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Peptides and Peptidomimetics in Medicinal Chemistry

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1. Introduction

Peptides show great pharmaceutical potential as active drugs and diagnostics in several clinical areas such as endocrinology, urology, obstetrics, oncology, etc. and as functional excipients in drug delivery systems to overcome tissue and cellular membrane barriers (Nestor, 2009). From a pharmaceutical point of view, peptides are situated somewhere between classical organic drug substances and high molecular weight biopharmaceuticals.

The importance of peptides to life is evident from the most primitive organism to man. In man, a myriad of roles is filled by peptides spanning hormonal, neuromodulatory, mucosal defense, et al.

Despite the obvious importance of peptides to homeostasis in man, there are few peptides resulting from medicinal chemistry that are commercialized pharmaceutical products in the USA and Europe. The shortcomings of peptides as pharmaceutical products have been long known: typically short duration of action, lack of receptor subtype selectivity, lack of oral bioavailability. However medicinal chemistry offers solutions to the first two limitations and oral bioavailability issues have been addressed by novel routes of administration (e.g. intranasal, inhalation) and injectable depot formulations (Nestor, 2009).

2. Peptide design considerations

The physical and chemical properties of peptides and proteins are determined by the nature of the constituent amino acid side chains and by the polyamide peptide backbone itself.

The structures of the 20 primary amino acids are given in Figure 1. Amino acids are divided into hydrophobic and hydrophilic residues. The first group includes those with aliphatic side chains (Ala, Val, Ile, Leu, Met) and those with aromatic side chains (Phe, Tyr, Trp). The hydrophilic group includes amino acids with neutral, polar side chains (Ser, Thr, Asn, Gln), those with acidic (Asp and Glu) or with basic side chains as Lys, Arg and His.

Two amino acids, Cys and Pro, have special properties that set them apart. Cys containing a thiol group that can be oxidatively couple to another Cys residue to form a disulfide bond that stabilizes secondary and/or tertiary structure or to hold two different peptide chains together. On the other hand, free thiols are present in some protein, where they serve as

ligand for metal chelation, as nucleophiles in proteolitic enzymes, or as carboxyl activators in acyl transferases. Pro is a cyclic residue that has specific conformational effects on the peptide or protein backbone. Indeed, cyclic structure locks Pro φ backbone dihedral angle at approximately -75°. Additionally, several residues can undergo post-traslational modification to yield new amino-acids.
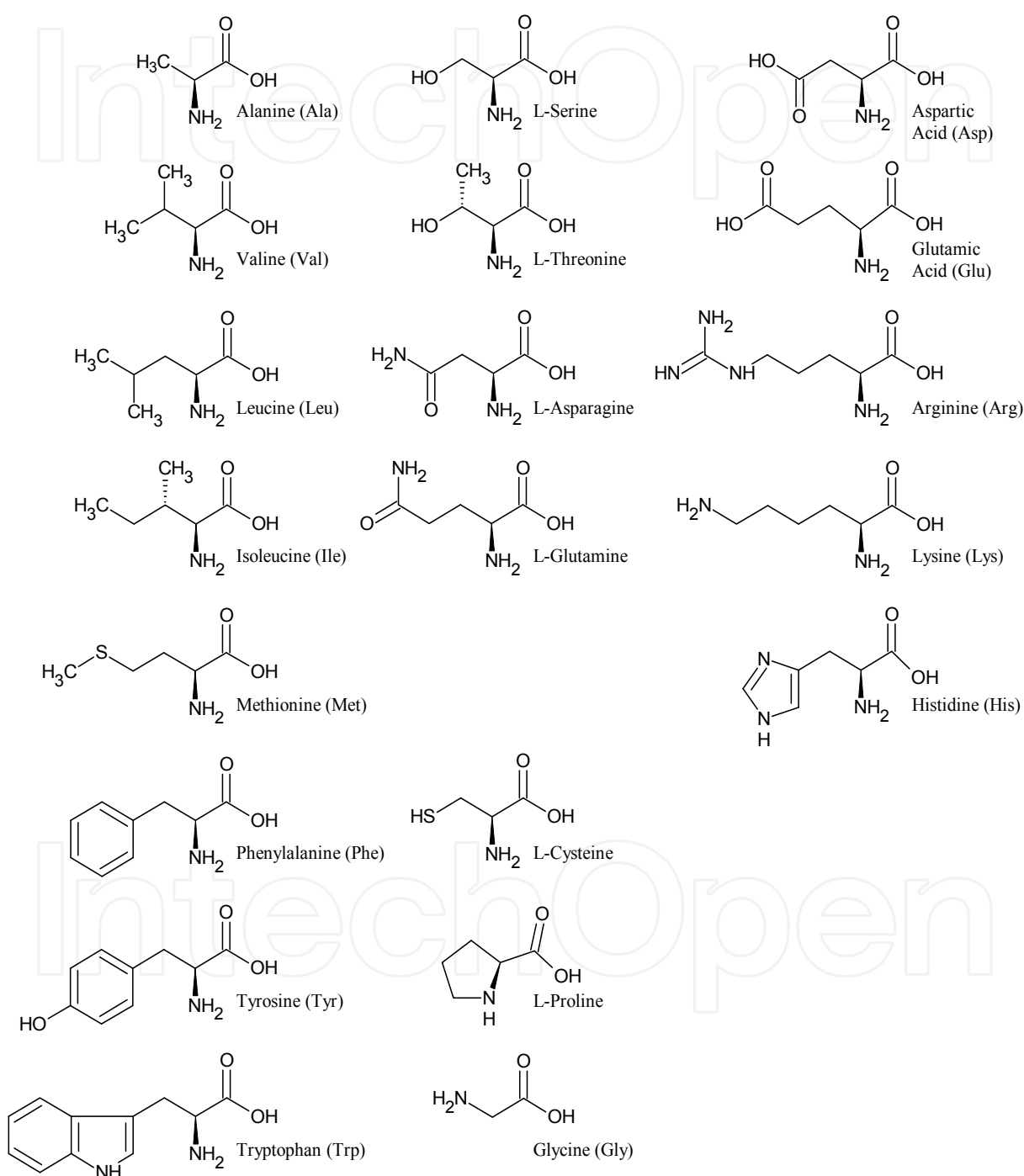

Fig. 1. L-Amino acid structures.

