

# Cyclic peptides in biological/medicinal chemistry

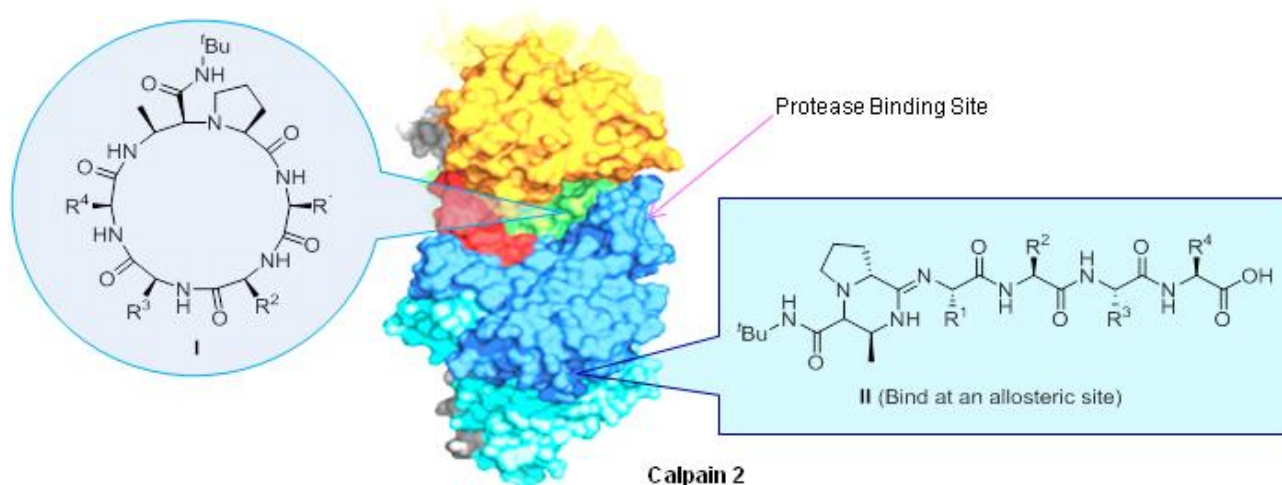
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In recent years there is a renewed interest in peptides as therapeutics and, in addition to the already marketed peptides for medical intervention, many of them are in preclinical studies and under clinical trials.<sup>1</sup> Moreover, peptides are being important tools for understanding complex biological processes, which in turn could render new biological targets for future medicines.<sup>2</sup> Most peptides suffer from shortcoming such as poor bioavailability, due to low metabolic stability, or high flexibility, which can result in low selectivity. However, advantages of using peptides as a primary source of biological/medicinal molecules are the easy creation of high diversity through relatively simple biologic or synthetic processes, thus increasing the possibility of discovering initial hits, and their efficiency and relatively low toxicity. In addition, there is an armamentarium of available modifications for conveniently controlling peptide metabolism and conformation, which can be applied at will to increase stability and to restrict peptide promiscuity.

Compared to their linear counterparts, cyclic peptides are less flexible, normally translated into higher selectivity and better resistance to hydrolysis by exo- and endopeptidases.<sup>3</sup> Therefore, cyclic peptides are considered good therapeutic agents and biochemical tools, although they still remain an underexplored class of compounds. Cyclization can be achieved through a range of strategies that mostly include head-to-tail and side-chain-to-side-chain (amide, disulfide, stapled, clicked, etc.) cyclizations, macrocyclization using organic frameworks, and grafted peptides. Here, I will comment on a few recent publications concerning cyclic peptide derivatives to illustrate their usefulness at different stages of the drug discovery process. Although there are many other recent examples of great interest, the selection understandably highlights articles that have first caught the attention of the writer.

