Chapter 1

Introduction to amino acids, peptides, heterocycles, urea/thiourea derivatives and importance of conjugation in medicinal chemistry

“Science is a way of thinking much more than it is a body of knowledge”

-Carl Sagan
1.1. Medicinal Chemistry: A brief introduction

1.1.1. The renewal of chemistry

The 18th century concluded its progress in chemistry with an enthusiastic environment. Joseph Priestley in the United Kingdom, Carl Wilhelm Scheele in Sweden, Antoine Laurent de Lavoisier in France gave a precise meaning to the chemical reactivity and promoted a large number of substances to the statute of chemical reagents. Scheele and Priestley prepared and studied oxygen. Both of them discovered nitrogen as a constituent of air, carbon monoxide, ammonia, and several other gases; manganese, barium and chlorine; isolated glycerin and many acids, including tartaric, lactic, uric, prussic, citric, and gallic. Lavoisier is generally considered as the founder of modern chemistry, as creating the oxygen theory of combustion. He should be known as one of the most astonishing 18th century “men of the Enlightenment”, the founder of modern scientific experimental methodology. By formulating the principle of the conservation of mass, he gave a clear differentiation between elements and compounds, something so important for pharmaceutical chemistry. Few years later, Antoine François de Fourcroy, Louis Nicolas Vauquelin, Joseph Louis Proust, Jöns Jakob Berzelius, Louis-Joseph Gay-Lussac, and Humphrey Davy introduced new concepts in chemistry. These scientists integrated the practical advancements of a new generation of experimenters. All these industrial innovations would have their own impact on other developments in industrial and then medicinal chemistry. Claude Louis Berthollet began the industrial exploitation of chlorine (1785). Nicolas Leblanc prepared sodium hydroxide (1789) and then, bleach (1796). Davy performed electrolysis and distinguished between acids and anhydrides.
Louis Jacques Thénard prepared hydrogen peroxide and Antoine Jérôme Balard discovered bromide (1826). The growing of therapeutic resources was mainly due to the mastery of chemical or physico-chemical principles proposed by Gay-Lussac and Justus Von Liebig. At the turn of the 19th century, as the result of a scientific approach, drugs were becoming an industrial item. This chemists’ generation, by realizing all these discoveries, established the concept of the therapeutic discoveries of the 19th century [1].

1.1.2. The dawn of organic chemistry crosses the birth of biology

The constitution of chemistry as a scientific discipline found a new turn few decades later by crossing the road of biology which included revolutionary works of Claude Bernard, Rudolph Virchow and Louis Pasteur. Besides these fundamental sciences, physiology, biochemistry, or microbiology were becoming natural tributaries of the outbreak of pharmacology. Thus, rational treatments were about to be designed on the purpose of new knowledge in various clinical or fundamental fields. After a period characterized by extraction and purification from natural materials (mainly plants), drugs would be synthesized in chemical factories or prepared through biotechnology (fermentation or gene technology) after a rational research, design and development in research laboratories. The birth of organic chemistry progressively led chemists and pharmacists toward organic synthesis performed in what would be called “laboratory” a new concept created by this generation of scientists. Even when those laboratories hosted discoveries like active principles extracted from plants, progresses in drug compounding and packaging made irreversible industrialization processes. At the same time, the economical dimension of growing pharmaceutical industry transformed drugs as strategic
items, mainly when it could interfere with military processes, for instance during colonial expeditions.

The “modern” word “pharmacology” became more and more often used by physicians after the works of François Magendie in France or Oscar Schmiedeberg in Germany. Progressively a clear dichotomy took place between those two entities. *Materia Medica* considered drugs with a static and conservative view as for their production and the compounding of medicines. It was somewhere considered as the natural history of drugs. At the contrary, pharmacology was embracing the creation of drugs through a more dynamic point of view, studying drugs with respect of their site and mechanism of action. At the same time, medicinal chemistry was becoming the application of chemical research techniques to the synthesis of new pharmaceuticals. During the early stages of medicinal chemistry development, chemists were primarily concerned with the isolation of medicinal agents found in plants. Today, in this field they are also equally concerned with the creation of new synthetic drug compounds. As a constant, medicinal chemistry is almost always geared toward drug discovery and development [1].

### 1.1.3. Early history of medicines to drug discovery evolution

Drug discovery and development has a long history and dates back to the early days of human civilization. In those ancient times, drugs were not just used for physical remedies but were also associated with religious and spiritual healing. Sages or religious leaders were often the administrators of drugs. The early drugs or folk medicines were derived mainly from plant products and supplemented by animal materials and minerals.
These drugs were most probably discovered through a combination of trial and error experimentation and observation of human and animal reactions as a result of ingesting such products.

Although these folk medicines probably originated independently in different civilizations, there are a number of similarities, for example, in the use of the same herbs for treating similar diseases. This is likely to be a contribution by ancient traders, who in their travels might have assisted the spread of medical knowledge.

Folk medicines were the only available treatments until recent times. Drug discovery and development started to follow scientific techniques in the late 1800s. From then on, more and more drugs were discovered, tested, and synthesized in large scale manufacturing plants, as opposed to the extraction of drug products from natural sources in relatively small batch quantities. After World War I, the modern pharmaceutical industry came into being, and drug discovery and development following scientific principles was firmly established. From discovery to marketing approval of a drug, the stages involved are shown in Figure 1.1.

Figure 1.1: The stages from drug discovery to marketing approval
Now the pharmaceutical industry is perhaps one of the most regulated industries in the world. From discovering a new drug to registering it for marketing and commercialization, pharmaceutical organizations have to negotiate through very complex and lengthy processes [2].

1.1.4. Changing trends in drug design

The broad field of drug development is poised for an interesting future given major changes in screening and design technologies, the regulatory environment, the lengthening time frames from drug discovery to drug registration, and the economics of pharmaceutical delivery. Our focus is specifically on the future potential of peptides in drug development. Table 1.1 summarizes some historical trends in drug development technologies, targets and product classes, as well as providing a broad overview of the changing regulatory environment.

