Review

Cyclic Peptides on a Merry-Go-Round; Towards Drug Design

Anthi Tapeinou,[1] Minos-Timotheos Matsoukas,[2] Carmen Simal,[1] Theodore Tselios[1]
[1]*Department of Chemistry, University of Patras, 26500, Patras, Greece*

[2]*Department of Biostatistics, Laboratory of Computational Medicine, Autonomous University of Barcelona, 08193, Bellaterra, Spain*

Received 30 January 2015; revised 14 April 2015; accepted 4 May 2015
Published online 13 May 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/bip.22669

ABSTRACT:

Peptides and proteins are attractive initial leads for the rational design of bioactive molecules. Several natural cyclic peptides have recently emerged as templates for drug design due to their resistance to chemical or enzymatic hydrolysis and high selectivity to receptors. The development of practical protocols that mimic the power of nature's strategies remains paramount for the advancement of novel peptide-based drugs. The de novo design of peptide mimetics (nonpeptide molecules or cyclic peptides) for the synthesis of linear or cyclic peptides has enhanced the progress of therapeutics and diverse areas of science and technology. In the case of metabolically unstable peptide ligands, the rational design and synthesis of cyclic peptide analogues has turned into an alternative approach for improved biological activity. © *2015 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 104: 453–461, 2015.*

Keywords: cyclic peptides; solid-phase peptide synthesis; rational drug design

This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of any preprints from the past two calendar years by emailing the Biopolymers editorial office at biopolymers@wiley.com.

Correspondence to: Theodore Tselios; e-mail: ttselios@upatras.gr
Contract grant sponsors: "Cooperation Greece-Israel Program" ISR_3148, [O. P. Competitiveness & Entrepreneurship (EPAN II), ROP Macedonia–Thrace, ROP Crete and Aegean Islands, ROP Thessaly–Mainland Greece–Epirus, ROP Attica], FICYT, the European Union (7th WP)

INTRODUCTION

Peptides constitute one of the most promising platforms for drug development due to their biocompatibility, chemical diversity, and resemblance to proteins.[1] Inspired by the protein assembly in biological systems, a large number of peptides have been designed using different amino acids and sequences, while forming unique folded structures ("fold-on-binding") and providing a broad spectrum of physiological and biological activities.[2] In this regard, peptides have triggered applications that currently range from drug discovery[3] to nanomaterials;[4] such as nanofibers for biomedical purposes, tissue engineering,[5] vaccines,[6] and medical imaging technologies,[7] among others. The computer-aided drug design approaches[8] along with efficient and economic peptide synthesis have contributed to revitalize peptide-based drugs in the current pharmaceutical market. Innovative tools for the identification and characterization of new protein binding sites[9] ("PPi" protein–protein interaction) have also evoked the discovery and optimization of novel drugs via protein–protein interaction specificities.[10] Notably, these binding areas in PPis are regularly nonadjacent, and the interaction interfaces are often found in exposed loop regions.[11] These regions are less accessible by small-molecule-based therapeutics (MW $<$ 500 Da) that usually target protein clefts and inhibit specific catalytic centers or binding sites of native structures.[12] Consequently, higher molecular weight peptides as therapeutics ought to be potentially more suitable owing to their ability to establish a broad number of non-covalent interactions, providing specific recognition to a given target.[10,13] Peptide-based drugs usually offer an alternative and synthetically appealing strategy in the field of rational drug

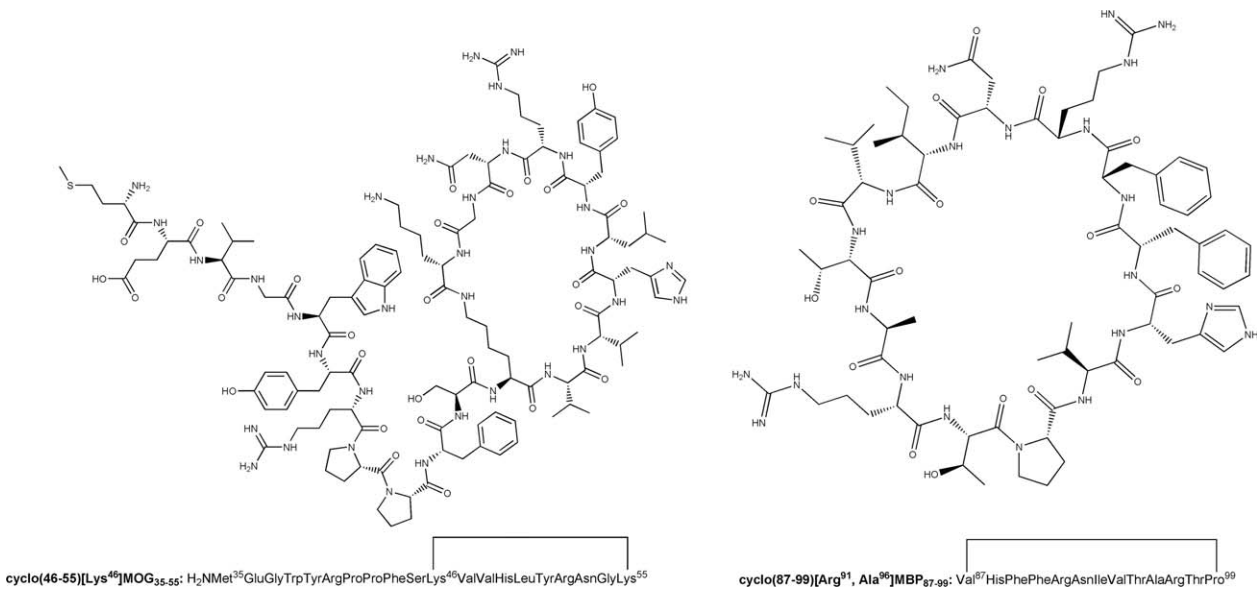

FIGURE 1 Representative 2D structures of side chain-to-tail cyclic MOG analogue and head to tail MBP analogue.[32,33] The cyclization was achieved via amide bond between the amine and carboxyl groups.

