1. 1. General

Today's search for safer, more effective pharmaceuticals continues as we look for a cure everything starting from the common cold to a life threatening cancer. Researchers around the world have spent countless hours and dollars on trying to find pharmaceuticals that could save the thousands, if not millions of lives a year. Today's physicians are also facing new challenges, new strains of diseases, and new viruses that can kill if a person is infected.

Thousands of new compounds are tested each year for their pharmaceutical activity. Of these thousands, a few pharmaceuticals do make it to the market each year. Although, there are advances being made in the field of pharmaceutical research, it is often quite empirical.

The development of new pharmaceuticals has always been exciting challenge for scientists and medical professionals. The discovery of new drugs can be a long and difficult process with no guarantee of success, although finding a compound showing positive biological activity is probably one of the most exhilarating feelings for the discoverer.

An essential part of the search for new leads in a drug designing programme is the synthesis of new molecules, which are novel yet to resemble the known biologically active molecules by virtue of the presence of some critical structural features. There are many approaches used for discovery of new pharmaceuticals such as trial and error, natural products or combinatorial chemistry. One other possibility in the discovery of new pharmaceuticals is the modification of existing, already proven bioactive molecules/pharmaceuticals.

In the field of biomedical research, heterocycles and amino acids/peptides deserves a unique and prominent place because of their wide variety of biological and pharmacological significance. Further, the conjugation of amino acids/peptides to heterocycles is most promising and successful strategy employed for developing new leads with enhanced potency and low toxicity. By taking the advantage of likeliness and

favored interaction of amino acids with the biological system, currently there is huge tendency for conjugating amino acid/peptide to heterocycles. Further, there are several successful evidences available in the literature signifying the importance of amino acid /peptide conjugation in improving the solubility, selectivity, stability and high cell permeability of the heterocycles.

With the above context, here we present a brief account on the general strategies and biological applications of amino acids, peptides and amino acids/peptides conjugated heterocycles in the filed of drug discovery.

1.2. A Quick Overlook on the Biological Roles of Amino Acids, Peptides/Proteins and their Medicinal Applications

Amino acids and peptides/proteins play a central role both as building blocks of proteins and as intermediates in metabolism. In terms of structure, an amino acid has at least one carboxyl (COOH) group, which is acidic, and one amino (NH_2) group, which is basic. Amino acids join together in long chains, the amino group of one amino acid linking with the carboxyl group of another to form peptide. The linkage is known as a peptide bond, and a chain of peptides is known as a polypeptide. Proteins are large, naturally occurring polypeptides.[1]

1.2.1. Amino acids:

Amino acids fulfill three broad classes of function in biology. They serve as building blocks in prokaryotes and plant and animal eukaryotes for the synthesis of peptides and proteins. Most peptides derived from the processing of proteins, but some such as glutathione, folate and peptide antibiotics are biosynthesized by specific nonribosomal routes. In contrast, particular amino acids, especially glycine, is required in the synthesis of a wide variety of small molecules, including alkaloids, purin and pyrimidine nucleotides, porphyrins and phosphocreatine. The second role of amino acids is to act as intermediates in incorporating or disposing of small molecules. For example, arginine is involved in various reaction sequences in the disposal of unwanted nitrogen as urea and

production of perhaps the most unexpected biomolecule, nitric oxide. Again, methionine makes its S-methyl group available for methylation reactions via the intermediate S-adenosylmethionine. Finally, some important biomolecules are derived by the metabolism of amino acids. Enzymatic decarboxylation of some of the coded amino acids or of hydroxylated derivatives gives rise to important cellular messengers and hormones. Alternatively an amino acid and alpha keto acids are important metabolic intermediates; this reaction offers a simple route to some of the nonessential amino acids. The amino group can also be removed oxidatively from an amino acid, giving rise to alpha keto acids. Some amino acids such as histidine and tryptophan undergo unique ring opening reactions the lead, through rather complex pathways, to glutamic acid and alanine respectively.[2]

In humans, non-protein amino acids also have important roles as metabolic intermediates, such as in the biosynthesis of the neurotransmitter, gamma-amino butyric acid.[3] Many amino acids are used to synthesize other molecules, for example:

- Tryptophan is a precursor of the neurotransmitter serotonin.[4]
- Glycine is a precursor of porphyrins such as heme.[5]
- Arginine is a precursor of nitric oxide.[6]
- Ornithine and S-adenosylmethionine are precursors of polyamines.[7]
- Aspartate, glycine and glutamine are precursors of nucleotides.[8]

1.2.2. Peptides/Proteins:

In the animal kingdom, peptides and proteins regulate metabolism and provide structural support. The cells and the organs of our body are controlled by peptide hormones. Insufficient protein in the diet may prevent the body from producing adequate levels of peptide hormones and structural proteins to sustain normal body functions. Individual amino acids serve as neurotransmitters and modulators of various physiological processes, while proteins catalyze most chemical reactions in the body, regulate gene expression, regulate the immune system, form the major constituents of muscle, and are the main structural elements of cells. Deficiency of good quality protein in the diet may contribute to seemingly unrelated symptoms such as sexual dysfunction, blood pressure

problems, fatigue, obesity, diabetes, frequent infections, digestive problems, and bone mass loss leading to osteoporosis. Severe restriction of dietary protein causes kwashiorkor which is a form of malnutrition characterized by loss of muscle mass, growth failure, and decreased immunity.[1]

Proteins (also known as polypeptides) are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code.[9] In general, the genetic code specifies 20 standard amino acids; however, in certain organisms the genetic code can include selenocysteine and in certain archaea-pyrrolysine. Shortly after or even during synthesis, the residues in a protein are often chemically modified by post-translational modification, which alters the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins. Proteins can also work together to achieve a particular function, and they often associate to form stable complexes.[10]

Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape.[11] Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle. Proteins are also necessary in animals diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food. Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism.[12]

A first step in the digestion of proteins is their cleavage by proteolytic (protein-splitting) enzymes into smaller chains of amino acids or peptides. These initial peptides are then cleaved into smaller and smaller peptides until free amino acids are available.

The amino acids are absorbed from the intestine by a complete biochemical process and are circulated by the blood to the tissues that utilize them. Some of the amino acids are used directly as the building blocks in the synthesis of new proteins unique to the species. Other amino acids may be used to supply energy, and still others, particularly when large amounts of proteins are consumed, may be excreted in the urine.[13]

Thus, peptides play a crucial role in fundamental, physiological and biochemical functions of life. Peptides (proteins) are present in every living cell and possess a variety of biochemical activities. For decades now, peptide research is a continuously growing field of science.