Small peptides typically show high conformational flexibility due to the multiple conformations that are energetically possible for each residue. The conformation of the

peptide backbone can be described by three torsional angles (Figure 2): φ, which is the angle defined by C(O)—N—Cα—C(O); ψ, which is defined by N—Cα—C(O)—N; and ω, which is defined by Cα—C(O)—N—Cα. The ω angle for the peptide bond is generally trans (ω = 180°) except for the Xaa-Pro bond, which can be cis (ω = 0°) or trans. Pioneering work of Ramachandran et al. (1965) resulted in the so-called Ramachandran plots which restrict the allowed values for the torsional angles φ and ψ to most amino acids. The conformational space accessible to the L-amino acids is about one third of the total structural space. Nevertheless the remaining degrees of freedom still make a prediction of structure extremely difficult. There are only few examples reported in the literature where short to medium sized peptides (<30-50 residues) adopt a stable structures in aqueous solution (Grauer & Köning, 2009). In most cases they exist in numerous dynamically interconverting conformations.
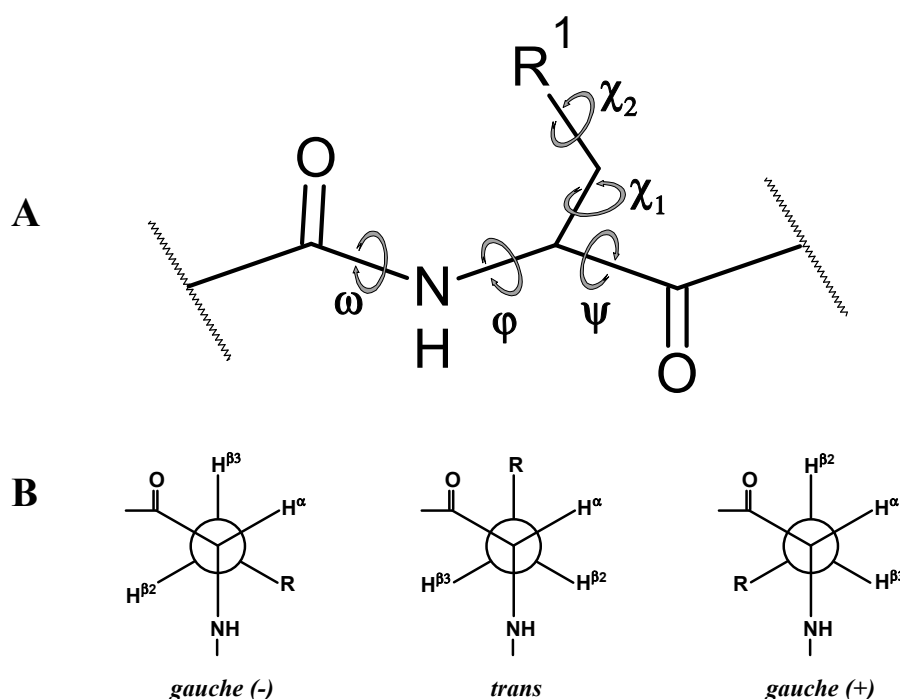

Fig. 2. (**A**) Backbone and side chain torsional angles; (**B**) Newman projection of three staggered rotamers in L-amino acids.

An equally critical area for the design of bioactive peptides, though much less explored, is that of the three-dimensional structures of the side chain moieties. This is described by the side chain χ torsional angle ($χ_1$ is defined by the N-Cα-Cβ-Cγ) (Figure 2). It can assume three low energy staggered conformations (rotamers): *gauche*(+); *gauche*(-); and *trans*. The energy differences between these conformations is not high, but the orientation of the side chain group of an L-amino acid residue relative to the peptide backbone is dramatically different: for *gauche*(-) $χ_1$, the side chain points toward the N-terminus of the peptide chain; for *trans*, the side chain points toward the C-terminus; and for *gauche*(+), the side chain points over the peptide backbone (Hruby et al., 1997). The consequences for both the surface that is created by the peptide ligand and its complimentarity for a receptor/acceptor are critical for successful recruitment of the target from small peptides as confirmed by structure-activity relationship studies (Hruby et al., 1997).

The most important technique for structural determination of biomolecules is the x-ray diffraction analysis on single crystal, even if it is questionable whether the solid state conformation determined by X-ray analysis are identical to those occurring in solution or during the interactions with the biological target (Kessler, 1982; Wuthrich et al, 1991). Over the last decade, NMR spectroscopy has emerged as an important tool for structural determination of biomolecule in solution. The increase in magnet technology and in the speed and data storage capacity of modern PCs allowed the development of multi-dimensional NMR methods which permit to solve in detail the resonance assignment of biomolecules and to determine proton-proton distances at the base of NMR structures determination.

In addition to these powerful techniques, also fluorescence and circular dichroism (CD) studies provide useful information about the peptide conformation in solution and its capability to interact with target molecules (Ruzza, 2001; Siligardi, 2011).

2.1 Methodologies for design peptides

Conformational considerations in peptide synthesis remain one of the greatest challenges in the design of biologically active peptides. Peptides will usually be an ensemble of conformation states in solution. If biological activity involves only one discrete conformer, this conformational ensemble represents a dilution of the biologically active species.

This problem is more acute for peptides designed to mimic a portion of a protein structure, where the intramolecular interactions characteristic of the protein structure are lost (Figure 3). A strategy for reducing the number of the accessible conformations and rendering the selectivity of synthetic peptides more stringent than that afford by the sequence could take advantage by the introduction of local or global constraints into peptide sequences.
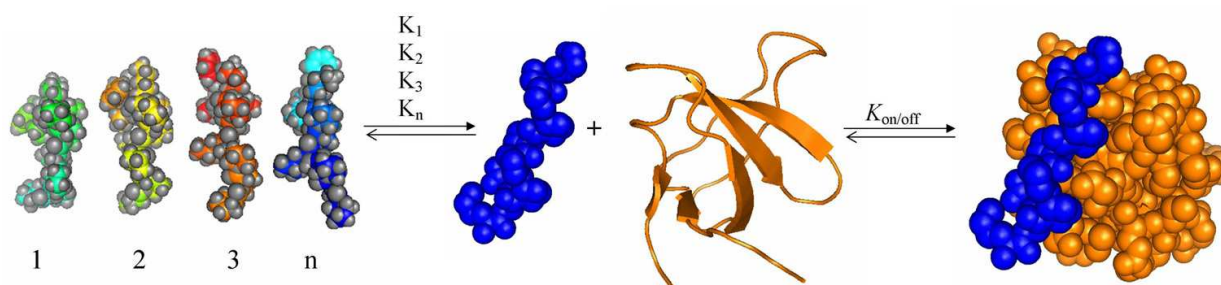

Fig. 3. Binding equilibrium involving a conformational manifold of the proline-rich peptide. The association process consists of the redistribution of the conformational ensemble from the binding-incompetent to the binding-competent (PPII) species, and then of the interaction of the latter with the SH3 domain (adapted from Ruzza et al., 2006).

