Design and Synthesis of Novel Peptidomimetic Scaffolds

1. General Introduction

Research on synthetic foldamers (peptidomimetics) has attracted considerable attention not only for discovering predictable and well defined structures, but also for harnessing the latent bioactivities in enzyme mimics as well as for drug design and development. The current efforts aimed at developing compounds containing non-peptide structural elements and capable of mimicking or antagonizing the biological action(s) of the natural peptide(s), represent the ultimate challenge in peptidomimetic research. The rational design of small-molecule non-peptide peptidomimetics of biologically active peptides requires a rather complete understanding of the chemical and spatial elements of the pharmacophore of interest. For understanding the biologically relevant conformations of the molecules, the synthesis of conformationally constrained analogues is the most common approach employed and the same is achieved in the case of peptides through insertion of conformationally constrained non-peptide scaffolds in the peptide backbone. Such modifications also help in understanding the central role of peptides and proteins in communication, regulation and metabolism of biological systems, because such conformationally restricted mimetics of natural ligands provide information about macromolecular receptor requirements, particularly, in the case of membrane bound receptors, whose structural elucidation is extremely difficult. Many a times, the de Novo design of a folded peptide helps in improving our understanding of the protein structure in general and provides a platform for further engineering of proteins with tailored structures.

Although many peptide sequences have been identified as potent bioactive agents, there are fundamental limitations associated with the development of peptides as therapeutics. The inherent flexibility of a small peptide results in a myriad of conformations adopted by the peptide. In addition, there are a number of metabolic limitations restricting the use of peptides as therapeutics. Poor permeability across membranes, proteolytic degradation, rapid clearance, poor solubility and a tendency to aggregate, all contribute to low oral bioavailability of peptide-based therapeutics.
lack of specific transport systems restricts the efficient passage of peptides to the desired site of action. The susceptibility of externally administered peptides to proteolytic degradation in the gastrointestinal tract, blood and other tissues results in rapid clearance, thus significantly reducing the effectiveness of these peptides in inducing a satisfactory response. The peptide must therefore survive in the pharmacologically active form under conditions of exposure to various proteolytic enzymes in the digestive and circulatory systems. Given that therapeutics are usually administered far from the site of action, there is a clear need for enhanced metabolic stability.

One way to overcome these disadvantages of natural short polypeptides is the use of peptidomimetics. IUPAC has issued the following definition of peptidomimetics: “A peptidomimetic is a compound containing non-peptide structural elements that is capable of mimicking or antagonizing the biological action(s) of a natural peptide. A peptidomimetic does no longer have classical peptide characteristics such as enzymatically scissile peptide bonds.”

Peptidomimetics are small protein-like molecules designed to mimic natural peptides or proteins. These mimetics should have the ability to bind to their natural targets in the same way as the native peptide sequences on whose structure their design is based and hence should produce the similar physiological outcome. It is possible to design these molecules in such a way that they show the same biological effects as the native peptide but with enhanced beneficial properties such as higher proteolytic stability, higher bioavailability and also, often with, improved selectivity or potency. Sometimes the peptidomimetics are designed to bind the same macromolecular target but to antagonize the action of native peptide/ inhibit enzymes; this constitutes an interesting approach for the discovery of new drug candidates. For the development of potent peptidomimetics it is necessary to understand the forces that lead to protein–protein interactions with nanomolar or often even higher affinities. These strong interactions between the peptides and their corresponding proteinaceous targets are mainly based on side chain interactions indicating that the peptide backbone itself is not an absolute requirement for high affinities.
Based on their functional and structural features, peptidomimetics may be classified into three categories:  

**Type I** mimetics: including peptides that mainly consist of the native peptide amino acid sequence and carry all the functionalities responsible for the interaction with an enzyme or a receptor with a well-defined spatial orientation. For instance, a thyrotropin-releasing hormone (TRH) mimetic based on a cyclohexane scaffold (1, Figure-1), have the same spatial orientation of amino acid side-chains as found in TRH hormone.  

**Type II** mimetics: involves modified type I mimetics with various non-natural amino acids based on the interaction with the target receptor or enzyme, without apparent structural analogies. For instance, the replacement of peptidic fragment in Somatostatin receptor binding cyclopeptide with a D-glucose scaffold (2, Figure-1).  