1.2.3. Importance of diverse functionalities of amino acids in preserving structure and functions of peptides/proteins:

The chemical properties of the amino acids of proteins determine the biological activity of the protein. Proteins not only catalyze all (or most) of the reactions in living cells, they control virtually all cellular process. In addition, proteins contain within their amino acid sequences the necessary information to determine how that protein will fold into a three dimensional structure, and the stability of the resulting structure.

Although, all amino acids have amino and carboxyl groups in common, they differ in the rest of the molecule having different substituents which determine their importance. Some have an additional amino or carboxyl group, and others have water-repelling (hydrophobic) groups. The shape and the overall properties of a protein are dependent upon its constituent amino acids. In some proteins, a change in just one amino acid in the polymer chain, out of a total of perhaps 250 amino acids, or even a change in its position, can cause the protein to become nonfunctional. Chemists are currently trying to relate the role of each of these amino acids to the way in which the protein works.

The most common natural amino acids are characterized by different charges, sizes, ability to form hydrogen bonds and by different chemical reactivities. A part of them have short, linear or branched non-polar aliphatic chains as side chains. Three amino acids have aromatic or heterocyclic side chains. Two amino acids contain a

hydroxyl-group, two further sulphur. Again two others carry additional carboxyl- or amino-groups and one has an imidazole group. These groups can be ionized. Two last amino acids have amidized carboxyl-groups so that the ability to become ionized is lost.[13]

Amino acids that are found within proteins convey a vast array of chemical versatility. During the biological interaction, the varied functional groups of amino acids ought to play an important role in non-covalent interaction such as ionic interactions, hydrophobic/hydrophilic interactions and hydrogen bonding. The varied functional groups of amino acid/peptidic side chains which can be a polar and/or non-polar, have both hydrophilic and lipophilic regions and have a negative, positive or neutral net charge, are capable of interacting with the various biological targets (enzymes, receptors, cells etc.,) and also can easily pass through the cell membrane.

Depending on the polarity of the side chain, amino acids vary in their hydrophilic or hydrophobic character. These properties are important in protein structure and protein-protein, protein-peptide interactions in the biological system. The importance of the physical properties of the side chains comes from the influence this has on the amino acid residue's interactions with other substituents within a single protein or between protein molecules. The distribution of hydrophilic and hydrophobic amino acids determines the tertiary structure of the protein, and their physical location on the outside structure of the proteins influence their quaternary structure.[14]

For example, soluble proteins have surfaces rich with polar amino acids like serine and threonine, while integral membrane proteins tend to have outer rings of hydrophobic amino acids that anchor them into the lipid bilayer. In the case part-way between these two extremes, peripheral membrane proteins have a patch of hydrophobic amino acids on their surface that locks onto the membrane. Similarly, proteins that have to bind to positively-charged molecules have surfaces rich with negatively charged amino acids like glutamate and aspartate, while proteins binding to negatively-charged molecules have surfaces rich with positively charged chains like lysine and arginine. Recently a new scale of hydrophobicity based on the free energy of hydrophobic association has been proposed.[15]

On the other hand, the range of posttranslational modifications can attach other non-peptidic chemical groups to the amino acids in proteins. For example, these modifications can produce hydrophobic lipoproteins[16] or hydrophilic glycoproteins.[17] These type of modifications allow the reversible targeting of a protein to a membrane. For example, the addition and removal of the fatty acid i.e., palmitic acid to cysteine residues in some signalling proteins causes the proteins to attach and then detach from cell membranes.[18]

1.2.4. Medicinal applications of amino acids, peptides/proteins:

1.2.4.1. *As dietary supplements:*

Insufficient levels of the essential amino acids can drastically interrupt the way our bodies work. For example, deficiencies of tyrosine, tryptophan, phenylalanine, and histidine can cause neurological problems and depression. The branched amino acids, leucine, isoleucine, and valine, provide recovery and energy needs. Because plants do not have all the amino acids humans need to survive (i.e. lysine), having a strict vegetarian diet is not in our best interests; we need protein that comes from meat, fish, eggs and dairy. A balanced diet prevents our body from breaking down other tissues to replenish the amino acid supply.

Similarly a wide range of activities have been described for bioactive peptides including antimicrobial and antifungal properties, blood pressure-lowering effects, cholesterol-lowering ability, antithrombotic effects, enhancement of mineral absorption, immunomodulatory effects, and localized effects on the gut. Although there is still considerable research to be performed in the area of food-derived bioactive peptides, it is clear that the generation of bioactive peptides from dietary proteins during the normal digestive process is of importance. Therefore, it will become necessary when determining dietary protein quality to consider the potential effects of latent bioactive peptides that are released during digestion of the protein.[19]

1.2.4.2. *As drugs:* [20]

In today's medicine amino acid/peptide based drugs continue to grow in popularity for their potential use in drug therapy. Amino acid/peptide based drugs are

any substance that uses the different amino acids to diagnose, prevent and treat diseases and conditions to restore or maintain normal body functions. These have led to the discovery of numerous amino acids based drugs of therapeutic potential, a number of which are already applied clinically. Peptide drugs are either naturally-occurring peptides or altered natural peptides. There are many naturally-occurring peptides that are biologically active. If the patients does not naturally produce a peptide that they need, this peptide can be synthesized and given to them. In addition, the amino acids in an active peptide can be altered to make analogues of the original peptide. If the analogue is more biochemical active than the original peptide it is known as an agonist and if it has the reverse effect is known as an antagonist.

1.2.4.3. *As diagnostics:*

Peptides can be designed that change colour under certain conditions, and these can be used for diagnostic purposes. For example, a chromogenic peptide substrate can readily detect the presence, absence and varying blood levels of enzymes that control blood pressure and blood clotting ability.

1.3. Amino Acids and Peptides in Modern Drug Discovery: *- A bird's eye view*

1.3.1. Fundamental aspects of amino acid/peptide based drug design:

As discussed above, amino acids and peptides are the fundamental components of living organisms playing a crucial role both as building blocks of proteins and as intermediates in metabolism. The diverse functionalities of amino acid residues which can be a polar and/or non-polar, have both hydrophilic and lipophilic regions and have a negative, positive or neutral net charge are ought to play very unique and important role in various protein-protein, peptide-protein, protein-receptor, enzyme interactions in the human body. Due to their low toxicity, biocompatibility, likeliness and favored interaction of amino acidic residues with the biological system, amino acids/peptides have become central to the development of new drugs.