2.1.1 Global restrictions

The simplest way to introduce a conformational constraint into a peptide sequence is by cyclization (Figure 4). This typically increases the *in vivo* stability of the cyclic peptides compared to their linear analogs. Cyclization can be obtained by connecting the N- with the C-terminus (head-to-tail) portion of the peptide sequence, or the couple of either the N- or the C-terminus with one of the side chains (backbone-to-side chain), or the couple of side chains not involved in specific interactions with other (side chain-to-side chain).

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 a limited section of the polypeptide is constrained. To overcome this problem several covalent bridges may be incorporated into one sequence (Grauer & Köning, 2009).
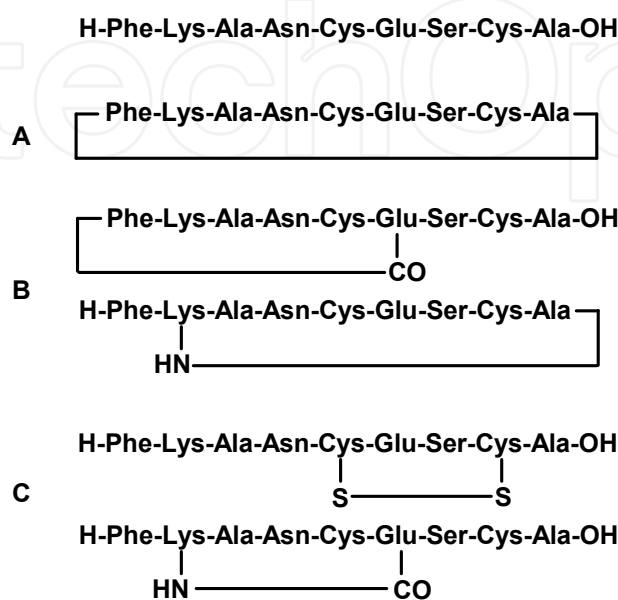
H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala-OH

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

Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala-OH
CO

B
H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala
HN

H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala-OH
S——————S

C

H-Phe-Lys-Ala-Asn-Cys-Glu-Ser-Cys-Ala-OH
HN —————— CO

Fig. 4. Examples of peptide cyclization: (**A**) head-to-tail, (**B**) backbone-to-side chain, and (**C**) side chain-to-side chain.

2.1.2 Local restrictions

The simplest local constraints that can be placed on a given residue involve the substitution of a methyl group for an hydrogen adjacent to a rotable bond (Figure 5). One substitution which have been extensively investigated in the last years involve the α-hydrogen yielding Cα-tetrasubstituted α-amino acids (Toniolo, 2001, and reference therein). For example, replacing the α-hydrogen on alanine with a methyl group gives α-aminoisobutyric acid (Aib). This residue was found in peptide sequences from a fungal source. The steric bulk of the methyl group reduced the rotational freedom of the two peptide backbone angles φ and ψ. In the case of Aib, the allowable φ and ψ backbone angles in peptides are restricted to values near –57°, -47° and +57°, +47° (Karle, 1996).

The introduction of an alkyl group either at the β-position or on aromatic ring of naturally occurring amino acids rigidifies the conformational flexibility of the side chain. Three of natural amino acids show β-disubstitutions: Val (two methyl groups), Ile (a methyl and an ethyl) and Thr (a methyl and a hydroxyl). Additionally, β-substitution leads to a second asymmetric center in the amino acid structure. These modifications do not greatly perturb the backbone, allowing the peptide backbone and the side chain some degree of flexibility, which often is crucial for the activity of peptide mimetic. Another advantage of these modifications is that the extra alkyl groups can enhance the lipophility of peptide, and therefore can help it to overcome membrane barrier (Hruby et al., 1997; and references therein).

Surely, the introduction of a covalent bond between the aromatic ring of an α-amino acid residue and the peptide backbone has proven to be a useful further conformation restriction. For example, 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) is a cyclic constrained analog of phenylalanine (Figure 5), in which a methylene bridge is placed between the α-nitrogen, and 2'-carbon of the aromatic ring (Kazmierski & Hruby, 1988).
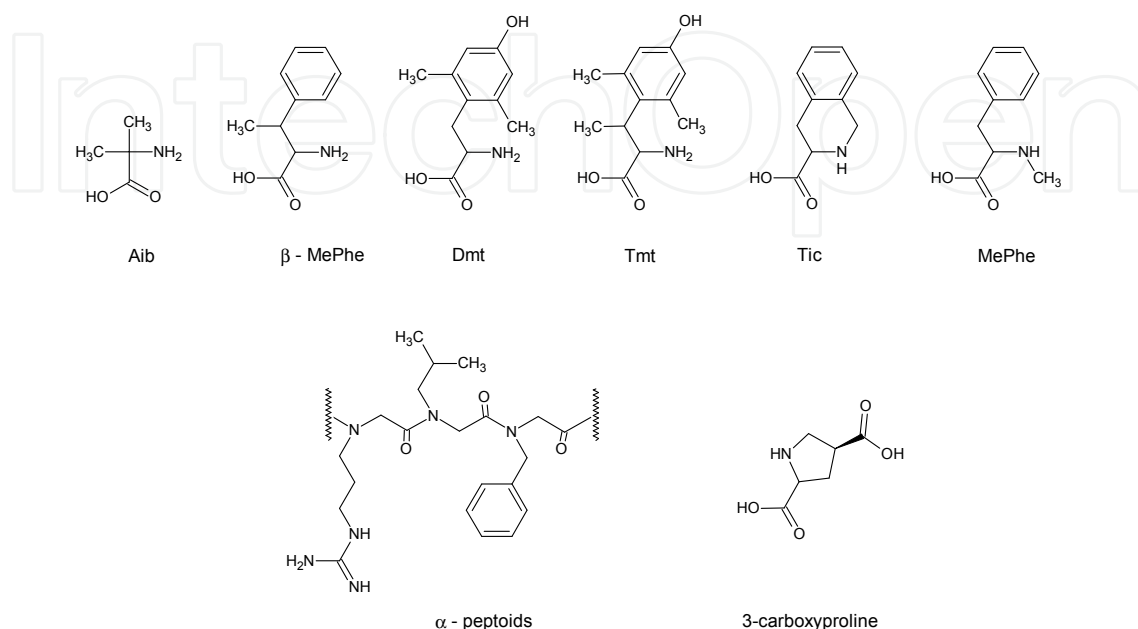