**Type III** mimetics includes highly modified scaffolds that replace the peptide backbone completely in such a way that all the functional groups needed for biological interactions are mounted in a well-defined spatial orientation. For instance, steroidal scaffold (3) that mimic the type II’ β-turn structure of RGD cyclopeptide (Figure-1).  

The most important requirement is that the scaffold is able to place the known side chains of amino acid in a derived 3D-position to allow interactions with the target protein. This can be achieved by conducting structure-activity relationship (SAR) investigations. By this method, the shortest active sequence in the natural protein–protein interaction can be identified. Towards this end, shorter analogues of the natural sequence are synthesized and tested against the target protein to identify the minimum sequence necessary for biological activity; now a days it is also aided by computerized data base and pharmacophore searching. The most significant parameters such as stereochemistry, charge and hydrophobicity can be examined by systematic exchange of single amino acids. As a result, the key residues, which are essential for the biological activity can be identified. As next step, the 3D arrangement of these key residues needs to be analyzed by the use of compounds with rigid conformations to identify the most active structure. When a clear model of the moieties necessary for the interaction and their location in 3D space has been gathered, these elements can then be reassembled by the use of peptidic or non-peptidic structures to form a peptidomimetic with the same biological activity as the natural ligand, which it should replace. This is a rather expensive and time-consuming method but the use of new techniques that allow the fast
synthesis and analysis of receptor binding of a great variety of peptides allows the whole process to become more efficient.

Figure-1: Peptidomimetics based on cyclohexane (1), glucose (2) and steroid (3) scaffolds

Since proteins tend to exert their biological activity through small regions of their folded surfaces, small functional units that are amenable to further chemical modifications could be designed to elicit the activity of the native protein. This approach involves mimicking specific secondary structures of the protein molecules such as α-helix, β-sheet, β-turn and loops that often constitute the bioactive surfaces involved in the receptor-ligand interaction. \(^{13}\)
An extended polypeptide strand presents contiguous residues on alternating sides of the strand, with the maximum possible distance separating their side chains. For example, the $i$ and $i+4$ residues in an extended strand are 14.5 Å apart (4). This separation minimizes steric clashes between side chains and the latter also have the maximum possible exposure to solvent (or receptor) in this structure. A single strand permits maximum exposure of the main chain atoms for hydrogen bonding to a receptor.

$\beta$-Sheets, key structural elements in the three-dimensional structure and important component of pharmacophore of peptides, 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.\textsuperscript{14} A strand within a $\beta$-sheet (5) has $i$ and $i+4$ residues 13.2 Å apart. The strands on the edges of the sheets still have half of their main chain atoms available for hydrogen bonding to a receptor or solvent. The side chains are exposed for potential enzyme interactions on both top and bottom surfaces. This presents a more three-dimensional recognition site with $i$ and $i+4$ residues being 13.2 Å apart on the same strand, and the C-C inter-strand distance of adjacent residues on adjacent strand being approximately 4.5-5.5 Å apart (distances as measured from a DNA binding antiparallel pleated sheet complex).\textsuperscript{15} The corresponding hydrogen-bonding distance from amide $N$ to carbonyl $O$ is approximately 3.0 Å. The $\beta$-strand thus offers three different recognition sites either via the top or bottom faces or via hydrogen bonding with main amide atoms on each side of the sheet. As the $\beta$-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, $\beta$-strand mimetics 5-amino-2-methoxybenzamides and hydrazides (6), serve as a central strand of the $\beta$-sheet to orient the hydrogen-bonding
functionality appropriately. In particular, rigid aromatic spacers force a hydrogen bonding bridge in cyclic peptides.

On the other hand, the α-helix is the most common peptide secondary structure, constituting almost half of the polypeptide structure in proteins. The helix has \( i \) and \( i+4 \) residues only ~ 6.3 Å apart (7). The helix is a compressed peptide chain with all main chain amidine, oxygen and NH atoms being involved in intramolecular hydrogen bonds. Consequently, main chain atoms are protected from intermolecular hydrogen bonding with a receptor and only the side chains are available for intermolecular interactions. Inhibitors based on the terphenyl scaffold 8 were developed, for instance, to inhibit the interaction of Calmodulin (CaM) with smooth muscle myosin light chain kinase (smMLCK). CaM has a variety of functions in the cell cycle and interacts with a number of proteins including smMLCK, which is supposed to play a significant role in the signaling cascade leading to muscle contraction, but it is also proposed to play a role in cancer.}