In continuation, peptide research on drug design and drug discovery is one of the most promising fields in the development of the new drugs. Peptide sequences are constituents of larger proteins, where they are responsible for molecular recognition and biological activities. Amino acids/peptides/proteins are involved in numerous biological processes and play important roles in the development and progression of various diseases. Protein-protein interactions, such as kinase activation and inhibition, and peptide-protein interactions, like various peptide hormones and their receptors, are just two examples of biological processes with high therapeutic importance that can best be mimicked by amino acid/peptide-based drugs. Thus, inhibition of protein-protein interactions by peptides and the evolution of peptide ligands to small molecule mimetics is a major goal of the field, with several notable successes. Peptides would therefore seem to be ideal drug leads.[21]

The list of peptides as potential drugs is huge: It is beyond the scope of this thesis to focus on older peptide therapies such as luteinizing-hormone-releasing hormone, growth hormone, arginine vasopressin or the very interesting peptide, cyclosporine. We will instead highlight one of the most exciting and established peptide therapies. For example, insulin was the first peptide to be isolated and administered therapeutically, and is still the most commonly prescribed peptide, having been used for over half a century. We have yet to find a chemical mimic; however, there is still ongoing research into novel analogues and methods of administration. Manipulation of the insulin molecule has allowed the development of shorter-acting insulins, such as Lispro insulin. This is rapidly absorbed, readily dissociates into insulin monomers, and produces plasma levels that more closely mimic the normal postprandial insulin profile. Thus, Lispro can be injected immediately prior to a meal, unlike conventional short-acting insulin that should be injected half an hour earlier.[22-23]

However, peptides are limited in that they are metabolically unstable due to the protease cleavage of the peptide backbone and have poor bioavailability, in part due to low membrane transport characteristics of the peptides amide backbone structure.

1.3.2. Peptidomimetics: *Turning peptides in to* drugs [24-25]

We are on the brink of a therapeutic revolution. There has been a rapid expansion in the use of peptides as drugs over the last decade, and this is likely to continue. Peptides regulate most physiological processes, acting at some sites as endocrine or paracrine signals and at others as neurotransmitters or growth factors. They are already being used therapeutically in such diverse areas as neurology, endocrinology and haematology.

Most peptides cannot be administered orally as they are rapidly inactivated by gastrointestinal enzymes, so that subcutaneous or intravenous administration is required. Therefore, research is focusing on alternative routes of delivery, including inhaled, buccal, intranasal and transdermal routes, as well as novel delivery systems such as the use of protective liposomes. Neuropeptide systems in the brain are being examined as potential targets for therapeutics, providing an exciting future development area. The dual problems of local targeted delivery and the blood-brain barrier prevent administered peptides from readily gaining access to the required site of action, although as we will discuss, solutions are on the horizon.

Peptides act by binding to specific cell surface receptors. The perfect therapeutic agent would be a small-molecular-mass chemical mimic of the receptor ligand, which would be cheap to manufacture and could get to the site of action after oral administration. However, receptors are large with many binding sites, and peptides have a complex tertiary structure, both of which improve specificity as well as affording protection from simple invading molecules, like bacterial toxins. Consequently, production of successful peptide mimics using chemical libraries is largely unsuccessful and we still rely on the native peptide for therapeutics.

After many years of stagnation, peptide related therapeutics once again became the focus of innovative drug development efforts. The new peptide based drugs overcome the unattractive pharmacological properties of native peptides and protein fragments by one of the facile modifications.

a) **Amide bond replacements:**

Replacement of peptide bonds by non-peptide analogs such as carbamate, urea, hydrazines etc.

b) **Backbone cyclization:**

Ring formation using appropriate building units, leads to cyclic peptides in which the amino acid side chains are unaltered.

c) **Incorporation of non peptide moieties:**

Insertion of various bi-functional, non-peptidic molecules such as heterocycles, nucleic acids, carbohydrates within the peptide sequence.

d) **Peptide side chain modification:**

Small molecules attachment to side chains, and use of *N*-alkylated and non-natural amino acid derivatives.

In contrast to the amide bond replacement and backbone cyclisation, we can in some cases insert various bioactive scaffolds to amino acid/peptidic residues for improving pharmacological properties of peptides. Amide bond replacements, incorporation of non-peptide moieties, peptide-small bioactive molecule conjugates and backbone cyclization. Among these modifications, peptide-small bioactive molecule conjugate is one of the most successfully targeted against various diseases.

1.3.3. Need for conjugating amino acids and peptides with other bioactive scaffolds in developing drug candidates:[26]

Peptide-small bioactive molecule conjugation systems are becoming powerful tools for the elucidation of complex interaction of bimolecules. Due to the facile synthetic procedure and the wealth of diversity available from varied functional groups of amino acid side chains, peptides-based biomimetic compounds are of particular interest with respect to high specificity and low toxicity profile. Additionally, because of their likeliness to the native bioactive molecules, peptide-small bioactive molecule conjugates of various amino acids lengths and compositions were synthesized and their interaction with targets was explored.

The starting point for a peptide mimetics research is the identification of a peptide or peptide sequence within a protein context that is active in the relevant assay. The process involves deconstructing the original peptide and reassembling the essential features on a new, mimetic scaffold that retains the ability to interact with the biological target, but circumvents the problems associated with a natural peptide. The deconstruction process begins by developing structure-activity relationships, then designing analogs to define a minimal active sequence and to identify the key residues and portions of the backbone in the peptide that are responsible for the biological effect. The structural constraints are added to check the effectiveness of these features.

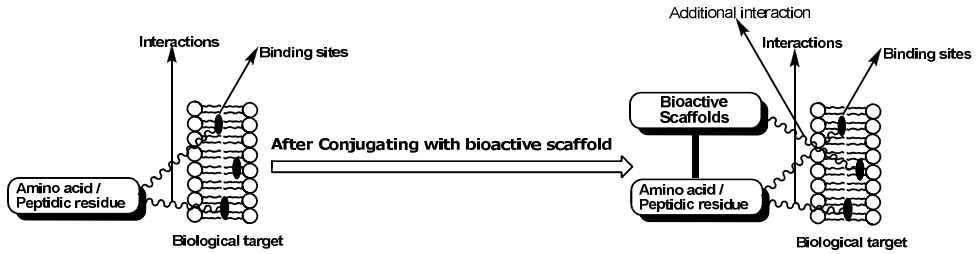
The interaction of a peptide with a biological target may occur via a direct binding of a linear sequence in any number of conformations accessible to a peptide. The modern peptide mimetics approach incorporates a production of small molecules which mimic peptides in order to overcome their ineffectiveness as drugs when administered orally. The small molecule mimetics retain the desired biological properties of the peptide lead, but are metabolically stable, have unlimited diversity, and can be designed to provide the new drugs.

