1.1. INTRODUCTION

The term bioactive peptides is referred to describe molecules of peptide nature or origin which display biological activity useful for pharmaceutical, diagnostic, chemical and agro-food applications (Shahidi et al., 2008). Bioactive peptides can also be defined as peptides with drug like activity that eventually modulate physiological function through binding interactions to specific receptors on target cells leading to induction of physiological responses. According to their functional properties, bioactive peptides may be classified as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding and antioxidative which play an important role in human health (Fitzgerald & Murray, 2006).

Peptide research has experienced considerable development during the past few decades as they influence a number of important physiological and biochemical functions of life (Sato et al. 2006). The majority of marketed peptide products and homologous compounds are peptide hormones or peptide derivatives that simulate the action of hormones. Peptides that are agonists or antagonists for receptors implicated in oncology and inflammation, as antibiotics and that act as enzyme inhibitors in a variety of therapeutic indications are increasing, being tested for efficacy at the discovery and preclinical stages suggesting that this class of drugs might soon occupy a larger niche in the marketplace (Vlieghe et al., 2010).

New therapeutic methods based on peptides for a series of diseases give rise to the hope that diseases, where peptides play a functional role, can be amenable to therapy (Lien S et al. 2003). Various chemical strategies like cyclic peptides or pseudo-peptides (modification of the peptide bond) and peptidomimetics (non-peptide molecules) preserving the biological properties of peptides are widely developed to increase the resistance to degradation and elimination, bioavailability and selectivity (targeting of protein receptor interaction). In fact from a model peptide of interest (lead peptide) it is often necessary to optimize its chemical structure (cyclization, bioisosteric replacement of peptide bonds changing the stereochemistry of an amino acid) to obtain a compound that can be therapeutically used (Bruckdorfer et al., 2004).
Thus, peptide-based drug discovery could be a serious option for addressing as yet unresolved problems. To date, hundreds of synthetic therapeutic peptides are in clinical development and even more are in advanced stages of preclinical development in the pipeline of pharmaceutical companies (Marx, 2005).

1.2. BRIEF REVIEW ON PEPTIDES AND PEPTIDOMIMETICS

1.2.1 Definition and classification of peptides

Peptides are the amides formed by the interaction between the amino group (-NH$_2$) of one amino acid and the carbonyl group (-COOH) present in another amino acid. The -CONH- in these compounds is referred as “peptide linkage”. A large number of natural and synthetic products are based upon a peptide framework and exhibit a spectrum of biological activity. Peptides are classified as linear and cyclic peptide, linear peptides with peptide linkages in linear structure and cyclic peptides contains peptide linkages in cyclic structure (Xiao-Jie Xu et al., 1996).

Linear peptides are classified with Greek prefixes as $di$-, $tri$-, $tetra$-, $penta$-, . . . $octa$-, $nona$-, $deca$ peptides, etc., according to the number of amino acid residues incorporated. In longer peptides, the Greek prefix may be replaced by Arabic figures; for example, a decapeptide may be called 10-peptide, while a dodecapeptide is called 12-peptide. According to the currently accepted nomenclature rules, “oligopeptides” are composed of fewer than 15 amino acids, “polypeptides” contain approximately 15–50 amino acids residues, and the expression “protein” is used for derivatives containing more than 50 amino acids. Cyclic peptides can be further classified as homodetic peptides, cyclic structure containing only peptide linkages such as 2, 5- piperazinediones and heterodetic peptides where cyclic structure contains both peptide bond and other linkages (Nobert Sewald et al., 2002).

The two components of amino acids are connected resulting in a dipeptide by a peptide (amide) bond formation with the elimination of water (Fig-1).
Ever since the early days of peptide chemistry, reactions involving peptide synthesis have been traditionally performed in solution, and many impressive results including the total synthesis of small proteins have been achieved using this conventional technique. Emil Fischer, the father of peptide chemistry introduced the concept of peptides and polypeptides and presented protocols for their synthesis in the early 1900s. One major advantage of solution-based synthesis is the high purity of the final product, though this is clearly dependent upon the purification of intermediate compounds (Guzman F et al. 2007).