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Another interesting approach involves synthesis of 1,4-dipiperazinobenzene 10, using a stepwise transition metal catalyzed \(N\)-arylation of chiral piperazines to a benzene core. The structure determined by X-ray crystallography revealed a geometric arrangement of the side chains resembling the orientation of \(\alpha\)-helical i, i+3 and i+7 residues.\(^{18}\)

Along with helices and sheets, an important feature of peptide and protein secondary structure is that instance where the polypeptide chain reverses direction. These reverse turns, as a consequence of their frequent appearance on the external surface of the proteins, are postulated to have a high probability of being part of a pharmacophore.\(^6\) A turn can be defined as the site where a peptide changes its overall direction. The turn conformation provides maximum two-dimensional exposure of its side chains to either solvent or receptor, but limits the exposure of the main chain atoms to hydrogen bonding. The average C-C distance of adjacent residues is 5.2 Å (11), which is similar to the distance between two adjacent side chains of a helix, but is distinguished by the presentation of the side chains in two dimensions for a turn compared with dimensions for a helix.

Different type of turns have been described, such as \(\delta\)-, \(\gamma\)-, \(\beta\)-, \(\alpha\)- and \(\pi\)-turns corresponding to the loops involving two to six residues, respectively.\(^7\) Numerous organic mimetics of the most common types of reverse turns, \(\beta\)- and \(\gamma\)-turns, have been constructed in order to provide conformationally relevant templates on which key pharmacophore elements may be hung. The \(\beta\)- and \(\gamma\)-turns have been known as structural elements which are involved in bimolecular recognition events.\(^6\) In proteins, \(\beta\)-turn\(^{19}\) (12), characterized by ten-membered hydrogen bonded rings, are more prevalent than \(\gamma\)-turns (13) forming seven-membered hydrogen bonded rings, which are found in small peptides but only rarely in larger proteins.
Thus, a peptide turn may be defined by 3 residues (γ-turn), 4 residues (β-turn), 5 residues (α-turn) that can form, respectively, 7-, 10- and 13- membered hydrogen bonded rings as shown in (14).

The β-turn (12) is the most common reverse turn found in polypeptides. The prevalence of β-turns in peptide surface suggests that they play essential roles in the molecular recognition events in biological systems, such as receptor-ligand, enzyme-substrate and antigen- antibody interactions. This has led to the challenge for the development of functionalizable β-turn mimics. β-Turns comprise a rather diverse group of structures and are classified according to the φ and ψ torsion angles of the i + 1 and i + 2 residues. β-Turn, which reverses the direction over four residues, often involves a hydrogen bond between the carbonyl group of residue i and the NH moiety of residue i + 3; the O1----H-N hydrogen bond is not an essential structural feature, but is often indicative of a β-turn structure. The turn structures of peptidomimetics involve
the restriction of the torsional angles $\phi$ (phi), $\psi$ (psi), $\omega$ (omega), which determine the 3D structure of the peptide backbone, as well as the $\chi$ (chi) torsional angles that define the position of the side chain functional groups.

An ideal $\beta$-turn 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 either scaffolds mimicking the whole peptide motif (15) or developing dipeptide isosteres capable of inducing a turn in a peptide motif (16). In addition, chemical tethers have been introduced as constraining elements to stabilize $\beta$-turn structures within a macrocyclic molecule (17). Therefore, ideal $\beta$-turn mimic is the one in which the four variable torsion angles $\phi_2$, $\phi_3$, $\psi_2$, and $\psi_3$ are constrained in a predictable manner such that a $\beta$-turn conformation exists over the pseudo tetrapeptide sequence.