By this process, the peptide has been reduced to its information content, the basis for a pharmacophore model that defines the critical features and their arrangement in space. This model supports the re-assembly of the critical elements and non-peptide variants on a modified scaffold that presents the optimized pharmacophore to the receptor. The optimized peptide-hybrid may be valuable as a first drug candidate, in addition to its role as a tool for further evolution to a mimetic. Mimetic scaffolds are designed to be resistant to the proteases that would destroy a natural peptide, and would have pharmaceutical properties consistent with a drug candidate.

It is possible to represent the biologically active sites of the peptides in the form of orally administered small-molecule mimetics that take all the advantages of evolutionally designed peptides on the one hand and have good drug properties, are stable, bioavailable, inexpensive in manufacture and convenient in use, on the other hand. There

is no way to get involved in modern drug discovery and drug design without peptide and their small molecule mimetics research.

For example, when interactions of amino acid/peptidic residue with certain specific binding sites of biological target is missing, various bioactive scaffolds can be inserted through side chains or one of the termini to exert additional interactions, there by improving pharmacological properties of peptides (**Scheme-1.1**). These modifications have shown to be responsible for enhanced metabolic stability and cell permeability of amino acid/peptides conjugated bioactive scaffolds when compared to their native peptides and free scaffolds.



Scheme-1.1: Interaction of amino acid/ peptidic residues and their conjugates with binding sites of biological target

This comprehensive platform is aimed to identify drug-like molecules that combine the highly specific binding ability of the original protein fragment or peptide and potency of bioactive molecules, with the expectation of improved pharmacological properties, and increased metabolic stability, oral availability and cell-permeability.

In literature, there are numerous examples of amino acid/peptide conjugated bioactive scaffolds including glycopeptides, lipopeptides, heterocyclic conjugated peptides, peptide nucleic acids, prodrugs etc., displaying wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, antimalarial, anti HIV, anticancer etc.[27-32] In light of these developments, conjugation of amino acids/peptides with bioactive scaffolds is of great importance in biomedical research.

The division of amino acid/peptide conjugated bioactive scaffolds has seen continuous expansion over the last decade. As our research is mainly focused on the main achievements which involve the heterocyclic-amino acids/peptide link, here we present

the current status of amino acid/peptide conjugated heterocycles as novel class of lead molecules in modern drug discovery.

1.4. Development of Amino Acid/Peptide Conjugated Heterocycles as Novel Class of Lead Molecules in Modern Drug Discovery: *A Concise Review*

1.4.1. A brief note on biological significance of heterocycles:

Heterocycles make up an exceedingly important class of compounds. All the available natural and synthetic heterocyclic compounds can and do participate in biochemical reactions in the human body. Furthermore, all biological processes are chemical in nature. Such fundamental manifestations of life as the provision of energy, transmission of nerve impulses, sight, metabolism and the transfer of hereditary information are all based on chemical reactions involving the participation of many heterocyclic compounds, such as vitamins, enzymes, coenzymes, ATP, DNA, RNA and serotonin.[33]

The heterocycles are able to get involved in an extraordinarily wide range of reaction types. Depending on the pH of the medium, they may behave as acids or bases, forming anions or cations. Some interact readily with electrophilic reagents, others with nucleophiles, yet others with both. Some are easily oxidized, but resist reduction, while others can be readily hydrogenated but are stable towards the action of oxidizing agents. Certain amphoteric heterocyclic systems simultaneously demonstrate all of the above-mentioned properties. The ability of many heterocycles to produce stable complexes with metal ions has great biochemical significance. The presence of different heteroatoms makes tautomerism ubiquitous in the heterocyclic series. Such versatile reactivity is linked to the electronic distributions in heterocyclic molecules. Evidently, all the natural products and the synthetic drugs available are good examples of nature's preference for heterocycles whose biological activity cannot be determined by one or a combination of two or three of the above-mentioned properties.[34]

The introduction of a heteroatom into a cyclic compound imparts new properties. Heterocycles are chemically more flexible and better able to respond to the many demands of biochemical systems. In fact more than half of all known organic compounds are heterocycles. Many natural drugs such as quinine, papaverine, emetine, theophylline, atropine, procaine, codeine, morphine and reserpine are heterocycles. Almost all the compounds we know as synthetic drugs such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocycles. Some dyes (e.g. mauveine), luminophores (e.g. acridine orange), pesticides (e.g. diazinon) and herbicides (e.g. paraquat) are also heterocyclic in nature.[35]

However, the majority of heterocycles have some major limitation with regard to the clinical toxicity and lower bioavailability due to their physicochemical properties. Due to their lower bioavailability, they can't pass across the cell membrane, resulting in the decreased potency than of capable. Where as, the varied functional groups of amino acid/peptidic side chains can be polar and/or non-polar, have both hydrophilic and lipophilic regions and have a negative, positive or neutral net charge that are capable of interacting with the biological targets (enzymes, receptors, cells etc.,) and also can easily pass through the cell membrane. Moreover, amino acids/peptides are less toxic and biocompatible. Thus, the conjugation of bioactive heterocycles to some amino acid/peptidic residues that would facilitate the easy passage of this conjugate across cell membrane for release into the cell's cytosol and can then exert the maximum potency. There are several successful evidences available in the literature indicating the importance of amino acid conjugation in improving the solubility, stability and high cell permeability of the bioactive molecules. Some of them are presented below.

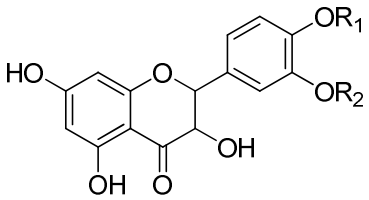
1.4.2. Amino acid and peptide conjugated heterocycles:

In recent years, the interest in amino acid/peptide conjugated small bioactive molecules is growing, because this conjugation methodology is highly utilized to form bi-functional compounds that were successfully used in several prodrugs, drug delivery systems, targeted imaging materials, targeted therapy agents, bi-substrate drugs that bind to two different binding sites of the target, and various carrier-drug systems. In

addition, conjugation of hydrophobic moieties like amino acids/peptides with bioactive molecules, may facilitate highly specific binding ability of the original protein fragment or peptide, with the advantages of improved pharmacological properties, and increased metabolic stability, oral availability and cell-permeability.

1.4.2.1. Amino acid conjugated heterocycles:

The most interesting application of heterocyclic conjugated amino acids is the study of Kim *et. al.,*[36] on *in vitro* solubility, stability and permeability of novel quercetin-amino acid conjugates. They have synthesized nine quercetin-amino acid conjugates and estimated their pharmacokinetic properties including water solubility, stability against chemical or enzymatic hydrolysis, and cell permeability. Among the synthesized quercetin prodrugs, quercetin-glutamic acid conjugates **1** showed remarkable increase in water solubility, stability, and cell permeability compared with quercetin, which warrants further development as a quercetin prodrug.