Table 1.1. Changing trends in drug design

<table>
<thead>
<tr>
<th>Year</th>
<th>Technological era</th>
<th>Molecular classes and/or approaches</th>
<th>Regulatory environment</th>
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<tbody>
<tr>
<td>1960</td>
<td>Chemistry</td>
<td>Natural products screening, rational design</td>
<td>Activity paramount</td>
</tr>
<tr>
<td>1980</td>
<td>Molecular Biology</td>
<td>Biologics (insulin, growth factors)</td>
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<tr>
<td>2000</td>
<td>Genome and Proteomics</td>
<td>New target identification and validation</td>
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<tr>
<td>2020</td>
<td>Peptide drug?</td>
<td>Personalized medicine</td>
<td>Safety paramount</td>
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<tr>
<td></td>
<td>Plant factories?</td>
<td>Increased specificity</td>
<td></td>
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<td></td>
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<td>Cheaper manufacture</td>
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</table>
Chemical biology is a relatively new scientific field spanning the traditional disciplines of chemistry and biology. It involves the application of synthetic chemistry to study and manipulate biological systems at a molecular level. Organic synthesis allows the construction of molecular assemblies with a wide variety of functional groups and 3-dimensional topologies. Combining synthetic compounds with biological macromolecules such as carbohydrates, proteins or oligonucleotides has resulted in a number of hybrid structures that are promising candidates for use in targeted imaging and drug delivery. The concept of using “magic bullets” that specifically seek, target and destroy diseased cells with minimal damage to normal tissue has been around since Ehrlich postulated more than 100 years ago the existence of specific receptors that bind antigens [3].

1.2. Biomolecules

Fifteen to twenty billion years ago, the universe arose as a cataclysmic eruption of hot, energy-rich subatomic particles. Within seconds, the simplest elements (hydrogen and helium) were formed. As the universe expanded and cooled, material condensed under the influence of gravity to form stars. Some stars became enormous and then exploded as supernovae, releasing the energy needed to fuse simpler atomic nuclei into the more complex elements. Thus were produced, over billions of years, the Earth itself and the chemical elements found on the Earth today. About four billion years ago, life arose-simple microorganisms with the ability to extract energy from organic compounds or from sunlight, which they used to make a vast array of more complex biomolecules from the simple elements and compounds on the Earth’s surface.
The structures of biomolecules or biologically active molecules usually contain more than one type of functional group. This means that the properties of these molecules are a mixture of those of each of the functional groups present plus properties characteristic of the compound. The latter are frequently due to the interaction of adjacent functional groups and/or the influence of a functional group on the carbon–hydrogen skeleton of the compound. This often involves the electronic activation of C–H bonds by adjacent functional groups. Many biological molecules are macromolecules, polymers of high molecular weight assembled from relatively simple precursors. Proteins, nucleic acids, and polysaccharides are produced by the polymerization of relatively small compounds with molecular weights of 500 or less [4].

1.2.1. Amino acids—Building blocks of body

Simple amino acids are the basic building blocks of proteins. Their structures contain an amino group, usually a primary amine, and a carboxylic acid. Amino acids may be classified as α, β, γ . . . .etc. depending on the relative positions of the amine and carboxylic acid groups but for most naturally occurring compounds the amino group is attached to the same carbon as the carboxylic acid as shown in Figure 1.2. α-Amino acids are the most common naturally occurring amino acids.

![Figure 1.2](image_url)

**Figure 1.2.** The general structural formula of α-amino acid
α-Amino acids and their derivatives are central to the chemistry and biology of peptides and proteins as well as versatile synthetic building blocks for pharmaceutical applications, precursors for the generation of molecular diversity, important templates in asymmetric catalysis, and common subunits in many bioactive compounds and natural products [5].

1.2.1.1. Biological significance of amino acids in nutrition and metabolism [6]

Based on growth or nitrogen balance (namely net synthesis of protein in the whole body), amino acids have traditionally been classified as nutritionally essential (indispensable) or non-essential (dispensable) for animals and humans. Nutritionally essential amino acids are those whose carbon skeletons are not synthesized by animal cells and, therefore, must be provided from the diet. Dietary essentiality of some amino acids (e.g. arginine, glycine, proline, and taurine) depends on species and developmental stage. In contrast, non-essential amino acids are those amino acids that are synthesized de novo in a species-dependent manner. It was tactically assumed, without much evidence, that animals or humans could synthesize sufficient amounts of all non-essential amino acids and did not need them in diets for optimal nutrition or health. However, growing evidence from cell culture and animal studies shows that some of the traditionally classified non-essential amino acids (e.g. glutamine, glutamate, and arginine) play important roles in multiple signaling pathways, thereby regulating gene expression, intracellular protein turnover, nutrient metabolism, and oxidative defense. Pathways for the synthesis of arginine, glutamine, glutamate, and proline and alanine are now well
documented and have important nutritional and physiological significance. Amino acids display remarkable metabolic and regulatory versatility as listed below:

✓ Nutrient absorption and metabolism (e.g., nutrient transport, protein turnover, fat synthesis and oxidation, glucose synthesis and oxidation, amino acid synthesis and oxidation and urea and uric acid synthesis for ammonia detoxification)
✓ Cellular signaling via mTOR, cAMP, and cGMP activation pathways, as well as the generation of NO, CO, and H$_2$S
✓ Hormone synthesis and secretion (e.g., insulin, glucagon, growth hormone, glucocorticoids, prolactin, placental lactogen, and epinephrine)
✓ Endothelial function, blood flow, and lymph circulation
✓ Immune function and health (e.g., T-cell proliferation and B-cell maturation, antibody production by B-cells, killing of pathogens, obesity, diabetes, and metabolic syndrome)
✓ Acid–base balance, neurotransmission, extracellular and intracellular osmolarity, antioxidative defense, and whole body homeostasis
✓ Fetal and postnatal growth and development, as well as tissue regeneration and remodeling
✓ Reproduction and lactation (e.g., embryo implantation, placental angiogenesis and growth, fetal growth and development, and lactogenesis)

They serve as essential precursors for the synthesis of a variety of molecules with enormous importance, and also regulate key metabolic pathways and processes that are vital to the health, growth, development, reproduction, and homeostasis of organisms. These findings exemplify the power of basic research on amino acids biochemistry and
nutrition to discover new knowledge of animal biology and solve significant practical problems in medicine and animal agriculture.