Recognition of a turn conformation normally involves interactions between side chain residues of the ligand with the receptor and thus the peptide turn can be considered to be a scaffold, which could potentially be either conformationally constrained or entirely replaced by an alternative rigid non-peptide scaffold designed to support moieties that may mimic peptide side chains. The conformational restriction of flexible bioactive molecules is a well-known technique for increasing their intrinsic activity or their selectivity for a particular receptor subtype or enzyme isoform. Such rigid ligands can also lead to variations in lipophilicity and/or increased stability toward metabolic enzymes; both factors contribute to improved bioavailability of a
given active substance and conformationally constrained amino acids have been the focus of both synthetic and medicinal chemistry, particularly, for their application in designing novel peptidomimetics.\textsuperscript{25}

Recently, considerable progress has been made on designing and synthesizing reverse turn scaffolds with diversity and biological applications in mind. Notable reverse turn scaffolds along with their biological applications are listed in Figure-2.\textsuperscript{26}

**Monocyclic $\beta$–turn Mimetics**

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**Bicyclic $\beta$–turn Mimetics**

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Global Restrictions through Cyclic Peptidomimetics

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. Cyclization can be obtained by connecting the N- with the C-terminus (head-to-tail) portion of the peptide sequence, or by coupling of either the N- or the C-terminus with one of the side chains (backbone-to-side chain), or by coupling of side chains not involved in specific interactions with other (side chain-to-side chain, Figure-3).27 The macrocyclic peptide has several advantages in improving the quality of the bioactive compound in terms of bioavailability and potency, as the high proportion of cis amide bonds and the absence of free C- and N-termini confer higher metabolic resistance.28 In addition, the limited conformational freedom results in higher receptor selectivity and binding affinity, by reducing unfavorable entropic effects. The most common side chain-to-side chain cyclization is the oxidation of two Cys residues with the formation of a disulfide bond. Alternatively, the formation of amide bonds between the side chains of Lys and Asp/Glu can occur. One limiting factor of side chain-to-side chain cyclization is that only a limited section of the polypeptide is constrained. To overcome this problem, several covalent bridges may be incorporated into one sequence.29
H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala-OH

A) Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala

B) Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala

C) H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala

**Figure: 3.** Examples of peptide cyclization: A) head to tail, B) backbone to side chain, and C) side chain to side chain

Another example includes Somatostatin, 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, leading to a lowering of blood glucose concentrations.\(^{30}\)

**Local Restrictions**

The introduction of local modification around a side-chain is aimed at modulating the conformational profile of the peptide, thus intervening on all the rotatable bonds present in the amino acid unit. The conformation of the peptide backbone can be described by three torsional angles (Figure-4): \(\phi\), which is the angle defined by C(O)—N—Ca—C(O); \(\psi\), which is defined by N—Ca—C(O)—N; and \(\omega\), which is defined by Ca—C(O)—N—Ca. The \(\omega\) angle for the peptide bond is generally *trans* \((\omega = 180^\circ)\) except for the Xaa-Pro bond, which can be *cis* \((\omega = 0^\circ)\) or *trans*.\(^{31}\)

Pioneering work of Ramachandran et al. resulted in the so-called Ramachandran plots which restrict the allowed values for the torsional angles \(\phi\) and \(\psi\) for most amino acids.\(^ {32}\) The conformational space accessible to the L-amino acids is about one third of the total structural space. Moreover, side-chain modifications have been introduced to explore pharmacophoric steric and electronic interactions, such as modulation of the hydrophobic content by adding aromatic moieties or the introduction of polar
appendages to address any polar or hydrogen-bonding interactions with the target receptor.

\[
\begin{align*}
\phi &= \text{C(O)}-\text{N-}\text{C}^\alpha-\text{C(O)} \\
\psi &= \text{N-}\text{C}^\alpha-\text{C(O)}-\text{N} \\
\omega &= \text{C}^\alpha-\text{C(O)}-\text{N-}\text{C}^\alpha \\
\chi &= \text{C}_\gamma-\text{C}_β-\text{C}^\alpha-\text{N}
\end{align*}
\]

