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Design, synthesis, and activity evaluation of selective inhibitors of anti-apoptotic Bcl-2 proteins: The effects on the selectivity of the P1 pockets in the active sites

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ABSTRACT

The anti-apoptotic Bcl-2 proteins are attractive targets for anti-cancer drug development, and the discovery of their selective inhibitors has become a research focus. In this Letter, obvious differences in the P1 pocket of the active site between Bcl-2, Bcl-x_L, and Mcl-1 proteins were proposed by the structural comparison of these proteins. As a result, the groups in their inhibitors binding to the P1 pockets may have significant effect on the selectivity for these proteins. Based on this hypothesis, five types of derivatives of the lead compound **B-1** were designed, and several highly selective inhibitors of Bcl-x_L (**E-1**) or Mcl-1 proteins (**G**) were found. The selective inhibitors of Mcl-1 protein found in this Letter provide new structural types for the development of novel antitumor agents.

Apoptosis is a process of natural, programmed cell death, which plays a key role in ontogenesis and homeostasis in multicellular organisms.[1,2] The Bcl-2 protein family plays a key regulatory role in this process. It contains both anti-apoptotic (e.g., Bcl-2, Bcl-x_L, Mcl-1, Bcl-w, A1) and pro-apoptotic members.[1,3] The overexpression of the anti-apoptotic members of the Bcl-2 family, which is found in a wide variety of human cancers, is associated with tumour progression and resistance to anti-cancer therapy.[3–5] This is why the anti-apoptotic Bcl-2 proteins have become attractive targets for anti-cancer drug development. In recent years, a series of small-molecule inhibitors of anti-apoptotic Bcl-2 proteins with different structure types have been reported, which show anti-cancer effects.[4–16] At present, three small-molecular inhibitors, AT-101,[17] ABT-263,[18,19] and GX15-070[20,21] are in clinical research (Fig. 1). ABT-199 (venetoclax)[22,23] has been approved recently by FDA for the treatment of patients with chronic lymphocytic leukemia (CLL) who have a chromosomal abnormality called 17p deletion.

Apoptosis can be triggered by BH3-only pro-apoptotic Bcl-2 proteins (Bad, Bim, Bmf, Bik, Hrk, Bid, Puma, Noxa), which are activated upon cellular stresses. These proteins antagonize anti-apoptotic Bcl-2 proteins or directly activate Bax-like pro-apoptotic Bcl-2 proteins (Bax, Bak, Bok), which can lead to cytochrome C release from mitochondria to activate apoptosis.[4,5,24,25] Different anti-apoptotic Bcl-2 proteins show diverse selectivity and preference for binding BH3-only pro-apoptotic Bcl-2 proteins. Bad and Bmf are selective for the Bcl-2, Bcl-x_L, and Bcl-w proteins, while Noxa is selective for the Mcl-1 and A1 protein, and Bim, Bid and Puma are able to bind all anti-apoptotic proteins well.[16,25–28] These observations suggest that apoptosis can be regulated by different anti-apoptotic Bcl-2 proteins by diverse ways. And they may have different physiological effect.[5,24] Furthermore, human cancers may have different expression spectrum of anti-apoptotic Bcl-2 proteins.[5,29,30]

To explore the exact role of each anti-apoptotic Bcl-2 protein, its selective inhibitors can be utilized as good molecular tools.[24] In addition, these selective inhibitors have the opportunity to be developed to anticancer drug candidates with fewer adverse side effects than general inhibitors. Therefore, the discovery of these selective inhibitors has become a research focus. However, the structural similarity of anti-apoptotic Bcl-2 proteins makes the design of its selective inhibitors challenging. Presently, not many structure types of selective inhibitors have been reported. ABT-263 was reported to have high selectivity for Bcl-2 and Bcl-x_L proteins.[10] ABT-199,[11] WEHI-539[12] and A-1210477[13,31] were reported

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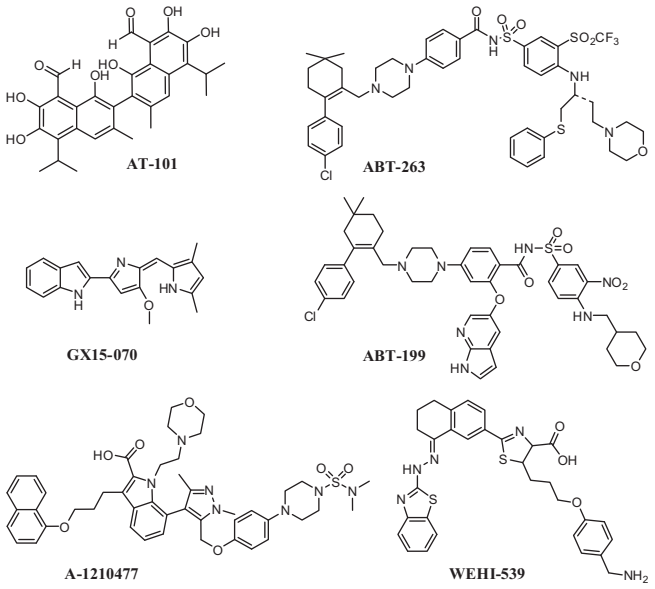

Figure 1. Representative small-molecule inhibitors of anti-apoptotic Bcl-2 proteins.

to have high selectivity for Bcl-2, Bcl-x_L and Mcl-1 proteins, respectively (Fig. 1).