Despite these restrictions, highly specialized and experienced research teams have successfully completed many ambitious syntheses following this technique (Mc Gregor DP 2008). Unfortunately, peptide synthesis in solution is highly labor-intensive and requires extensive knowledge with regard to the strategy and tactics in choosing protecting groups and coupling methods, as well as solving problems of solubility (Bruckdorfer et al., 2004)

The indigenous concept of peptide synthesis on a solid support which is now known as SPPS (Solid Phase Peptide Synthesis)was developed by Robert Bruce Merrifield in 1963 and provided a major breakthrough in peptide chemistry (Merrifield, B.1963). Merrifield was awarded the Nobel prize in 1984 for this unique
invention that has revolutionized organic chemistry during the past 20 years. In SPPS the peptide chain is assembled in the usual manner, starting from the C-terminus. The amazingly simple concept is that the first amino acid of the peptide to be synthesized is connected via its carboxy group to an insoluble polymer that may be easily separated from either reagents or dissolved products by the use of filtration.

Unfortunately, it does suffer from several limitations. The final product of a synthesis carried out on a polymeric support is only a homogeneous compound, if all deprotection and coupling steps proceed quantitatively. A large excess of each amino acid component is required in the corresponding coupling reaction in order to achieve complete conversion. Monitoring the reaction progress and analysis of complete conversion are difficult to perform in heterogeneous reaction systems, and are hampered by experimental error. Swelling properties of the polymeric resin and diffusion of the reagents are important parameters for the success of a solid-phase synthesis. Aggregation phenomena of the growing peptide chain may complicate the synthesis. Sometimes, drastic conditions required to cleave the peptide from the polymer may also damage the final product (Amblard M et al, 2006).

Today, the concept has been extended and generalized to organic synthesis on polymeric supports, which includes not only heterogeneous reactions involving an insoluble polymer, but also the application of soluble polymeric materials which allow homogeneous reactions (liquid-phase peptide synthesis) to be conducted. A combination of these two variants is also possible, being referred to as alternating solid-liquid-phase peptide synthesis (Miguel Castanho et al., 2011).

1.2.2 Biological activities of peptides

Therapeutic peptides traditionally have been derived from three sources: (i) Natural or bioactive peptides produced by plants, animal or human (derived from naturally occurring peptide hormones or from fragments of larger proteins. (ii) Peptides isolated from genetic or recombinant libraries and (iii) Peptides discovered from chemical libraries (Latham 1999). The isolation and targeted application of these endogenous substances as potential intrinsic drugs is gaining importance for the treatment of pathologic processes (Lien et al., 2003).
Peptides and their homologous compounds (proteins and antibodies can be used in multiple pathologies, including allergy and asthma, arthritis, baldness, cardiovascular diseases (coronary syndrome and angina) diabetes, gastrointestinal dysfunction, growth problem homeostasis, immunity disease, impotence incontinence, infective diseases (bacterial, fungal and viral inflammation), obesity, oncology, cancer and tumor imaging osteoporosis (calcium metabolism dysfunction), pain, vaccine and so on which represent important markets (Table 1) (Loffet, 2002).

Peptide-based vectors (cationic or cationized peptides or proteins which cross the BBB by adsorptive mediated transport developed for CNS drug targeting will certainly contribute to the development of peptide based prodrugs with facilitated access to the CNS (Stevenson, 2009).

Opioid peptides may be produced by the body itself, for example endorphins, or be absorbed from partially digested food. The effect of these peptides varies, but they all resemble opiates. The body’s own opioids are generally much longer. Brain opioid peptide systems are known to play an important role in motivation, emotion, attachment behavior, the response to stress and pain, and the control of food intake (Hollt, 1983). Casomorphins are peptides, i.e., protein fragments, derived from the digestion of milk protein casein. The distinguishing characteristic of casomorphins is that they have an opioid effect (Jinsmaa et al., 1999). Some examples of opioid peptides are