Fig. 5. Structures of some local constraints.

N-alkylated residue, and in particular N-methylation is an important modification of the peptide bond that influence the conformational freedom of the peptide backbone. The steric constraints introduced by the N-alkyl group have also effect on the side chain freedom of the neighboring amino acids. Additionally, the number of inter- and intra-molecular hydrogen bonds decreases 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.

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 5) (Zuckermann & Kodadek, 2009). The conformational change in the N-substituted glycines makes the α-carbon achiral so that peptoids are less restricted in their spatial conformation. Neither peptoids can 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.

A further investigated group of local constraints are derivatives of the natural amino acids proline. Proline analogs displaying the characteristics of other amino acids are referred to as proline-amino acid chimeras, and have been used to study the spatial requirements for receptor affinity and biological activity of both natural amino acids and peptides. For example, β-substituted-prolines such as 3-carboxyproline, 3-phenylproline, and 3-di-methylproline combine amino acid side chain functionality with proline's conformational

rigidity. In these cases, replacement of the natural amino acids in peptides by proline-amino acid chimeras provided better understanding of the bioactive conformations of peptides binding to receptors (Quancard et al., 2004; and references therein).

2.1.3 Backbone modification

The backbone of a peptide can be modified in various ways by isosteric or isoeletronic substitution (Cudic & Stawikowski, 2007; and reference therein). Figure 6 summarizes the most important ways to modify the peptide backbone.
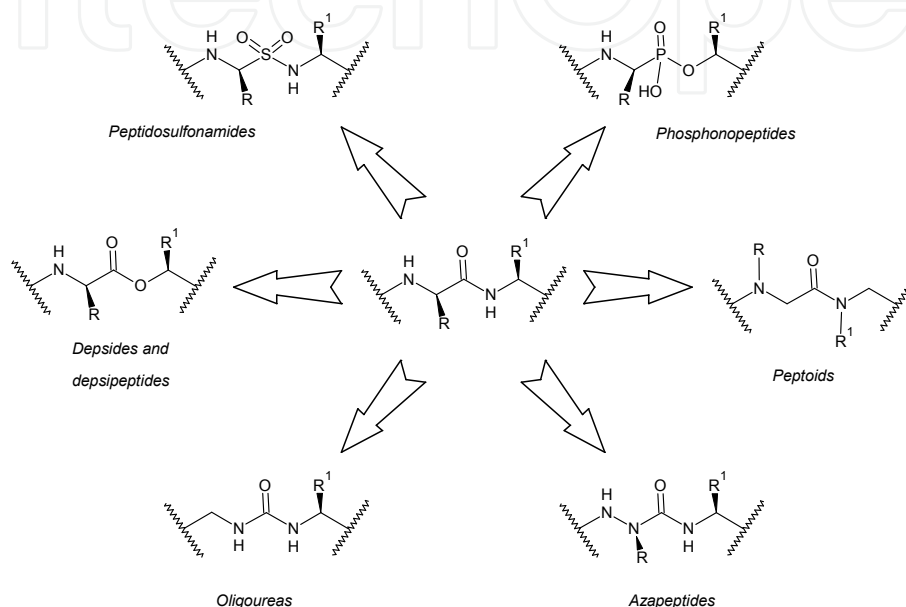

Fig. 6. Most important peptide bond surrogates (adapted from Cudic & Stawikowski, 2007).

Various peptidomimetics containing pseudopeptides or peptide bond surrogates, in which peptide bonds have been replaced with other chemical groups, are designed and synthesized with the aim to obtain peptide analogs with improved pharmacological properties. This is mainly because such approaches create an amide bond surrogate with defined three dimensional structures and with significant differences in polarity, hydrogen bonding capability and acid-base character. Also important, the structural and stereochemical integrities of the adjacent pair of α-carbon atoms in these pseudopeptides are unchanged. The psi-bracket (ψ[ ]) nomenclature, introduced by A. Spatola (1983), is used for this type of modification. The introduction of such modifications to the peptide sequence is expected to completely prevent protease cleavage of amide bond and significantly improve the peptides metabolic stability. However, such modifications may also have some negative effects on peptides biophysical and biochemical properties, in particular their conformation, flexibility and hydrophobicity. 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 (Cudic & Stawikowski, 2007). The ability of the surrogate 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. From the synthetic point of view, the methods for assembly of peptidosulfonamides, phosphonopeptides, oligoureas, depsides, depsipeptides, peptoids

and azapeptides are parallel those for standard solid-phase peptide synthesis, although different reagents and different coupling and protecting strategies need to be employed.

2.2 Methodologies for peptide synthesis

Methods for synthesizing peptides are divided into two classes: solution or liquid phase (classical) and solid phase (SPPS). The classical methods have evolved since the beginning of the last century, and they are described amply in several books and reviews (Goodman et al., 2004; Bodansky, 1993). The SPPS was conceived and elaborated by R.B. Merrifield beginning in 1959, and it has also been covered compressively (Merrifield, 2006; Chan and White, 2000).

Solution synthesis retains value in large-scale manufacturing and for specialized laboratory application. However, the need to optimize reaction condition and purification procedures for the different intermediate renders this method time-consuming and laboratory-intensive. Consequently, most workers, now requiring peptides for their research, opt for the more accessible SPPS approach.