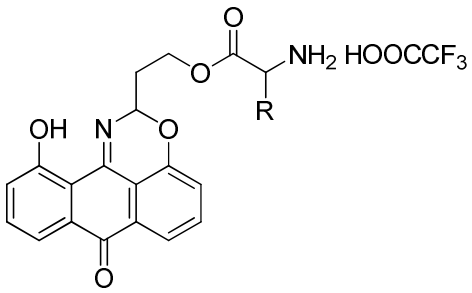


1

		Quercetin(Qu)	Qu-E
Structure		R_1=R_2=H	R_1=(S)CONHCH($CH_2CH_2CO_2H$)CO_2H,R_2=H , R_1=H, R_2=(S)-CONHCH($CH_2CH_2CO_2H$)CO_2H
Solubility in water(Mm)		50	2649
Stability	PBS buffer	-	>17h
	Cell lysate	-	180 min
Relative cell permeabiity		1.0	5.2

In another study, Mincher *et al.,*[37] synthesized a new series of oxoazabenzo[de]anthracenes conjugated to amino acids **2** as DNA-binding antitumor

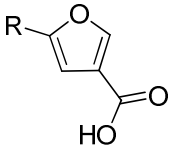
agents. The lysine conjugate was found to be over 20 times more cytotoxic to CEM human leukemia cells with an IC_{50} in the submicromolar range than the other Pro, Ala, and Gly conjugates, whereas the Phe analogue showed the lowest DNA binding capacity. The authors discovered that these compounds form intercalation complexes with DNA, evidenced from electric linear dichroism and topoisomerase based DNA unwinding experiments. Altogether, the work provides interesting structure-activity relationships in the oxoazabenzo[de]anthracene-amino acid conjugate series and identifies the lysine derivative as a promising candidate for further *in vivo* evaluation and drug design.



R= Side chains of amino acids Ala, Lys, Pro, Phe, Gly

2

Stanchev *et al.,* [38] have synthesized Oxazole **3** and thiazole containing amino acids (leucine and alanine) and peptides and reported as they have showed moderate antimicrobial activity.

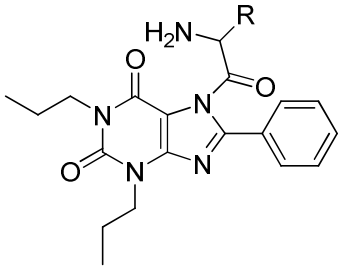


R = $(CH_3)_2$-CH-CH_2-CH (NH-Boc), CH_3-CH-(NH-Boc)-

3

Jacobson *et al.,*[39] have conjugated a series of amino acids covalently to 1,3-dipropyl-8-phenylxanthine **4**, a potent antagonist of adenosine at A1 and A2-adenosine receptors. The potency (subnanomolar range) and selectivity for A1-receptors (up to 200-fold) suggest that this approach can yield a versatile class of "functionalized congeners" of adenosine receptor antagonists. The water solubility in many of the more potent analogs has been enhanced by two orders of magnitude over that of simple, uncharged 8-phenyl

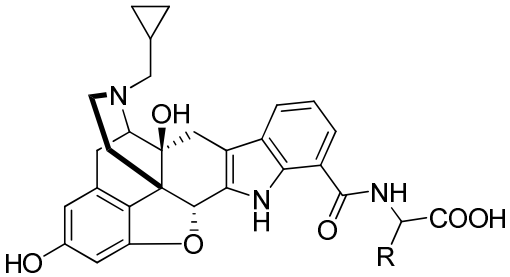
xanthine derivatives. The functionalized congener approach is potentially applicable to other drugs and for the development of prodrugs.



R = Side chains of amino acids

4

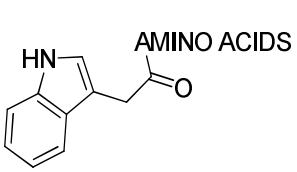
Portuguese *et al.,*[40] have synthesized a series of amino acid conjugates of naltrindole **5** in order to obtain delta antagonists that would have minimal access to the central nervous system (CNS) upon peripheral administration. All of the ligands were tested in smooth muscle preparations and found to be potent and selective delta opioid antagonists. Two of the more selective conjugates, the glycinate and aspartate, were evaluated by the iv and icv routes in mice, and they afforded very high iv/icv dose ratios consistent with poor CNS penetration.



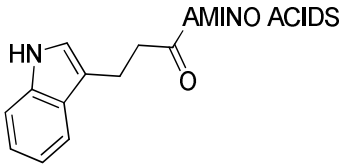
R = Side chains of 24 amino acids

5

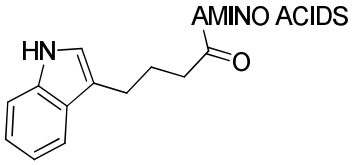
Ana *et al.,*[41] have studied the affinity of indole-3-acetic acid (IAA) **6**, indole-3-propionic acid **7**, indole-3-butyric acid **8** and their conjugates of amino acid to immobilized human serum albumin. They found that the retention factor k (determined by HPLC), was dependent on (1) lipophilicity, (2) chirality and (3) functional groups in the amino acid moiety; in some cases conformation plays an additional role. Most compounds examined are possible metabolic precursors of IAA, an experimental tumor therapeutic.



6

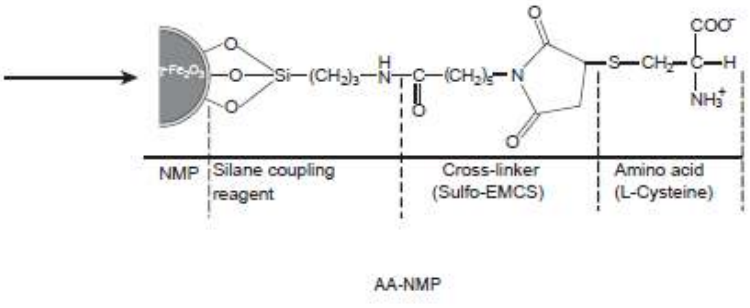


7



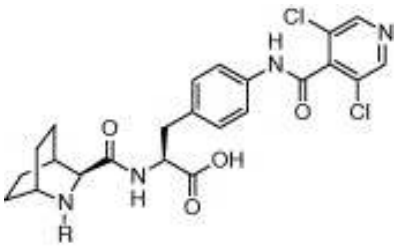
8

For the purpose of functionalizing the surface of the particles with amino groups for subsequent cross-linking with pharmaceuticals and biomolecules by cells, Shinji *et al.,*[42] have conjugated the functionalized nano-magnetic particles (NMPs) with an amino acid via a cross-linker, N-(EPSILON-maleimidocaproyloxy)sulfosuccinimide ester. They reported that the amino acid-conjugated NMPs **9** have been a great potential for use in cell-selective delivery systems involving amino acid transporter proteins.