design.[14] Linear peptides are not very promising therapeutic agents owing to their low stability towards proteolysis, consequently reducing their feasibility and profitability for the pharmaceutical industry. This approach has been coincidentally amended by the discovery and development of novel constrained peptides over the past decades.[3] Diverse chemical modification protocols that have evolved to diminish the mentioned drawbacks[15] include cyclization (increased stability), *N*-methylation (increased membrane permeability and stability), incorporation of unnatural amino acids (increased specificity and stability), PEGylation (reduced clearance), assorted structural constraints (e.g., disulfide bonds) as well as recent progress in "stapled" peptides (improved potency and specificity) as a promising new modality for future therapeutics.[16] This novel generation of cyclic peptides, which are less prone to proteolysis,[17] provides superior binding affinities and entropy advantage in receptor binding compared to their more flexible linear counterparts.[9c,18] Confining a peptide into a cyclic structure reduces the conformational freedom of its parent structure and enhances its metabolic stability, bioavailability and specificity,[10] providing promising lead compounds for drug development. These cyclic mimetics force the molecule into an ordered secondary structure,[19] preventing off-target side effects,[20] and leading to harmless metabolic products in contrast to small-molecule drugs. Therefore, these preferred cyclic peptidomimetics have increased cell permeability and oral bioavailability, besides maintaining the potency and selectivity of larger proteins with lower immunogenicity. Furthermore, they are significantly smaller compared to proteins and more accessible due to lower manufacturing costs through various chemical methods.[21] Solid-phase peptide synthesis (SPPS) has become the prevailing technique for peptide synthesis in a solid support and is now a routine in numerous research laboratories.[22] Current synthetic strategies are based on orthogonally protected precursors at specific functional groups (amino acids), which are selectively deprotected during peptide synthesis.[23] In addition, novel straightforward protocols involving solid-phase peptide synthesis, followed by in-solution fragment coupling have been introduced to improve the yield and facilitate the synthesis of large polypeptides.[24] This progress has been crucial for the re-emergence of these privileged motifs as prospective drug candidates.[3,15]

This article reviews the current state-of-the-art of peptide-based therapeutics and concentrates on diverse strategies for cyclization of peptides, recent rational drug design approaches in conjunction with a number of representative contributions to the current medicinal and pharmaceutical chemistry.

Cyclic Peptides as Therapeutic Agents

The versatility and modularity of peptides derives from the chemical diversity of amino acids and their availability to integrate chemically modified building blocks into peptide synthesis, featuring modifications on the peptide backbone and/or side chain. Cyclic peptides have been broadly exploited over the past 20 years in medicine as active ingredients of natural extracts (bacteria, fungi, plants, animal venoms); some

Table I Cyclic Peptides in the Global Drug Market

Synthetic Cyclic Therapeutic Peptides	Primary Structure	Indications
Calcitonin (Human) (one disulfide bond)	H-cyclo[Cys-Gly-Asn-Leu-Ser-Thr-Cys]-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-NH_2	Osteoporosis, hypercalcaemia
Calcitonin (Salmon) (one disulfide bond)	H-cyclo[Cys-Ser-Asn-Leu-Ser-Thr-Cys]-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2	
Ziconotide (three disulfide bonds)	[Cys^1-Cys^{16}, Cys^8-Cys^{20}, Cys^{15}-Cys^{25}]-tricyclo H-[Cys^1-Lys-Gly-Lys-Gly-Ala-Lys-Cys^8-Ser-Arg-Leu-Met-Tyr-Asp-Cys^{15}-Cys^{16}-Thr-Gly-Ser-Cys^{20}-Arg-Ser-Gly-Lys-Cys^{25}]-NH_2,	Severe chronic pain
Oxytocin (one disulfide bond)	H-cyclo[Cys-Tyr-Ile-Gln-Asn-Cys]-Pro-Leu-Gly-NH_2	Bleeding or hemorrhage

representative examples in the current pharmaceutical market include the well-known cyclosporine A,[25] gramicidin-S,[26] vasopressin, oxytocin,[27] vancomycin,[28] and insulin.[29] Nevertheless, a large number of natural occurring peptides exhibit a limited applicability due to their poor stability in physiological conditions, which have inspired scientists to develop the so-called peptidomimetics (nonpeptide mimetics or cyclic peptides). Thus, novel, cutting-edge recombinant protein techniques (e.g., high-throughput screening, HTS) along with additional organic chemistry, computational techniques, and molecular biology tools, have assisted the design and development of peptide-like drugs.[8,30] Moreover, the continuous advances on solid-phase peptide synthesis and purification strategies, besides a considerable price reduction of the monomers (amino acids), have enabled the pharmacological progress and competitiveness of cyclic peptides as therapeutically relevant targets.[31] In this rationale, the advancement of biologically active cyclic peptides has experienced sustained growth; antidiabetics agents, cardiovascular, calcitonins among others have progressed to the pharmaceutical industry (selected examples are outlined in Table I). Recent directions of these modified peptides in applied research have resulted in the design and synthesis of cyclic peptide analogues of immunodominant epitopes of myelin basic protein (MBP)[32] and myelin oligodendrocyte glycoprotein (MOG)[33] providing novel therapies for Experimental Autoimmune Encephalomyelitis (EAE, the best well-studied animal model of Multiple Sclerosis, MS) (Figure 1). Also, gonadotropin-releasing hormone (GnRH)[34] cyclic peptides have been designed and synthesized for their ability to act as agonists or antagonists. Moreover, grafting techniques are being applied to cyclotides, in order to attach biologically active fragments on their loops, therefore enhancing bioavailability and stability. Such cyclic peptides have resulted in potent oxytocin agonists and CXCR4 antagonists as efficient HIV-1 cell-entry blockers. Molecular modeling and NMR studies on the bioactive conformations of these peptides, which have emerged as promising platforms for drug design, will allow novel investigations on peptide-based drugs.

Synthetic Strategies for Cyclization

Chemical synthesis strategies, in solution and solid-phase, have progressively entered the peptide-based therapeutics market.[24] The suitability of modern orthogonal monomeric building blocks in combinatorial chemistry has enabled the ring closure on solid support with standard coupling reagents. Among the plethora of cyclization protocols, the backbone constraint has been traditionally considered as crucial in the lead optimization process for enhancing drug-like properties. In addition, backbone constraint approaches are regularly adopted for the stabilization of peptide sequences into a specific secondary structure, including α-helices, β-sheets, reverse turns, and loops, that are involved in the molecular recognition process by the target.[19]

Solution-Phase Peptide Synthesis (SPS). Since pioneering studies by du Vigneaud in 1953,[35] the SPS method has been broadly used to generate peptides. Classical protocols provide flexible and convergent synthesis where individual peptides can be readily synthesized, purified and coupled to access polypeptides or proteins. Nevertheless, the tedious and demanding steps of purification associated with solution phase synthesis that frequently involved reduced overall yield have diminished its popularity over solid-phase strategies.

