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STUDY OF PROTEINS AND PEPTIDOMIMETICS

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1. INTRODUCTION

Peptides have proved useful not only in biological chemistry, but also in chemistry, pharmacology, medicinal chemistry, and biotechnology and gene technology.

A number of important physiological and chemical functions of life are influenced by peptide. Peptides are involved as neurotransmitter, neuromodulators, and hormones in receptor-mediated signal transduction. More than thousands of peptides with function in the central and peripheral nervous systems, in immunological processes, in cardiovascular systems, and in the intestine are known. Peptides are short fragments of proteins obtained either by enzymatic or chemical hydrolysis. The size of peptides ranges from few amino acids to few hundreds of amino acids. The therapeutic methods based on peptides are playing a functional and vital role for a series of diseases. Chemical peptide synthesis is the classical method which has been mainly developed during the past five decades, although foundation was laid in the early 20^{th} century by Theodor Curtius and Emil Fischer.

The demand for synthetic peptides in biological applications is steadily increasing. The new targets do not allow for an isolated position of peptide chemistry exclusively oriented toward synthesis. Studies on structure-activity relationships, involves a large number of synthetic analogues with sequence variations and the introduction of nonproteinogenic building blocks. The methods of combinatorial peptide synthesis allow for the simultaneous creation of peptide libraries which contain at least several hundreds of peptides. The high purities enable both *in vitro* and *in vivo* screening of biological activities to be carried out. Peptide drugs can be applied only to limited extent because of their chemical and enzymatic liabilities, but many are inactive when applied orally, however, application via mucous membrane is promising. The development of orally active peptides is an important target. A major strategy in peptide chemistry is directed towards chemical modification in order to increase its chemical and enzymatic activity and also selectivity towards the receptor. The synthesis of analogues of bioactive peptides with unusual amino acid building blocks, linker or spacer and modified peptide bond is directed towards the development of potent agonists and antagonists of endogenous peptides. Several hetero-cyclic compounds show remarkable bio-activity, and are very useful for therapeutic purposes. Actually, I am going to target some of these types of compounds like Bonzothiazole, Benzoxazole, and Benzimidazole. In our lab, we have synthesized derivatives of benzothiazole because benzothiazole is a very good antitumor compound and also exhibit several other bioactivities

such as, immunosuppressive, immunomodulatory and antiviral properties. 2-(4-aminophenyl)-Benzothiazole was originally discovered in a program of screening for tyrosine kinase inhibitors. Their simple structures belie remarkable and intriguing antitumor properties, and their biological profile is unlike that of any known investigational anticancer agent. Analysis of structure-activity relationships identified the benzothiazole nucleus as being essential for potent activity, further substitution at the 3c'-position of the phenyl ring (with alkyl or halogen groups) increased the potency and spectrum of activity against tumor cell lines in vitro. Keeping these facts in mind, we have synthesized different tripeptides which are substituted with 2-(4-aminophenyl)-benzothiazole.

2. BIOMOLECULES

Bio-molecules are essential components of all living organisms. All known forms of life are composed solely of biomolecules. A biomolecule is a molecule that naturally occurs in living organisms. Biomolecule may be defined as complex lifeless chemical substances such as carbohydrate, fats, nucleic acids and protein which are responsible for growth and maintenance of living organisms. Biomolecules consist primarily of carbon and hydrogen, along with nitrogen, oxygen, phosphorus and sulphur. Other elements sometimes are incorporated but are much less common. There are various types of bio-molecules. Mainly these can be divided as follows:

Types of Biomolecules: The macromolecules can be divided into four classes;

1. Carbohydrates

It includes simple sugar (or monosaccharide) and all larger molecules constructed of sugar building block. Most of the sugar has general formula $(CH_2O)_n$.Each sugar molecule consists of backbone of carbon atoms linked together in a linear array by single bond. Each of the carbon atoms of the backbone is linked to a single hydroxyl group, except for one which bears a carbonyl(C=O) group. If the carbonyl group is located at an internal position the sugar is a ketose, such as fructose, and if located at the other terminal of the sugar, it forms an aldehydic group and known as aldose, such as glucose. On the basis of monomer unit sugars can be divided into monosaccharide, disaccharide and polysaccharide.

2. Lipids

Lipids are a diverse group of non biological molecules. They are soluble in organic solvents such as, chloroform, benzene and insoluble in water. The most common example of lipids is fats, steroid, and phospholipids, glycolipids and lipoproteins (Figure 1).