The concept of the SPPS can be illustrated in Figure 7 where the C-terminal protected amino acid is attached to an insoluble polymeric support via a labile linker. Subsequently, the anchored peptide is extended by a series of deprotection/coupling cycles, which are required to proceed with high yields and fidelities. The reactions are driven to completion by the use of excess soluble reagents, which can be removed by simple filtration and washing without losses. Once peptide has been accomplished, it is necessary to release protected residues and to cleave the crude peptide from the solid support. These two operations can be performed simultaneously or in separate step, according to the successive destine of the peptide. Finally, the synthetic peptide must undergo purification step and characterization to verify the desired structure.

The two major chemistries for SPPS involve the use of either base labile α-amino protecting group (Fmoc) or acid labile α-amino protecting group (Boc). Each method involves different side chain protecting group, and consequent cleavage/deprotection methods and resins (Table 1).

Peptide purification and peptide quality evaluation is performed by HPLC. Most crude peptides are purified by reversed phase HPLC to achieve the desired purity. The combination of cation or anion exchange HPLC purification followed by RP-HPLC provides a powerful technique to purify a crude peptide with inferior quality. Mass spectra by MALDI or ESI-TOF and mono- and bi-dimensional NMR are the standard analytical procedure to assess peptide identity.

Recently, convergent synthesis strategies for the generation of highly complex branched peptides or scaffolded peptides and proteins have been developed. Such ligation reactions include the formation of thiazolidines or oximes from mutually reactive precursors, as well as native chemical ligation through reaction of a peptide thioester with an N-terminal cysteine in aqueous buffers, or the generation of [1,2,3]-triazoles through 1,3-dipolar cycloadditions of alkynes to azides, which belongs to a group of reactions referred to as click chemistry, which proceed at room temperature in the presence of copper(I) as a catalyst.
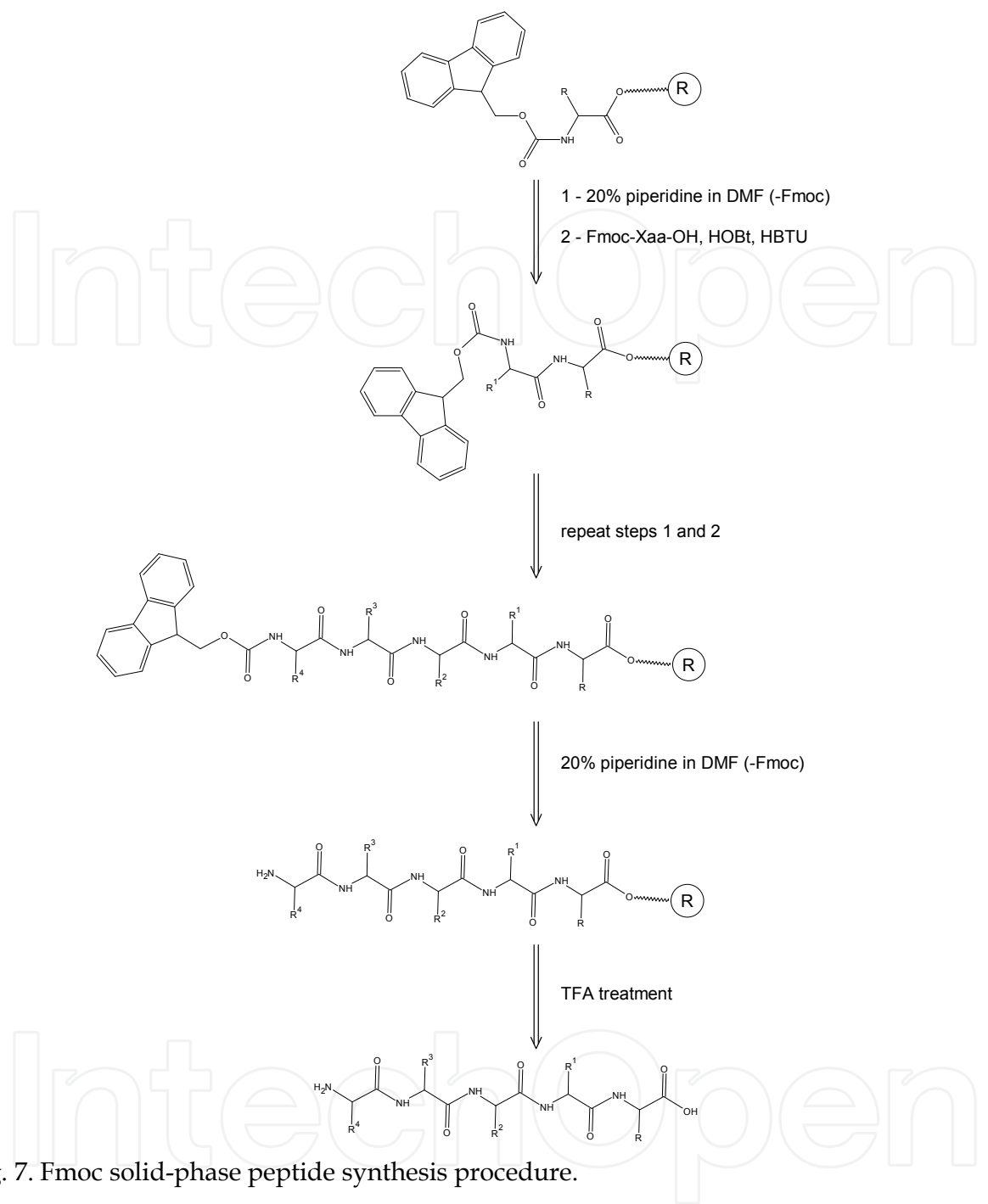
Fig. 7. Fmoc solid-phase peptide synthesis procedure.

Topic	Fmoc chemistry	Boc chemistry
Side chain protection	Acid sensitive	Strong acid sensitive (HF)
N$^\alpha$-deprotection	20% piperidine in DMF	50% TFA in DCM
Final cleavage	TFA in SPPS vessel	HF (special equipment)
Automation	Yes	Yes
Synthetic steps	Deblock, wash, couple, wash	Deblock, neutralization, wash, couple, wash
Resin	Acid or super-acid sensitive	Merrifield type

Table 1. Comparison of Fmoc- and Boc-SPPS.