Solid-Phase Peptide Synthesis (SPPS). First described by Merrifield in 1963,[22] the SPPS method is an important

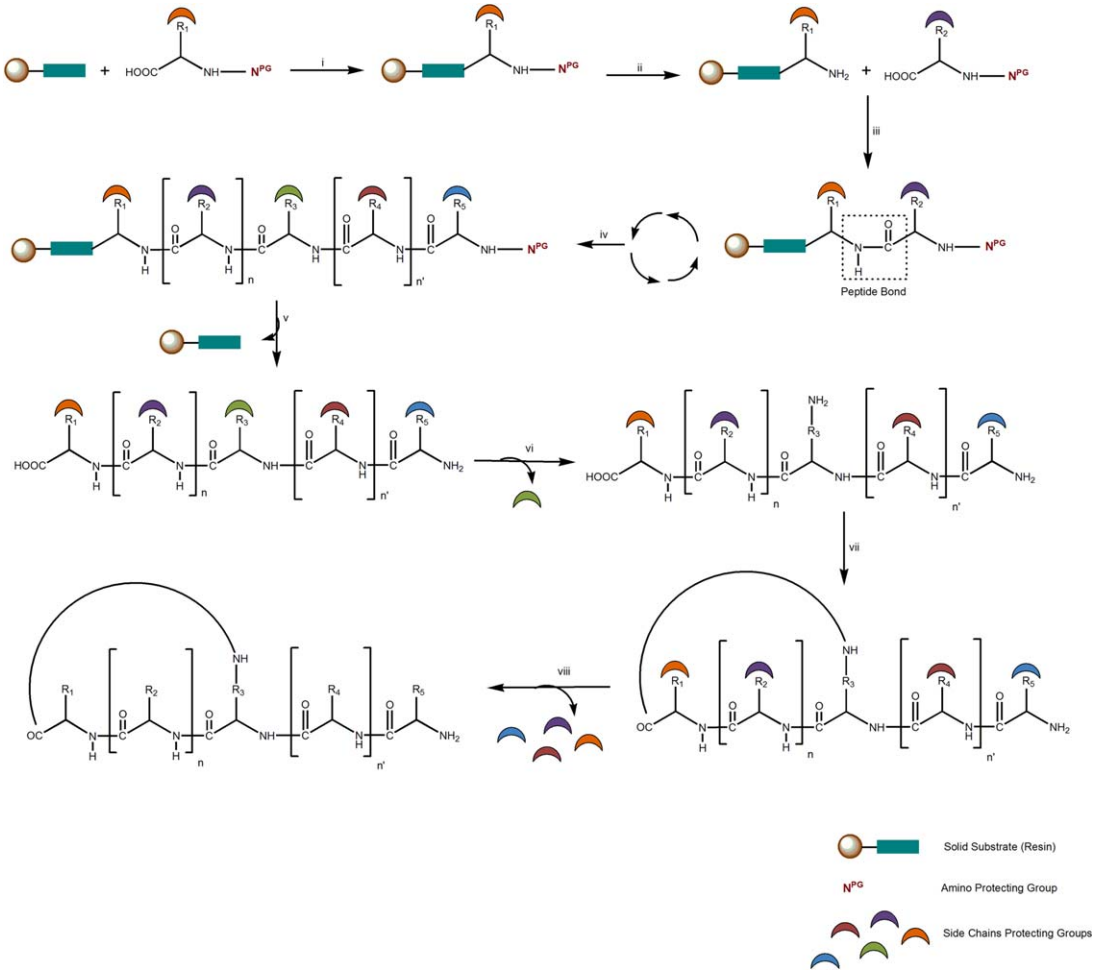

FIGURE 2 Representative procedure for the synthesis of side chain-to-tail cyclic peptides: (i) loading of the first amino acid to the solid support via the carboxyl group; (ii) removal of the N^{α}-amino protected group; (iii) coupling of the amino group with the activated carboxyl group of the second amino acid; (iv) subsequent coupling of the following amino acids; (v) cleavage from the resin; (vi) selective removal of the side chain amino protected group; (vii) cyclization between the side chain amino group and the *C*- terminal carboxyl group; (viii) final deprotection of side chain protected groups.

cornerstone of peptide chemistry. Based on an insoluble and activated solid support (polymer), the synthetic process begins from the covalent binding to the *C* or *N*-terminal of one amino acid. Consecutively, the stepwise synthetic procedure of a peptide sequence includes the activation of the carboxyl group and coupling to an appropriate protected amino acid (Figure 2). Certainly noteworthy is the ability of polymer-supported synthesis to offer much faster transformations over liquid-phase procedures with experimental simplicity, where excess reagents and by-products can be selectively separated from the growing and insoluble peptide through simple filtration rather than liquid-liquid extraction and chromatographic purification. Moreover, with the development of automation and high-throughput peptide production, novel chemically modified building blocks and amino acids have extended the resources for solid-phase synthesis, where the polymer substrate plays a critical role in the coupling achievement. In this manner, a feasible solid support should incorporate mechanical strength, good swelling in common solvents, and facile access to the active sites of the polymer by the reagents.[36] Notably, the selected N^{α}-protected amino acid residues in combination with the Boc/Bzl or Fmoc/tBu synthetic methodologies, which clearly define two broad categories on the solid-phase peptide synthesis, are vital to discriminate the appropriate type of resin. Particularly, the Fmoc/tBu is the most efficient and attractive strategy based on the mild acidic conditions for final deprotection.[37]

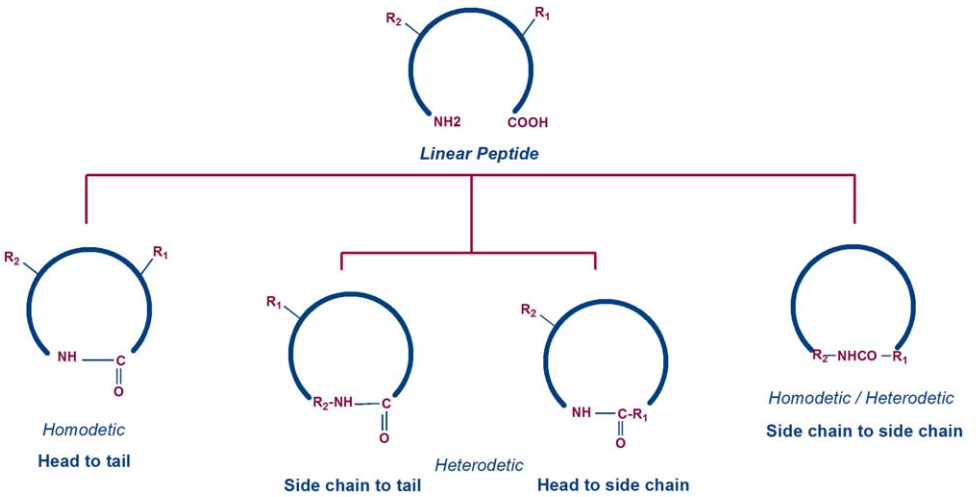

FIGURE 3 Homodetic and heterodetic types of cyclization.