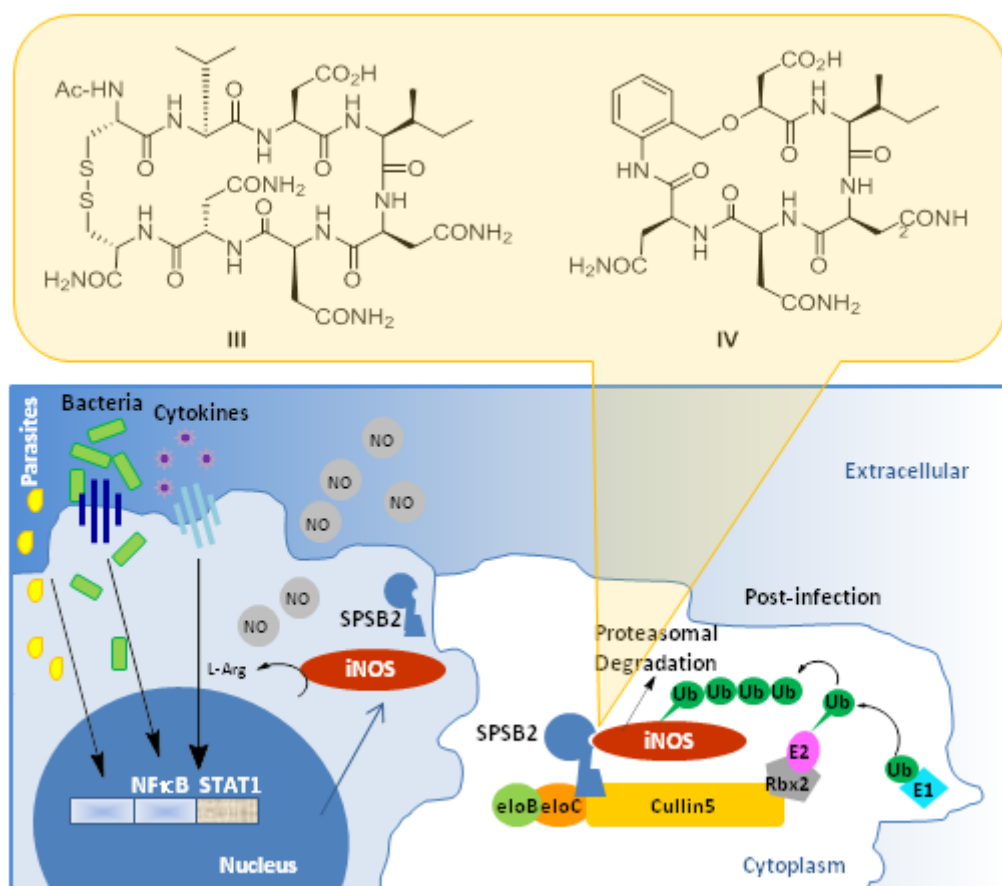
Based on the structure of calpastatin, the group of Zaretsky developed libraries of medium-sized cyclic peptides **I** and related cycle-tail peptidomimetics **II** (Figure 1), both with ability to function as calpain inhibitors (mid to low  $\mu\text{M}$  range).<sup>4</sup> A key step in the preparation of compounds **I** is a head-to-tail aziridine aldehyde-mediated peptide cyclization, an improved procedure over other conventional peptide cyclization methods, since the formation of dimers and polymers is almost avoided. As expected these compounds adopt  $\beta$ -turn type conformations, important for enzyme recognition. A competing intramolecular reaction of the same multicomponent reaction of aziridine aldehyde dimers, peptides, and isocyanides is responsible for the formation of compounds **II**, obtained in more or less amount depending on the configuration of residues. The procedure allowed the easy preparation of different peptide derivatives for SAR studies, leading to the identification of compounds displaying competitive, noncompetitive, or mixed inhibition of calpain, with some of them showing good selectivity against other related proteases. The combination of molecular modeling studies and the preparation and study of analogues containing photoreactive cross-linking groups allowed the authors to locate the binding sites for these two types of molecules, including a calpain allosteric site that could open a new strategic way for inhibiting the enzyme.



**Figure 1.** Calpain inhibitor cyclic peptides and proposed points of interaction

Inhibition of the SPSB2–iNOS protein-protein interaction could have interest towards novel anti-infective agents, because prolonged iNOS expression or enhanced NO production improves the killing of persistent pathogens. Following an initial proof of concept with the  $\beta$ -turned cyclic peptide c[CVDINNNC]-NH<sub>2</sub> (**III**), able to potently

interact with SPSB2, the group of Bael developed a series of analogues cyclized through different organic linkers designed *in silico* to maintain the model conformation.<sup>5</sup> A combination of biophysical techniques, like SPR, ITC and 19F-NMR, served to establish that the optimal organic link to join the *N*- and *C*-termini of the DINNN linear peptide was a *O*-(2-aminobenzyl)malate, affording cyclic analogue **IV** (Figure 2), with nanomolar affinity for SPSB2. In addition, this compound inhibits the interaction between full length iNOS and SPSB2 in a complex macrophage cellular lysate. The organic linker provides this compound with a better redox stability compared to the disulfide-based cyclic peptide. Although permeability is still a pharmacokinetic property to be improved in compound **IV**, it could have potential to be developed into a new type of anti-infective drugs. The development of innovative agents against community-acquired pathogens, having different mechanisms of action with respect to marketed drugs, remains a strong need because antibiotic resistance continues to grow and constitutes a significant public health problem. Peptides and especially cyclic peptides could play an important role here.