9

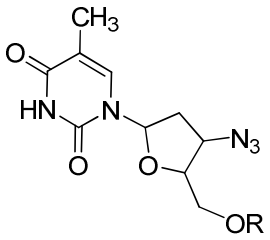
Dyatkin *et al.,*[43] have developed aza-bicyclic amino acid carboxamides **10** as $\alpha_4\beta_1/\alpha_4\beta_7$ integrin receptor antagonists. Several derivatives demonstrated nanomolar balanced $\alpha_4\beta_1/\alpha_4\beta_7$ *in vitro* activity. Two compounds were selected for *in vivo* leukocytosis studies and demonstrated increase in circulating lymphocytes up to 250% over control.



R = Side chains of amino acids

10

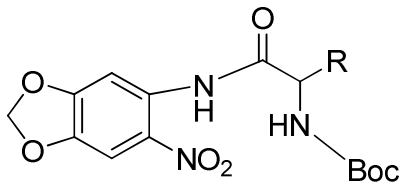
Agarwal *et al.,* [44] have synthesized a series of prodrugs of zidovudine (AZT) in an effort to enhance the uptake of the prodrugs by the HIV-1 infected cells and to increase the plasma half-life of AZT. All the five amino acid substituted esters of AZT **11** were generally less cytotoxic than AZT except the retinoic acid ester. Among the five amino acid substituted esters of AZT, the phenylalanine and isoleucine analogues were more lipophilic, as expected, than the tyrosine, lysine, and glutamic acid esters.

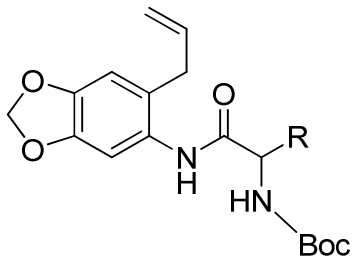


11

where R= *N*-α-t-Boc-*L*-phenylalanyl, *N*-α-t-Boc-*L*-tyrosinyl, *N*-α-t-Boc-*L*-isoleucinyl, *N*-α,*N*-ε-bis-t-Boc-*L*-lysinyl, *O*-t-butyl-α-t-Boc-*L*-serinyl, phenylalanyl, tyrosinyl, isoleucinyl, lysinyl, *N*-α-t-Boc-γ-glutamyl-α-ter-butyl ester, gluatamyl.

Leite *et al.,*[45] have synthesized two series of 5 and 6-substituted-1,3-benzodioxole amino acids derivatives **12**, **13** and evaluated as antitumour and antimicrobial agents. All the products of the series were able to inhibit the growth of the tumor mass on the assays. The products leucine, valine, glutamic acid and glycine derivatives, presented an antitumor profile with reduction of the tumor mass. The other tested products phenylalanine, lysine, alanine and aspartic acid derivatives were less active.

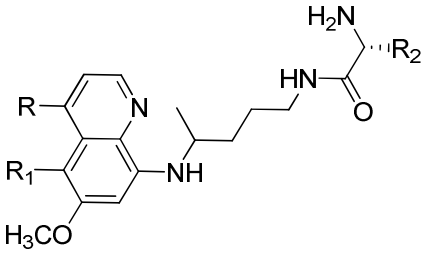




12 **13**

where R= -H (Gly), -CH_3 (Ala), -CH-$(CH_3)_2$ (Val), -CH_2-$CH(CH_3)_2$ (Leu), -CH_2-Ph (Phe), -$(CH_2)_4$-Fmoc (Lys), $CH_2COOBzl$ (Asp), $(CH_2)_2$-COOBzl(Glu).

Vangapandu *et al.,*[46] have synthesized amino acids (alanine, lysine ornithine and valine) conjugated 8-quinolinamines **14** and primaquine and evaluated for their blood-schizontocidal antimalarial activity. Among the synthesized anologues, amino acid conjugated 8-quinolinamines have exhibited potent *in vivo* antimalarial activities much superior when compared to peptide analogues reported earlier. Furthermore, in general, antimalarial activities of amino acid conjugated 8-quinolinamines are superior compared to the activities of respective 8-quinolinamines.



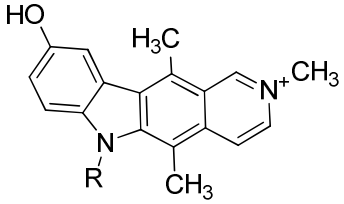
R = H, C_2H_5

R_1 = H, OC_3H_7, OC_4H_9, OC_5H_{11}, OC_6H_{13}, OC_7H_{15}, OC_8H_{18}

R_2 = CH_3, $(CH_2)_4NH_2$, $(CH_2)_3NH_2$, CH $(CH_3)_2$

14

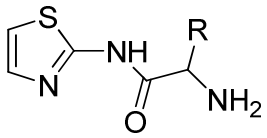
Auclair *et al.,*[47] have synthesized series of amino acids conjugated antitumor drug N^2-methyl-9-hydroxyellipticinium (NHME) **15** through a peroxidase-catalysed oxidation reaction. All aliphatic amino acids (Gly, Ala, Val and Leu) NHME adducts exhibit a higher lipophilicity property than NHME directly correlated with the length of the aliphatic chain of amino acids. The presence of amino acids moiety linked to NHME results (1) slight decrease of cytotoxicity (2) in a decrease of the antitumor efficiency (3) in a suppression of antitumor effect (4) strong increase in bacteriostatic activity. The bacteriostatic effect is directly correlated with the lipophilic property of the drugs.



