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Antagonists of Protein−Protein Interactions Made Easy?

Maurizio Pellecchia*

Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, California 92037, United States

In the realm of drug discovery, the design or discovery of selective and effective antagonists of protein−protein interactions (PPIs) is considered both an opportunity and a challenge for innovative targeted therapies. The opportunity resides in the continuous unraveling of the complex molecular mechanisms at the basis of cellular functions in the normal and diseased states. For example, alterations of apoptosis, or programmed cell death, a process that normally ensures a proper balance between cell production and cell loss, are associated with both neurodegenerative diseases (excessive apoptosis) and cancer (defective apoptosis). In normal cells, central to this balancing act are antiapoptotic proteins such as Mcl-1 and its closest relatives such as Bcl-2, Bfl-1, Bcl-xL, Bcl-W, and Bcl-B that promote cell survival, while the structurally similar proapoptotic relatives such as Bak, Bax, Bad, Bim, Noxa, or Bid promote cell death. The critical mechanism by which pro- and antiapoptotic members modulate the apoptotic machinery involves their direct interactions, mediated by a large hydrophobic groove on the surface of the antiapoptotic members and an amphipathic α-helical domain (known as the BH3 domain) on the proapoptotic counterparts.[1] Overexpression of antiapoptotic Bcl-2-family proteins in cancer cells confers tumor resistance to chemotherapy or radiation; hence, these proteins represent valid targets for cancer drug discovery. A viable way to inhibit the antideath activity of Bcl-2 proteins is to design small molecule inhibitors of their interactions with proapoptotic proteins, presumably deriving small molecules mimicking the prodeath α-helical BH3 domain. Fesik and co-workers[2] report on a novel and effective small molecule that possesses a remarkable potency, selectivity, and ligand efficiency against Mcl-1.

Earlier and most intuitive approaches to derive BH3 mimetics consisted in designing stapled or stabilized version of these helices, and significant success has been reported recently in this regard against Mcl-1.[3] However, the quest for small organic compounds peptide-mimetics has remained fervid. The development of simple assay platforms for the detection of compounds capable of displacing protein−peptide interactions opened the way to several high-throughput screening campaigns. However, to date, little success has been reported using these screening approaches. The failure of HTS campaigns in discovering protein−protein interactions antagonists can be attributable to several converging factors. The nature of the chemical libraries, for example, may not be suitable for such task, as likely these compound collections are not populated by molecules that mimic a protein surface. Other even more critical factors, however, are that protein−protein interaction surfaces are usually shallower and larger than the typical and more "druggable" binding pockets for small molecule cofactors and that the interface contacts are made up of a discrete and distant set of weak interactions. Hence, intuitively, small molecule compounds may not be suitable to entirely recapitulate these interactions. To this end, fragment-based drug discovery (or fragment-based ligand discovery, FBLD) approaches[4,4b] seemed particularly suitable to the stepwise identification of initial weakly interacting low molecular weight binders (fragments) that are subsequently optimized into more potent hit compounds. Previous efforts in this field resulted in Abbott inhibitor ABT-737, a BH3 mimetic targeting Bcl-2 and Bcl-xL (Figure 1A)[5] designed using the SAR by NMR approach pioneered by Dr. Fesik and co-workers.[4b] Despite this success, the relatively large molecular weight and relatively complex chemical structure of ABT-737 raise some concerns about the general applicability of this approach to derive PPIs antagonists with druglike properties comparable to, for example, typical kinase inhibitors. One critical observation about protein−peptide interfaces is that essential interactions reside usually in few amino acids, as often suggested by alanine scanning studies. Modifying or eliminating a single side chain often results in a peptide that may completely lose the ability to bind to its target. Backbone or side chain atoms located on either a linear or an α-helical peptide may be considered as protruding teeth of a zipper which may interdigitate with corresponding elements from the protein target, linking them. Therefore, even a small object impeding the proper location of one protruding tooth may jam the zipper, precluding its closure. Hence, following this analogy, it should be possible to derive small molecule inhibitors of protein−protein interactions without necessarily designing compounds that recapitulate the entire peptide. The work of Fesik and co-workers[2] typifies this simple concept deriving a highly potent and efficient small molecule inhibitor of Mcl-1/BH3 interactions. Using a typical NMR-based screening approach using [^{15}N,^{1}H]HMQC spectra of Mcl-1 collected in the absence and presence of test compounds from a library of fragment-like molecules, the authors identified two small molecules that recapitulate two critical interactions present in the Mcl-1/BH3 complex. These involve a carboxylate, mimicking a critical salt bridge, and two of the several hydrophobic interactions observed in the protein−peptide complex (Figure 1B,C). NOE-based docking studies suggest that while the ligands present distinct binding modes, some portions of the compounds occupy overlapping regions. These structural studies suggested that merging these two fragments rather than linking them together could lead to compounds with higher affinity. Simple direct optimization of a single fragment and linking or merging two fragments are alternative possible critical optimizations paths in FBLD,[4] and the decision on what approach to perform often requires some structural information. NMR, followed by fluorescence polarization

