

# 1

## The Basics of Peptidomimetics

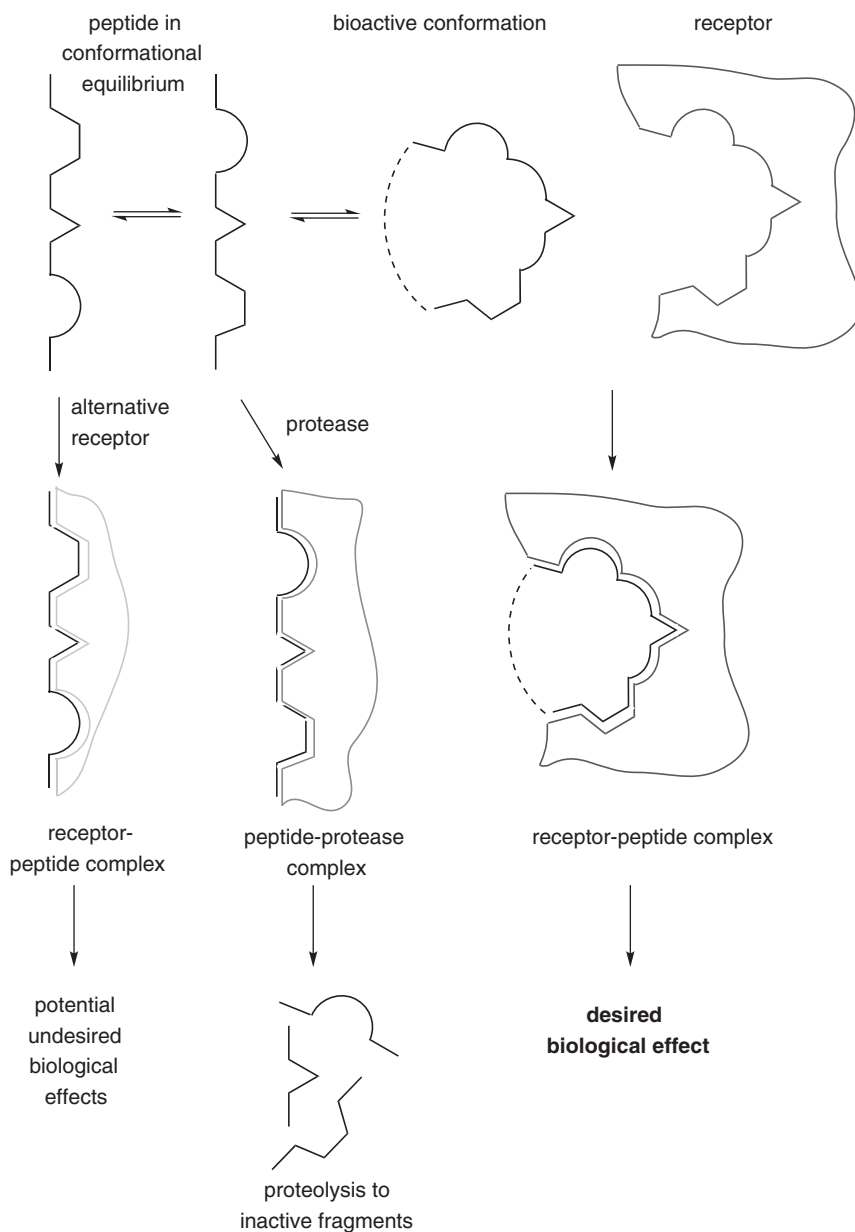
### 1.1 Introduction

During the last three decades an important number of biologically active peptides has been discovered and characterized, including hormones, vasoactive peptides and neuropeptides. As a consequence of interaction with their membrane-bound receptors, these bioactive peptides influence cell–cell communication and control a series of vital functions. Thus, they are of great interest in the biomedical field, and the number of native and modified peptides used as therapeutics is ever increasing. Many bioactive peptides have been prepared on a large scale and tested both in pharmacology and the clinic, thus allowing for the development of new therapies for various pathologies.

However, the use of peptides as therapeutics is limited due to several factors, including low metabolic stability towards proteolysis in the gastrointestinal tract, poor absorption after oral ingestion, low diffusion in particular tissue organs (i.e. the central nervous system, CNS), rapid excretion through liver and kidneys and undesired effects due to interaction of flexible peptides with several receptors (Figure 1.1) [1]. In particular, the flexibility of medium-sized polypeptides (<30 amino acids) is due to the multiple conformations that are energetically accessible for each residue constituting the peptide. The flexibility of each residue constituting a peptide is due to two degrees of conformational freedom addressed by N-C $\alpha$  and C $\alpha$ -CO rotational bonds and described by  $\varphi$  and  $\psi$  dihedral angles, respectively, which result in a population of local conformations, all contributing to the overall flexibility of the peptide in dynamically interconverting equilibria in aqueous solution.

Besides all these drawbacks, biomedical research is constantly oriented towards the development of new therapeutics based on peptides and proteins, by introducing both structural and functional specific modifications and maintaining the features responsible for biological activity.

These requirements are all matched in the development of *peptidomimetics* [2, 3]. In this approach, peptides and proteins are considered as tools for the discovery of other classes of compounds.