3. Peptides and molecular recognition

In the post-genomic era, the importance of protein-protein interactions is becoming even more apparent. Proteins continuously interact each other to govern signaling pathways within and between cells. Biological signaling requires that protein complexes are formed and activated at the right time and in the right place, and that their formation is both reversible and transient. The strength and duration of a signal may be critical for the effects of hormones, cytokines and growth factors, and a large number of specific protein interaction domains are known which mediate this machinery (Pawson, 2004).

Although it is possible to derive some general principles of protein-protein recognition from experimentally determined structures, recent structural studies on protein complexes formed during signal transduction illustrate the remarkable diversity of interactions, both in term of interfacial size and nature. There are two broad classes of complexes: "domain-domain," in which both components comprise pre-folded structural units, and "domain-peptide," in which one component is a short motif that is unstructured in the absence of its binding partner.

Structure-based drug design seeks to identify and modulate such interactions. This optimization process requires knowledge about interaction geometries and approximate affinity contributions of attractive interactions that can be gleaned from crystal structure and associated affinity data.

3.1 Peptide-receptor ligands in cancer imaging

Since the first histological evidence of the high expression of somatostatin receptors in pituitary adenomas (Reubi, 1984), many other human cancers were found to overexpress peptide receptors in vitro (Table 2). Based on these results, peptide receptor ligands labeled with different probes (radionuclides, magnetic and optical probes) have been started to be developed for the in vivo targeting and imaging of tumors.

Receptor	Tumor type
Somatostatin (hSSTR1 – hSSTR5)	Neuroendocrine, non-Hodgkin's lymphoma, melanoma, breast, pancreatic, gastric, colon, prostate, lung, SCLC, MTC
Cholecystokinin (CCK-A and CCK-B)	MTC, SCLC, pancreatic, astrocytoma, ovarian
Vasoactive Intestinal Peptide (VPAC1 and VPAC2)	SCLC, colon, gastric, breast, pancreatic, prostate, urinary bladder, lymphoma, meningioma
Neurotensin (NTR1 – NTR3)	SCLC, colon, exocrine pancreatic, prostate
Bombesin (BRS-1 – BRS-4)	SCLC, glioblastoma, colorectal, gastric, prostate, ovarian, breast
$\alpha_v\beta_3$ Integrin	Melanoma, neuroblastoma, breast
α-MSH (MC1R – MC5R)	Melanoma
Neuropeptide Y (Y1 – Y6)	Neuroblastoma, glioblastoma, breast
Substance P	SCLC, MTC, glioblastoma, astrocytoma, breast

Table 2. Peptide receptors overexpressed in tumors (adapted from Ruzza & Calderan, 2011).

The distribution and significance of the different peptide receptors as well as the properties and characteristics of their ligands have been discussed in manifold reviews (Bolzati, 2010; Lee, 2010; and references therein).

Development of labeled peptide probes relied on isolation of naturally occurring peptides (Table 3), screening of synthetic or phage libraries, and structure-based rational design. Generally, peptide-based probes are designed starting from naturally occurring peptide hormones, which, excepting the indispensable amino acids involved in biological activity, are modified to prolong their half-lives in vivo.

Peptide	Sequence
Somatostatin (14)	H-Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH
Somatostatin (28)	H-Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH
Chlocystokinin	H-Lys-Ala-Pro-Ser-Gly-Arg-Met-Ser-Ile-Val-Lys-Asn-Leu-Gln-Asn-Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr(SO$_3$H)-Met-Gly-Trp-Met-Asp-Phe-NH$_2$
Gastrin	Pyr-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$
CCK8	H-Asp-Tyr(SO$_3$H)-Met-Gly-Trp-Met-Asp-Phe-NH$_2$
Minigastrin	H-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$
Vasoactive Intestinal Peptide (VIP)	H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH$_2$
Neurotensin	Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
Bombesin	Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$
Gastrin Releasing Peptide	H-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$
Neuromedin B	H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH$_2$
RGD	$_c$[Arg-Gly-Asp-D-Phe-Lys]
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH$_2$
β-MSH	H-Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH
γ-MSH	H-Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-NH$_2$
ACTH	H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH
GLP-1	H-His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-NH$_2$
Neuro Peptide Y	H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH$_2$
Calcitonin	H-$_c$(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$_2$

Table 3. Peptide receptor ligand sequences.

A linker is usually introduced between the imaging probe and the ligand, with the aim to preclude or minimize unwanted interferences between these two moieties. Linkers may act as a pharmacokinetic modifier and have a profound impact on the biodistribution of the whole molecule (Rufini, 2006; Schottelius, 2004). The most popular linkers are short amino

acid sequences (i.e., dimer o trimer of β-Ala, Gly or N^ϵ-amino hexanoic acid) or low molecular weight polyethylene glycole (PEG) or hydrocarbon chains. Lipophilic molecules are cleared from the body by the hepatobiliary route, whereas hydrophilic probes are mainly cleared via the renal system.

Natural occurring peptide-ligands are usually recognized by more than one receptor subtype. A great debate is ongoing about the opportunity to increase the ligand-specificity towards receptor subtypes expressed on tumor cells. Several studies demonstrated that there are significant variations in the expression profile of subtype peptide receptors also between the primary tumor and its metastases or even within the same tumor (Reubi, 2002; Reubi, 2003) and so, an increase of receptor subtypes selectivity might be counterproductive in the development of imaging molecules. On the other hand, subtype-selective ligands are useful tools for functional imaging purposes as well as to study the changes in receptor expression and response to therapeutic interventions.

Until recently the use of receptor-antagonists has not been considered for in vivo targeting of tumors overexpressing G-protein coupled receptors and this due to the low capability of antagonist to internalize into tumor cells. Indeed, the cell internalization of imaging probes has been regarded for a long time as a fundamental prerequisite of labeled peptides, providing high contrast imaging. Recently, interesting results have been obtained with two somatostatin antagonists towards SST2 and SST3 receptor subtypes, which showed high tumor accumulation and retention, resulting in matchless imaging properties (Ginj, 2006). The benefit of the use of antagonist peptide ligands has a considerable impact on those systems, in which the binding of agonist-ligand either stimulates tumor growth or exerts unwanted pharmacological action.