Types of Cyclization. Cyclic constrained peptidomimetics enhance the hydrophobicity of native linear counterparts conferring conformational stability and improved affinity to a certain target. They are normally synthesized in a multi-step synthetic procedure. Cyclization also increases the resistance to cleavage by proteolytic enzymes and membrane permeability that delivers superior bioavailability. According to the type of bond between the two amino acids that furnishes the cycle ring, cyclic peptides can be classified in two major categories: *homodetic* (containing only peptide bonds) and *heterodetic* (diverse functional groups are additionally used to connect the amino acids). Assorted cyclization strategies are summarized below (Figure 3).

- *Head-to-tail cyclization* (homodetic); the peptide bond is formed between the *N*-terminus amino group and the *C*-terminus carboxyl group.[38]
- *Cyclotides*; the peptide bond is formed *head-to-tail* and the ring is usually strengthened by three disulfide bonds.[39,40]
- *Side chain-to-one of the termini cyclization: head-to-side chain or side chain-to-tail* (heterodetic); the bond is formed between the *N*- or *C*- terminus and the side chain functional group of amino acids.[41]
- *Side-chain-to-side-chain cyclization*; the bond is formed between two side chains of amino acids (homodetic and heterodetic).[42]
- *Disulfide*; the disulfide bond is formed between two thiol groups (cysteine).[43]
- *Thioether;* the thioether bond is formed between the side chain thiol group of a cysteine and the α-carbon atom of an amino acid.[44]

Cyclic Peptides and Drug Design

Drug Design Methodologies. Since 1998, in which 2058 structures were deposited in the protein data bank (PDB), each year there has been a ~7.5% increase, resulting in 9681 structures in 2014 for a total of 105, 465 structures from which only 9.7% are NMR derived. Undoubtedly, structural biology provides protein structures that are cornerstone for drug design. Cybase,[45] offers an online database of known natural and non-natural head to tail cyclic proteins (Figure 4). As of January 2015, it contains 813 cyclic protein sequences and 67 PDB entry structures with NMR or X-ray data. This database includes several cyclotide entries, plant-made defense proteins, such as the kalata proteins, primate rhesus θ-defensins (RTDs) with increased biological activities, such as anti-HIV activity, which are used as lead molecules for drug design.[40] Disulfide linked peptides are also considered cyclic analogues displaying conformational rigidity and retaining a level of resistance to hydrolysis by endopeptidases. Another category of cyclic peptides found in natural hormones form rigid structures by making disulfide bonds, such as oxytocin, mostly targeting G protein coupled receptors (GPCRs).[46]

There are two distinct approaches to the drug design of cyclic peptides. The first is the use of known cyclic analogues that may or may not have affinity against a target. The recent growth in the use of natural cyclic peptides for drug design has established them as scaffolds for customization against various targets. For example mutation or grafting of cyclotides has been applied in several cases.[47] Also, modification of cyclic hormones to obtain enhanced compounds for their target has been widely employed. These include altered peptides to target oxytoxin or vasopressin,[27,48] melanocortin,[49] gonadotropin

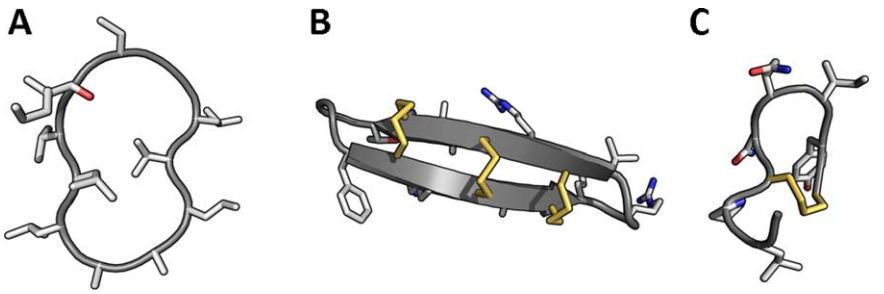

FIGURE 4 Structures of naturally occurring cyclic peptides; (A) Crystal structure of cyclosporine in its FAB binding conformation (PDB id 1ikf); (B) NMR structure of rhesus θ-defensin 1 (RTD-1), (PDB id 2lyf); (C) Crystal structure of deamino-oxytocin (PDB id 1xy1). Peptides are shown in gray cartoon representation, disulfide bridges in yellow and amino acid side chains in white.

releasing hormone,[50] somatostatin,[51] receptors and many more. In the second approach, systems with no evidence of cyclic protein involvement are targeted using several techniques of cyclization of that have yielded potent molecules against clinical targets. This approach usually involves synthesis of libraries based on linear peptides, or mutagenesis studies and are driven through trial and error. These two different approaches are the main approaches used for cyclic peptide drug design today.

Cyclization for Stability and Potency. Many integrins recognize the RGD peptidic motif and are also able to discriminate among distinct natural ligand containing this same recognition pattern.[52] On this basis, Kessler *et al.* synthesized a series of cyclic pentapeptides with the sequence RGDFV and by combining a series of cyclization, screening and *N*-methyl scans concluded to the discovery of the cyclic peptide cyclo(-RGDf(NMe)V).[53] Cilengitide, was developed by Merck-Serono, and entered Phase III clinical trials, however, it did not get final approval.[54]

Protein–protein interactions (PPis) have always had an increased difficulty to target because of the large interaction areas that can hardly become targeted by small molecules. An extra element of difficulty is the selection of the binding pocket from a large surface. However, cyclic analogues can possess a level of selectivity against protein surfaces as well as increased bioavailability. A recent successful example of a PPi disruption by cyclic analogues is the inhibition of the YAP (Yes-associated protein)−TEAD complex, in order to abolish the oncogenic function of YAP, which is overexpressed or activated in certain human cancers.[55] Hu *et al.* narrowed down the minimal region of YAP for significant binding to TEAD (^{81}PQTVPMRLRKLPDSFFKPPE^{100}) and furthermore identified the key residues by alanine scanning.[56] Based on the crystal structure of the complex, cysteine and homocysteine residues were introduced at positions 87 and 96, forming a disulfide bond as a conformation restraint. This resulted in a highly potent cyclic peptide inhibitor.