**Figure 1.1** Conformational flexibility of peptides and their affinity with proteases cause off-target interactions and degradation, respectively, resulting in undesired biological effects, and inactive fragments from proteolytic events. (Reproduced with permission from Reference [1]. Copyright 1993 Wiley-VCH Verlag GmbH & Co. KGaA.) (See plate section for colour version)

## 1.2 Definition and Classification

A peptidomimetic compound may be defined as a substance having a secondary structure, besides other structural features, similar to native peptide, such that it binds to enzymes or receptors with higher affinity than the starting peptide. As an overall result, the native peptide effects are inhibited (antagonist or inhibitor) or increased (agonist). Since their introduction as a new concept for developing drug candidates, peptidomimetics have shown great promise both in organic and medicinal chemistry. Apart from being much more selective and efficient than native peptides, thus resulting in fewer side effects, peptidomimetics show greater oral bioavailability and the biological activity is prolonged due to lowered enzymatic degradation [4, 5]. The generation of peptidomimetics is basically focussed on knowledge of the electronic and conformational features of the native peptide and its receptor or active site of an enzyme. Thus, the development of peptidomimetics as compounds with potential biological activity must take account of some basic principles [6], including:

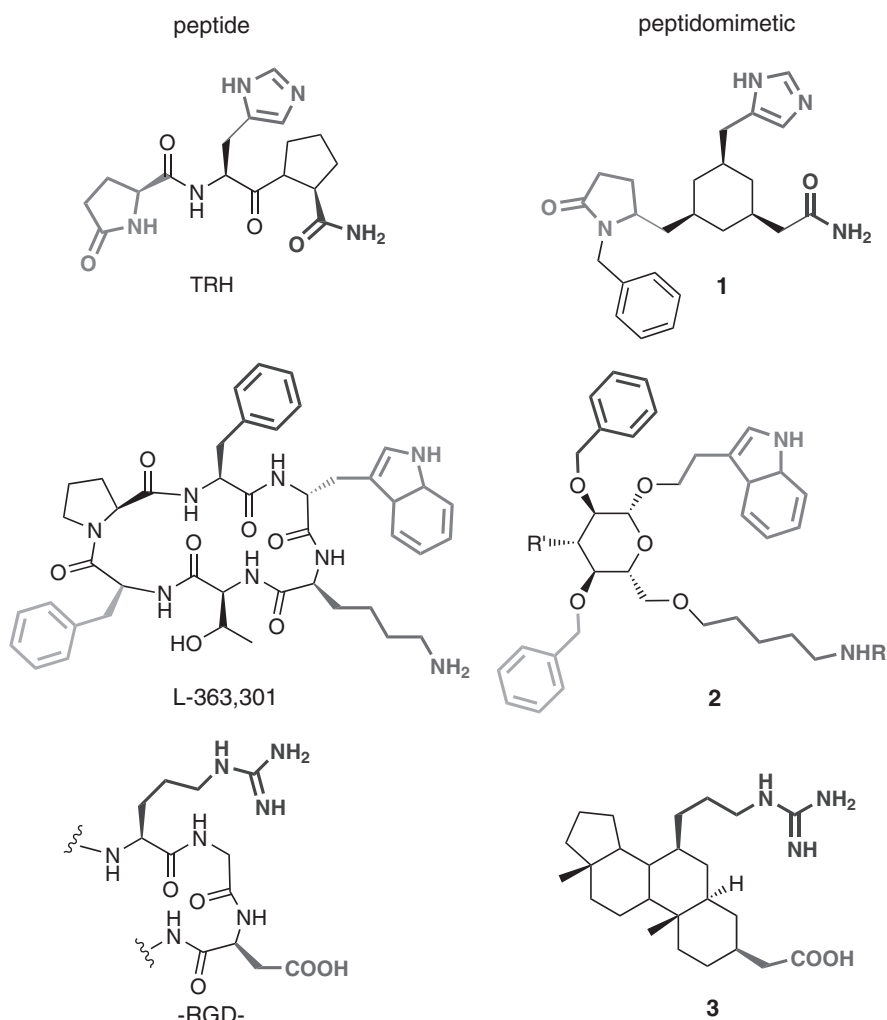
- Replacement of peptide backbone with a non-peptide framework: if an amide bond substitution does not change the biological activity or amide bonds are not exposed to the active site, then the template may be designed to eliminate peptide bonds.
- Preservation of side-chains involved in biological activity, as they constitute the pharmacophore. In the development of second-generation mimetics, several modifications may be introduced to improve biological activity, including chain length modification, introduction of constraints, cyclopeptide bond replacement with a covalent one and introduction of isosteric replacements [7].
- Maintenance of flexibility in first-generation peptidomimetics: if biological activity is observed for a flexible mimetic, then the introduction of elements of rigidity to side-chains is a rational approach to improve the preliminary activity observed.
- Selection of proper targets based on a pharmacophore hypothesis. In other words, knowledge of the structure–activity relationship or the three-dimensional structure of bioactive conformation is a promising route to rapidly achieve the best compound, without generating a huge number of compounds with poor biological activity.