Many authors have demonstrated that different peptide receptors are co-expressed on tumors in a heterogeneous manner, e.g., breast cancer (expressing concomitantly GRP- and NPY-receptors), gastrointestinal stromal tumor (GRP, CCK-B, and VPAC2 receptors), gastroenteropancreatic neuroendocrine tumors (GLP-1, CCK, and VPAC1 receptors), and the prostate cancer (prostate-specific membrane antigen or PSMA, and integrin or GRP receptors) (Ruzza & Calderan, 2011). These suggest that targeting more than one receptor class simultaneously would greatly enhance the sensitivity of tumor detection. In addition, some peptide receptors (e.g., αvβ3 integrin) are characterized by a low ligand internalization capacity, which improves in the presence of multimer ligands (Liu, 2010).

These aspects (co-expression on tumors and low internalization capacity) stimulate the development of multivalent and/or multireceptor ligands able to bind either to multiple homo (multivalent) or to hetero (multireceptor) receptors present on the surface of the tumor cell. The presence of more ligands induces a number of peculiar biological characteristics to the targeting molecules that are not present in the monovalent ligand. Polyvalent interactions are generally much stronger than the corresponding monovalent and offer the basis for mechanisms of agonizing or antagonizing biological activities that are fundamentally different from those available in monovalent system. Multivalent interactions may become particularly attractive and biologically relevant when the ligand-receptor binding is weak or the receptor density is low.

Linkers and/or scaffolds structure is important because it must present ligands simultaneously to their cognate receptors with minimal entropic penalty. The development

of suitable linkers and/or scaffold structures, permitting the correct simultaneous presentation of ligands to their receptors, as well as the choice of opportune ligands for multivalency and/or multireceptor approaches may provide both new targets and strategies for designing new imaging agents.

3.2 Peptides in protein-protein interactions: The SH3 domain

From the huge array of "domain-peptide" complex class, a super-family of small domains is represented by the SH3 domains that it is identified in more than 350 different proteins in organisms ranging from yeasts to humans, showing a completely conserved structure.

The SH3 domain is a 60-amino acids non-catalytic protein domain identified in the sequence similarity region between the Src and Abl families of non-receptor tyrosine kinases (Mayer, 1988). This domain is structured on five β-strands arranged into two sheets packed at right angle. The first sheet is formed by strands A, E and the first half of B, while the second is formed by β-strands C, D, and the second half of B. The βA-strand is connected to the βB-strand by the long RT loop, which contain the ALY(F)DY(F) motif. The loops connecting strands βB and βC and strands βC and βD are called N-Src and Distal loop, respectively. The βC strand contains the WW dipeptide motif. After the βD strand, the polypeptide chain adopts a 3_{10} helix containing the PxxY motif that connects βD and βE strands (Marchiani, 2009; and references therein).

Crystal and solution structures of SH3 domain-ligand complexes reveal that the SH3 region involved in the interaction with the proline-rich peptide is a surface patch formed by the side chains of a few well-conserved residues: 1) one of the two aromatic residues in the ALY(F)DY(F) motif; 2) the first tryptophan of the WW dipeptide; 3) the PxxY motif. These residues are aligned to form a surface patch, quite hydrophobic, in which the aromatic side chains are stacked against each other (Figure 8).

The majority of SH3 domains recognize proline-rich peptides that adopt a left-handed poly-proline type II (PPII) helix containing two XP dipeptides, separated by a scaffolding residue (often a proline), forming the XPxXP core element (Feng, 1994). A basic residue, Lys or more frequently an Arg, is present either at the N-terminal or at the C-terminal to the XPxXP motif, permitting to classify Pro-rich ligands into two distinct groups named classes I and II, respectively.

The capability to recognize Lys or Arg residue into the binding motif is a selectivity mechanism found in SH3-ligands. For example, the Crk SH3 domain is selective for class II peptide-ligands containing a Lys residue (Knudsen, 1995). High resolution crystal structures of this domain bound to proline-rich peptides containing either lysine or arginine residue shown that while the lysine side-chain is in a extended configuration and the ε-amino group makes strong hydrogen bonds with three acidic residues (Asp147, Glu149 and Asp150), the arginine side chain does not adopt a low energy extended configuration and is involved in only two hydrogen-bonding interactions with proteins (Wu, 1995).

Structural analysis of either class I or class II peptides – SH3 complexes showed that these two ligand classes bind to SH3 domains in opposite orientations at the same binding site. The fact that ligands can bind either N-to-C or C-to-N orientations is a consequence of the two-fold rotational pseudo-symmetry possessed by the left-handed PPII helix (Siligardi &

