Ribosomal synthesis of peptide libraries containing cyclic γ-amino acids for drug screening

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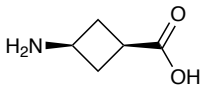
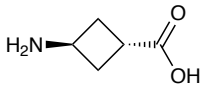
γ-Amino acids can induce unique secondary structures of peptides, such as C_{14}-helix, $C_{12/10}$-helix, and C_{12}-turn. [1] γ-amino acids are found in some natural bioactive peptides and play important roles in their biological activities and proteolytic stability.[2] Especially, cyclic $\gamma^{2,4}$-amino acids (cγAA, Figure) are ideal building blocks that stabilize structures of peptides, and thereby can improve binding affinity to target proteins due to their constrained rigid conformations. Therefore, incorporation of cγAAs into a random peptide library and its application to in vitro selection of active ligands would be powerful approach for developing novel therapeutic peptides.

Recently, our group reported the ribosomal incorporation of cγAAs into nascent peptide chains using a reconstituted translation system assisted by Flexizyme technology, referred to as the Flexible In vitro Translation (FIT) system.[3] Here we report the ribosomal synthesis of macrocyclic peptide libraries containing two cγAAs, *cis*- and *trans*-3-aminocyclobutane carboxylic acid by means of the FIT system, and its application to an in vitro screening methodology, referred to as the Random nonstandard Peptides Integrated Discovery (RaPID) system.[4] Consequently, we obtained inhibitor peptides against interferon-gamma receptor 1 (IFNGR1) that showed strong binding affinity and inhibitory activity with low nM K_D and IC_{50} values. Moreover, the peptides containing cγAA exhibited up to 27-fold longer half-lives in human serum than their alanine-substituted mutants.



cis-3-aminocyclobutane carboxylic acid



trans-3-aminocyclobutane carboxylic acid

Figure Cyclic γ-amino acids used in this study

[1] Hintermann, T., Gademann, K., Jaun, B., Seebach, D., *Helv. Chim. Acta* **1998**, *81*, 983.
[2] Frackenpohl, J., Arvidsson, P.I., Schreiber, J.V., Seebach, D., *Chembiochem*, **2001**, *2*, 445.
[3] Katoh, T.; Suga, H. *J. Am. Chem. Soc.* **2020**, *142*, 4965.
[4] Yamagishi, Y.; Shoji, I.; Miyagawa, S.; Kawakami, T.; Katoh, T.; Goto, Y.; Suga, H. *Chem. Biol.* **2011**, *18*, 1562.