1.2.2. Peptides and proteins

Peptides and proteins are found throughout biology, and possess a range of physiological and cellular functions. Their structure is often complex, capable of presenting in many different conformations, depending upon their environment. They consist of amino acid residues linked together by amide functional groups, which in peptides and proteins are referred to as peptide links. The basic structure of peptides and proteins is twisted into a conformation (time dependent overall shape) characteristic of that peptide or protein. These conformations are dependent on both the nature of their biological environment as well as their chemical structures. The ability of peptides and proteins to carry out their biological functions is normally dependent on this conformation. Any changes to any part of the structure of a peptide or protein will either change or destroy the compound’s biological activity. For example, sickle-cell anemia is caused by the replacement of a glutamine residue by a valine residue in the structure of haemoglobin. Peptides, typically classed as molecules containing between two and fifty amino acids bonded together, and proteins (larger peptide molecules containing over fifty amino acids, ‘polypeptides’) have long been regarded as crucial to offering solutions to mounting and increasingly difficult world health issues, and the possibility of patient-specific therapy [5].
1.2.3. *Significance of amino acids/peptides as therapeutics*

Amino acids/peptides are involved in a variety of physiological and pathological processes and play very important roles in modulating various cell functions. They have certain bio-functionalities that make them excellent leads in drug design, including exquisite selectivity for their molecular targets and high potency and may therefore serve therapeutic roles in body systems. **Thus they may act as alternatives to small molecule drugs. They offer a lot of advantages over conventional small molecule due to their high bioactivity and biospecificity to targets, wide spectrum of therapeutic action, low levels of toxicity, structural diversity and absence (or low levels) of accumulation in body tissues.**

Peptides offer a particularly versatile platform in the drug-design process because many biological interactions are mediated by protein-protein interactions and peptides may be derived from knowledge of the protein sequence and binding motif to yield a starting point for drug design. The versatility of peptides derives from the chemical diversity of naturally occurring amino acids and the ready availability of chemically modified building blocks for peptide synthesis, featuring modifications on the peptide backbone and/or the side chain. With the development of high-yield solid phase synthesis procedures, these building blocks and amino acids offer a vast resource for exploring chemical space in terms of functionality and chirality [7]. Bioactive peptides may induce functionalities such as antioxidative, antimicrobial, antihypertensive, cytomodulatory and immunomodulatory effects in living systems and these multifunctionalities enhance their potential use as therapeutic aids. The role of bioactive peptides in modulating innate immune responses and boosting natural immunity while controlling microbial host
invaders is well documented. ‘Traditional’ peptide therapeutics are manufactured by transgenic, recombinant, or synthetic methods but these approaches are known to be very expensive and thus are prohibitive for large scale applications.

Bioactive peptides are protein hydrolysates that can induce beneficial physiological responses in the human body. They are inactive within the sequence of the parent protein molecule and can be released by \textit{in vivo} or \textit{in vitro} enzymatic hydrolysis of the parent proteins. The functional abilities of bioactive peptides have therefore aroused a lot of scientific, technological, and consumer interest. Research continues to uncover novel peptide sequences for potential application in the control or prevention of ill health. Currently, there are around 60-70 approved peptide drugs in the global market, with 100-200 more in clinical trials, 400-600 more in pre-clinical studies and possibly hundreds to thousands more on the laboratory bench. With the increase of approved peptide-based drugs and the advance in peptide-associated technologies, we believe that peptide-based drug therapeutics will become more significant and will open up more commercial opportunities for treating human diseases. With the barriers having been broken, it will be open season for peptide drugs and their hunters for the future [8].

\section*{1.3. Heterocycles: Small molecule based drug discovery}

The importance of heterocyclic compounds in drug discovery and development is well recognized over the last decades with multiple heterocyclic novel chemical entities approved as drugs or currently in clinical development [9]. The syntheses of heterocyclic systems are comprehensively and periodically reviewed on both novel synthetic routes
and novel types of the compounds, as well as their applications [10]. Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic while countless additives and modifiers used in industrial applications ranging from cosmetics, reprography, information storage and plastics are heterocyclic in nature. One striking structural features inherent to heterocycles, which continue to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations. For more than a century, heterocycles have constituted one of the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life. Among the approximately 20 million chemical compounds identified by the end of the second millennium, more than two-thirds are fully or partially aromatic and approximately half are heterocyclic. The presence of heterocycles in all kinds of organic compounds of interest in electronics, biology, optics, pharmacology, material sciences and so on is very well known. Between them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis [11]. However, heterocycles with other heteroatoms such as oxygen [12], phosphorus [13] and selenium [14] also appears. Many natural drugs [15-18] such as papaverine, theobromine, quinine, emetine, theophylline, atropine, procaine, codeine, reserpine and morphine are heterocycles. Pyrimidine (cytosine, thymine and uracil) and purine (adenine and guanine) derivatives are monocyclic and
bicyclic heterocycles with two and four nitrogen atoms, respectively. They are key components of the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules which participate directly in the encoding of genetic information [19, 20]. The essential amino acids like proline, histidine and tryptophan [21], photosynthesizing pigment chlorophyll; the oxygen transporting pigment haemoglobin [22], the hormones kinetin, heteroauxin, cytokinins [23], neurotransmitter serotonin, histamine respectively are successful application of heterocyclic compounds. Almost all the compounds known 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. All these natural and synthetic heterocyclic compounds can and do participate in chemical 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, nucleic acids, ATP and serotonin [24]. Why does nature utilize heterocycles? The answer to this question is provided by the fact that 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 toward 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 mentioned above 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.

Synthetic heterocycles have widespread therapeutic uses such as antibacterial, antifungal, antimycobacterial, trypanocidal, anti-HIV, antileishmanial agents, genotoxic, antitubercular, antimalarial, herbicidal, analgesic, antiinflammatory, muscle relaxants, anticonvulsant, anticancer and lipid peroxidation inhibitor, hypnotics, antidepressant, antitumoral, anthelmintic and insecticidal agents [25-31].

