Development of thiazole-containing cyclic peptide ligands by an mRNA-display-coupled post-translational chemoenzymatic modifications

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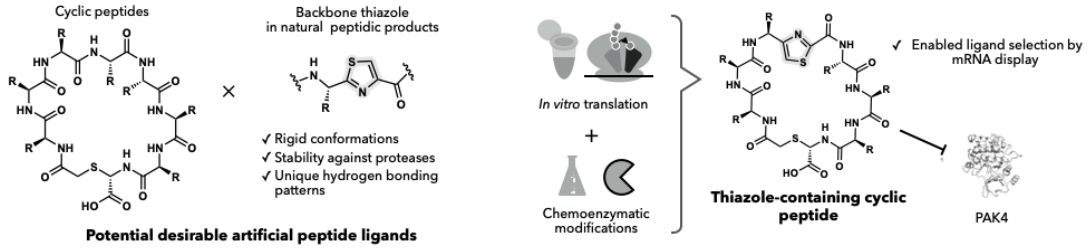
Backbone thiazole moieties are widely found in peptidic natural products, possibly due to their rigid conformations, resistance to protease and hydrolysis[1], and unique hydrogen-bonding patterns[2]. These intrinsic attributes confer an advantage to the presence of backbone thiazoles in artificial cyclic peptides, enhancing their potential for ligand development. However, exploring *de novo* thiazole-containing peptide ligands with high efficiency and reliability has proven challenging. In this study, we focus on the synthesis of thiazole-containing peptides by *in vitro* post-translational chemoenzymatic modifications, which can synthesize diverse sequences under mild aqueous conditions. By applying this approach to mRNA display, we aim to establish a methodology to obtain cyclic peptide ligands containing backbone thiazoles. The synthetic method involves ribosomal incorporation of thioamides into peptides[3], spontaneous heterocyclization of thioamide and adjacent Cys, to form thiazoline, followed by thioether macrocyclization and enzymatic oxidation by GodE.

To achieve the specified goal, we first improved the synthesis method by focusing on the translation step in the post-translational chemoenzymatic modification method to increase the efficiency of desired product formation, making the method more versatile. We then constructed diverse thiazole-containing cyclic peptide libraries using the versatile synthetic method. Through *in vitro* selection of ligands using mRNA display, we obtained thiazole-containing cyclic peptide ligands with high binding affinities and inhibitory activities against p21-activated kinase 4 (PAK4), demonstrating their potential for drug development applications. This study established a selection system which expedite *de novo* discovery of desirable cyclic peptide ligands containing backbone thiazoles.



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