- γ-Endorphin: Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu
- β-Endorphin: Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys
- Casomorphin: Tyr-Pro-Phe-Pro-Gly-Pro-Ile
- Met-Enkephalin: Tyr-Gly-Gly-Phe-Met
- Leu-Enkephalin: Tyr-Gly-Gly-Phe-Leu
- Endomorphin-1: Tyr-Pro-Trp-Phe
- Endomorphin-2: Tyr-Pro-Phe-Phe

Among the immunomodulating peptides isolated from bovine caseins, the β-casein (f191-f193), αs1-casein C-terminal hexapeptide (f194-f199) stimulated macrophages. β-casein f63-f68 stimulated \textit{in vitro} phagocytosis, but this peptide as well as β-casein f191-f193 failed to exert protection of mice \textit{in vivo} (Fiat et al. 1989).

- α-casein : Arg-Tyr-Leu-Gly-Tyr-Leu-Glu
- β-casein : Phe-Gln-Glu-Gln-Gln-Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys

Synthetic peptides derived from milk proteins have been shown to enhance proliferation of human peripheral blood lymphocytes. These peptides were Tyr-Gly and Tyr-Gly-Gly and they correspond to fragments of bovine k-casein and α-lactalbumin.

Whey protein-derived opioid peptides (β-lactorphin and α-lactorphin) have been shown to moderately inhibit ACE activity. The N-terminal dipeptide (Tyr-Leu) of β-lactorphin was found to be the most potent inhibitor (Mullally et al, 1997)

- α-lactorphin : Tyr-Gly-Leu-Phe
- β-lactorphin : Tyr-Leu-Leu-Phe

Tryptic β-lactoglobulin peptide, corresponding to β-lactoglobulin (f142-f148), was found to be the most active ACE-inhibitory whey peptide so far reported. α-lactalbumin and β-lactoglobulin have also been considered as potential precursors of bactericidal fragments. Similarly, antibacterial fragments have also been derived from αs1, αs2 and k-casein. These peptides have been found to be active against broad range of pathogenic organisms such as \textit{Escherichia}, \textit{Helicobacter}, \textit{Listeria}, \textit{Salmonella} and \textit{Staphylococcus}, yeasts and filamentous fungi (Lahov et al. 1996).
Therapeutic peptides also offer several advantages over other organic molecules that make up traditional medicines. The first advantage is that often representing the smallest functional part of a protein they offer greater efficacy, selectivity and specificity (limited nonspecific binding to molecular structures other than the desired target) than other organic molecules. A second advantage is that the degradation products of peptides are amino acids, thus minimizing the risks of systemic toxicity (minimization of drug–drug interactions). Thirdly because of their short half life few peptides (receptor agonists) accumulate in tissues, generally small quantities of these peptides agonists are necessary to activate the targeted receptors (Leader B et al. 2008).

Compared with proteins and antibodies, peptides have the potential to penetrate further into tissues owing to their smaller size. Moreover, therapeutic peptides even synthetic ones are generally less immunogenic than recombinant proteins and antibodies. Peptides have other advantages over proteins and antibodies as drug candidates including lower manufacturing costs, greater stability (lengthy storage at room temperature is acceptable), reduced potential for interaction with the immune system and better organ or tumour penetration (Abba Kastin, R.C. et al. 2004).

In France, the 2008 Mercer Life Science Compensation Survey - Medical Devices Report (MEDEC) MEDEC prize of the year was awarded to a synthetic therapeutic peptide, the new antidiabetic drug Byetta (exenatide). Other therapeutic peptides, such as antimicrobial peptides, with broad-spectrum antimicrobial activity against bacteria, viruses and fungi, are promised a great future, especially in counteracting the loss of efficiency of conventional antibiotics (Rossi et al. 2008).