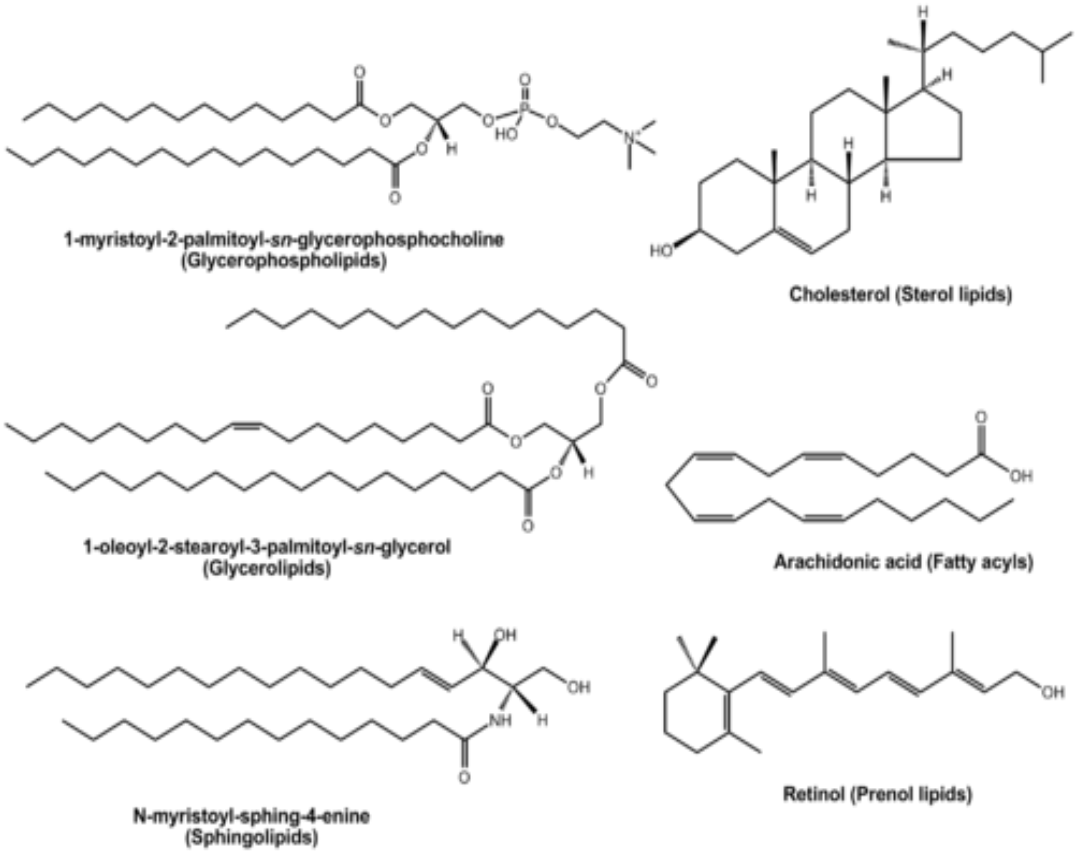


Figure 1 Different form of lipids (http://en.wikipedia.org/wiki/Lipid)

3. Nucleic acids

Nucleic acids are macromolecules constructed as a long chain (strand) of monomers called as nucleotides. Nucleic acids act as storage of genetic information from one generation to other generation. Nucleic acids are mainly of two types; Deoxiribonucleic acid and ribonucleic acid (Figure 2).

Nitrogenous bases:

1- Purine

Adenine (A)

Guanine (G)

2- Pyrimidine

Thymine (T)

Cytosine (C)

Uracil (U)