In conclusion, it can be stated that 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. The constantly accelerating rate of research and development in heterocyclic chemistry suggested that enormous numbers of heterocyclic systems are well known and this number is increasing very rapidly. The nitrogen, oxygen and sulphur heterocycles (thiazetidines, thiazoles, isothiazoles, thiazolines, thiazolidines, dithiazinanes, thiadiazines, etc. and their derivatives) are an attractive source of compounds for the identification of new biological probes and are widely used in industry and in medical practice. The main aim is to design and synthesize molecules involving the use of structural motif commonly found in
majority of well-established drug molecules. Preparations with antimicrobial activity, diuretics, mitodepressants, and antihistamine, antiparasitic, antiviral, and antipyretic agents have been developed and introduced on the basis of these types of heterocycles [32].

1.3.1. Therapeutic utility of thiazole & benzisothiazole derivatives

Amongst different heterocyclic systems, the chemistry of five membered heterocycles with more than one heteroatom has gained significance as many of them exhibit pronounced bioactive nature. Molecules that possess nitrogen and sulfur atoms are important in living organisms. In this context, thiazole (I) is one important class of heterocyclic compound. Hence, attempts to study their detailed chemistry would add new dimensions to the existing knowledge.

Thiazole and related compounds are called 1,3-azoles (nitrogen and one other heteroatom in a five-membered ring). They are isomeric with the 1,2-azoles, the nitrogen and sulfur compound being called isothiazole. Thiazoles being important class of heterocyclic compounds are found in many potent biologically active molecules such as Sulfathiazole (antimicrobial drug), Ritonavir (antiretroviral drug), Abafungin (antifungal drug) with trade name Abasol cream and Tiazofurin (antineoplastic drug). It has been
noticed continuously over the years that interesting biological activities were associated with their derivatives [33, 34]. Thiazoles are found in a variety of specialized products, often fused with benzene derivatives, are so-called benzothiazoles (II). Two isomeric forms of benzisothiazole are known, depending on the position of the ring fusion. Benz[d]isothiazole is better known as 1,2-benzisothiazole (IIa) and benz[c]isothiazole as 2,1-benzisothiazole (IIb). 2,3-Dihydro-1,2-benzisothiazole (IIc) is also described as 1,2-benzisothiazoline, and 1,3-dihydro-2,1-benzisothiazole (IId) as 2,1-benzisothiazoline.

Benzisothiazole is a unique heterocyclic structure that has been visualized as an important pharmacophore of some bioactive molecules. The main interest in benzisothiazoles over the years has been the sweet taste of the compound saccharin, 1,2-benzisothiazol-3(2H)-one 1,1-dioxide. Five hundred times sweeter than sugar in dilute solution, saccharin has been the subject of many patents and also much development work aimed at reducing its metallic aftertaste [35]. Figure 1.3 enlists few drugs and drug like candidates with thiazole and benzisothiazole in their molecular framework. Other benzisothiazoles have pharmacological importance and value as agrochemicals.
Figure 1.3. Some commercially important drugs and drug-like candidates with Thiazole/Benzisothiazole scaffold in their molecular framework.
For example, ipsapirone is an axiolytic serotonin receptor agonist and probenazole is used against rice blast fungus. Benzothiazole and its derivatives have been reported to possess many other interesting biological activities such as antibacterial [36], anti-HIV [37], antiproliferative activities of B-lymphoblastic leukemia cells [37] and others [38, 39]. As an example, Ziprasidone, which contains a key benzothiazole subunit, is an FDA-approved antipsychotic drug for the treatment of schizophrenia [40-42]. Due to the importance of this scaffold in drug discovery, the synthesis of benzothiazoles has received considerable attention from organic chemists.

Due to its varied applications in the field of chemistry, several researchers have extensively worked on isothiazole nucleus. In this context, it was felt worthy to give a brief description about its utility in biological chemistry. Some of the foregoing pages get a look into various thiazole containing moieties which have been shown to posses different therapeutic properties.

Popsavin et al., [43] described a divergent de novo synthesis of 2-(2, 3-anhydro-β-D-ribofuranosyl) thiazole-4-carboxamide (2',3'-anhydro-tiazofurin) derivatives and screened them for antitumor activity. Remarkably all the analogs exhibited sub-micromolar cytotoxicity against K562 malignant cells, with IC_{50} values ranging from 0.09-0.49 μM. The most active compound against these cells is the α-homo-C-nucleoside III, being 33-fold more cytotoxic than tiazofurin, which was recently been approved as an orphan drug for treatment of the corresponding malignant disease.
Kumar et al., [44] synthesized a group of 3-[4'(p-chlorophenyl) thiazol-2'-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethyl]-6-bromoquinazolin-4-ones and screened them for anti-inflammatory and analgesic activities. IV was found to be highly active in both the activities. They found that the presence of thiazolidinone ring have shown much better anti-inflammatory as well as analgesic activity at 50 mg/kg po as compared to their parent compounds. Compound substituted with chloro group at 2nd position of phenyl ring has shown almost equal anti-inflammatory activity to that of the standard drug phenylbutazone at 50 mg/kg body weight.

Holla et al., [45] reported different series of arylaminothiazoles, arylidene/5-aryl-2-furfurylidene hydrazinothiazoles and screened them for their antibacterial and anti-
inflammatory activities. Two of the newly synthesized compounds V and VI showed excellent anti-inflammatory activity comparable with that of ibuprofen.

