Development of cyclic peptides that can be administered orally to inhibit disease targets

Cyclic peptides can bind challenging disease targets, but their oral application is hindered by digestion and absorption issues. We developed a versatile method for the synthesis and functional screening of vast numbers of synthetic cyclic peptides and identified peptides with high inhibitory activity, stability and oral bioavailability in rats.

This is a summary of:
Merz, M. L. et al. De novo development of small cyclic peptides that are orally bioavailable. *Nat. Chem. Biol*. https://doi.org/10.1038/s41589-023-01496-y (2023).

Published online: 28 December 2023

The mission

A major challenge in drug development is that for many important disease targets, it is difficult to generate inhibitors that can be administered orally[1]. The reason for this is that small molecules that are suited for oral application often cannot bind to protein targets due to the lack of deep clefts or pockets, and monoclonal antibodies that could bind are too large and unstable for oral application. A solution to this problem could be small cyclic peptides, which have excellent binding properties that allow them to interact with shallow and featureless protein surfaces while still being membrane permeable and orally available. In fact, a small number of peptides derived from natural sources have been developed into oral drugs, such as cyclosporine A (used as an immunosuppressant), desmopressin (an antidiuretic) and a somatostatin analog[2], indicative of the feasibility of orally available peptides. However, the generation of orally available cyclic peptides directed against new targets has remained challenging, as in addition to binding the target protein, they need to be highly stable to resist proteases and sufficiently small and nonpolar to cross the epithelial layer of the gastrointestinal tract.

The solution

Recently, we established a strategy for the synthesis and functional screening of thousands of small cyclic peptides at a nanomolar scale[3], and we speculated that this could be used to develop orally applicable peptides. However, our original method generates peptides cyclized by disulfide bridges that are metabolically labile and not suited for oral application. We thus modified the method to yield more-stable cyclic peptides based on thioether bridges. In this new combinatorial synthesis strategy, '*m*' random peptides are cyclized by '*n*' linkers to obtain thioether-cyclized peptides, which are then acylated at a peripheral amine with '*o*' carboxylic acids to yield $m \times n \times o$ cyclic peptides (Fig. 1a). By monitoring the stability and breakdown of such thioester-linked peptides, we found this to be a metabolically more stable peptide format than disulfide-linked molecules. Our new approach also enabled the generation of structurally more diverse peptides due to the diversification of three elements (m, n, o) rather than only two (m, o) as in previous approaches, which is important for maximizing structural diversity in the search for good ligands.

We synthesized a large library of 8,448 cyclic peptides with an average molecular mass of approximately 650 Da, which is slightly above the maximum limit of 500 Da recommended for orally available small molecules, according to Lipinski's rule of five. Screening of the library against the disease target thrombin (a serine protease important for blood coagulation that has been implicated in thrombotic disorders) identified several inhibitors. These peptides showed good inhibitory activity and proteolytic stability, but had low membrane permeability and metabolic stability and thus were not suitable for oral use. However, our cyclic peptide synthesis method allowed several iterative cycles of library synthesis and testing on the basis of strong candidate peptides from the first round. We obtained cyclic peptides with nanomolar affinity, high stability and oral bioavailability (%F) of up to 18% in rats. This proof-of-concept work shows that peptides that are specific for targets of interest and orally available can be developed de novo from random libraries.

Future directions

Our work provides a set of synthesis and screening tools, as well as a workflow that can be implemented for each target interaction with a functional readout, and moves the field closer to the longstanding goal of developing peptide-based drugs that can be administered orally.

To apply the method to more-challenging disease targets, such as protein–protein interactions, we anticipate that larger libraries will need to be synthesized and screened. Recently, we have applied the methods to synthesize and screen libraries on 1,536-well plates, which enabled the screening of tens of thousands of cyclic peptides. With the automation of more steps of the methods, libraries of more than one million molecules seem to be within reach.

As next steps, we have selected several intracellular protein–protein interaction targets for which it has been difficult to develop inhibitors based on classical small molecules. We are confident that orally applicable cyclic peptides can be developed for at least some of them.

Manuel L. Merz & Christian Heinis
École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

EXPERT OPINION

"The most significant contribution of this work is the discovery of a novel thrombin inhibitor that has the characteristics of a potential therapeutic agent. Another important contribution is a method of synthesizing fairly large cyclic peptide libraries in solution in a format that is compatible with functional screening — i.e., enzyme inhibition and membrane permeability. This method should be applicable to other target proteins."
Dehua Pei, Ohio State University, Columbus, OH, USA.