Peptidomimetics may be divided into three classes depending on their structural and functional characteristics [8]:

- Type I mimetics, or *structural* mimetics, show an analogy of a local topography with the native substrate, and they carry all the functionalities responsible for the interaction with an enzyme or a receptor in a well-defined spatial orientation.
- Type II mimetics, or *functional* mimetics: here the analogy with the native compound is based on the interaction with the target receptor or enzyme, without apparent structural analogies.
- Type III mimetics, or *functional-structural* mimetics, are generally conceived as possessing a scaffold with a structure different from that of the substrate, in which all the functional groups needed for biological interactions are mounted in a well-defined spatial orientation. Many examples have been reported in the literature in which an unnatural framework substitutes the peptide backbone and carries the required functional groups for biological activity.

An elegant example of a peptidomimetic scaffold is given by a thyrotropin-releasing hormone (TRH) mimetic based on a cyclohexane scaffold (**1**, Figure 1.2), which replaces the peptide backbone, and the three functional groups that constitute the pharmacophore are placed on the scaffold with the same spatial orientation of amino acid side-chains found in TRH hormone [5]. Other examples include replacement of peptidic fragment in somatostatin receptor binding cyclopeptide with a D-glucose scaffold (**2**) [9], and steroidal scaffold **3** to mimic the type II'  $\beta$ -turn structure of RGDfV cyclopeptide (Figure 1.2) [10].

The workflow towards the development of peptidomimetics has been proposed within the drug discovery process in the case of peptide molecules as hit compounds towards an identified target [11].



**Figure 1.2** Peptidomimetic compounds consisting of cyclohexane (**1**), glucose (**2**) or steroid (**3**) scaffolds. (Reproduced with permission from Reference [2]. Copyright 1994 Wiley-VCH Verlag GmbH & Co. KGaA.) (See plate section for colour version)

Accordingly, the first step in a drug discovery process is hit identification; this is generally carried out by scanning peptide libraries for binding affinity (i.e. by phage display or combinatorial chemistry of synthetic peptide libraries). Molecular biology techniques, such as sequencing, cloning and site-directed mutagenesis experiments, are essential to achieve structural information regarding receptor residues responsible for peptide recognition in combination with molecular modelling calculations. Such information is very important in selecting a bioactive peptide to be successively processed in a hierarchical way, also taking advantage of structural data. The hierarchical approach takes advantage of several steps, which are important in giving insight into structure–activity relationships with respect to the starting bioactive peptide hit compound to be converted into a peptidomimetic lead:

- alanine scanning
- size reduction
- D-amino acid scanning
- introduction of local and global constraints to define the bioactive conformation.

The so-obtained first-generation peptidomimetics are then subjected to further conformational studies aimed at defining the rationale for ligand–receptor (or enzyme–inhibitor) key interactions. The results are then applied for the optimization of hit peptidomimetics towards improved compounds possessing a non-peptide framework.

Chapter 2 presents a detailed overview of the design principles and applications to peptidomimetics.

### 1.3 Strategic Approaches to Peptidomimetic Design

A major effort in peptidomimetic chemistry is connected to the development of compounds capable of replacing one or more amino acids in a peptide sequence without altering the biological activity of the native peptide. The overall result of this structural intervention is to stabilize the molecule with respect to metabolic processes that occur *in vivo*, thus giving access to orally available drugs and compounds with improved pharmacokinetics/pharmacodynamics (PK/PD) properties.

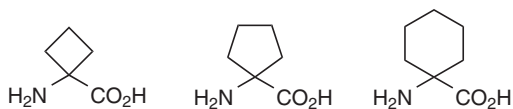
The development of peptidomimetics has generally been approached by synthesizing novel amino acids possessing several features, including synthetic accessibility from commercially available enantiopure reagents, such as amino acid and sugar derivatives, or straightforward synthetic methods for asymmetric synthesis, to access a wide array of novel compounds. Moreover, the need to achieve partially rigid compounds has been pursued to probe a limited number of conformations with the aim of understanding the bioactive topology and giving insight into requisites to design improved bioactive compounds. Access to novel amino acids as peptide isosteres has been pursued by either modifying the atoms involved in backbone formation of a peptide or in manipulating the side-chain moiety, for example by introducing chemical tethers as rigidifying elements. Moreover, peptidomimetic chemistry has been oriented to the development of higher isosteres, taking into account di-, tri- or tetrapeptides motifs to be replaced by more complex molecular architectures. Finally, the approach to intervening in terms of the overall peptide structure has been accessed by working on global restrictions of the native peptide conformation.

### 1.3.1 Modification of Amino Acids

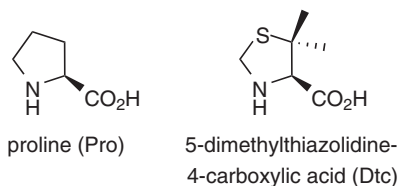
Manipulation of the peptide structure with aim of reducing molecular recognition by proteases and of introducing conformational restrictions is achieved locally by intervening on either backbone or side-chains by introduction of modified amino acids. Accordingly, a well-established approach is to replace proteinogenic amino acids locally and systematically with their corresponding D-variants, C $_{\alpha}$ -alkylated, C $_{\beta}$ -alkylated or N $_{\alpha}$ -alkylated amino acids. For example, substitution of  $\alpha$ -aminocycloalkane carboxylic acids varying in ring size (Figure 1.3) into various positions of enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), a peptide responsible for modulating pain response, resulted in a peptidomimetic with greater *in vivo* activity [12].