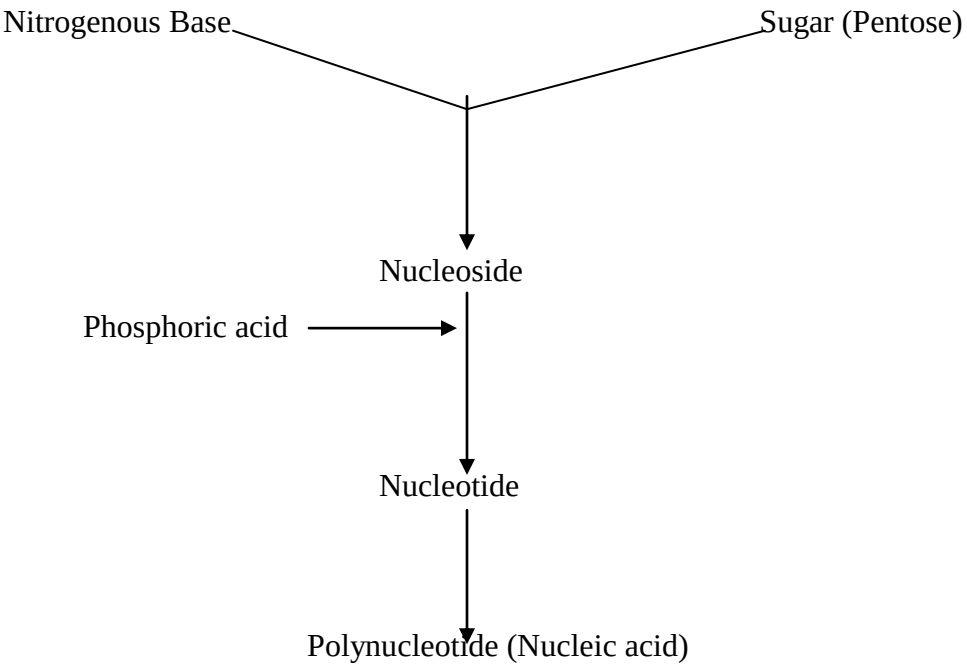


Figure 2

4. Proteins

The word protein comes from the Greek word πρώτειος (proteios) means "primary", first discovered and named by Jöns Jakob Berzelius, in 1838. James B. Sumner showed in 1926 that the enzyme urease was a protein. The first protein to be sequenced was insulin, by Frederick Sanger, who won the Nobel Prize for this achievement in 1958. In 1958, Max Perutz and Sir John Cowdery Kendrew solved haemoglobin and myoglobin. Perutz and Kendrew also reported the three dimensional structure of these proteins by x-ray diffraction method and shared the Nobel Prize in Chemistry in 1962. A protein is a macromolecule made up of long chain of amino acids linked together by peptide bond.

Proteins have different functions as they can provide structural material of bodily tissues both in animal and plant, help in digestion (stomach enzymes), movement (muscles), and play a part in our ability to see (the lens of our eyes is pure crystalline protein). Enzymes, hormones, and

immunoglobulins (makes antibodies and hemoglobin), etc. are also made up of proteins. Functionally proteins involved in oxygen transport, muscle contraction, electron transport, other activities throughout the body and in photosynthesis. In short we can say that, important function of protein is to build up, keep up, and replace the tissues in the body. Now the question is that how can one type of molecule have so many varied function? Actually each protein has a unique and highly ordered structure that enables it to carry out a particular function.

Proteins have different shapes and surfaces that allow them to interact selectively with other molecules. Proteins, in other words, exhibit a high degree of specificity. Some residues must be left in order to carry out a particular biochemical function, and around 40-50 residues appears to be the lower limit for a functional domain size. Protein size ranges from this lower limit to several hundred residues in multi-functional proteins. Large protein complexes with RNA are found in the ribosome particles, which are in fact known as 'ribozymes'.

3. PEPTIDOMIMETICS

Peptides and proteins play a very crucial role in the biological system. However, their use as a therapeutic agent is limited due to their susceptibility to protease degradation and their poor absorption through cell membranes. This problem can be solved by developing peptidomimetics.

Isosteric replacements of a scissile peptide bond represent a viable and popular approach in the rational design of peptidomimetices (Table 1). Peptidomimetics have great application in drug, in protein engineering and so on. In peptidomimetic the peptide segment is replaced by a scaffold. These mimetics does not resemble with peptides except the side chains attached to the rigid core. The purpose of these modifications is to;

1. Increase the potential and selectivity of peptides.
2. Bio availability and stability.
3. Increase its lipophilic character so that can be used for the drug properties.

Peptide	Peptide Mimetic
Poor absorption through cell membrane	Highly absorbed due to high lipophilic character
Not stable	Highly stable

How Bioavailability	Bio available
Not good for therapeutic purpose	Drug candidate

Table 1 Comparison of peptide and peptide mimetic

4. MODIFICATION OF PEPTIDE BACKBONE

Backbone modification includes isosteric or isoelectronic replacement of amide functionality in the peptide chain and / or introduction of additional groups. Although there are number of amide bond replacements reported but we are going to focus on that which are widely used.