R= Side chain of amino acids

15

Karade *et al.,*[48] have synthesized a new series of thiazole-derived *L*-amino acids **16** and evaluated as targeted potential antimalarials against *plasmepsins II* enzyme of malaria parasite *Plasmodium falciparum.* They reported that *a*ll the compounds showed moderate to good activity. The tryphtophan and proline conjugates were found to have highest the 50% inhibitory concentration (IC_{50}) values (3.45 µM and 4.89 µM, respectively) against *Plasmodium falciparum.*

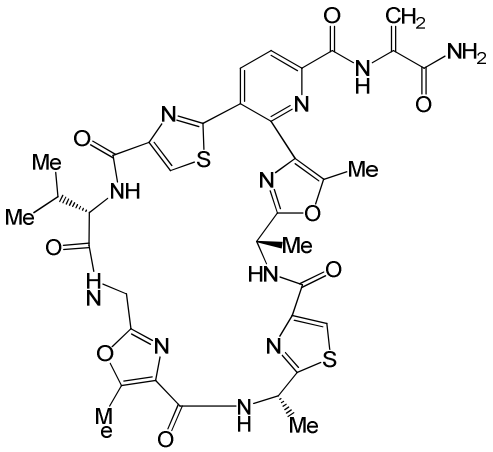


R= Side chain of amino acid

16

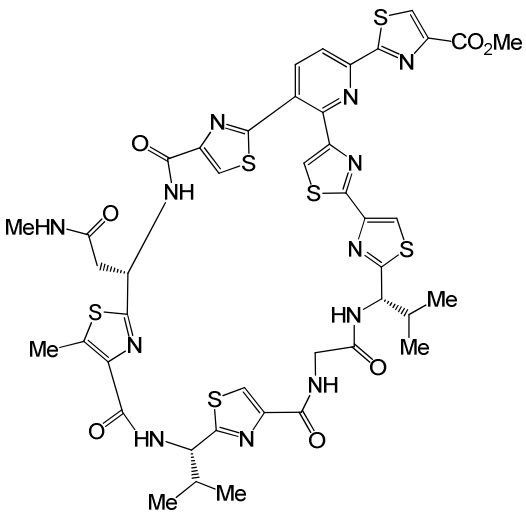
1.4.2.2 . Peptide conjugated heterocycles:

The term "peptide conjugated heterocycle or heterocyclic peptide" is used in several ways in the literature especially to define a group of natural products that are peptidic in origin, that is, derived from amino acids, but consist largely of heteroaromatic rings, often in a macrocyclic array. The most important heteroaromatic rings are thiazoles, oxazoles, indoles, and pyridines, and they occasionally occur as their di- or tetrahydro derivatives. The thiopeptide antibiotics, with their complex molecular architectures, are wonderful examples. There are numerous classes of thiopeptides, well known natural "heterocyclic peptides" embedded with thiazole moiety have been isolated and are now the most promising lead structures in many drugs such as Bleomycin A_2, Antibiotic GE2270A, Antibiotic A 10255, Thiangazole, BE 10988, Nostocyclomide, Promothiacin **17** and Amythiamicin **18**.[49]



Promothiocin A

17

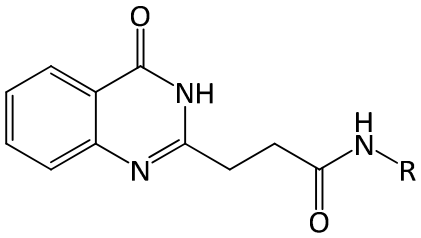


Amythiamicin D

18

The most interesting and wonderful application of heterocyclic conjugated peptides is 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid conjugated peptides **19** as novel class of potent antimicrobials which were recently investigated in our laboratory.[50] The quinazolinone conjugated peptides were synthesized by coupling 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid with VP, GVP, VGVP and GVGVP peptides. All the

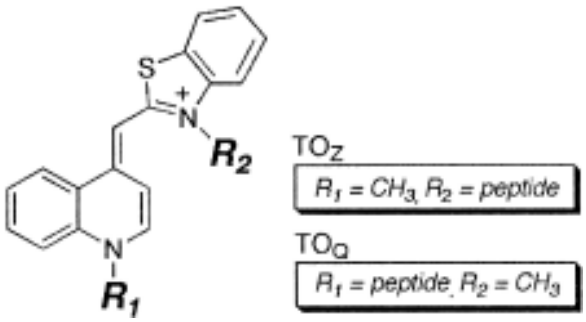
synthesized compounds were characterized for their antibacterial activity against bacterial strains viz., *B. substilis (gram positive) and E. coli, P. fluorescens, X. campestris pvs and X. oryzae (gram negative).* It was noticed that quinazolinone conjugated VGVP and GVGVP peptides showed increase in activity by nearly two fold compared to conventional antimicrobials. All the quinazolinone conjugated peptides showed enhanced activity, even though peptides and quinazolinone moiety which was taken in isolation were inactive towards those bacterial strains.



R= Shorter elastin peptides *ie,* VP, VGP, VGVP, GVGVP

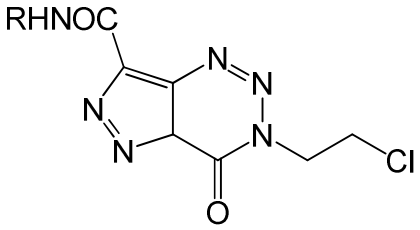
19

Carreon *et al.,*[51] have synhesised a new series of Thiazole Orange(TO)-peptide conjugates **20** and studied the sensitivity of DNA binding to chemical structure. Interestingly, the binding of many of the TOQ derivatives was more sensitive to the presence of distamycin than the TOZ counterparts. The appended peptide appears to significantly alter the DNA binding properties of a conjugate. A comparison of the properties of TO-K and TO-WK indicates that even more subtle changes in peptide sequence affect the binding mode and photophysical properties of the conjugates.



20

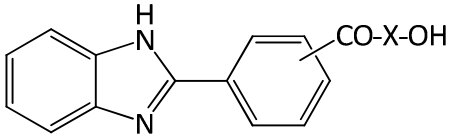
Arrowsmith *et al.,*[52] reported the carboxylic acids derived from the amido groups of the antitumor agents mitozolomide and temozolomide have been conjugated to simple amino acids and peptides by carbodiimide coupling. Attachment of the acids to imidazole polyamidic lexitropsins gave a series of potential DNA minor groove binding ligands. *In vitro* biological evaluation of a limited number of these novel conjugates **21** failed to demonstrate enhanced growth-inhibitory activity compared to the unconjugated drugs; sites of alkylation at tracts of multiple guanines were also unaffected.



R= Peptide residue

21

Dahiya and Pathak [53] have coupled four substituted benzimidazolyl-benzoic/salicylic acids with different amino acid ester hydrochlorides/dipeptide/tripeptide/tetrapeptide methyl esters to afford novel benzimidazolopeptide derivatives **22.** All peptide derivatives were screened for their antimicrobial, anthelmintic and cytotoxic activities. Almost all newly synthesized peptide conjugates of all the compounds have shown moderate to good anthelmintic activity against all three earthworm species and good antimicrobial activity against pathogenic fungal strains *C. albicans* and *A. niger*, gram negative bacterial strains *P. aeruginosa* and *E. coli*. Some of them possessed significant cytotoxic activity against Dalton's lymphoma ascites (DLA) and Ehrlich's ascites carcinoma (EAC) cell lines.