1.2.3 Limitations to the use of peptides as drug candidates

Bioavailability and bio distributions of drug candidates which include absorption, transport, passage of biological membranes and cellular barriers are determined by a combination of their physicochemical properties such as aqueous solubility, lipophilicity, H-bond formation, chemical stability and metabolic stability (proteolytic and or enzymatic degradation) (Veber et al. 2002).
With a few exceptions, peptides composed of natural amino acids are not very good drug candidates because of their intrinsic physicochemical properties and pharmacokinetic profiles. The main limitations generally attributed to therapeutic peptides are low oral bioavailability (injection is generally required), a short half life because of their rapid degradation by proteolytic enzymes of the digestive system and blood plasma, rapid removal from the circulation by the liver (hepatic clearance) and kidneys (renal clearance), poor ability to cross physiological barriers because of their general hydrophilicity high conformational flexibility resulting sometimes in a lack of selectivity involving interactions with different receptors or targets (poor specific biodistribution) causing activation of several targets and leading to side effects (Rossi et al., 2008).

The greatest threat to therapeutic peptides lies in the lumen of the small intestine, which contains gram quantities of peptidases secreted from the pancreas (e.g. α-chymotrypsin, trypsin, pancreatic elastase, carboxypeptidases A, B, D, N and U and so on), as well as cellular peptidases from mucosal cells. The second major enzymatic barrier is the brush border membrane of the epithelial cells, which contains at least 15 peptidases like dipeptidyl-peptidase IV, prolyltripeptidyl-peptidase, angiotensin-converting enzyme, leucyl-aminopeptidase, aminopeptidase M, aminopeptidaseA, neprilysin, etc., that together have a broad specificity and can degrade both peptides and proteins (Woodley, 1994).

Lysosomal peptidases (leukocyte elastase, cathepsins B and D, and so on) will also target peptides or proteins endocytosed by epithelial or endothelial cells. In the matrix metalloproteinase family (zinc-dependant endopeptidases) known to degrade extracellular matrix proteins, interstitial collagenase is also able to cleave specific small molecules such as peptides. Among proteases, carboxypeptidase C sometimes referred to as Y is the only enzyme that exhibits both the esterase and the amidase activities typical of serine proteases (Kumar et al., 2005).

1.2.4 Chemical Strategies to improve peptide biological activity

As discussed previously the low bioavailability of peptides is due in part to high biodegradability by gastrointestinal, plasma and tissue peptidases. Moreover, their rapid removal from the circulation can also limit their therapeutic use.
Absorption, distribution, metabolism and excretion processes play a pivotal part in defining the disposition of a drug candidate and thus its therapeutic effect. To develop a peptide as a therapeutic agent its biological effect, pharmacokinetic profile and low immunogenicity are crucial parameters (Kerns et al., 2003).

The chemical optimization strategy of a therapeutic peptide is based on structure activity relationship and/or quantitative structure activity relationship studies of newly synthesized peptide derivatives, with the aim of improving bioavailability, reducing elimination and biodegradation and increasing selectivity or affinity to its receptor or target. Lead peptide chemical optimization usually required different strategies developed by peptide chemists (Hummel et al., 2006)

1.3 PEPTIDOMIMETICS

A peptidomimetic is a compound that incorporates secondary structural features analogous to a naturally occurring peptide, thus enabling it to mimic the biological function of the parent peptide. Peptidomimetics have become increasingly important in the development of therapeutic agents due to their ability to resist enzymatic degradation and hydrolysis, while displaying enhanced bioavailability and bioselectivity. The advantageous therapeutic profile of peptidomimetic compounds has made this class of compounds an attractive synthetic target and encouraged their isolation as natural products (Dorner, 1997)

Modern advances in the development of peptidomimetics as medicinal agents and as biological probes have been attributed primarily to the development of two key areas of modern drug discovery. Combinatorial chemistry, which allows the generation of vast libraries of organic compounds, in conjunction with the hardware for mass screening of these libraries to identify new lead compounds. The advent of solid phase organic chemistry has been critical to the preparation of these compound libraries, which range from those purely peptide in nature to libraries of non-peptide mimetic.

The activity of lead compounds, identified through mass-screening, can in turn be optimized by structural and functional variation of the original peptidomimetic platform. Mass screening can also be used to identify lead
compounds derived from libraries of natural products, which in turn can undergo refinement through investigation of the structure-activity relationship. The key development in the rise in popularity of peptidomimetic agents has been the development of modern chemical tools that allow the structural biologist to visualise target receptors and native peptides. With this technology comes an understanding of the topochemical, conformational and electronic properties of native peptide ligands, or correspondingly, the receptors (Silverman et al., 2004).

