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Discovery of Stapled Peptides as Efficient BCL-x_L Inhibitors

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Introduction

Protein-protein interactions (PPIs) play essential roles in regulating cellular processes, thus represent an important class of targets for drug discovery. The B cell CLL/lymphoma-2 (BCL-2) family of proteins control the intrinsic apoptotic pathway and can be divided into two major classes: proapoptotic members and prosurvival members [1]. In cancer cells, the overexpression of pro-survival proteins can block proapoptotic signaling by sequestering the BH3-only proteins (proapoptotic). Selective inhibition of BCL-x_L, a prosurvival member, is of interest due to its functional roles in solid tumors and drug resistance [2]. Using peptides as inhibitors of PPIs is a promising approach because of their larger size and ability to directly mimic native binding domains. Although small molecules have been reported to inhibit BCL-x_L, selective inhibition of BCL-x_L using stapled peptides remains underexplored. Recently, our group developed a stapling method using dibromomaleimide (DBM) for crosslinking two cysteines/homocysteines [3]. In this study, we performed a DBM staple scan on a peptide sequence based on a potent BH3 domain in combination with virtual alanine scanning, to identify efficient and selective peptide-based BCL-x_L inhibitors. One stapled peptide showed improved inhibitory potency (IC_{50}) in a fluorescence anisotropy (FA) competition assay compared to the wild-type and the linear precursor.

Results and Discussion

We designed a number of peptides by inserting a pair of cysteines into a 23-mer BH3 sequence at *i*, *i*+4 positions. After Fmoc-based solid-phase peptide synthesis, the peptides were cleaved and purified by a prep-HPLC. The linear peptides were treated with TCEP and each maleimide-staple was installed using dibromomaleimide (Figure 1).

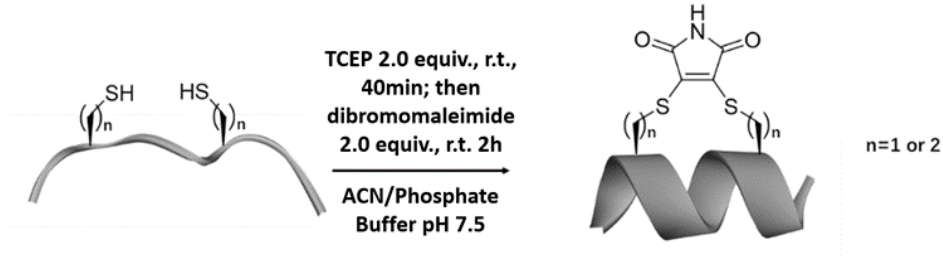

Fig. 1. A representative scheme of synthesis of stapled peptide using dibromomaleimide.

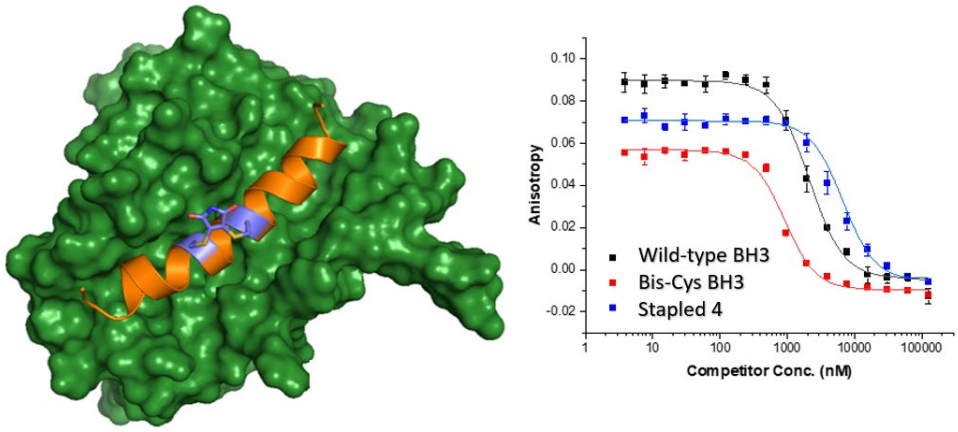

Fig. 2. A representative diagram of a stapled peptide binds to BCL-x_L and FA results of the peptides.

To establish a fluorescence anisotropy assay for screening, we prepared a tracer peptide, FAM-Ahx-BH3, by adding an aminohexanoic (Ahx) linker and a carboxyfluorescein (FAM) group at the *N*-terminus of the 23-mer BH3 sequence. In our direct titration experiment, the tracer bound to the BCL-x_L with an affinity of K_d = 38 (± 6) nM. Thereafter, the designed peptides were tested in competition assays. Surprisingly, one peptide, *Staple 4*, showed slightly improved potency in comparison to the Wild-Type BH3, whereas the linear peptide, Bis-Cys BH3, showed decreased potency (Figure 2).

Thus we have broadened the scope of the maleimide constraint in the construction of PPI inhibitors in this work and identified promising starting points for identification PPI inhibitors as potential therapeutics for treatment of BCL-x_L-overexpressed cancers.

Acknowledgments

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References

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