Another case of cyclization of linear peptides to obtain enhanced pharmacological agents is the myelin basic protein (MBP) 87-99 series of cyclic peptides. CD4+ T cells, major determinants in autoimmunity, including multiple sclerosis (MS) have been found to be stimulated with the self-antigen, myelin basic protein (MBP) 83-99 epitope. A series of head to tail cyclized mutants of the 83-99 MBP residues resulted in a more stable peptide, cyclo(83-99)[Ala^{91}]MBP$_{83\text{-}99}$, which conjugated to mannan, showed increased *in vivo* efficacy in SJL/J mice compared to the linear epitope.[57] Further investigations and molecular modeling studies led to cyclo(87-99)[Ala^{91,96}]MBP$_{87\text{-}99}$, which showed promising results in mice against the experimental autoimmune encephalomyelitis (EAE).[58]

Studies on cyclization of peptides targeting the gonadotropin releasing hormone receptor (GnRHR), have resulted in potent cyclic analogues.[50] NMR studies on the endogenous 10-residue hormone, GnRH and its analogues have identified a bend around the central amino acid at position 6.[50e,59] Further mutational studies, on the potent peptide leuprolide, which is used in the clinical setting, resulted in analogues with inhibition of cell proliferation.[34,59] This information was used to design a series of cyclic peptides with non-natural amino acids, resulting in a potent analogue, cyclo(1-10)GnRH[Pro^{1}, $_{D}$Leu^{6}, BABA^{10}], which showed enhanced proteolytic stability and improved pharmacokinetic properties.[50]

Cyclic Peptides as Templates for Drug Design. Several hormone receptors, in their majority G protein-coupled receptors (GPCRs), have been targeted by cyclized peptides. GPCRs are drug targets for 30% of the currently marketed drugs, however, only ~10% of all GPCRs are targeted by approved drugs.[60] A chemokine receptor, CXCR4 was recently co-crystalized with CVX15 (Figure 5A), a 16-residue peptide cyclized by disulfide

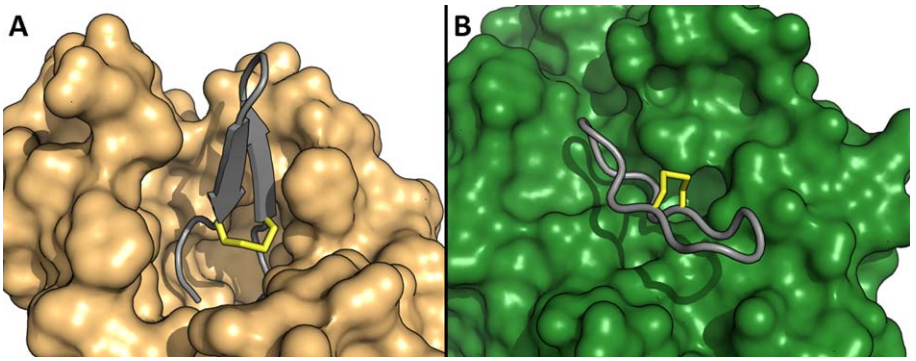

FIGURE 5 Protein co-crystalized cyclic peptides; (A) CXCR4 Chemokine receptor and CVX15 cyclic analogue (PDB id 3oe0); (B) Crystal structure of sunflower trypsin inhibitor I (SFTI-1) bound to trypsin (PDB id 1sfi). Peptides are shown in gray cartoon representation and disulfide bridges in yellow. Proteins appear as van der Waals surfaces in colors wheat (CXCR4) and in green (trypsin).

bridge, previously characterized as an HIV-inhibiting and anti-metastatic agent.[61] By combining medium throughput screening and a rational design approach based on the CXCR4-CVX15 complex structure, researchers at Eli Lilly identified a cyclic peptide, LY2510924, cyclo[Phe-Tyr-Lys(iPr)-$_D$Arg-2-Nal-Gly-$_D$Glu]-Lys(iPr)-NH_2.[62] LY2510924 is now in phase II clinical studies against cancer.

In a strategy described by Gruber *et al.*, the cyclotide Kalata B7 was used as a template to design grafted potent peptidic analogues against the oxytocin and vasopressin 1A (V_{1A}) receptors. Kalata B7 was found to be a partial agonist at both receptors, and was examined to identify the bioactive region responsible for this receptor activating effect. Particularly, loop 3 of kalata B7 (-CYTQGC-), homologous to an oxytocin region, was used to graft four peptides (nonapeptides), which were NMR characterized and found to have improved binding affinity to the oxytocin and vasopressin receptors.[63] In contrary to the endogenous agonist oxytocin, the nonapeptide linear analogues of oxytocin retain flexibility, adopting several conformations that lead to increased binding to the pocket of their target receptors. Another latest example of cyclic peptide-grafting is the fusion of modified CVX15 based peptides onto the loop 6 of cyclotide MCoTI-I. MCoTI-I, a plant-derived cyclotide with no affinity for CXCR4, was used as a stable template to produce potential CXCR4 antagonists. One of the resulting grafted cyclotides was a potent CXCR4 antagonist and an efficient HIV-1 cell-entry blocker.[64] Another naturally occurring highly rigid cyclic peptide, the sunflower trypsin inhibitor 1 (SFTI-1), derived from sunflower seeds, has been extensively used as a drug lead. It binds to trypsin, with which it has been co-crystallized[65] (Figure 5B) and type II serine protease matriptase with high affinity (0.002 and 200 n*M*, respectively).[66] Daly *et al.* used the 14 residue SFTI-1 as a scaffold to develop cyclic peptides with angiogenic activity. Three major known angiogenic sequences were grafted onto the trypsin-inhibitory loop of SFTI-1. One of the resulting cyclic peptides, SFTI-OPN was stable against the enzymes thrombin and MMP-9 and was proven to enhance the binding integrin $\alpha_9\beta_1$ relative to the linear OPN peptide.

CONCLUSIONS AND PERSPECTIVES

Cyclic peptides are envisaged to overcome the limitations of their linear counterparts by addressing biological problems such as the enzymatic hydrolysis and poor oral bioavailability. Improvement of their pharmacological properties in conjunction with the current advances on rational drug design, peptide synthesis, and structure determination have assisted the development of novel and more potent cyclic peptides. The growing interest in peptide-based drugs in medicinal research, as well as the promising *in vitro* and *in vivo* efficacy of cyclic peptides, provides many opportunities for the development of cyclic peptides towards the treatment of several diseases.