By combining modern nuclear magnetic resonance (NMR) spectroscopy, computer modelling and the data from X-ray structural analysis of peptides, it has become possible to design tailor-made peptidomimetic compounds (Stevenson et al., 2009).

1.3.1 Lead Peptide Chemical Optimization

- Search for the minimum active sequence from N-and / or C-terminal truncated analogues.

- Significance of the N-and C-terminus towards biological activity.

- Deletion of one or more consecutive amino acid(s) and combinatorial deletion with two or more positions omitted independently throughout the sequence.

- Simplification and/or optimization of the structure after alanine scanning (Ala-Scan) and or D-Scanning (D-scan) to eliminate potential sites of cleavage (notably by endopeptidases) and to determine important functional groups involved in the interaction with the target of interest.

- Cyclization of the peptide sequence (between side chains or ends of the peptide sequence): head to tail, N-backbone to N-Backbone, end to N-Backbone, end to side chain, side chain to N-backbone side chain to side chain through disulfide (disulfide-bond cyclization scan) lanthionine, dicarbahydrazine or lactam bridges to decrease the conformational flexibility of linear peptides, to reduce hydrogen bonding to enhance membrane permeability and importantly to increase their stability to proteolysis by endo and exopeptidases.
✓ Substitution of a natural amino acid residue by an unnatural amino acid (d-configuration) an N-methly-amino acid, a non-proteogenic constrained amino acid or β amino acid to increase plasma stability (e.g. resistance to endopeptides) of the peptide.

✓ Isosteric, amide bond replacement between two amino acids: NH-amide alkylation, the carbonyl function of the peptide bond can be replaced by CH₂ (reduced bond - CH₂-NH-), C(=S) (endothiopeptide, -c(=S)-NH-) or PO₂H (phosphonamide. –P(=O)OH-NH). NH-amide bond can be exchanged by O (depsipeptide, -CO-O-). S (thioester, -CO-S) or CH₂ (ketomethylene. –CO-CH₂-). The peptide bond can also be modified: retro-inverso bond (-NH-CO-). Methylene-oxy bond (-CH₂-O) thiomethylene bond (-CH₂-S-), carba bond (-CH₂-CH₂-), hydroxyethylene bond (-CHOH-CH₂) and so on, to increase plasma stability of the peptide sequence (notably towards endopeptidases).

✓ Blocking N- or C-terminal ends by N-acylation, N-pyroglutamate, C-amidation and so on, or addition of carbohydrate chains (glycosylation: glucose, xylase, hexose and so on) to increase plasma stability (notably, resistance towards exopeptidases).

✓ N-terminal esterification (phosphoester) or pegylation modifications to enhance plasma stability (e.g. resistance to exopeptidases) and to reduce immunogenicity. Pegylation is also designed to make the peptide larger to retard excretion through the kidneys (renal clearance).

1.3.2 Conformational restriction

A stretched out or randomly orientated protein is generally devoid of biological activity. Protein function is achieved through the spatial arrangement of the peptide backbone, which in turn is defined by the amino acid sequence. This highlights an important concept applicable to peptidomimetic research. Enhanced biological activity can be achieved by mimicking the biologically active conformation of the native substrate. Additional structural elements can be incorporated into peptidomimetic design to force rigidity and achieve the desired bioactive conformation (Grauer et al., 2009).
These rigid structural features ensure the correct positioning of specific functionalities in order to optimize hydrogen bonding, electrostatic and hydrophobic interactions between the peptidomimetic ligand and the receptor. To this effect, peptidomimetic ligand can be designed to be preorganised into a bioactive conformation by the inclusion of rigid structural elements. Rigid peptidomimetic analogs play a lower entropy cost upon binding to the target receptor, and therefore should bind more avidly and assume a preorganised placement of pharmacophoric residues (Victor J. Hruby, 1982).