$\beta$ -Methylamino acids have been reported for restricting the conformations of a bioactive peptide through the insertion of a stereocenter at the  $\beta$ -position. Indeed, four configurations are accessible by varying the two stereocenters; as an exemplificative entry to this approach, the systematic incorporation of  $\beta$ -MePhe into somatostatin peptidomimetics has resulted in a model for the ligand–receptor interaction, based on the changes in activity induced by different configurations at the  $\beta$  centre [13]. Proline analogues are important for introducing strong conformational bias to the peptide, as the  $\varphi$  angle corresponding to the rotation of the N–C $_{\alpha}$  bond is constrained to  $-65 \pm 15^\circ$ , preventing  $\alpha$ -helix formation and encouraging the formation of  $\beta$ -turns. Moreover, as the barrier to proline *cis/trans* isomerism is  $\sim 2 \text{ kcal mol}^{-1}$ , compared to that of secondary amide ( $10 \text{ kcal mol}^{-1}$ ), proline analogues have been proposed with the aim of orienting the equilibrium towards a preferred geometry, generally the *cis* form owing to its importance in peptide folding. This has been approached by varying the ring size, the substitution pattern around the cyclic backbone and introducing heteroatoms. For example, the substitution of 5,5-dimethylthiazolidine-4-carboxylic acid (Dtc) for Pro (Figure 1.4) in angiotensin II, a key peptide in blood pressure regulation, resulted in a peptidomimetic with 39% greater agonist activity than the natural peptide [14].

Moreover, many applications consisting of other unnatural amino acids have been proposed, including unsaturated, cyclic and  $\beta$ -amino acids, which allowed for the addition of conformational bias to the overall structure. For example, proline analogues and



**Figure 1.3**  $\alpha$ -Aminocycloalkane carboxylic acids varying in ring size



**Figure 1.4** 5,5-Dimethylthiazolidine-4-carboxylic acid (Dtc) as a proline analogue in angiotensin II peptidomimetics

conformationally locked phenylalanine amino acid isosteres have been proposed with aim of introducing conformational restriction to a native peptide locally.

More complex local modifications have considered the introduction of dipeptide isosteres, with aim of mimicking amide bond and side-chains with suitable chemical moieties. The dipeptide fragment is commonly addressed with cyclic compounds possessing chemical tethers for imposing restricted conformations. In addition, retro-inverso isomeric moieties, double bond fragments and cyclic *cis*-amide bond isosteres have been proposed with aim of replacing the amide bond without altering the topology of the adjacent side-chains of the corresponding dipeptide.

### 1.3.2 Compounds with Global Restrictions

The introduction of global restrictions into the peptide by cyclization of the peptide strand typically results in a higher *in vivo* stability of the cyclic peptidomimetics compared to their linear analogues. Nature has also taken advantage of this opportunity, as many biologically active macrocyclic peptides are found in nature. For example, somatostatin is a macrocyclic peptide hormone, formed in the hypothalamus, which regulates the release of growth hormone. It also acts in the pancreas, preventing the release of glucagon and insulin, leading to a lowering of blood glucose concentrations [15]. The regulatory action of somatostatin is due to interaction of the macrocyclic loop with a receptor site.

The approach of tethering the native peptide sequence with aim of reducing the conformational flexibility of the structure is different from the introduction of cyclic scaffolds as it does not modify the parent peptide locally, but involves the modification of the overall conformational profile of the target peptide compound.

The relatively restricted conformation of the macrocyclic compounds can offer enhanced binding selectivity with receptors as the availability, and orientation, of peptide side-chains is constrained within a macrocyclic ligand. As with all constrained peptidomimetics, the macrocyclic motif offers an entropic advantage over an acyclic peptide, as the bioactive conformation of the constrained peptide is reached from a smaller population of random conformers.

The cyclization strategies can be classified with respect to backbone and side-chains according to the chemical moieties used for the introduction of the constraint. Cyclization between backbone elements is approached in several ways:

- by tethering two amide nitrogen atoms with a linker (backbone to backbone);
- by introducing a chemical junction between a C $_{\alpha}$  and a nitrogen atom (backbone to backbone);
- by linking a *N*-terminal amino group with an amide nitrogen atom with a spacer (head to backbone);
- by cyclizing the two *N*- and *C*-terminal ends of a peptidomimetic structure with an amide bond (head-to-tail);
- by a chemical bond involving two side-chains.

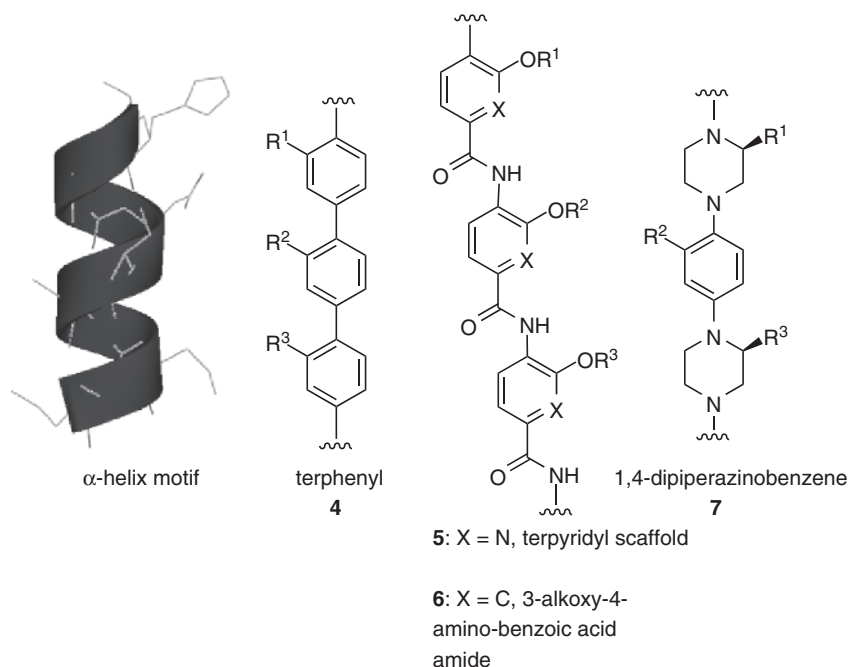
The latter is by far the most popular approach for the generation of a cyclic peptidomimetics. Specifically, cyclization is achieved by exploiting basic and amino acid residues for the formation of an amide bond or by taking advantage of cysteine amino acids for the development of cyclic peptidomimetics through disulfide bridges between the two side-chains.