REFERENCES

1. (a) Purcell, A. W.; Croft, N. P.; Dudek, N. L. In Handbook of Biologically Active Peptides; Kastin, A. J., Ed.; Elsevier Inc.: Oxford, U.K. 2013; pp 580–589; (b) Joo, S. H. Biomol Ther 2012, 20, 19–26.
2. (a) Newman, D. J.; Cragg, G. M. J Nat Prod 2012, 75, 311–335; (b) Watt, P. M. Nat Biotechnol 2006, 24, 177–183; (c) Paterson, I.; Anderson, E. A. Science 2005, 310, 451–453; (d) Clardy, J.; Walsh, C. Nature 2004, 432, 829–837.
3. (a) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. Chem Biol Drug Des 2013, 81, 136–147; (b) Kaspar, A. A.; Reichert, J. M. Drug Discov Today 2013, 18, 807–817; (c) Sun, L. Mod Chem Appl 2013, 1, 1000–1103; (d) Vlieghe, P.; Lisowski, V.; Martinez, J.;

Khrestchatisky, M. Drug Discov Today 2010, 15, 40–56; (e) Zompra, A. A.; Galanis, A. S.; Werbitzky, O.; Albericio F. Future Med Chem 2009, 1, 361–377; (f) Otvos, L. Peptide-Based Drug Design: Here and Now Ed.; Humana Press: Totowa, NJ, 2008.
4. Mandal, D.; Shirazi, A. N.; Parang, K. Org Biomol Chem 2014, 12, 3544–3561.
5. Collier, J. H.; Segura, T. Biomaterials 2011, 32, 4198–4204.
6. (a) Moyle, P. M.; Toth, I. Chem Med Chem 2013, 8, 360–376; (b) Zaman, M.; Abdel-Aal, A. B.; Fujita, Y.; Ziora, Z. M.; Batzloff, M. R.; Good, M. F.; Toth, I. J Med Chem 2012, 55, 8515–8523.
7. (a) Moore, S. J.; Leung, C. L.; Cochran, J. R. Drug Discov Today Technol 2012, 9, 3–11; (b) Liu, D.; Overbey, D.; Watkinson, L. D.; Daibes-Figueroa, S.; Hoffman, T. J.; Forte, L. R.; Volkert, W. A.; Giblin, M. F. Anticancer Res 2009, 29, 3777–3783.
8. (a) Vanhee, P. M.; van der Sloot, A.; Verschueren, E.; Serrano, L.; Rousseau, F.; Schymkowitz, J. Trends Biotechnol 2011, 29, 5231–239; (b) Reina, J.; Lacroix, E.; Hobson, S. D.; Fernandez-Ballester, G.; Rybin, V.; Schwab, M. S.; Serrano, L.; Gonzalez, C. Nat Struct Biol 2002, 9, 621–627.
9. (a) Henrich, S.; Salo-Ahen, O. M. H.; Huang, B.; Rippmann, F. F.; Cruciani, G.; Wade, R. C. J Mol Recognit 2010, 23, 209–219; (b) Lummis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; roadhurst, R. W.; Dougherty, D. A. Nature 2005, 438, 248–252; (c) Nakanishi, H.; Kahn, M. In Design of Peptidomimetics; The Practice of Medicinal Chemistry, 2nd Ed.; Wermuth, C.G., Ed., Academic Press: London, 2003.
10. (a) Cini, E.; Bifulco, G.; Menchi, G.; Rodriquez, M.; Taddei, M. Eur J Org Chem 2012, 2133–2141; (b) Grigoryan, G.; Reinke, A. W.; Keating, A. E. Nature 2009, 458, 859–864; (c) Grauer, A.; König, B. Eur J Org Chem 2009, 5099–5111; (d) Mandell, D. J.; Kortemme, T. Nat Chem Biol 2009 5, 797–807.
11. Jubb, H.; Higueruelo, A. P.; Winter, A.; Blundell, T. L. Trends Pharmacol Sci 2012, 33, 241–248.
12. (a) Wells, J. A.; McClendon, C. L. Nature 2007, 450, 1001–1009; (b) Drews, J. Science 2000, 287, 1960–1964.
13. (a) Benyamini, H.; Friedler, A. Future Med Chem 2010, 2, 989–1003; (b) Zinzalla, G.; Thurston, D. E. Future Med Chem 2009, 1, 65–93.
14. Ovadia, O.; Greenberg, S.; Laufer, B.; Gilon, C.; Hoffman, A.; Kessler, H. Expert Opin Drug Discov 2010, 5, 655–671.
15. (a) Goodwin, D.; Simerska, P.; Toth, I. Curr Med Chem 2012, 19, 4451–4461; (b) Ahrens, V. M.; Bellmann-Sickert, K.; Beck-Sickinger, A. G. Future Med Chem 2012, 4, 1567–1586; (c) Mason, J. M. Future Med Chem 2010, 2, 1813–22; (d) Gentilucci, L.; De Marco, R.; Cerisoli, L. Curr Pharm Design 2010, 16, 3185–3203.