Shiradkar et al., [46] reported a series of $N$-[4-[(4-amino-5-sulphonyl-4$H$-1,2,4-triazol-3-yl) methyl]-1,3-thiazol-2-yl]-2-substituted amide derivatives. The compounds were tested for their preliminary in vitro antibacterial activity and then were screened for antitubercular activity against $M. tuberculae$ H37Rv strain. Compound VII and VIII showed best activity showing more than 90% inhibition. The antimycobacterial screening of the novel series has demonstrated emergence of potent derivatives that have highly electronegative part at sulfhydryl group.
Zitouni et al., [47] reported new thiazole derivatives of triazoles and evaluated for antifungal and antibacterial activities. Most of the compounds were active against *C. albicans* (NRRL Y-27077). The most and effective compound against clinic isolate of *C. albicans* was found to be IX which includes chlorine substitution on phenoxy moiety.

\[ \text{IX} \]

Narayana et al., [48] prepared a series of 5-{2-[(N-substituted aryl) amino]-1,3-thiazol-5-yl} 2-hydroxy benzamides by reacting 5-(bromoacetyl) salicylamide with thiourea, thioformamide, thioalkylamide and substituted thiureas in absolute ethanol. These compounds were converted to 5-(2-substituted-1,3-thiazol-5-yl)-2-alkoxybenzamides and 5-(2-N-(substituted aryl)-1,3-thiazol-5-yl)-2-alkoxy benzamides by reacting with N-alkylbromides in presence of a base. The resulting compounds were screened for their antifungal activity. The derivative X exhibited significant activity.

\[ \text{X} \]

Clark et al., [49] presented new series of phenoxy thiazolyl derivatives and screened them for their acetyl-Co-A carboxylase inhibitory profile. Compound XI was found to be highly active in the inhibition of acetyl-Co-A carboxylase isozyme.
The *in vitro* antimicrobial activity of 2-amino-benzo[d]isothiazol-3-one and of several 2-arylideneamino derivatives carrying in the second position a substituted or unsubstituted aromatic ring or an arylalkenyldiene moiety was determined by the broth dilution method against several strains selected to define their spectrum and potency. It was reported that the parent 2-amino-benzo[d]isothiazol-3-one XII was the most effective agent, with minimum inhibitory concentration (MIC) values ranging from 0.07 to 6 μg/mL [50].

Vicini et al., [51] described the synthesis and evaluation of benzo[d]isothiazole hydrazones as antiproliferative agent. Compound XIII bearing a hydroxyl group at position 2 of the benzylidene moiety, resulted as being the most potent, showing 6- to 32-fold more potency than 6-MP against skin melanoma and lung squamous carcinoma, heptatocellular and prostrate carcinoma.
In another study, new analogs of benzisothiazole hydrazones were synthesized and tested in MT-4 cells cultures for their anti-HIV properties against wild type HIV-1 and HIV strains carrying clinically relevant mutations (EFVR, Y181C and K103/Y181C). All the tested compounds showed good activity against wild type HIV-1 and against the EFVR mutant. In terms of SAR the relevant result was that, in the class of benzisothiazole hydrazones, the benzo[d]isothiazol-3(2H)-one moiety is an essential structural requirement for the antiretroviral activity. Compounds XIV and XV showed good activity against HIV-1 wild type, XVI showed significant activity against the Y181C mutant [52].

Sharma et al., [53] reported the synthesis of 1,2-benzisothiazole derivatives, having 2-thiazolyl-4(5)-acetic acid moiety attached to position-3 of 1,2-benzisothiazole nucleus, and evaluated them as anti-inflammatory agents. Amongst, compounds XVII and XVIII possessed excellent level of anti-inflammatory activity, higher than that of the standard drug ibuprofen, in carrageenan-induced rat paw edema assay.
A series of novel cholinesterase inhibitors based on 2-substituted 6-fluorobenzo[d]thiazole were synthesised by Imramovsky et al., [54]. The novel carbamates were tested for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The toxicity of the most active compound XIX was investigated using a standard *in vitro* test with HepG2 cells, and the ratio between biological activity and toxicity was determined. In addition, the toxicity of the most active compounds was evaluated against MCF7 cells using the xCELLigence system. It was found that substitution with an *n*-propyl chain is more advantageous for high AChE/BChE inhibition. R substitution with a branched long-chain alkyl is crucial for potent AChE inhibition, while R substitution by a short unbranched alkyl chain seems to be preferred for potent BChE inhibition.
1.3.2. Piperazine: An honored scaffold in medicinal chemistry

Piperazine is a six membered cyclic secondary diamine with nitrogen in 1 and 4 positions. It can also be called diethylenediamine for convenience; its molecular formula can be written $C_4H_{10}N_2$, and structural formula shown as under (XX).

![Piperazine structure]

Piperazines are a broad class of chemical compounds used widely in human and veterinary medicines. They are also used in industry to make plastics, resins, pesticides, brake fluid and a variety of materials and products. Some piperazine compounds act as effective worming agents for pets and farm animals. The piperazine motif appears in many drugs encompassing a broad range of activities displaying anti-allergenic antibacterial, anti-anxiety, antiemetic, antimigraine, etc., [55]. Some drugs and drug candidates with piperazine scaffold in their molecular framework are listed in Figure 1.4.

The piperazine framework is observed in a large number of compounds of pharmaceutical interest. In 2001 the MDDR (MDL Drug Data Report) listed 2271 phenylpiperazines which totaled 65 structures in phase II clinical trials or higher across 23 therapeutic areas. This total includes antibacterials, $\alpha$-1-adrenergic blockers, $\alpha$-2-adrenergic agonists, antidepressants, serotonin receptor (5-HT2A) antagonists, phosphodiesterase III inhibitors, antitussives, antifungals, antivirals, anxiolytics, antipsychotics, lipooxygenase inhibitors, antiaggregants, endothelin antagonists, hypolipidemic compounds, and also molecules that treat cognition disorders [56].
Figure 1.4. Drugs and drug like candidates with piperazine scaffold in their molecular framework
The piperazine scaffold has been classified as a privileged structure [57] capable of multiple receptors with high affinity. It is a recurring structural motif in a large number of biologically active molecules [58]. In this context, a brief literature survey of the compounds containing piperazine scaffold is presented in the following section.

The synthesis and preliminary pharmacological evaluation of a series of potential atypical antipsychotic agents based on the structure of 1-(1,2-dihydro-2-acenaphthylene)l-piperazine is described by Srinivas et al., [59]. Amongst, XXI showed significant affinities at the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and moderate affinity at the D$_2$ receptor. It exhibited a high reversal of catalepsy induced by haloperidol indicating its atypical antipsychotic nature.