FIGURE

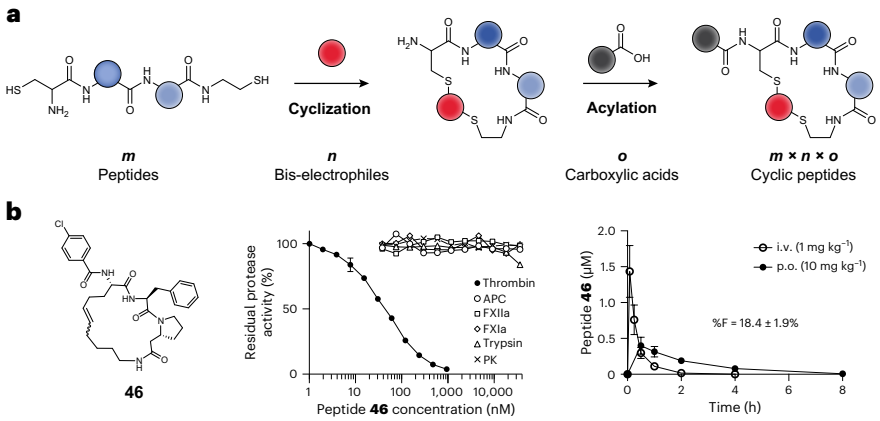

Fig. 1 | Strategy for the combinatorial synthesis of cyclic peptide libraries to enable the development of a thrombin inhibitor. a, Short peptides composed of random amino acids (*m*; blue) are cyclized with linker reagents (*n*; red) and are further diversified by carboxylic acids (*o*; black) to generate large libraries arrayed in microwell plates ready for functional screens. **b**, Chemical structure (left) of the novel cyclic peptide inhibitor of thrombin (peptide 46 after iterative cycles of our screen), its inhibitory activity and specificity for thrombin compared with that of unrelated enzymes (middle), and data showing its oral availability (%F) in rats (right). APC, activated protein C; FXIa, activated factor XIa; i.v., intravenous; PK, plasma kallikrein; p.o., per os (by mouth).

BEHIND THE PAPER

Our lab has developed peptide-based ligands over many years using bicyclic peptide phage display[4]. These peptides were quite large, typically 1–2 kDa, and were not membrane permeable, which tremendously limited their application due to the restriction to extracellular targets, the need for injection and their propensity for rapid renal clearance. For this reason, a few years ago we decided to take up the challenge of developing membrane-permeable and orally available peptides. In order to generate structurally diverse molecules, we moved from phage display to chemical synthesis, as this gave us access to more than 500 different commercially available amino acid building blocks to produce the libraries. We established methods for synthesizing a large number of small cyclic peptides at a nanomole scale that can be tested in functional screens[3], which formed the basis for the de novo development of target-specific, orally available cyclic peptides in this current work. **M.L.M. & C.H.**

REFERENCES

1. Scott, D. E. et al. Small molecules, big targets: drug discovery faces the protein–protein interaction challenge. *Nat. Rev. Drug. Discov.* **15**, 533–550 (2016).
   This review article discusses challenging disease targets and their structural features.
2. Naylor, M. R. et al. Cyclic peptide natural products chart the frontier of oral bioavailability in the pursuit of undruggable targets. *Curr. Opin. Chem. Biol.* **38**, 141–147 (2017).
   This review article presents cyclic peptides identified in nature that are orally available.
3. Habeshian, S. et al. Synthesis and direct assay of large macrocycle diversities by combinatorial late-stage modification at picomole scale. *Nat. Commun.* **13**, 3823 (2022).
   This paper describes a method for nanoscale synthesis and high-throughput screening of disulfide-cyclized peptides.
4. Heinis, C. et al. Phage-encoded combinatorial chemical libraries based on bicyclic peptides. *Nat. Chem. Biol.* **5**, 502–507 (2009).
   This paper describes a method for the generation of bicyclic peptides by phage display.

FROM THE EDITOR

"There are many methods for creating libraries of cyclic peptides; however, most peptides cannot be delivered orally because they are rapidly digested or are not absorbed in the gastrointestinal tract. This paper stood out because the screening and selection process considered stability and permeability as well as activity."
Russell Johnson, Chief Editor, *Nature Chemical Biology*.