16. (a) Verdine, G. L.; Hilinski, G. J. Methods Enzymol 2012, 503, 3–33; (b) Chang Y. S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K. H.; Olson, K. A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; Baker T.; Darlak, K.; Elkin, C.; Filipovic, Z.; Qureshi, F. Z.; Cai, H.; Berry, P.; Feyfant, E.; Shi, X. E.; Horstick, J.; Annis, D. A.; Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. Proc Natl Acad Sci USA 2013, 110, 3445–3454.
17. Hussack, G.; Hirama, T.; Ding, W.; MacKenzie, R.; Tanha, J. PLoS ONE 2011, 6, e28218.
18. (a) Craik, D. J. Science 2006, 331, 1563–1564; (b) Kessler, H. Angew Chem Int Ed Engl 1982, 21, 512–523.
19. Guharoy, M.; Chakrabarti, P. Bioinformatics 2007, 23, 1909–1918.
20. Lindgren, M.; Hällbrink, M.; Prochiantz, A.; Langel, U. Trends Pharmacol Sci 2000, 21, 99–113.
21. Terrett, N. Med Chem Commun 2013, 4, 474–475.
22. Merrifield, R. B. J Am Chem Soc 1963, 85, 2149–2154.
23. (a) Lunquist, J. T. IV; Pelletier, J. C. Org Lett 2002, 4, 3219–3221; (b) Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. Tetrahedron Lett 1993, 34, 1549–1552.
24. (a) Koopmanschap, G.; Ruijter, E.; Orru, R. V. A. Beilstein J Org Chem 2014, 10, 544–598; (b) Smith, J. M.; Frost, J. R.; Fasan, R. J Org Chem 2013, 78, 3525–3531; (c) White, C. J.; Yudin, A. K.; Nat Chem 2011, 3, 509–524; (d) Pattabiraman, V. R.; Bode, J. W. Nature 2011, 480, 471–479; (e) Smith, J. M.; Vitali, F.; Archer, S. A.; Fasan, R. Angew Chem Int Ed 2011, 50, 5075–5080.
25. (a) Fahr, A. Clin Pharmacokinet 1993, 24, 472–495; (b) Borel, J. F.; Feurer, C.; Gubler, H. U.; Stähelin, H. Agents Actions 1976, 6, 468–475.
26. (a) Lunquist, J. T. IV; Pelletier, J. C. Org Lett 2002, 4, 3219–3221; (b) Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. Tetrahedron Lett 1993, 34, 1549–1552.
27. Emanuele, E.; Arra, M.; Pesenti, S. Med Hypotheses 2006, 67, 1250–1251.
28. Mohr, J. F.; Murray, B. E. Clin Infec Dis 2007, 44, 1536–1542.
29. Bellary, B.; Barnett, A. H. Int J Clin Pract 2006, 60, 728–734.
30. (a) Wang, C. K.; Northfield, S. E.; Colless, B.; Chaousis, S.; Hamernig, I.; Lohman, R-J.; Nielsen, D. S.; Schroeder, C. I.; Liras, S.; Priceb, D. A.; Fairlie, D. P.; Craik, D. J. Proc Natl Acad Sci USA 2014, 111, 17504–17509; (b) Yin, H.; Slusky, J. S.; Berger, B. W.; Walters, R. S.; Vilaire, G.; Litvinov, R. I.; Lear, J. D.; Caputo, G. A.; Bennett, J. S.; DeGrado, W. F. Science 2007, 315, 1817–1822; (c) Pei, D.; Wavreille, A. S. Mol Biosyst 2007, 3, 536–541.
31. Baeriswyl, V.; Heinis, C. Chem Med Chem 2013, 8, 377–384.
32. Tselios, T.; Apostolopoulos, V.; Daliani, I.; Deraos, S.; Grdadolnik, S.; Mavromoustakos, T.; Melachrinou M.; Thymianou, S.; Probert, L.; Mouzaki, A.; Matsoukas, J. J Med Chem 2002, 45, 275–283.
33. Tselios, T.; Aggelidakis, M.; Tapeinou, A.; Tseveleki, V.; Kanistras, I.; Gatos, D.; Matsoukas, J. Molecules 2014, 19, 17968–17984.
34. Keramida, M. K.; Tselios, T.; Mantzourani, E.; Papazisis, K.; Mavromoustakos, T.; Klaussen, C.; Agelis, G.; Deraos, S.; Friligou, I.; Habibi, H.; Matsoukas, J. J Med Chem 2006, 49, 105–110.
35. Du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C.W.; Katsoyannis, P.G.; Gordon, S. J Am Chem Soc 1953, 75, 4879–4880.
36. Erickson B. W; Merrifield R. B. In The Proteins, 3rd ed.; Neurath, H.; Hill, R. L.; Boeda, C.-L., Eds.; Academic: New York, 1976; 2, pp 255–527.
37. (a) Barlos, K.; Gatos, D.; Koutsogianni, S.; Schäfer, W.; Stavropoulos, G.; Wenqing, Y. Tetrahedron Lett 1991, 32, 471–474; (b) Rink, H. Tetrahedron Lett 1987, 28, 3787–3790; (c) Tjoeng F. S.; Heavner G. A. Tetrahedron Lett 1982, 23, 4439–4442; (d) Wang, S. S. J Am Chem Soc 1973, 95, 1328–1333.
38. (a) Satoh, T.; Li, S.; Friedman, T. M.; Wiaderkiewicz, R.; Korngold, R.; Huang, Z. Biochem Biophys Res Commun 1996,