Although disulfide and lactam bridges, which are also found in naturally occurring peptides, effectively stabilize three-dimensional structures, such structural elements are not always stable *in vivo*. Thus, additional cyclization approaches have been developed that aim to overcome this limitation, and crosslinks consisting only of hydrocarbons have been developed as effective linkers, for example taking advantage of the ring-closing step that can be performed by a metathesis reaction using a Grubbs' catalyst [16].

### 1.3.3 Molecular Scaffolds Mimicking the Peptidic Backbone

The goal of creating peptidomimetics of peptide secondary structures is a well-established approach in drug discovery, with the aim of fixing those bioactive conformations characterized to a high degree by such structural elements. This resulted in the development of peptidomimetic molecules of  $\alpha$ -helix and  $\beta$ -sheet secondary structures, as well as in the design of a wide array of molecular scaffolds capable of replacing turns and loops, which are essential conformational components for peptides and proteins.

The  $\alpha$ -helix is the most common peptide secondary structure, constituting almost half of the polypeptide structure in proteins. A remarkable entry to  $\alpha$ -helix mimetics was first proposed by Hamilton, who reported molecular templates based on the terphenyl (**4**) [17] and terpyridyl (**5**) scaffolds [18] (Figure 1.5). Subsequently, Boger described 3-alkoxy-4-aminobenzoic acid-based oligoamides **6** with the aromatic building block varying in number from 1 to 3 units [19] (Figure 1.5). The latter constitute a rigid framework from which the subunits are joined by an amide bond, and the three-substituents

