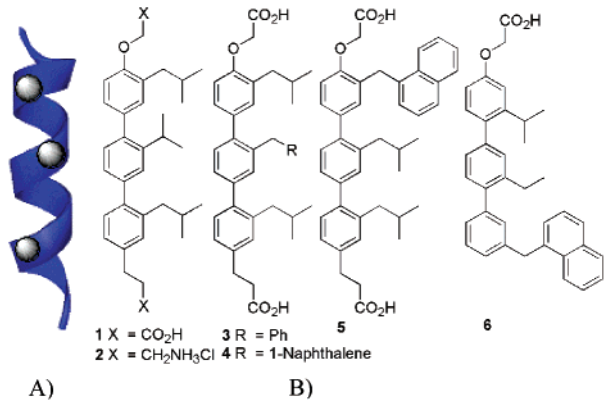

Figure 1. (A) *i*, *i* + 3, and *i* + 7 substituents of an α-helix. (B) Compounds tested in the fluorescence polarization assay.

Docking studies with the AutoDock program using the Bcl-x_L conformation found in the complex with Bak showed an optimal location for **4** in the same hydrophobic cleft as the helical peptide but in a slightly different orientation (Figure 2B). Further support for this binding site came from HSQC NMR experiments with ^{15}N-labeled Bcl-x_L protein. Addition of **4** (1.2 equiv, 460 μM) led to shifts in a number of residues on the surface of Bcl-x_L (F146, L130, I140, R139, W137, E193, S203, Y195, A104) near the predicted binding site. These affected residues all lie in the shallow cleft on the protein into which the Bak helix binds. Overlay of **4** and the Bak peptide within the binding pocket suggests that the terphenyl is indeed mimicking the cylindrical shape of the helix with the

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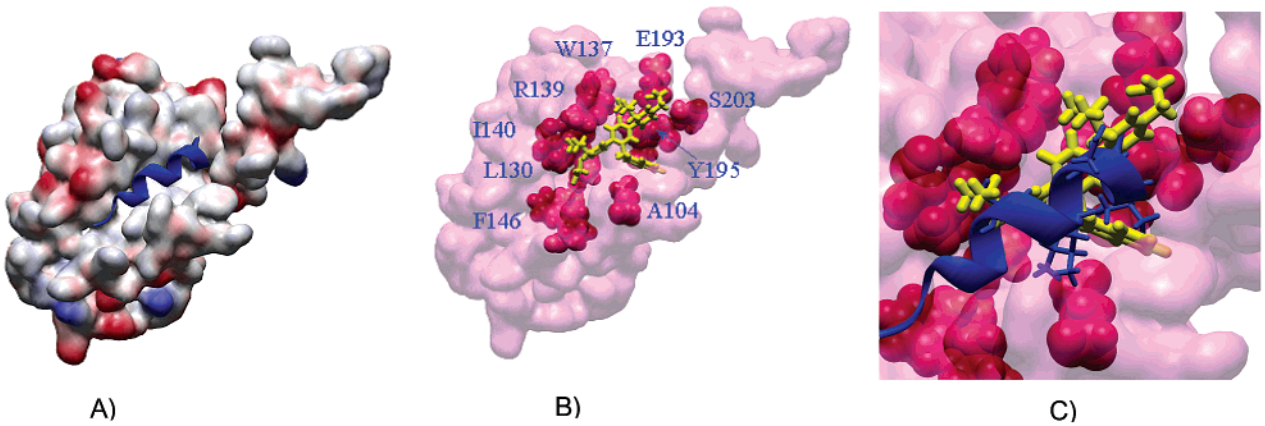

Figure 2. (A) Bak-BH3-peptide/Bcl-x_L complex. (B) Docking results and residues shifted in the NMR experiment of **4** and Bcl-x_L. (C) Overlay of peptide and postulated binding location for **4**.

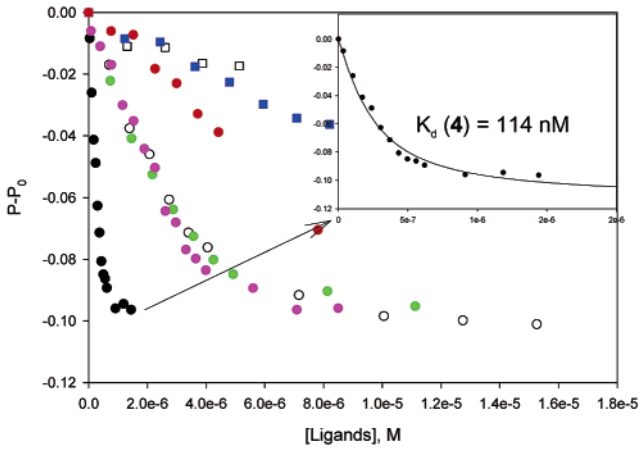

Figure 3. Results of the fluorescence polarization assay. Unfilled circle = **1**; green circle = **3**; black circle = **4**; magenta circle = **5**; red circle = **6**; unfilled square = **7**; blue square = **2**.

substituents making a series of hydrophobic contacts with the protein (Figure 2C).

In conclusion, a strategy of helix mimicry based on a substituted terphenyl scaffold was successfully applied to the design of a Bcl-x_L antagonist with binding affinity in the lower nM region. Previous small-molecule inhibitors of Bcl-x_L that were discovered by screening of large libraries,[8,12−15] or by serendipity,[16] only have K_D values in the μM range.[11] On the basis of the promising in vitro results for the inhibition of Bcl-x_L by **4**, preliminary cell studies on breast cancer cells are currently under investigation.

Acknowledgment. We thank the NIH for partial support of this work, and O.K. is grateful for a research fellowship of the *Deutsche Forschungsgemeinschaft* (*DFG*). We also thank Professor Gerhard Wagner and Dr. Alexey A. Lugovskoy (Harvard Medical School) for providing HSQC assignments of Bcl-x_L before publication, Sang-gyu Lee for assistance with the NMR experiments, and Professor J. Yuan for help with protein expression.

Supporting Information Available: Experimental details, characterization data (NMR, MS), docking parameters, and results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA026861K