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1.2 ABSTRACT

Cancer remains one of the leading causes of death worldwide, and its effective treatment poses a significant therapeutic challenge. Peptide prodrugs and antibody-drug conjugates (ADCs) activated by proteases represent promising therapeutic strategies. However, commonly used linkers, such as Val-Cit, exhibit limited selectivity, leading to adverse side effects. The aim of my study was to develop, synthesize, and analyze a new generation of peptide-drug conjugates and ADCs selectively activated by specific proteases, including cathepsins B, L, and S, and to evaluate their efficacy against breast cancer cells.

I synthesized a series of prodrugs, initially employing doxorubicin (DOX) as the cytotoxic payload. However, due to its degradation under acidic conditions, it was replaced with the more stable monomethyl auristatin E (MMAE). I optimized the synthesis conditions and assessed the enzymatic hydrolysis of the prodrugs by cathepsins, monitoring the process using LC-MS. Cytotoxicity studies demonstrated that prodrug activation occurred through enzymatic hydrolysis, in which MMAE strongly binds to tubulin dimers, preventing microtubule polymerization. However, if the proteolytic activation did not occur, the entire peptide conjugate weakly binds to tubulin, leading to reduced cytotoxicity, although MMAE still exhibits activity on microtubules.

This discovery regarding the impact of proteolytic activation on cytotoxicity led me to design and synthesize antibody-drug conjugates (ADCs) incorporating both the conventional Val-Cit linker and novel tetrapeptide linkers containing unnatural amino acid residues. Their application enhances the selectivity and activity of prodrugs, potentially reducing toxicity toward healthy cells. Cytotoxicity analyses confirmed that the linker structure plays a crucial role in therapeutic efficacy. The results highlight the potential of the selectively designed peptide sequences in the development of novel cancer therapies based on peptide prodrugs and ADCs.

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