![XXI](image)

Kimura et al., [60] synthesized a new series of diphenylalkyl piperazine derivatives with high affinities for the Dopamine Active Transporter (DAT) and were evaluated for their inhibitory activities against auto-oxidative lipid peroxidation in canine brain homogenates. Amongst, 4-hydroxyphenyl derivative XXII showed the most potent antioxidative activity with an IC$_{50}$ value of 0.32 µM, exhibiting approximately 5-fold more potent activity than α-tocopherol, a standard.
Within the series of piperazine derivatives (with various substituents in position 3) synthesized by Bedürftig et al., [61], the phenylacetamide XXIII revealed considerable affinity towards $\sigma_1$ receptors, which is in the range of the $\sigma_1$ receptor affinity of ditolylguanidine ($K_i = 164$ nM).

Chonan et al., [62] reported the synthesis and evaluation of novel unsymmetric disubstituted (4-piperidinyl)-piparazine derivatives as Acetyl-CoA carboxylase 1/2 (ACC 1/2) inhibitors. XXIV and XXV displayed excellent potency as non-selective ACC 1/2 inhibitors.
Nozawa et al., [63] reported the design, synthesis, and structure-activity relationships of the novel bis-piperazines as MC4 receptor antagonists. During the investigation of antagonists for the MC4 receptor, it was found that compound **XXVII** and **XXVIII** having a naphthyl group showed almost the same binding affinity for the MC4 receptor as that of the lead compound **XXVI** with a benzoyl group. In particular, **XXVIII** exhibited the highest affinity for the MC4 receptor with an IC$_{50}$ value of 8.13 nM.

Orjalis et al., [64] synthesized new 4-(diphenylmethyl)-1-piperazine derivatives with a terminal heteroaryl or cycloalkyl amide fragment and evaluated for their antihistaminic, anticholinergic and antiallergic activities. Tested compounds were found to be moderate to potent *in vitro* (guinea-pig ileum) histamine H$_1$-receptor antagonists. In the preliminary pharmacological studies, compound **XXIX**, has been demonstrated to be an excellent histamine H$_1$-receptor antagonist, highly active when orally administered, with antiallergic properties and without anticholinergic and sedative CNS side effects.
Sapa et al., [65] reported the synthesis of 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl) propyl]-pyrrolidin-2-one and its enantiomers which were later tested for electrocardiographic, antiarrhythmic and hypotensive activities. The racemic mixture and its S-enantiomer significantly decreased systolic and diastolic blood pressure and possessed antiarrhythmic activity. The S-enantiomer XXX displayed marked effect.

Pietrzycka et al., [66] reported preliminary evaluation of antioxidant activity of some 1-(phenoxyethyl)-piperazine derivatives. The antioxidant profile of 1-(phenoxyethyl)-piperazine derivatives were compared to Trolox and Resveratrol. The piperazine derivatives possessing 4-(methyl) or 1-[2,6-(dimethyl)phenoxyethyl] moiety XXXI showed significant activity.
Aytemir et al., [67] reported the synthesis of 3-hydroxy-6-methyl-2-substituted 4-\textit{H}-pyran-4-one derivatives for the evaluation of their potential anticonvulsant activity. Among the synthesized compounds carrying 4-(3-trifluoromethylphenyl)piperazin-1-ylmethyl group at position 2, XXXII on the pyranone ring was found to have significantly high anticonvulsant activity against the scMet seizures, while XXXIII which carry a 4-chlorophenyl moiety was found to be protective against MES at all doses of half an hour. Although XXXII showed neurotoxicity at the high dose, XXXIII did not. Hence, XXXIII is the most promising compound among these Mannich bases.

A novel series of 4-substituted-piperazine-1-carbodithioate derivatives of 2,4-diaminoquinazoline were synthesized by Cao et al., [68] and tested for their antiproliferative activity against five human cancer cell lines. Most of the synthesized compounds showed broad spectrum antiproliferative activity (IC$_{50}$ = 1.47-11.83 mM), of which XXXIV, XXXV and XXXVI were the most active members with IC$_{50}$ values in the range of 1.58-2.27, 1.84-3.27 and 1.47-4.68 µM against five cancer cell lines.
examined, respectively. Further investigations revealed that these compounds exhibited weak inhibition against dihydrofolate reductase and no activity against thymidylate synthase, while induced DNA damage and activated the G2/M checkpoint in HCT-116 cells.

![Chemical structure]

**XXXIV** $R = \text{OCH}_3$

**XXXV** $R = \text{F}$

**XXXVI** $R = \text{NO}_2$

### 1.4. Bioconjugation

Improved efficacy of therapeutics, particularly through enhanced local delivery to the cellular compartment of the diseased cell, is a strong focus in pharmaceuticals R&D labs. These techniques look to solve traditional drug delivery challenges, such as poor cellular uptake and/or non-specific toxicity. Targeted delivery involves the use of a biological vector that is covalently bound to a therapeutic agent, such as a drug, biologic, toxin, radionuclide, etc., to form a bio-conjugate hybrid or, simply termed, a conjugate. Delivery vectors include cell-penetrating peptides, proteins or enzymes and monoclonal antibodies (mAbs) or antibody fragments.

Bioconjugation, a novel technique is usually exploited to improve the biopharmaceutical aspects of a bioactive as well as afford its spatial and temporal distribution. The field of bioconjugate chemistry is a growing research activity, with the
potential for scientific breakthroughs and lies at the core of many biomedical and biotechnological endeavors. It could be described as the science (and art!) of joining two different molecular functions by chemical means. This includes the conjugation of antibodies (and their fragments), nucleic acids (and their analogs), other biomolecules (such as receptor binding proteins, hormones, peptides) with each other, or with any molecular group that adds useful properties such as drugs, radionuclides, toxins, fluorophores, photoprobes, inhibitors, enzymes, and ligands [69]. The strategy enlightens newer vistas for delivery of drugs, peptides, enzymes, and oligonucleotides. Site specific delivery may be obtained by tailoring the conjugates as an inactive prodrug and designing polymer drug linkages susceptible to cleavage by specific enzymes or pH. These prodrugs substantially change the mechanisms of cellular entry, pharmacokinetic disposition and ultimately target the drug. The conjugate vehicles are being exploited for targeting pharmacological agents to visceral tissues viz brain, colon etc. These biomaterials are bringing into play, novel drug delivery systems for selectively and specifically ferrying drugs to the desired organ. Noteworthy contributions reported with bioconjugated nanoparticles for biosensing and bioimaging incorporate cell staining, DNA detection, separation and recombination relevance in DNA protection. Only recently, these tailor-made polymers have also gained impetuous for enzyme therapy, gene therapy, insulin therapy, cancer therapy and management of AIDS with the interception of minimal side effects [70].