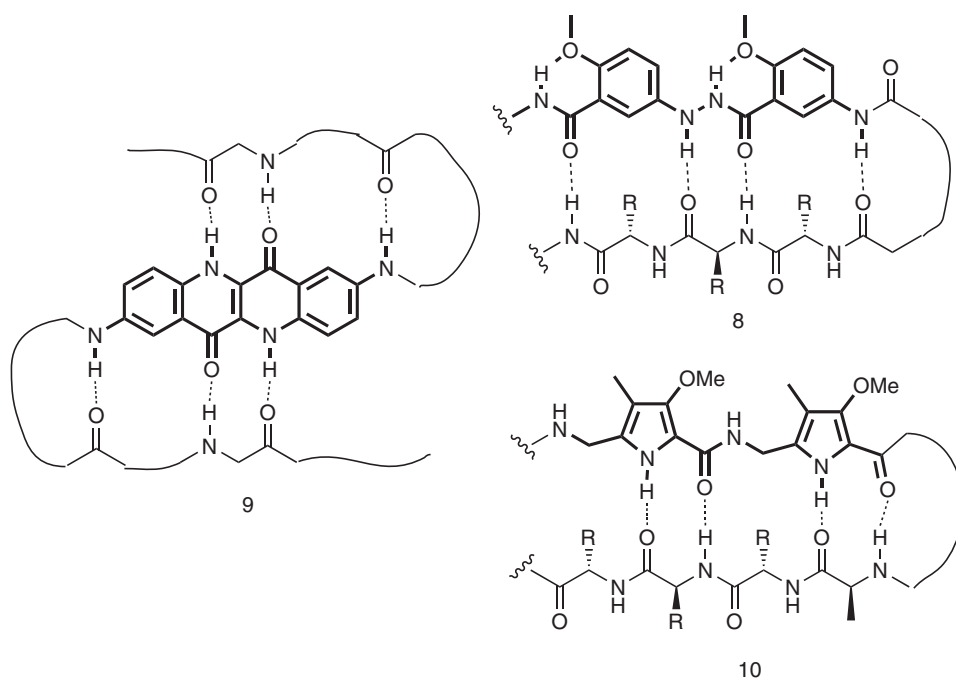


**Figure 1.5** Representative entries to  $\alpha$ -helix mimetics

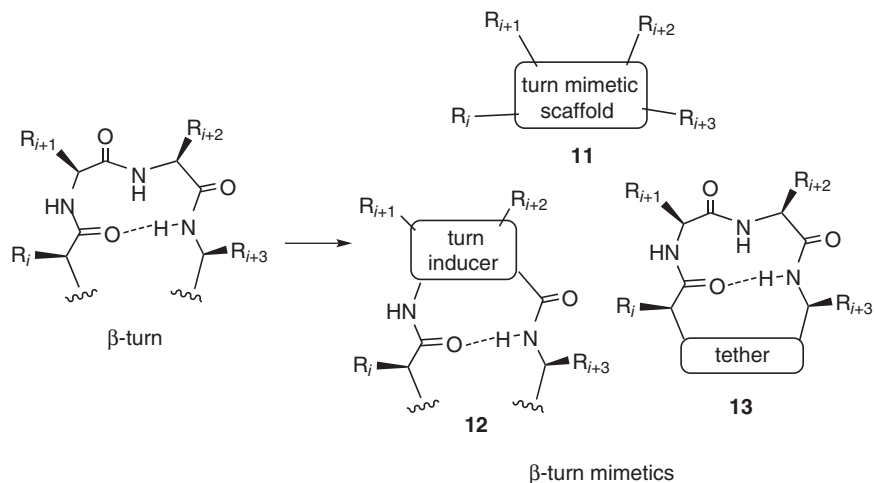


are conceived as mimicking the side-chains at the  $i$ ,  $i+4$  and  $i+7$  positions of an  $\alpha$ -helix, and carrying the side-chain diversification. An additional entry to helix mimetics composed of aromatic rings was proposed by Koenig and collaborators, who reported a 1,4-dipiperazinobenzene (**7**) as a short helix mimetic containing side-chain isosteres at the two piperazines [20] (Figure 1.5).

$\beta$ -Sheets, key structural elements in the three-dimensional structure and biological activity of proteins, are characterized by a regular array of intramolecular hydrogen-bonds connecting adjacent  $\beta$ -strands, and side-chain hydrophobic interactions; interest is growing especially in the field of neuropathies involving aggregation of oligomeric species possessing flat structures of this type [21]. Nevertheless, the structure and stability of  $\beta$ -sheets are still not as well understood as those of  $\alpha$ -helices; thus  $\beta$ -sheet-like molecules are employed as model structures to study such folding propensities in peptides and proteins.  $\beta$ -Sheet structures have been developed taking advantage of specific moieties, such as urea bonds or designed molecular scaffolds, possessing a flat structure and the capability of mimicking  $\beta$ -strands and establishing parallel hydrogen-bonds both as donors and acceptors [22]. This is the case for the  $\beta$ -strand mimetics 5-amino-2-methoxybenzamides and hydrazides (**8**) for stabilizing antiparallel  $\beta$ -sheets [23], epindolidione (**9**) [24] and methoxypyrrole-based amino acids (**10**) [25], all serving as a central strand of the  $\beta$ -sheet to orient the hydrogen-bonding functionality appropriately (Figure 1.6). In addition, aromatic scaffolds have been demonstrated to induce  $\beta$ -sheet-like structures between two attached peptides [26]. In particular, rigid aromatic spacers force a hydrogen bonding bridge in cyclic peptides.



**Figure 1.6** Representative entries to  $\beta$ -sheet mimetics



**Figure 1.7** Chemical approaches to  $\beta$ -turn mimetics

$\beta$ -Turns are the most frequently mimicked protein secondary structures. A  $\beta$ -turn is defined as a tetrapeptide sequence where the distance between  $C_{\alpha i}$  and  $C_{\alpha i+3}$  is  $\leq 7$  Å. The turn can be stabilized by chelation of a cation or intramolecular hydrogen bonds. An ideal mimic will have a rigid scaffold that orients the side-chain residues in the same direction as the natural peptide, while conferring better solubility and/or resistance to enzymatic degradation. The generation of  $\beta$ -turn mimetics has been approached by proposing either scaffolds mimicking the whole peptide motif (**11**) or developing dipeptide isosteres capable of inducing a turn in a peptide motif (**12**). In addition, chemical tethers have been introduced as constraining elements to stabilize  $\beta$ -turn structures within a macrocyclic molecule (**13**) (Figure 1.7). Chapter 9 gives a more detailed overview of synthetic approaches and applications of  $\beta$ -turn mimetics.

## 1.4 Successful Examples of Peptidomimetic Drugs

The most successful application of the concept of peptidomimetics in drug discovery is in the development of enzyme inhibitors. In this field, proteases have been found as an attractive therapeutic target for several pathologies, as they are crucial for a number of processes, including the regulation of peptide hormones and neuromodulators through proteolytic activation of inactive precursors. Since the mechanism of action of proteases is crucial for the design of peptidomimetic inhibitors, a classification of proteases has been taken into account and, according to Gante [2], the main proteases as therapeutic targets have been divided into four groups, namely, serine, cysteine, aspartic and metalloproteases. In addition, the nomenclature proposed by Schechter and Berger has been considered as standard in describing the cleavage site of a substrate ( $P_1 - P_1'$  at the cleavage site and  $P_n - P_n'$  for the flanking amino acids) with respect of amino acid sequence, and the corresponding enzyme sites ( $S_1 - S_1'$  at the cleavage site and  $S_n - S_n'$  for the flanking sites) [27].

The most representative entries to peptidomimetic drugs acting as protease inhibitors are illustrated by angiotensin-converting enzyme (ACE) inhibitors, thrombin inhibitors and

human immunodeficiency virus (HIV) protease inhibitors. The first two entries are herein briefly reported, whereas the topic of HIV protease inhibitors possessing a peptidomimetic nature is described thoroughly in Chapter 11.