1.3.3 Modification of the peptide backbone

Modification of the peptide backbone refers to the generally isosteric or isoelectronic exchange of units in the peptide chain and the introduction of additional fragments. Conformational restriction is imparted from these modifications by electronic and steric effects of the backbone replacements. Backbone modifications lower the peptide character of the ligand, thereby increasing proteolytic stability, whilst retaining favorable properties of the parent peptide (Giannis, 1993).

1.3.4 Modifications to amino acid side-chains

A well established method of incorporating conformational restriction into peptidomimetics is through the modification of naturally occurring amino acid side chains. Conformational restriction is possible by introducing sterically demanding groups so as to limit free rotation of the amino acid residue. Modification of the tyrosine(1) side chain by introducing methyl groups in the 2',6', and β-positions hinders free rotation about the Cβ-Cα bond restricting the analogue(2) to a preferred conformation. Phenylalanine (3) derivative has been incorporated into potent peptidomimetic ligands of the angiotensin II receptor as a constrained phenylalanine mimic (4). Compound (5) is a phenylalanine analogue that is conformational constrained, so that the dihedral angle about the C-C bond has a very narrow range. This unnatural amino acid has been incorporated into various opioid antagonists (Hsieh et al., 1989).
1.3.5 Conformationally-stabilising rings

The conformation of ligands can be fixed by the introduction of short and long-range cyclisations within the peptidomimetic backbone. The bridging can occur within a single amino-acid residue (6) or may involve several amino acid residues (7). In general the bridging unit will link two side chains (8) or a side chain bridged to the backbone (9) or between two backbone units (10) (Abell, 2002).

1.3.6 Macrocyclic peptidomimetics

Many biologically active macrocyclic peptides are found in nature. Their biological activity, in contrast to their acyclic counterparts, is due in part to the inherent conformational restriction provided by a cyclic system. The relatively restricted conformation of the macrocyclic peptides 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 (Victor Hurby, J., 2000).

The notion of macrocyclic peptidomimetic inhibitors has been successfully applied to the inhibition of protease (HIVp), a key proteolytic enzyme in the replicative cycle of immune deficiency virus (IDV). This proteolytic enzyme selectively recognizes and cleaves extended substrate conformations. Structural information derived from the X-ray structure of IDVp-inhibitor complexes has enabled researchers to design and synthesize existing IDVp inhibitors that are constrained within a macrocyclic array. IDVp inhibitors (11) and (12) have been incorporated into cyclic analogues (13) and (14).

The N-terminal macrocycles of these new IDVp inhibitors were designed to link residues that are spatially close when bound to the active site of IDVp. Researchers have also been able to generate C-terminal macrocyclic replacements and bicyclic hexapeptide analogues. This has resulted in cyclic arrays that constrain segments of the peptidomimetic inhibitor in a bioactive conformation (Edmonds, 2001).
1.3.7 Some advances in applications of conformational constrictions

A variety of stable, small-molecule peptidomimetic ligands containing lactam (15), bicyclic (16) and spiro-bicyclic (17) scaffolds have been developed to elucidate the mechanism by which the neuropeptide Pro-Leu-Gly-NH (PLG) modulates dopaminergic neurotransmission. These conformational restrictions yielded molecules that were able to modulate dopamine receptors because of their ability to place the carboxamide NH pharmacophore in the same topological space (Swapna bhagwanth et al, 2013).
Arg-[3-amino-3(1-naphthyl)-propionic acid]-Phe (18) exhibited anti-proliferative activity with IC₅₀ values in micromolar range in breast cancer cell lines. Conformational constraints were initiated in the peptidomimetic with introduction of a proline residue in the peptidomimetic sequence (19) exhibited antiproliferative activity in the lower micromolar range than (18) (Sashikanth et al., 2011).
Caffeic acid, a natural antioxidant was conjugated to histidine containing dipeptides to develop better antioxidants. Caffeic acid-Proline-Histidine amide (20) exhibited the highest activity in both free radical scavenging and lipid peroxidation tests (Hyo- Suk Seo, 2010)