**Figure: 4.** Dihedral descriptors for conformational flexibility around the peptide chain

\[ \alpha-\text{Substituted amino acids are a common approach to reduce the torsional freedom around the backbone of such amino acids. }\alpha-\text{Methyl-}\alpha-\text{amino acids possess a methyl at the C}^\alpha, \text{which greatly reduces the rotational freedom around N-}\text{C}^\alpha \text{and C}^\alpha-\text{CO bonds.}^{31} \text{As an example, the introduction of } \alpha-\text{Me-alanine in a peptide reduces the rotational freedom around its backbone bonds by about 90\%. This is the most widely studied } \alpha-\text{alkylated amino acid, which is able to restrict the dihedral angles } \phi, \psi \text{ to values encountered in } \alpha \text{ or } 3_{10} \text{ helices. Moreover, for these quaternary amino acids the preferred conformation is an extended structure with dihedral angles } \phi, \psi \text{ being about } 180^\circ.^{31} \text{ Other entries to } \alpha-\text{substituted-}\alpha-\text{amino acids are represented by both glycine derivatives and cyclic amino acids. } \alpha,\alpha-\text{Dialkyl glycines are characterized by an extended conformation (Figure-5), whereas cyclic amino acids contribute to constrain the folded conformation to various degrees as a function of the ring size, as well as improving the potency of the bioactive peptide compound.}^{33}
\]

**Figure: 5.** Structure of alanine (Ala)-, glycine (Gly)- and phenylalanine (Phe)-derived \(\alpha\)-substituted amino acids.
β-Substituted analogues of the naturally occurring amino acids are one example for rigidification in the side chain. Various analogues of natural amino acids alkylated at the β-carbon can be found in the literature (Figure-6). For example, introduction of a methyl group into the side chains of phenylalanine or tryptophan leads to β-MePhe (33) and β-MeTrp (34); often resulting in a comparably higher activity and an increased biological stability of the modified peptides. The introduction of three methyl groups at the 2′-, 6′- and β-position of natural tyrosine hinders the free rotation around the C\(^\beta\)–C\(^\gamma\) bond often result in the formation of biologically active conformations of type 35.

![Chemical structures of valine, isoleucine, threonine, 33, 34, and 35.](image)

**Figure: 6.** Three β-methylated natural amino acids valine, isoleucine and threonine; and some selected examples of unnatural β-methylated amino acids 33-35.

\(N\)-Alkylation, and in particular \(N\)-methylation, is an important modification of the peptide bond that influences the conformational freedom of the peptide backbone. The number of inter- and intra-molecular hydrogen bonds decrease due to the removal of the backbone NH groups, destabilizing both α-helix and β-sheet conformations. Finally, the attached carbonyl group shows an increased basicity and decreased polarity.

β-Alanine amino acids (36) can be rigidified to improve bioavailability through the cyclopropane ring (37-39). The cyclopropane ring system is particularly interesting because it affects the chemical and biological properties in peptides through significant conformational restrictions in the amino acids residues.
The $\alpha,\alpha$-disubstituted $\alpha$-amino acids (40) are non-proteinogenic amino acids\(^{37}\) and have attracted attention of many synthetic and medicinal chemists and those involved with protein engineering, because of their properties such as conformational restriction of side chains, the stable secondary structure of the peptides involving them, and their characteristic biological activities.\(^{37}\) The side chain of $\alpha,\alpha$-disubstituted $\alpha$-amino acids have been further constrained with cyclopropyl ring (41) as well as other cycloalkane rings (42). Among the cyclic $\alpha,\alpha$-disubstituted $\alpha$-amino acids, 4-aminopiperidine-4-carboxylic acid (Pip, 43), which is an achiral $\alpha$-amino acid bearing a $\delta$-nitrogen atom, has been focused upon because of the anti-microbial activity it confers on peptides.\(^{38}\)

Aminomalonic acid (44) is a $\alpha$-amino acid, which is attracting increasing attention due to its inherent biological activity and as an efficient building block for unnatural amino acid derivatives.\(^{39}\) The rigid conformation of (44) is achieved by developing nitrogen containing heterocycles incorporating an aminomalonic acid moieties (45).\(^{40}\)
Proline (46) has a special place among the proteinogenic amino acids.\textsuperscript{41} Besides proline itself, numerous derivatives were found in proteins as results of post-translational modifications. \textit{Cis}-4-Methyl-L-proline (47) was discovered in hydrolysates of different leucinostatines.\textsuperscript{42} The proline derivative 48 and 49 were first found in hydrolysates of Mediterranean sponge and later also in several other organisms.\textsuperscript{43} Additionally to the naturally occurring proline analogues, countless proline derivatives have been synthesized by the introduction of alkyl chains or aromatic groups in the 3-, 4- and 5-position of the ring. Derivatives with additional heteroatoms and halogenated prolines have also been synthesized and extensively studied.\textsuperscript{42-43}