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., [71-76]. In light of these developments, conjugation of amino acids/peptides with bioactive scaffolds is of great importance in biomedical research.

Amide bonds have a half life of ca. 600 years in neutral solution at 25 °C. This extraordinary stability makes amide linkages highly attractive for bioconjugation. The random introduction of amide linkages in biomolecules is trivial [77].

1.4.1. Bioconjugation of amino acids/peptides with heterocyclic scaffold as therapeutic agents

Pharmacotherapy or medication is by far the most widely used therapy for mitigating maladies and involves the use of chemical substances in the diagnosis, cure, treatment, or prevention of disease. The 18th to 20th centuries saw the discovery of antibiotics, vaccination and the use of asepsis treatment methods. Since then, different classes of drugs have been available for the treatment of different medical conditions. However, most of these drugs pose side effects upon intake. Furthermore, there is progressive development of resistance by pathogenic microorganisms against small molecule drugs thus necessitating a search for new therapeutics with reduced side risks. Several studies in the past few decades have established that bioactive peptides have certain bio-functionalities and may therefore serve therapeutic roles in body systems [78-81]. Thus they may act as alternatives to small molecule drugs. They offer a lot of advantages over conventional small molecule due to their high bioactivity and biospecificity to targets, wide spectrum of therapeutic action, low levels of toxicity, structural diversity and absence or low levels of accumulation in body tissues [82].
Peptide research has seen progressive growth over the past few decades, in particular with respect to ‘peptide therapeutics’. Many companies specializing in their manufacture, along with companies developing peptide-based products ranging from new drug candidates to medical diagnostic devices, through to cosmetics and food technologies, have come to the forefront of pharmaceutical acquisitions and venture capital groups [83-85].

Peptides are involved in a variety of physiological and pathological processes and play very important roles in modulating various cell functions. Peptide drugs have been successfully applied in treating certain human diseases. For instance, Goserelin (a synthetic gonadotropin-releasing hormone analog, marketed as Zoladex) is applied to treat breast cancer and prostate cancer. Glatiramer acetate (a synthetic peptide with four amino acids, Copaxone) is used for multiple sclerosis and Exenatide (a synthetic glucagon-like peptide-1 analog, Byetta) for type 2 diabetes. The synthetic somatostatin analogs such as octreotide (Sandostatin) and lanreotide (Somatuline) are the most common drugs used in treating neuroendocrine tumors while conventional chemotherapy and radiotherapy have very limited effects. Unfortunately, the annual sales of all the approved peptide drugs are only a small amount (approximately 2%) of the huge drug market. However, the approval rate for peptide drugs may be twice as high as that for small molecules [86]. The peptide drug market is also growing twice as fast in the worldwide drug market [87]. Compared to the small molecules that dominate the worldwide drug market, with advantages such as small size, low cost and low price, oral availability, ready synthesis, membrane-penetrating ability and stability, peptides are at a disadvantage [86-90]. However, peptides are still small compared to large molecules such
as proteins and antibodies. Due to this smaller size, peptides can be readily synthesized, optimized, evaluated and do not cause serious immune responses. Peptides are potent and could be metabolically cleaved and rapidly cleared from body. Peptides do not accumulate in specific organs and this can minimize their toxic side effects. In contrast, small molecules are not selective and can accumulate in specific organs such as the kidney and liver, resulting in severe toxic side effects. To be more stable, peptides could be modified or made as a cyclic peptide pro-drug.

There are still some challenges for us to be able to bring a peptide to commercial drug status and to expand the peptide drug market despite more peptides that have been successfully brought to market. The oral drug administration route is the most convenient and comfortable way, but is the most difficult challenge for peptide drugs. The poor oral bioavailability limits the commercial applications of these drugs. Peptides are easily degraded and have difficulty passing through the intestinal mucosa. Gastric acid in the stomach and peptidases in the blood could easily chop peptides into single amino acids while poor permeability blocks intestinal absorption [89]. Also, different administration routes could affect the peptides’ pharmacokinetics and biological activity [91]. Compared to cheap and small molecules, the production cost and the market price are still high for commercializing a peptide drug besides costing more to synthesize longer peptides than shorter ones. There are some other challenges, like the stability of recombinant peptides (recombinant peptides may be readily digested by enzymes in body), peptide antigenicity (peptide antigenicity may result in immune responses) and production scales (different production scales may require a completely different technology for synthesis and purification). We also need to consider the challenges in searching and identifying novel
peptides and the associated technologies. All these show us there is a long way to go before peptides fit well with commercial requirements.

Despite the many challenges we face, the advances made in the fields of peptide drug development give us more confidence and more willingness to develop novel peptide-based therapeutics. The new phage display technology now is used for peptide discovery. This is completely different from the traditional way and may open a new window for finding completely new peptide drugs.

Due to the severe toxic side effects of small molecules, the advances in receptor-targeted therapeutics in which peptides and mAbs are used as receptor-specific drug delivery carriers is catching scientists’ interests. Many peptides target the family of G-protein coupled receptors (GPCRs), some of which are aberrantly expressed in some specific diseased cells/tissues [90]. These peptides, especially the chemically modified and long-acting peptide analogs, have been used as drug delivery carriers to couple the small molecule drugs at the N- or C-terminus to form new drug peptide conjugates. The new conjugates could bind to specific GPCR members on the cell surface and deliver drugs into target cells. More examples come from cancer treatments due to many cancer cells highly expressing certain GPCRs, such as SSTR2 and GRPR. These receptor specific conjugates such as AN215, AN238 and JF-10-81 display much more potent and specific anti-tumor efficacy while reducing toxic side effects and multi-drug resistance [92, 93]. This kind of receptor targeted therapeutics has been named as a new generation approach. These synthetic peptides, used as delivery carriers, also have been widely used to couple with siRNAs, oligoDNAs, oligoPNAs, other peptides, to deliver them into target cells, and thus increase their internalization and efficacy [94-97].
These suggest us that peptide-based therapeutics are an exceptional arena in the present day scenario for the development of new disease curing molecules provided the disadvantages associated with them are met out especially stability. In order to improve the stability of the peptides, there are several approaches of which conjugation is in one. Several research groups are utilizing this technique to develop newer therapeutics. Hence, in order to get an insight into this, a brief note of amino acids/peptides conjugates has been presented below.