Synthetic C-terminal amidated cationic ferrocenoyl peptide bioconjugates Fc-Orn-Orn-Orn (21) and Fc-Tyr-Orn-Orn (22) were rationally designed as superoxide dismutase (SOD) mimics based on the structure of the iron SOD from *E.Coli* (Laurent Soulere, 2009)

Argpyrimidine (23) was synthesized by mixing L-arginine with 3-acetoxypentane-2,4-dione under acidic conditions and purified by chromatography. Argpyrimidine inhibited lipid peroxidation of rat brain homogenates catalyzed by hydroxyl radicals, metal ions, and autooxidation in a concentration- and time-
dependent manner. In addition, argpyrimidine scavenged superoxide anion, 1,1-diphenyl 2-picryl-hydrazyl-stable free radical, intracellular-hydrogen peroxide, and inhibited free-radical-mediated nicking of plasmid-DN (Nair Sreejayan, 2008).

![Chemical structure](image)

**Table 1: Some peptide based therapeutics of clinical use in the market**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peptide/peptidomimetic</th>
<th>Brand name</th>
<th>Therapeutic application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insulin</td>
<td>HUMULIN</td>
<td>Diabetes</td>
</tr>
<tr>
<td>2</td>
<td>Enalapril maleate (24)</td>
<td>RENITEC</td>
<td>Hypertension</td>
</tr>
<tr>
<td>3</td>
<td>Lisinopril (25)</td>
<td>PRINIVIL ZESTRIL</td>
<td>Hypertension, congestive heart failure</td>
</tr>
<tr>
<td>4</td>
<td>Saralasin acetate</td>
<td>SARENIN</td>
<td>Hypertension</td>
</tr>
<tr>
<td>5</td>
<td>Exenatide</td>
<td>BYETTA</td>
<td>Glycemic control in patients with type 2 diabetics</td>
</tr>
<tr>
<td>6</td>
<td>Liraglutide</td>
<td>VICTOZA</td>
<td>Type 2 diabetics</td>
</tr>
<tr>
<td>7</td>
<td>Pramlintide acetate</td>
<td>SYMELIN</td>
<td>Both type 1 and 2 diabetes</td>
</tr>
<tr>
<td>8</td>
<td>Enfuvirtide</td>
<td>FUZEON</td>
<td>AIDS/HIV infection</td>
</tr>
<tr>
<td>9</td>
<td>Human Calcitonin</td>
<td>CIBACALCIN</td>
<td>Post menopausal osteoporosis</td>
</tr>
<tr>
<td>10</td>
<td>Bivalirudin hydrate</td>
<td>ANGIOX</td>
<td>Anticoagulant</td>
</tr>
<tr>
<td>11</td>
<td>Eftifibatide</td>
<td>INTEGRILIN</td>
<td>Acute coronary syndrome, unstable angina</td>
</tr>
<tr>
<td>12</td>
<td>Ceruletide</td>
<td>TYMRAN</td>
<td>Gall bladder and pancreatic diagnosis</td>
</tr>
<tr>
<td>S.No</td>
<td>Peptide/peptidomimetic</td>
<td>Brand name</td>
<td>Therapeutic application</td>
</tr>
<tr>
<td>------</td>
<td>------------------------</td>
<td>------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>13</td>
<td>Ziconotide acetate</td>
<td>PRIALT</td>
<td>Severe chronic pain</td>
</tr>
<tr>
<td>14</td>
<td>Talterin hydrate</td>
<td>CEREDIST</td>
<td>Ataxia</td>
</tr>
<tr>
<td>15</td>
<td>Somatorelin acetate/ GHRH/GRF</td>
<td>STIMU-GH</td>
<td>Growth hormone deficiency</td>
</tr>
<tr>
<td>16</td>
<td>Bortezomib (26)</td>
<td>VELCADE</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>17</td>
<td>Glutathion(Gly-Cys-Gly) (27)</td>
<td>GLUTATHIOL</td>
<td>Hepatic insufficiency, wound healing</td>
</tr>
<tr>
<td>18</td>
<td>Somatostatin acetate</td>
<td>STILAMIN</td>
<td>Acute bleeding</td>
</tr>
<tr>
<td>19</td>
<td>Oxytocin</td>
<td>PITOCIN</td>
<td>Initiation/improvement of uterine contraction</td>
</tr>
<tr>
<td>20</td>
<td>Histrelin acetate</td>
<td>SUPPRELIN</td>
<td>Advanced prostate cancer</td>
</tr>
<tr>
<td>21</td>
<td>Argipressin (28)</td>
<td>PITRESSIN</td>
<td>Diabetes insipidus</td>
</tr>
<tr>
<td>22</td>
<td>Vasoactive intestinal peptide</td>
<td>AVIPTADIL</td>
<td>Sarcoidosis and acute lung injury (phase III)</td>
</tr>
<tr>
<td>23</td>
<td>Thymopentin (29)</td>
<td>MEPENTIL</td>
<td>Primary and secondary immune deficiencies</td>
</tr>
<tr>
<td>24</td>
<td>Thymalfasin</td>
<td>ZADAXIN</td>
<td>Chronic hepatitis B&amp;A</td>
</tr>
<tr>
<td>25</td>
<td>Buserelin acetate (30)</td>
<td>SUPERFACT</td>
<td>Advanced prostate cancer</td>
</tr>
<tr>
<td>26</td>
<td>Gonadrel acetate/ GNRH/LHRH</td>
<td>STIMU-LH</td>
<td>Stimulate secretion of gonadotropin</td>
</tr>
<tr>
<td>27</td>
<td>Secretin</td>
<td>SECREFLO</td>
<td>Diagnosis of pancreatic exocrine dysfunction</td>
</tr>
<tr>
<td>28</td>
<td>Desmopressin (31)</td>
<td>STIMATE</td>
<td>Haemophilia treatment</td>
</tr>
<tr>
<td>29</td>
<td>Spaglumat</td>
<td>RHINAXIA</td>
<td>Allergic rhinitis</td>
</tr>
</tbody>
</table>
Fig 2: Some peptide based therapeutics