224, 438–443; (b) Deber, C. M.; Madison, V.; Blout, E. R. Acc Chem Res 1976, 9, 106–113.
39. Craik, D. J.; Daly, N. L.; Bond, T.; Waine, C. J Mol Biol 1999, 294, 1327–1336.
40. Craik, D. J.; Swedberg, J. E.; Mylne, J. S.; Cemazar, M. Expert Opin Drug Discov 2012, 7, 179–194.
41. Shibata, K.; Suzawa, T.; Soga, S.; Mizukami, T.; Yamada, K.; Hanai, N.; Yamasaki, M. Bioorg Med Chem Lett 2003, 13, 2583–2586.
42. Piserchio, A.; Salinas, G. D.; Li, T.; Marshall, J.; Spaller, M. R.; Mierke, D. F. Chem Biol 2004, 11, 469–473.
43. Hahn, M.; Winkler, D.; Welfle, K.; Misselwitz, R.; Welfle, H.; Wessner, H.; Zahn, G.; Scholz, C.; Seifert, M.; Harkins, R.; Schneider-Mergener, J.; Hohne, W. J Mol Biol 2001, 314, 279–295.
44. Akamatsu, M.; Roller, P. P.; Chen, L.; Zhang, Z. Y.; Ye, B.; Burke, T. R., Jr. Bioorg Med Chem 1997, 5, 157–163.
45. Wang, C. K. L.; Kaas, Q.; Chiche, L.; Craik, D. J Nucleic Acids Res 2008, 36, 206–210.
46. (a) Naganathan, S.; Ray-Saha, S.; Park, M.; Tian, H.; Sakmar, T. P.; Huber, T. Biochemistry 2015, 54, 776–786; (b) Shpakov, A. O.; Shpakova, Elena A.; Derkach, Kira V. Curr Top Pept Protein Res 2013, 14, 1–12.
47. (a) Poth, A. G.; Chan, L. Y.; Craik, D. J Pept Sci 2013, 100, 480–491; (b) Conibear, A. C.; Bochen, A.; Rosengren, K. J.; Stupar, P.; Wang, C.; Kessler, H.; Craik, D. J. Chem Bio Chem 2014, 15, 451–459; (c) Chan, L. Y.; Gunasekera, S.; Henriques, S. T.; Worth, N. F.; Le, S.-J.; Clark, R. J.; Campbell, J. H.; Craik, D. J.; Daly, N. L. Blood 2011, 118, 6709–6717.
48. Manning, M.; Misicka, A.; Olma, A.; Bankowski, K.; Stoev, S.; Chini, B.; Durroux, T.; Mouillac, B.; Corbani, M.; Guillon, G. J Neuroendocrinol 2012, 24, 609–628.
49. (a) Eliasen, R.; Daly, N. L.; Wulff, B. S.; Andresen, T. L.; Conde-Frieboes, K. W.; Craik, D. J. J Biol Chem 2012, 287, 40493–40501; (b) Mayorov, A. V.; Cai, M.-Y.; Palmer, E. S.; Liu, Z.-H.; Cain, J. P.; Vagner, J.; Trivedi, D.; Hruby, V. J. Peptides 2010, 31, 1894–1905.
50. (a) Laimou, D.; Katsila, T.; Matsoukas, J.; Schally, A.; Gkountelias, K.; Liapakis, G.; Tamvakopoulos, C.; Tselios, T. Eur J Med Chem 2012, 58, 237–247; (b) Koerber, S. C.; Rizo, J.; Struthers, R. S.; Rivier, J. E. J Med Chem 2000, 43, 819–828; (c) Rivier, J. E.; Jiang, G.; Struthers, R. S.; Koerber, S. C.; Porter, J.; Cervini, L. A.; Kirby, D. A.; Craig, A. G.; Rivier, C. L. J Med Chem 2000, 43, 807–818; (d) Rivier, J. E.; Struthers, R. S.; Porter, J.; Lahrichi, S. L.; Jiang, G.; Cervini, L. A.; Ibea, M.; Kirby, D. A.; Koerber, S. C.; Rivier, C. L. J Med Chem 2000, 43, 784–796; (e) Bienstock, R. J.; Rizo, J.; Koerber, S. C.; Rivier, J. E.; Hagler, A. T.; Gierasch, L. M. J Med Chem 1993, 36, 3265–3273.
51. D'Addona, D.; Carotenuto, A.; Novellino, E.; Piccand, V.; Reubi, J. C.; Di Cianni, A.; Gori, F.; Papini, A. M.; Ginanneschi, M. J Med Chem 2008, 51, 512–520.
52. Ruoslahti, E.; Pierschbacher, M. D. Cell 1986, 44, 517–518.
53. Mas-Moruno, C.; Rechenmacher, F.; Kessler, H. Anticancer Agents Med Chem 2010, 10, 753–768.
54. Tucci, M.; Stucci, S.; Silvestris, F. Lancet Oncol 2014, 15, 584–585.
55. Zhao, B.; Wei, X.; Li, W.; Udan, R. S.; Yang, Q.; Kim, J.; Xie, J.; Ikenoue, T.; Yu, J.; Li, L. Gen Dev 2007, 21, 2747–2761.
56. Zhang, Z.; Lin, Z.; Zhou, Z.; Shen, H. C.; Yan, S. F.; Mayweg, A. V.; Xu, Z.; Qin, N.; Wong, J. C.; Zhang, Z.; Rong, Y.; Fry, D. C.; Hu, T. ACS Med Chem Lett 2014, 5, 993–998.
57. Katsara, M.; Deraos, G.; Tselios, T.; Matsoukas, J.; Apostolopoulos, V. J Med Chem 2008, 51, 3971–3978.
58. (a) Katsara, M.; Deraos, G.; Tselios, T.; Matsoukas, M.-T.; Friligou, I.; Matsoukas, J.; Apostolopoulos, V. J Med Chem 2009, 52, 214–218; (b) Deraos, G.; Chatzantoni, K.; Matsoukas, M.-T.; Tselios, T.; Deraos, S.; Katsara, M.; Papathanasopoulos, P.; Vynios, D.; Apostolopoulos, V.; Mouzaki, A.; Matsoukas, J. J Med Chem 2008, 51, 7834–7842.
59. (a) Laimou, D.; Katsara, M.; Matsoukas, M. T.; Apostolopoulos, V.; Troganis, A.; Tselios, T. Amino Acids 2010, 39, 1147–1160; (b) Digilio, G.; Bracco, C.; Barbero, L.; Chicco, D.; Del Curto, M. D.; Esposito, P.; Traversa, S.; Aime, S. J Am Chem Soc 2002, 124, 3431–3442; (c) Meyer, J. D.; Manning, M. C.; Vander Velde, D. G. J Pept Res 2002, 60, 159–168.
60. (a) Lee, S. Biomol Ther 2011, 19, 1–8; (b) Barnacal, P. Innov Pharm Technol 2011, 39, 48–50.
61. Wu, B.; Chien, E. Y. T.; Mol, C. D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F. C.; Hamel, D. J.; Kuhn, P.; Handel, T. M.; Cherezov, V.; Stevens, R. C. Science 2010, 330, 1066–1071.
62. Galsky, M. D.; Vogelzang, N. J.; Conkling, P.; Raddad, E.; Polzer, J.; Roberson, S.; Stille, J. R.; Saleh, M.; Thornton, D. A. Clin Cancer Res 2014, 20, 3581–3588.
63. Koehbach, J.; O'Brien, M.; Muttenthaler, M.; Miazzo, M.; Akcan, M.; Elliott, A. G.; Daly, N. L.; Harvey, P. J.; Arrowsmith, S.; Gunasekera, S.; Smith, T. J.; Wray, S.; Göransson, U.; Dawson, P. E.; Craik, D. J.; Freissmuth, M.; Gruber, C. W. Proc Natl Acad Sci USA 2013, 110, 21183–21188.
64. Aboye, T. L.; Ha, H.; Majumder, S.; Christ, F.; Debyser, Z.; Shekhtman, A.; Neamati, N.; Camarero, J. A. J Med Chem 2012, 55, 10729–10734.
65. Luckett, S.; Garcia, R. S.; Barker, J. J.; Konarev, A. V.; Shewry, P. R.; Clarke, A. R.; Brady, R. L. J Mol Biol 1999, 290, 525–533.
66. Quimbar, P.; Malik, U.; Sommerhoff, C. P.; Kaas, Q.; Chan, L. Y.; Huang, Y.-H.; Grundhuber, M.; Dunse, K.; Craik, D. J.; Anderson, M. A.; Daly, N. L. J Biol Chem 2013, 288, 13885–13896.