Another interesting case is that of arginine (50), the endogenous substrate of NO synthase, of which a number of conformationally restricted analogues have been prepared. Such rigid analogues have generally taken three forms. In the first form, the essential guanidine function (or an isosteric equivalent) is incorporated in a chain-terminating heterocycle. These include, for instance, the N\textsuperscript{δ}-N\textsuperscript{ω} ethylene bridged analogue (51)\textsuperscript{44} and the 2-aminopyrimidine derivative (52).\textsuperscript{45} In the second class of compounds, the 3-carbon tether is locked into a more rigid conformation either by introduction of a double bond (53) or by incorporation in a ring (guanidinophenylalanine derivative, 54).\textsuperscript{46} Another approach to rigid arginine analogues consists in linking one of the nitrogen atoms of the guanidine functionality to one of the methylene groups. One such molecule is the piperidine derivative (55), designed as a specific thrombin inhibitor.\textsuperscript{47}
Glutamic acid (56) is the main excitatory neurotransmitter in the central nervous system, involved in the physiological regulation of processes such as learning and memory. Homologation of the glutamic acid backbone embedded in selective ligands is expected to lead to a significant modification of their pharmacological profile. Consequently, the homo-derivative of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acids (57) behaves as a selective agonist at the metabotropic receptor. Bicyclic acidic amino acids (58) and (59), which are conformationally constrained homologues of glutamic acid, have been prepared through 1,3-dipolar cycloaddition. Among the proteinogenic aromatic α-amino acids, phenylalanine (60) is an important structural element present in large number of bioactive peptides. Phenylalanine (60) has been modified to constrained analogues (61-63); these well-defined structures have been observed to display interesting biological properties.
Based on the understanding of the receptor requirements, highly specific non-peptide ligands, generally incorporating specific conformationally constrained mimics of peptide (amide) linkage, have been developed, which not only helped in circumventing the known drawbacks of peptides as potential drugs, such peptidomimetics are able to bind with enhanced affinity and hence display beneficial biological activities with potential medicinal applications.

Oligomers of N-substituted glycines, where the side chain is attached to the amine nitrogen instead of the α-carbon, are called α-peptoids (Figure-7). The conformational change in the N-substituted glycines makes the α-carbon achiral so that peptoids are less restricted in their spatial conformation. Neither can peptoids form intramolecular hydrogen bonds through backbone–backbone interactions, because of the lack of amide protons that help peptides stabilize both α-helical structures and β-sheet conformations. However, the same backbone structure renders the peptoids highly resistant to proteases.

![α-peptoids](image)

**Figure: 7. Structure of α-peptoids**

The backbone of a peptide can also be modified by isosteric or isoelectronic substitution. Various peptidomimetics containing pseudopeptides or peptide bond isosteres are designed and synthesized with the aim to obtain peptide analogs with
improved pharmacological properties (Figure-8). The introduction of these peptide bond isosteres to the native peptide sequence is expected to prevent protease cleavage of amide bonds and remarkably enhance the peptides metabolic stability, while such replacements may also limit the biophysical and biochemical properties of peptides. Therefore, the choice of an amide bond surrogate is a compromise between positive effects on pharmacokinetics and bioavailability and potential negative effects on activity and specificity. The ability of the peptide isostere to mimic the steric, electronic and solvation properties of the amide bond is certainly the most important characteristic in determining the potency of pseudopeptide analogs.

Such isosteres were at the basis of the development of transition state analogue enzyme inhibitors, which led to a breakthrough in the development of inhibitors for several therapeutically important enzymes. Other backbone modifications include the replacement of the α-carbon with nitrogen, leading to achiral azapeptides. In addition, the insertion of extra atoms into the backbone chain by using β-homo-amino acids, γ-amino acids or even longer-chain amino acids has been explored (Figure-9).
replace \(\alpha\)-carbon: \(\alpha\)-aza-amino acid
move side chain to N: N-peptoid

\[ \beta^3, \beta^2, \beta^{2,3}, \beta^{2,2}, \beta^{3,3}, \beta^{2,3,3} \] -amino acids