24 Enalapril
25 Lisinopril
26 Bortezomib
27 Glutathione
28 Argipressin
29 H-Arg-Lys-Val-Tyr-D-oh
30 Thymopentin
31 Buserelin
32 Desmopressin
1.4 AIM AND OBJECTIVES

Peptides and their conjugates constitute the major classes of bioactive ligands for drug design and development. The insertion of conformationally constrained non-peptide scaffolds such as cyclic structures and the isosteric replacement of a peptide bond represent an important tool in the design of peptide based drugs (Silverman, 2004). Replacement of the amide bond with isosteric groups prevent proteolysis, promote metabolic stability and enhance the bioavailability. There are many examples in the literature of peptide based therapeutics that resulted from the incorporation of heterocyclic rings into peptides or peptide-like structures (Abell, 2002).

Thus, the present study was aimed with the following objectives

- Rational design and synthesis of some peptide analogs by the introduction of rigid scaffolds possessing the functionalities of peptides such as quinazolinonyl peptide derivatives, quinazolinyl azapeptide derivatives, dipeptidyl oxadiazole derivatives, dipeptidyl imidazolinone derivatives and N-dehydrodipeptidyl- N, N'-dicyclohexylurea analogs.

- To purify the compounds by appropriate physicochemical methods and characterize the title compounds by spectral analysis.

- To perform acute toxicity studies and evaluate the title compounds for anti-inflammatory, analgesic, antimicrobial activities and screen the selected compounds for preliminary cytotoxic activities.

- To evaluate the title compounds for in vitro antioxidant activities such as reduction of DPPH stable free radical, nitric oxide scavenging activity, inhibition of iron induced lipid peroxidation, superoxide anion scavenging activity, hydroxyl radical scavenging activity, reducing power assay and CUPRAC assay (Cupric ion Reducing Antioxidant Capacity).

- To perform in silico evaluation by molecular properties predictions such as oral bioavailability, druglikeness and bioactivity scores.

- To perform molecular docking studies on selected active compounds for unraveling the mode of action along with target identification which can be used for further designing.