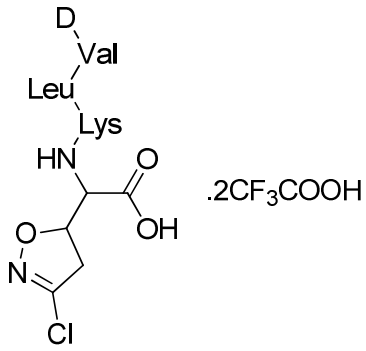


X= *L*- amino acids, di/tri/tetrapeptides

22

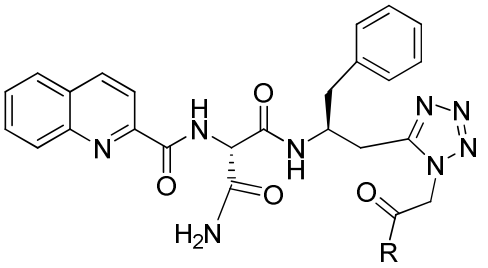
A new series of peptidic compounds, having peptide chains linked to bi- and tricyclic heterocycles (peptide-heterocycle hybrids), have been synthesized by Mann *et al.,*[54] The heterocyclic compounds have been used as acylating agents in coupling reactions with short *N*-unprotected peptides. They have used short (2-4 amino acids) peptides with hydrophobic amino acids of the two enantiomeric series. They reported that the inhibition of calpain by peptide-heterocycle hybrids with IC_{50} values in the nanomolar range.

Chakravarty *et al.,*[55] have synthesized the peptide conjugates of the anticancer drugs (αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (acivicin, AT-125) and *N,N*-bis(2-chloroethyl)-*p*-phenylenediamine(phenylenediamine mustard) **23** by mixed anhydride coupling of the parent drug with the protected tripeptide, The conjugates showed an increased selective *in vitro* cytotoxicity for Rous sarcoma virus transformed chicken embryo fibroblasts (which produce elevated levels of plasminogen activator) compared to nontransformed fibroblasts (which produce low levels of plasminogen activator). The prodrug of phenylenediamine mustard was also slightly less toxic than the parent drug.



23

In another study May and Abell [56] designed and synthesized tetrazole based peptidomimetics, assay results on these compounds suggest that the longer the C-terminus substitution, greater will be the potency. Among the synthesized compounds **24-26** proved to be as HIV protease inhibitor.

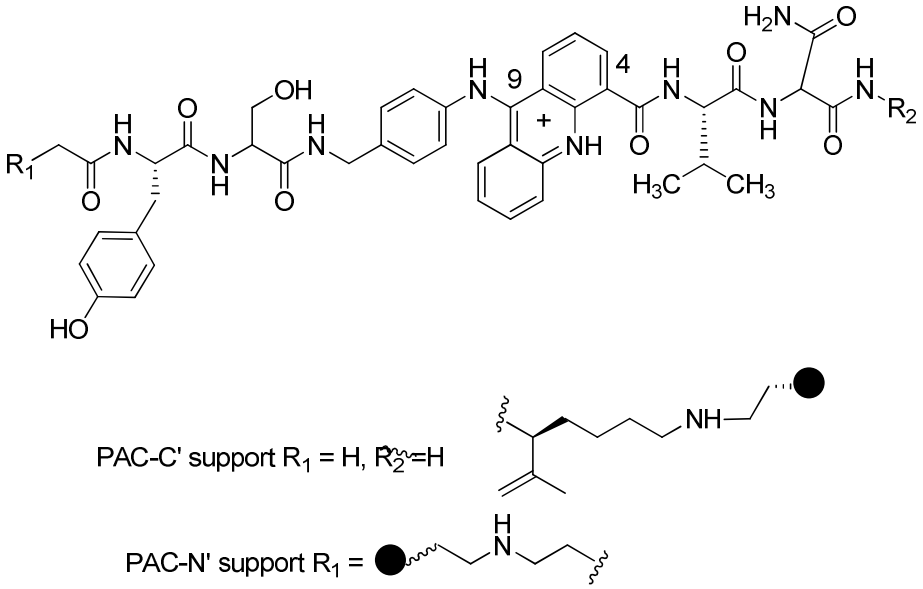


24. R = $NHCMe_3$

25. R= Ile-OMe

26. R= Ile-Val-OMe

Carlson and Beal [57] have synthesized libraries of peptide-acridine conjugates (PACs) **27** featuring a novel 9-anilinoacridine amino acid and screened for high-affinity RNA ligands using spatial arraying strategies. The compounds have been shown to bind nucleic acids by threading intercalation. One end of these molecules must pass between base pairs to allow maximum stacking of the acridine intercalator, linkage of this threading substituent to a solid support may significantly slow binding or prevent it altogether.

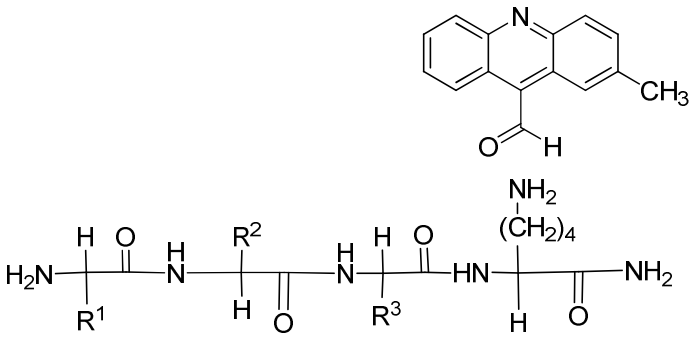


27

Tung *et al*.,[58] have synthesized a series of peptide-acridine conjugates **28** based on three features of the proposed catalytic mechanism of RNase A: 2'-proton abstraction by His-12, proton donation to the leaving 5'-oxygen by His-119, and stabilization of the

pentacoordinated phosphorous transition state by Lys-41. The substrate binding capability of RNase A was mimicked by the intercalator, acridine. Lysine served as a linker between acridine and the catalytic tripeptide. Cleavage of target RNA was monitored by agarose gel electrophoresis and by gel-permeation chromatography. The carboxyl-amidated conjugates HGHK(Acr)-NH_2, HPHK(Acr)-NH_2, and GGHK(Acr)-NH_2 (where Acr indicates 2-methyl-9-acridinemethylene) all had similar hydrolytic activity.

Intercalating Acridine



Catalytic Tripeptide Connecting Lysine

28

1.5. References

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