**Figure: 9.** Various Strategies used in Pseudopeptide design

Various heterocyclic moieties have been employed to impart conformational restriction in peptidomimetics and to control chemical reactivity. Peptidomimetics of these types have found widespread use as enzyme inhibitors and biological probes.\(^{56}\) Peptides are flexible biopolymers, the structure and conformation of which are strongly influenced by the nature of constituent amino acids and also the environment in which they exist. However, this high conformational flexibility presents a potential problem in generating therapeutics and biological probes since a peptide must attain a certain conformation in order to bind to its biological target, be it a receptor or an enzyme. Therefore, the pre-organization of peptide shape, via the introduction of a structural motif e.g \(64-70\) (Figure-10) that imparts conformational restriction, can enhance binding and hence therapeutic potential.\(^{57}\)

**Figure: 10.** Some rigid aromatic scaffolds for peptidomimetics
Examples of Peptidomimetics

Although β-amino acids are generally regarded as unnatural amino acids, there are a number of examples of naturally occurring peptides containing substituted β-amino acids isolated from marine organisms and various prokaryotes. Recently, the bacterial species *Rhodococcus* has been found to produce a family of antifungal cyclic tetrapeptides containing a lipid-like β-amino acid.\(^{58}\) β-Alanine is the most commonly occurring β-amino acid and is found in a number of biological peptides such as crytophycin, a tumour-selective depsipeptide isolated from blue-green algae.\(^{59}\) An example of a mammalian peptide containing a β-amino acid is the dipeptide carnosine, with the sequence β-Gly-His.\(^{60}\) This dipeptide is a highly abundant constituent of muscle and other excitable tissues and possesses strong and specific antioxidant properties. Interestingly, the enzyme that cleaves this dipeptide, carnosinase, has been shown to cleave a number of peptides containing β-Ala. α-Hydroxy-β-amino acids are also common constituents of natural products such as taxol (71).\(^{61}\) Taxol is a potent antitumour drug that acts by interfering with the formation of microtubules in tumour cells during mitosis ultimately leading to cell death and is currently on the market for the treatment of breast and ovarian cancer.

![2-Pyridone](71)

2-Pyridone based peptidomimetics inhibit hepatitis C virus (HCV) in an extended β-sheet conformation.\(^{62}\) They also act as inhibitors of human rhinovirus (HRV) 3C protease.\(^{63}\) In general, ring-fused 2-pyridone frame-works are also present in numerous compounds with diverse biological application such as ACE-inhibitors, anticancer agents etc. One example includes 2-pyridone based pilicide (72) which mimics PapG, an adhesin involved uropathogenic infections.\(^{62}\) 2-Pyridones (72) prevent
pilus assembly in uropathogenic *Escherichia coli* and were chosen for further derivatization into extended peptidomimetics.\textsuperscript{62}

![Chemical structure](image)

The benzazepin-3-one (73) based human melanocortin-3 receptor ligand that mimics the tetrapeptide (His-Phe-Arg-Trp) that has been found to contain the principal pharmacophore groups of α-melanocyte stimulating hormone (α-MSH).\textsuperscript{64}

![Chemical structure](image)

Recently, a more rational approach to the design of receptor agonists was reported wherein the Somatostatin analogue sandostatin (74) was used as a scaffold for constructing a peptide containing exclusively β-amino acids to mimic the receptor binding activity.\textsuperscript{65} The aim of this study was to design an analogue with higher bioavailability than sandostatin (74). It was found that the cyclic β-tetrapeptide (75, Figure-11), exhibited significant sandostatin activity.\textsuperscript{66} This study therefore demonstrated that β-peptides can interact with proteins and mimic the receptor binding properties of α-peptides.
Some examples of biologically active molecules possessing a pyrrolidine scaffold have also been developed. Molecule (76) is an inhibitor of human coagulation factor Xa, whose crystal structure in the bound conformation has also been worked out and utilized for further designing.\textsuperscript{67} Similarly, the crystal structure of inhibitor (77) bound to MMP-3 (stromelysin) has revealed a hydrogen-bonding pattern between the enzyme and the hydroxamic acid and sulfonamide moieties,\textsuperscript{68} which is a consequence of the positioning of these groups by the pyrrolidine scaffold.