### 1.4.1 ACE Inhibitors

ACE inhibitors are an important class of drugs that have found successful application for the treatment of blood pressure dysfunction, specifically related to hypertension, which is a major issue in the population worldwide [28]. The inhibition of ACE is crucial in influencing the renin–angiotensin system, which is characterized by a sequence of enzymatic proteolytic cleavages starting from angiotensinogen II and ending with angiotensin II. Specifically, renin, an endoprotease of the aspartic acid proteases family, cleaves the angiotensinogen peptide to produce the biologically inactive decapeptide angiotensin I. Such a peptide is successively cleaved at the C-terminal by ACE, which removes a dipeptide fragment to give the bioactive octapeptide angiotensin II, which has strong hypertensive properties by inducing vasoconstriction and augmenting the levels of aldosterone, which in turn promotes the retention of water and sodium ion, ultimately resulting in the increase of blood pressure.

ACE is a metalloprotease possessing a  $\text{Zn}^{2+}$  ion in the active site, and has been the starting point for the identification of ACE inhibitors in a study of the inhibition properties of the bioactive peptide teprotide. Subsequent studies to identify the fragments responsible of the inhibition allowed for the identification of the Ala-Pro dipeptide unit as the pharmacophore. Two different elaborations of this dipeptide resulted in two different ACE inhibitors, namely, captopril and enalapril (Figure 1.8).

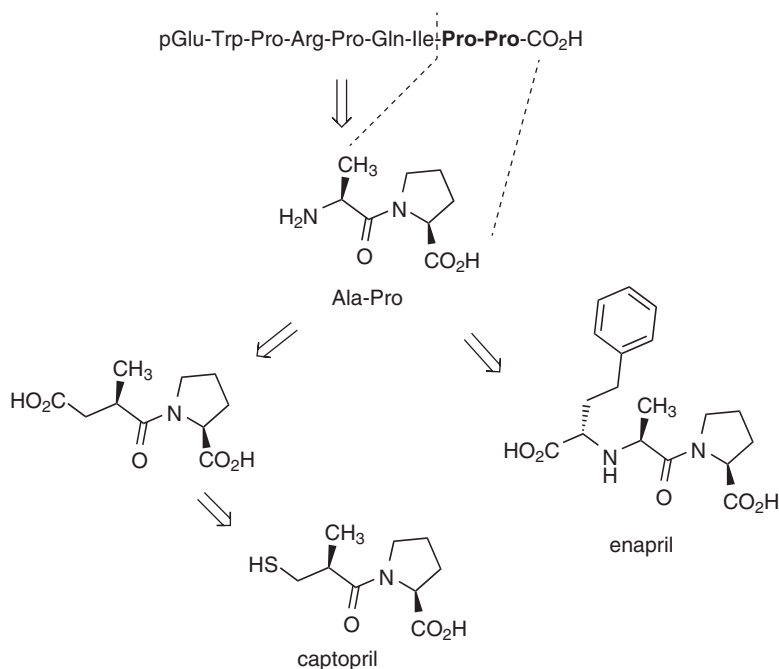
Captopril, which is a successful orally active drug for treating high blood pressure, resulted from matching the structure of the Ala-Pro unit with that of alkyl-succinic acids, which are well-known carboxypeptidase inhibitors. Thus, replacement of the amino group of Ala with an acetyl group resulted in the corresponding  $\alpha$ -methylsuccinylproline, which demonstrated major inhibition with respect to Ala by a factor of 100 due to improved coordination of the second carboxylic group with the zinc ion. Indeed, further improvement of such interaction resulted in the development of captopril, which has a SH group in place of the carboxy unit, thereby possessing stronger coordinating activity towards the metal ion.

Enalapril resulted from the addition of a carboxyalkyl group to the nitrogen atom of Ala. In this case, the improved inhibition was due to a hydrophobic interaction between the phenylethyl group at the nitrogen atom of Ala with  $\text{S}_1$  of ACE's active site, rather than an improved coordination with the metal ion.

Further advances in this field consisted of the generation of bicyclic compounds with reduced peptide-like structure, and of other peptidomimetic compounds consisting of proline analogues and appendages bearing both hydrophobic and metal-coordinating moieties.

### 1.4.2 Thrombin Inhibitors

Among novel approaches to the treatment of thrombosis, since the 1980s major efforts have been devoted to the development of thrombin inhibitors. Thrombin is a trypsin-like serine protease formed through the cleavage of prothrombin by the serine protease factor Xa. Thrombin is the central enzyme that controls the balance between haemostasis and