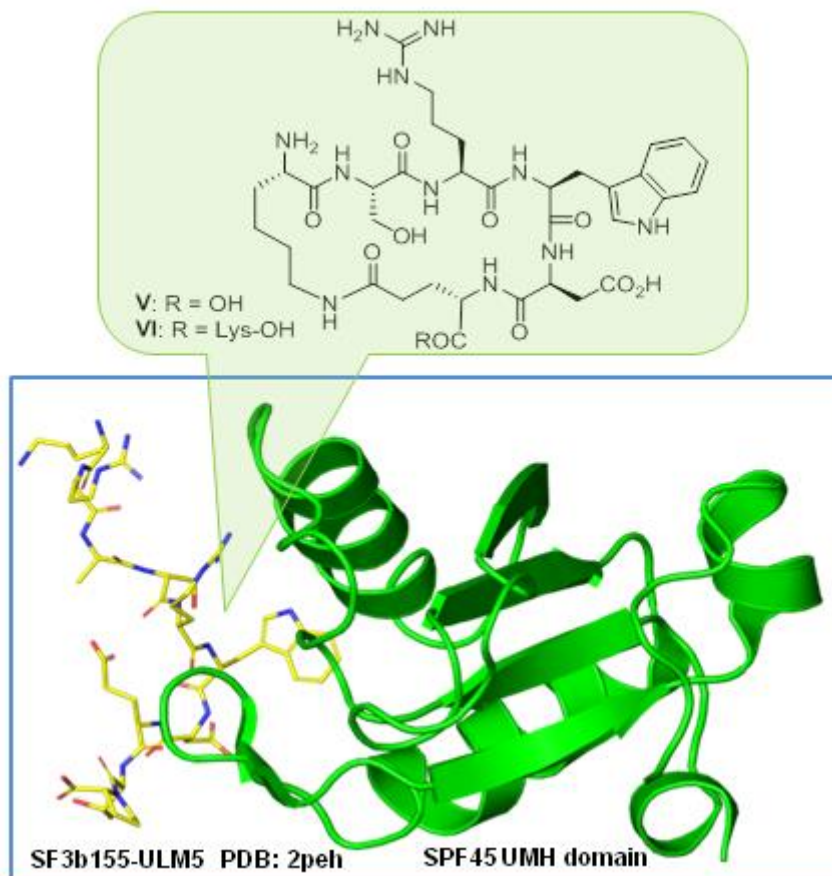


**Figure 2.** Cyclic peptides inhibiting iNOS-SPSB2 protein-protein interaction

Also related to the modulation of protein-protein interactions, the group of Sattler exploited the use of cyclic peptides for studying the relatively unknown molecular mechanisms of splicing regulation during early stages of spliceosome assembly. Recent knowledge is correlating aberrant splicing regulation and a growing number of pathological conditions, such as cancer and neurodegenerative diseases. SPF45 is an alternative splicing factor, a component of the spliceosome, implicated in certain cancers. The interaction of the SPF45 UHM domain and ULM ligand motifs in constitutive splicing factors is behind the splicing regulation of apoptosis-linked pre-mRNAs by SPF45. Using a rational design strategy, based on the X-ray structure of the SPF45 UHM-SF3b155 ULM5 complex, the authors defined a minimum linear peptide and some side-chain-to-side-chain analogues to fix the b-turn conformation found in the complex.<sup>6</sup> This led to cyclic peptide **V** (Figure 3), showing micromolar binding affinity and a >3-fold improvement with respect to the linear analogue. Further structure-based optimization afforded peptide **VI**, in which the presence of an extra Lys residue, which enable a cation- $\pi$  interactions with Tyr376 in the UHM domain, enhanced the affinity by one order of magnitude compared to **V**. Despite the low activity of this compound in cellular assays, the obtained results are a valuable first proof of concept demonstrating that targeting UHM-ULM protein-protein interactions could constitute a new objective for modulating initial stages of spliceosome assembly, and therefore for intervention in associated pathophysiological processes.

The commented papers are just a few examples of a growing field with good prospects in coming years. The advancement of synthetic methods, such as the recently published late-stage C(sp<sup>3</sup>)-H activation for peptide stapling,<sup>7</sup> along with a better understanding of conformational preferences and factors governing cell permeability, still to be

resolved, will undoubtedly contribute to increasing the significance of cyclic peptides in biological and medicinal chemistry. The role of cyclic peptides will be especially important in the case of the so-called “difficult targets” like protein-protein interaction. Also, their potential application as drug delivery carriers, as for the functional delivery of siRNA,<sup>8</sup> and the application of emerging technologies for peptide drug delivery and alternative administration routes, could also contribute to this field expansion. Taking all of the above into account, I am convinced that peptides in general, and cyclic peptides in particular, could offer enormous potential as biological tools for better understanding essential biological pathways, as well as future therapeutics for the treatment of unmet medical needs.



**Figure 3.** Cyclic peptides designed from ULM motifs to interact with SPF45 UHM

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