Drake, 1995). This apparent ambiguity is resolved by the observation that important determinant for the binding to the two XP pockets is the surface presented by the alkylated amide nitrogen of the proline residue, and the carbonyl, the α-carbon and the side-chain of the preceding residue (Fernandez-Ballester, 2004).
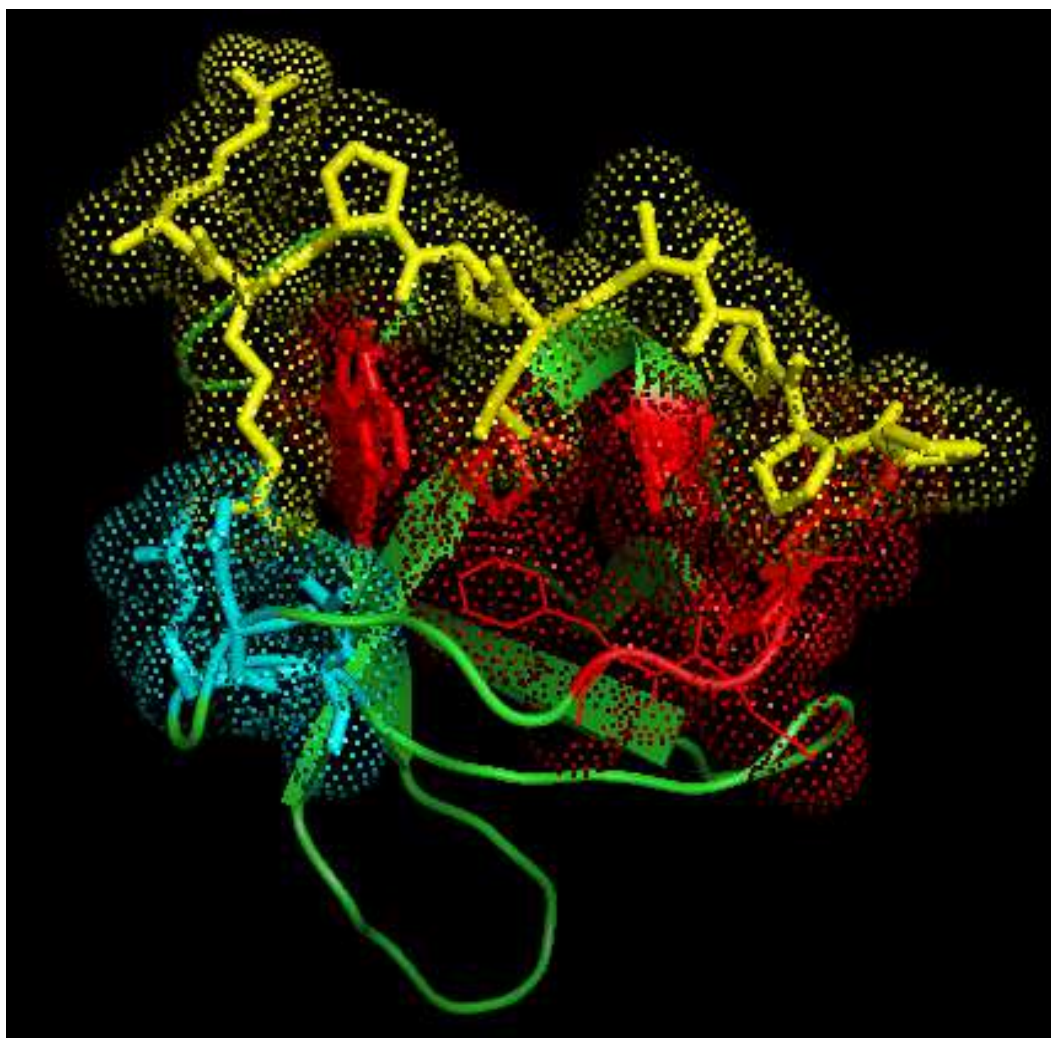

Fig. 8. The interactions occurring between the N-terminal SH3 domain of c-Crk and a lysine-containing proline-rich peptide are shown (PDB file 1CKA). The the ligand-peptide is highlighted in yellow. The aromatic surface patch is depicted in red, while the anionic side chain of the Asp and Glu residues involved in the interaction with the Lys residue in cyan. The Figure was drawn using the program PYMOL (K.L. DeLano, www.pymol.org).

Studies on the SH3 Fyn tyrosine kinase domain revealed that the Trp residue in the binding pocket can adopt two different orientations, determining the type of ligand able to bind to the domain. The motion of this residue is closely related to the presence of specific residues located in a key position; e.g. the residue numbering 132 of Fyn. When the conserved Trp residue can move, upon ligand binding, the structural conformation of the ligand fixes the conserved Trp either to SH3 class I or class II orientations. Unlike, when the motion of conserved Trp is hindered, the SH3 domain invariably presents the conserved Trp residue in a SH3 class II orientation, either in free or ligand-bound state (Fernandez-Ballester, 2004).

A recent finding demonstrated as some SH3 domains recognized not only the XPxXP sequences but that also other contacts are involved in the recruitment (Li, 2005), which may be classified into three arbitrary categories: (1) interactions that involve the XPxXP-motif docking surface of the SH3 domain to a peptide that does not conform to a classic XPxXP consensus; (2) interactions that do not involve the XPxXP-motif docking surface of the SH3 domain and (3) complex interactions, which may or may not involve residues contributing to the PxxP docking surface, but differ strongly from typical SH3 target peptide recognition.

4. Conclusion

The focus of medicinal chemistry is on the design of molecules that can manipulate disease-related biological targets for beneficial effects with low toxicity. As we have seen, peptides show great potential as both active drugs and diagnostics. The discovery and development of peptide-based drugs have both rational and empirical aspects. Random screening procedures can be used to identify ligands for known functional domains of target proteins, which can be followed by successive structural and computational analysis.

The principal medicinal chemistry challenges for a peptide chemist are to design molecules characterized by a sufficient duration of action, sufficient receptor specificity, and a both stable and appropriate formulation. Recently, studies of self-organizing peptides (amyloids) yielded important information for the development of long-acting peptides. Peptide constraint has been used both to prevent proteolysis and to bias binding toward particular receptor subtypes. The latter activity appears still to be evolving into a rational design approach but still requires attention to an appropriate strategy for successful commercial development.

Many of these issues are reminiscent of the "rocky road" which other biotecnology drugs, e.g., monoclonal antibodies, had to take before they became a commercial success. However, the development of peptides into tools for diagnostic purposes and drugs, based on their specificity of target recognition and their versatility of mechanisms, offers enormous promise. While peptides as drugs is a concept that still involves considerable challenge, encouragement may be gleaned from the words of Arthur C. Clarke (1917 – 2008) "...we do not have all the answers, but we have plenty of questions certainly worth thinking about; and when the technology is finally sufficiently advanced it is indistinguishable from magic".
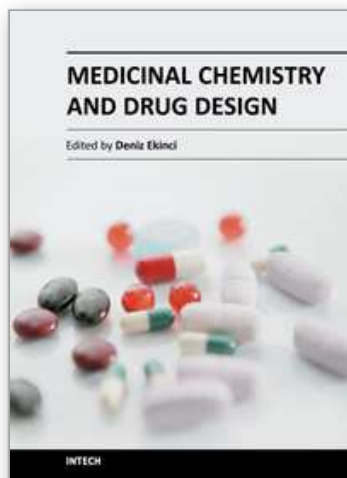
5. Acknowledgment

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Medicinal Chemistry and Drug Design

Edited by Prof. Deniz Ekinci

Over the recent years, medicinal chemistry has become responsible for explaining interactions of chemical molecules processes such that many scientists in the life sciences from agronomy to medicine are engaged in medicinal research. This book contains an overview focusing on the research area of enzyme inhibitors, molecular aspects of drug metabolism, organic synthesis, prodrug synthesis, in silico studies and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in medicinal chemistry and drug design. Particular emphasis is devoted to both theoretical and experimental aspect of modern drug design. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in associated areas. The textbook is written by international scientists with expertise in chemistry, protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some medicinal approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of medicinal chemistry and drug design.

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