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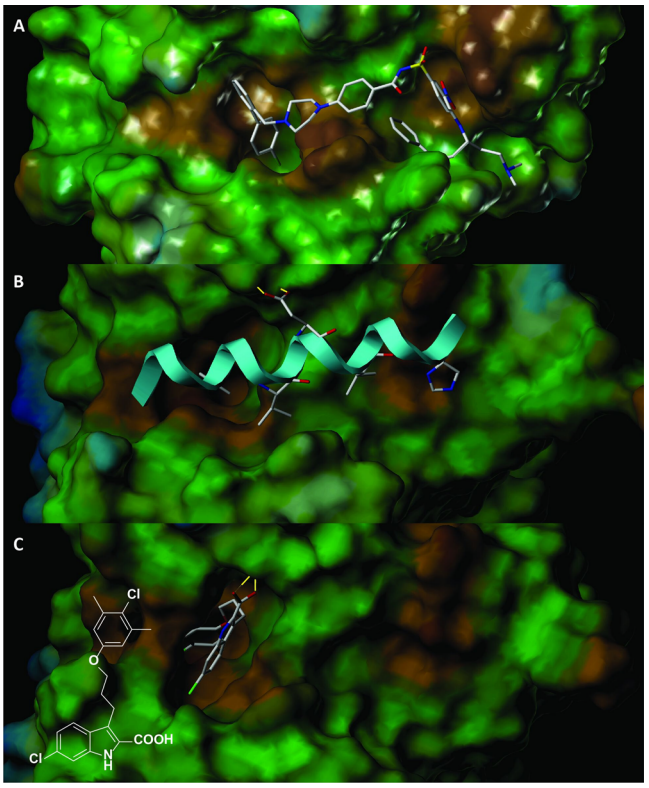

Figure 1. Chemical inhibitors of Bcl-2 family proteins. (A) Model of the interactions of ABT-737 in the complex with Bcl-xL (PDB code 2YXJ).[6] The protein is displayed as surface and color coded according to the lipophilic potential (brown, more lipophilic; blue, less lipophilic; green, intermediate), while the ligand is depicted as stick model. The compound occupies virtually the entire BH3 binding pocket of the protein. (B) Surface representation of Mcl-1 in complex with a BH3 peptide, shown as a ribbon (PDB code 4HW2).[2] Critical side chains of the BH3 peptide are displayed as stick model. Dashed yellow lines indicate hydrogen bonds between the side chain carboxylate of the BH3 residue Asp218 and the Mcl-1 side chain of Arg263. (C) Chemical structure of the indole-2-carbolylate compound **53** and a model of its structure in complex with Mcl-1 (PDB code 4HW3).[2] The protein is displayed as surface and color coded according to the lipophilic potential (brown, more lipophilic; blue, less lipophilic; green, intermediate), while the ligand is depicted as stick model. Dashed yellow lines indicate hydrogen bonds between the small molecule carboxylate and Mcl-1 side chain Arg263. Note that the unlike ABT-737 (A), compound **53** occupies only a smaller portion of the space occupied by the α-helical BH3 peptide (B). The artwork was generated with MOLCAD.

displacement assays with FITC-BH3 peptides, guided structure–activity relationships studies that resulted in the identification of merged compounds that bind to Mcl-1 with dissociation constants of <100 nM with selectivity for Mcl-1 over Bcl-xL and Bcl-2.[2] Moreover, the X-ray crystallography structures of the complex between Mcl-1 and merged compounds are also reported, providing detailed information about the molecular recognition of small molecule ligands binding Mcl-1 (Figure 1C).[2] The compounds reported by Fesik and co-workers not only represent viable starting points for the development of novel Mcl-1 based therapies but also demonstrate that FBLD approaches are suitable for designing small druglike compounds capable of effectively disrupting a protein–protein interactions by targeting only critical hot spots on the target surface. These studies should provide further impetus to attempt targeting PPIs with small molecules using FBLD approaches, and we anticipate that such efforts would most likely yield high return in several classes of targets.

AUTHOR INFORMATION

Corresponding Author

*Phone: 858-6463159. E-mail: mpellecchia@burnham.org.

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