4.1 Aminomethylene, ψ[CH_2NH]

This is one of the simplest isosteres of amide bond. Reduction of peptide bond introduces a new basic centre as a form of secondary amine group. Peptides containing the ψ [CH_2NH] replacement demonstrated an increased cell permeability and high stability towards destructive enzymes.

4.2 Oxomethylene, ψ [CH_2O] and Thiomethylene, ψ[CH_2S]

Other simple amide bond replacement contains methylene group instead of carbonyl group and substitution of amine with other heteroatom at oxygen and sulphur. Oxomethylene offers a polar, flexible proteolytically resistance to amide bond. Furthermore, due to the less electro negativity and larger size of the sulphur atom the ψ[CH_2S] modification is less polar and it acts as poor bond acceptor.

4.3 Sulphoxide, ψ[CH_2SO]

The sulphoxide modification of peptide bond contains a new chiral centre and provides enzymatic stability. The sulphoxide replacement also provides strong hydrogen bond acceptor.

4.4 Ketomethylene, ψ [$COCH_2$]

Replacement of amide nitrogen with methylene group gives the ketomethylene mimetic. This modification induces high inhibitory activity in peptides and stability towards proteolytic degradation.

4.5 Ester, ψ[-COO-]

This results in a depsipeptide in which one or more amide bonds are replaced by ester bond. Depsipeptide have often been used in research to probe the importance of hydrogen bond networks in protein folding, kinetics and thermodynamics. They are also found in nature as natural product. An infamous example is the L-Lys-D-Ala-D-Lac motif bound in vancomycin resistant bacteria's cell wall building block. Depsipeptide is an HDAC inhibitors (Histone decetylase inhibitors, HDI), are a class of compounds that interfere with the function of Histone deacetylase.

4.6 Sulfonamide, ψ [CH_2SO_2NH] and Thioamid, ψ[CSNH]

The sulfonamide group was also investigated and increases stability towards protease catalyzed degradation. Thioamides also been used as amide bond surrogate and inhibitors of angio tensis-converting enzyme inhibitor and HIV-I protease inhibitor.

4.7 Trans Carbon-Carbon double bond Isostere, ψ[(E)–CH=CH]

Among the many double bond surrogates, trans carbon-carbon double bond best mimics the transoid nature of the amide bond in terms of rigidity, bond angle and bond length.

4.8 1, 5–disubstituted Terazole, ψ [CN_4]

1, 5–disubstituded tetrazole rings, ψ[CN_4], has been proposed as a surrogate for cisamide bonds, making it a valuable tool in design of conformationally constrained peptide receptor probes, unfortunately, significantly reduced binding affinity by all of the pseudo peptides having tetrazole suggest that cis amide conformer is disfavored in peptides.

4.9 Azoles

1,2,4–oxadiazole, 1, 3, 4–oxadiazole and 1, 2, 4–triazole have been used for replacement of peptide backbone. Due to these modifications markedly different structure–activity profile was observed.

4.10. Retro-inverso, ψ[NCHO]

This modification reverses the order of carbonyl and amine functional groups in an amide bond resulting in a more closely related isosteric replacement for the original peptide bond. This modification enhances protection over hydrolysis and enzymatic attack.

No.	Type	Application

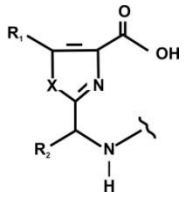
1.	ψ [CH_2NH]	HIV-1 protease inhibitor, neurokinin antagonist, thrombin inhibitor
2.	ψ[CH_2O]	gastrin releasing peptide antagonist
3.	ψ [CH_2S]	Reverse turn stabilizers
4.	ψ [$COCH_2$]	Thrombin inhibitor, neurostensin analogue
5.	ψ [CSNH]	HIV-1 protease inhibitor
6.	ψ [(E)–CH=CH]	HIV-1 protease inhibitor
7 .	ψ [(E)–CF=CH]	Opioid agonist
8.	ψ [CN_4]	Somatostatin and bradykinin
9.	 X=O, S, NH	Endothelin receptor anatagonist
10.	ψ [NHCO]	initiate or modulate cellular immune response
11.	ψ [COCONH]	HIV-1 protease inhibitors
12.	ψ [CH(OH)CONH] ψ [CH(CN)NH]	HIV-1 protease inhibitors

Table 2 Amide bond Replacement and their application

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