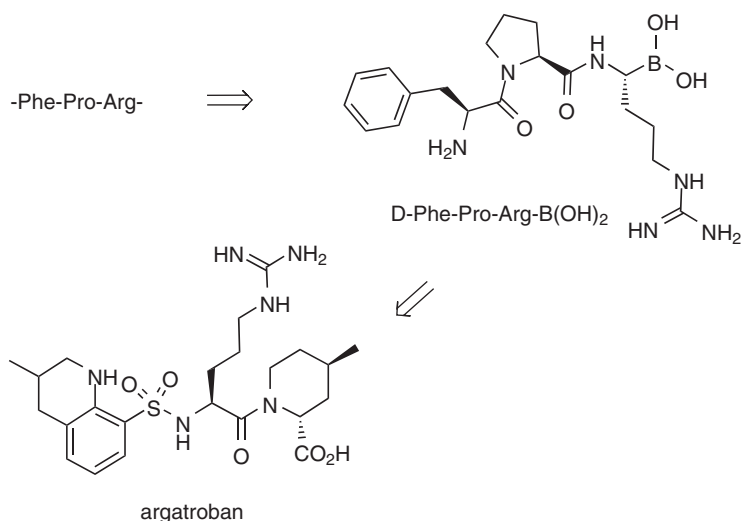


**Figure 1.8** Peptidomimetic ACE inhibitor drugs

fibrinolysis. It catalyses the conversion of fibrinogen into fibrin by cleaving the peptide bond between arginine and glycine in the fibrinogen sequence Gly-Val-Arg-Gly-Pro-Arg. Thrombin also cleaves other substrates such as factors V, VIII and XIII. It also activates the platelet thrombin receptor, being a potent stimulant of platelet aggregation [29].

Thrombin is inhibited by heparin, warfarin and other non-peptidic compounds. Among bioactive peptide exerting inhibition towards thrombin, hirudin was found as the best inhibitor, with an inhibition constant ( $K_i$ ) of 20 fM. In addition, hirudin fragments possessing inhibition, although with less potency, were identified. Nevertheless, intense research has been focussed on thrombin inhibitors according to a substrate-based approach rather than taking advantage of the structure of the natural peptide inhibitor hirudin.

The peptidomimetic approach resulted in the design and synthesis of a large array of compounds mimicking the fibrinogen sequence that interacts with the thrombin active site – this approach also took advantage of the three-dimensional structure of human thrombin that inspired structure-based design. Specifically, starting from the tripeptide fragment Phe-Pro-Arg of fibrinogen, which is recognized by the catalytic triad within the site of thrombin, several compounds have been developed with varying degree of mimetism. Replacement of the carboxylic end at the Arg amino acid with boronic acid resulted in a marked improvement in inhibition, taking advantage of the tetrahedral intermediate. The observation that the simple N-tosyl-arginine methyl-ester retained inhibition activity allowed development of the highly potent peptidomimetic drug argatroban by replacing the methyl ester with a pipercolic acid moiety [30].



**Figure 1.9** Peptidomimetic approach to argatroban

Argatroban is a potent synthetic thrombin inhibitor with a  $K_i$  of 40 nM (Figure 1.9). It is derived from L-arginine and was developed by Mitsubishi (Japan) from a rational stepwise modification of the lead compound *N*-tosyl-L-arginine methyl ester, including replacement of the ester group with tertiary amides and optimization of the arylsulfonamide moiety, and it was achieved before having the crystal structure of human thrombin solved.

It was first introduced in Japan in 1990 for treating peripheral vascular disorders, and successively approved by the FDA (US Food and Drug Administration) in 2000 as an anticoagulant for the prevention or treatment of thrombosis in patients with heparin-induced thrombocytopenia, and in 2002 as an anticoagulant in patients with or at risk of heparin-induced thrombocytopenia while undergoing percutaneous coronary intervention [31]. The binding mode of this peptidomimetic compound is characterized by the guanidino group of arginine interacting within the  $S_1$  binding pocket through hydrogen bonding with Asp189; however, the presence of such a basic guanidine moiety ( $pK_a = 13$ ) is the main cause for the lack of absorption from the gastrointestinal tract and, thus, argatroban must be applied parenterally. Intense research oriented to the development of an orally active thrombin inhibitor as an anticoagulant therapeutic resulted in ximelagatran, the first approved orally active direct thrombin inhibitor (France, 2003). This molecule was developed as a double prodrug of melagatran, another peptidomimetic thrombin inhibitor containing a benzamidine group as an arginine side-chain isostere. Melagatran, a tripeptidomimetic thrombin inhibitor ( $K_i$  of 2 nM) mimicking the D-Phe-Pro-Arg sequence, is characterized by a benzamidine arginine side chain mimetic and azetidine 2-carboxylic acid as proline mimetic. It has served as a lead compound for numerous peptidomimetic thrombin inhibitors [32].

Replacement of the benzamidine and carboxylic group of melagatran with a benzamidoxime and an ethyl ester in ximelagatran, respectively, was conceived to improve the poor bioavailability and absorption of melagatran upon oral dosing.

## 1.5 Conclusion

Several decades after the introduction of the concept of peptidomimetics, this approach in drug discovery is still timely, owing to the never-ending interest in new compounds based on peptides and proteins. Besides the development of biotechnological therapeutics based on antibody-derived compounds, the field of small molecules encompassing the panorama of peptide drugs is still covered by the generation of peptidomimetics, with the aim of obtaining hit compounds that possess optimal bioactivity and pharmacokinetics profile. During recent decades the basic concepts and approaches to peptidomimetic compounds have evolved to cover diverse compounds and synthetic strategies spanning from combinatorial chemistry to solid-phase synthesis and heterocyclic chemistry. Accordingly, diverse research fields within organic and medicinal chemistry have been matched to produce novel peptidomimetic entries with improved technology, such as ‘click chemistry’ to generate a diverse array of enzyme inhibitors and receptor ligands (Chapter 5). The following chapters give a picture of the panorama of peptidomimetic chemistry from early years to more recent reports dealing with synthesis and applications in drug discovery.

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