As a variation of the above theme, in the case of trans-lactam (78), the pyrrolidine-\textit{N}-side chain is forced into a constrained conformation, conferring good shape complementarity for the active site.\textsuperscript{69}
3,5-Disubstituted piperidine rings such as (79) represent a conformationally constrained \( \beta;\gamma \)-diamino acid derivative that mimics a hydrogen-bonding pattern of \( \beta \)-strand.\(^{70}\) In the case of the 1,4-disubstituted- piperidines, \( \beta \)-sheet hydrogen bonding has been observed e.g. in the crystal structure of 80, bound with trypsin.\(^{71}\) Similarly, the piperidine ring of 81 occupies the enzyme pocket, with a short \( \beta \)-ladder formed via two hydrogen bonds between the adjacent amino and carbonyl groups of cyclohexylalanyl/cyclohexylglycyl, respectively, and Gly-216 CO and Gly-216 NH of the trypsin.\(^{71}\)

![Chemical structure of 79](image1)

![Chemical structure of 80](image2)

![Chemical structure of 81](image3)

The rigidity, planarity, and solubility of pyridines have made them useful as scaffolds to position side chains in the constrained conformation. In the case of 82 and 83, the benzamidine group\(^{72}\) fits into the enzyme pocket and is tethered to the 2,4-substituted pyridine scaffold, and the second basic/hydrophobic moiety, the methyl imidazoline, fits snugly in the other subsite of the enzyme.
Incorporation of a ketone into the five-membered ring, such as pyrrolinones and pyrrolidinones, has a dramatic impact on the hydrogen-bonding capacity of the scaffold. Different types of mimetics have been developed using pyrrolinones (84, 85, 86, 87) as novel scaffolds that replace the hydrolyzable backbone of bioactive peptides.

In pyrrolidinones such as (88), the ring is incorporated into the peptidic backbone. Crystallographic studies of (88) and (89) have clearly identified a constrained conformation. The lactam (90) also binds in a constrained conformation to trypsin.
As example of involvement of six-membered piperidinone moiety in the peptide backbone, the crystal structures of the selective thrombin inhibitors (91) and (92) bound to trypsin have revealed that the main chains make three antiparallel hydrogen bonds with the Ser-214 and Gly-216 residues of trypsin.79

Similar observations were made on the seven membered lactams (93) and (94). The cis diastereomer (94) in this series was observed by crystallography to have an extended conformation, while trans isomer (93) formed the expected β-turn. More interestingly, NMR solution studies of the cis isomer (94) in non-coordinating solvents indicated head-to-tail self-association. This type of interaction was only weakly apparent for the trans isomer (93). These observations suggest that cyclic constraints of this type, although generally associated with turn structures, are also able to promote extended conformations.80
A variation of the \(N\) containing bicyclic is represented by the 6-amino-5-oxotetrahydroindolizine present in a potent nonpeptidic inhibitor (95) of the hepatitis C virus-NS3 protease (HCV-NS3). \(^8^1\) Herein, the heterocyclic ring C=O group and NH of the sulfonamide form a hydrogen bonds with the enzyme as confirmed by related crystal structures \(^8^2\) and thus effectively mimic the conformation of peptides.

Another approach has been to incorporate a bicyclic scaffold that mimics the conformation of a dipeptide constrained within a \(\beta\)-strand. \(^8^3\) A cyclic vinyl amide scaffold (96a) has been designed to mimic the segment Phe43-Leu44 (96b) of CD4, the cellular receptor protein for HIV-1, with constraints made from a single side. \(^8^4\)

Fused bicyclic dipeptide mimetics have been developed to inhibit the cellular receptor for HIV. In a variation of compound (96), the compound (97) was designed to mimic the Thr45-Lys46 module. \(^8^4\) Although (96) and (97) have been coupled to form a four-residue analogue, the efficacy of these cycles is yet to be determined.
Using a similar rationale, bicyclic scaffolds have been employed as thrombin inhibitors (98). 

Introduction of a further heteroatom into scaffolds with two rings has also been considered. Molecular modeling and conformational searches indicated that the 5,6-fused bicyclic scaffolds (99, 100, 101) would meet the spatial requirements of a peptide mimetic, while facilitating versatile incorporation of substituents.

A few other bicyclic heterocyclic ring systems have also been used as scaffolds to append substituents. Pyridopyrimidine trifluoromethyl ketones have been designed to extend the concept of the related pyridine trifluoromethyl ketones. Among a series of pyridopyrimidines, analogue (102) displayed potent inhibition of elastase.
2. References