Determining the structural difference in the active site between anti-apoptotic Bcl-2 proteins is useful to rationally design of its selective inhibitors. In this Letter, important differences were proposed by the structural comparison of Bcl-2, Bcl-x_L, and Mcl-1 proteins, three most studied anti-apoptotic Bcl-2 proteins. Based on this hypothesis, several types of derivatives of the lead compound were designed. Among them, several selective inhibitors of Bcl-x_L protein or Mcl-1 protein were found. These results validated our hypothesis and provided several lead compounds that merit further research into anti-cancer therapeutics.

All of the anti-apoptotic Bcl-2 proteins have a similar active site, which is a narrow and long groove on the protein surface. The bottom of it is bound by α5, and on the side, it is bound by α2, α3, α4, α7 and α8. The active site comprises three main areas: the P1 and P2 are two big and deep pockets in the active site, and the L1 is a narrow and shallow channel linking P1 and P2 (Fig. 2).[9,32,33] In the three-dimensional pharmacophore for Bcl-2 inhibitors we constructed, hydrophobic centers H1, H2 and H3 were three points bound in L, P1 and P2, respectively. They were found to serve as the linear molecular scaffold that satisfies the overall geometric and steric requirements for inhibitor binding.[16,32]

At present, several high-resolution three-dimensional structures of Bcl-2, Bcl-x_L, and Mcl-1 proteins in different states were reported, which offer good templates for determining the structural difference in their active sites. The structures of these three proteins which are free (PDB entry: 1GJH, 1LXL, and 1WSX) or complexed with a same substrate (BH3 of Bax) (PDB entry: 2XAO, 3PL7 and 3PK1) were superimposed and compared (Fig. 2).[34,35] Obvious difference in the opening of the P1 pocket could be observed because of the difference of the residues in the active site among these proteins (Fig. S1). The size order of free proteins was: Bcl-x_L < Bcl-2 < Mcl-1, that of complexed proteins was: Mcl-1 < Bcl-x_L < Bcl-2.

The different opening size order of free proteins and complexed proteins also suggested that there was difference in the flexibility when binding to substrates of the P1 pocket in the active site. Therefore, the root mean square deviation (RMSD) values of the changes between active sites of Bcl-2, Bcl-x_L, and Mcl-1 proteins in free and complexed states were calculated (Table 1). The order of the value of the changes between active sites in whole was: Mcl-1 < Bcl-2 < Bcl-x_L. In addition, the RMSD values of the changes between each domain of the active site were also calculated (Table 1). In Bcl-2 and Bcl-x_L proteins, the α3 domain appeared most flexible among the active site. While the α4 domain appeared most flexible in Mcl-1 protein. This conclusion is consistent with the finding of the molecular dynamic simulations to investigate the conformational flexibility of Bcl-x_L and Mcl-1 proteins.[36]

On basis of above analysis, there were obvious differences in the P1 pocket of the active site between Bcl-2, Bcl-x_L, and Mcl-1 proteins. As a result, the groups in their inhibitors binding to the P1 pocket may have significant effect on the selectivity for these proteins. In order to validate the hypothesis, a broad-spectrum inhibitor **B-1** we found, with a benzamide group binding to the P1 pocket predictively, was chosen as the lead compound (Fig. 3).[16] Then five types of derivatives **C**–**F** were designed by changing the benzamide group to other groups with diverse lengths and volumes, using different carboxylic acids from our in-house library (Fig. 3).

The synthetic routes of target compounds were shown in Scheme 1. The key intermediates **3** and **6** were synthesized by known procedures.[16] The target compounds **C** and **G** were

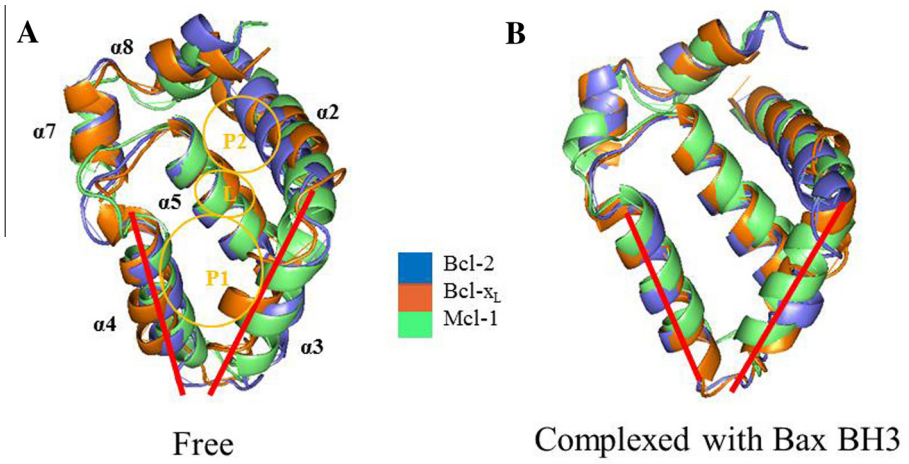

Figure 2. Comparison between the activity sites of Bcl-2, Bcl-x_L and Mcl-1 proteins. The PDB entries of the structures of free Bcl-2, Bcl-x_L and Mcl-1 proteins (A) are 1GJH, 1LXL, and 1WSX. Those of Bcl-2, Bcl-x_L and Mcl-1 proteins complexed with Bax BH3 (B) are 2XAO, 3PL7 and 3PK1. They are rendered as ribbons with different colors. The three main areas of the active site are labeled as P1, L and P2.