Kim et al., [98] 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 conjugates, quercetin–glutamic acid conjugate XXXVII and XXXVIII showed remarkable increase in water solubility, stability, and cell permeability compared with quercetin, which warrants further development as a quercetin prodrug.

![XXXVII](image1.png) ![XXXVIII](image2.png)

Nayyer et al., [99] reported the synthesis, antimycobacterial activity and 3D-QSAR study of two series of 4-(adamantan-1-yl) group containing quinolines conjugated to amino acids. The most potent analog XXXIX displayed 99% inhibition at 1.00 mg/mL against drug-sensitive strain, while it exhibited 99% inhibition at 3.125 mg/mL against drug-resistant strain.
Stanchev et al., [100] have synthesized oxazole and thiazole containing amino acids and peptides. The compounds showed antibacterial activity in vitro at concentrations of 0.4–0.8 mM. The activity revealed that variations in the side-chain led to the enhanced antimicrobial activity of the tested compounds. XL was found to possess excellent potency against microbial strains.

Carboxylic acids derived from the amido groups of the antitumor agents mitozolomide and temozolomide had been conjugated to simple amino acids and peptides by Arrowsmith et al., [101]. Attachment of the acids to imidazole polyamidic lexitropsins gave a series of potential DNA minor groove binding ligands. To assess the comparative in vitro activities of mitozolomide and the series of simple amino acid conjugates, compounds were evaluated against the mouse TLX5 lymphoma. Amongst, XLI was found to be potent.
Recently, Singh et al., [102] synthesized several piperoyl-amino acid ester conjugates and evaluated for antileishmanial activity in vitro and in vivo. Piperoyl-valine methyl ester XLII showed an IC$_{50}$ of 0.075 mM against the amastigotes and was further evaluated in vivo in the golden hamsters. Molecular docking studies indicated that XLII binds to the active site of L. donovani protein 1QB8.

In another study, Dias et al., [103] synthesized a new series of oxoazabenzo[de]anthracenes conjugated to amino acids (Ala, Phe, Pro, Lys and Gly) as DNA-binding antitumor agents. The lysine conjugate XLIII was found to be over 20 times more cytotoxic to CEM human leukemia cells with an IC$_{50}$ in the submicromolar range. These compounds were found to form intercalation complexes with DNA as 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 \textit{in vivo} evaluation and drug design.

![Chemical structure of XLIII](image)

In a continuous effort to develop novel amino acid/peptides conjugated heterocycles, earlier investigations of our laboratory have revealed that heterocycles upon conjugating to different amino acids/peptides have shown promising activity compared to heterocycles or amino acids/peptides tested alone.

Shivakumara \textit{et al.}, [104] have synthesized novel benzylpiperazine conjugated amino acids \textbf{XLIV} and screened them for antibacterial and antifungal activities. Among the synthesized compounds, benzylpiperazine conjugated Phe, Trp, His and Pro showed more potency compared to rest of the analogs.

![Chemical structure of XLIV](image)

where, $R$ = side chain of different amino acids

In another report, Shivakumara \textit{et al.}, [105] have worked on the synthesis and antimicrobial activity of amino acids conjugated diphenylmethylpiperazine derivatives
and shown that compounds bearing Phe, Trp, His and Pro residues XLV have shown enhanced activity compared to other amino acid conjugates.

Suresha et al., [106] synthesized 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid conjugated peptides (VP, GVP, VGVP and GVGVP) XLVI as a novel class of potent antimicrobial agents. 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 when taken in isolation were inactive towards those bacterial strains.

Suhas et al., [107] have synthesized elastin based peptides and conjugated to benzisoxazole moiety XLVII and evaluated them as potent antimicrobial agents. The study revealed that as the length and hydrophobicity of the peptide chain increases, the activity also increases.
In another attempt, Suhas et al., [108], synthesized elastin peptides and conjugated to 1-(2,3-dichlorophenyl)piperazine XLVIII to study the effect of conjugation on the antimicrobial activity. The sequences of elastin peptides chosen are tetrapeptides, pentapeptides and tricosamers and also aromatic amino acids. The study revealed that all the conjugates have exhibited enhanced activity than the conventional drugs. Further, the conjugates of tricosamers have shown extraordinary activity against the fungal species with MIC value of 3–5 µg/mL which is fivefold more potent than the antibiotic used.

1.5. Urea/thiourea derivatives as potential pharmacophores

It has been well established that urea derivatives have got a significant place in modern medicinal chemistry [109]. In particular substituted ureas have attracted attention
due to their range of applications as agricultural pesticides [110], dyeing material for hair and cellulose fibres, antioxidants in gasoline and additives in detergents to prevent carbon deposits and corrosion inhibitors [111]. Unsymmetrical ureas can serve as active herbicides and pharmaceuticals [109, 110].

Urea and thiourea have been used as purification agents for the effluent of organic and inorganic, industrial, agricultural and mining wastes [112]. Their related compounds have been studied for the systematic control of tuberculosis [113-115] as well as anti-inflammatory activity during carrageenan induced edema in rats [116]. A few compounds of urea and thiourea (2-amino-1,3-thiazines) have shown bactericidal, fungicidal, herbicidal and algaecide activities [117, 118]. These compounds are useful in agriculture, spinning mixtures, paper and paints [119]. It has been described that acyl urea derivatives act as cation surfactants [120], while $\text{S-[w-(carboxamidino) alkyl]}$ isothiourea was used as radiation protector in mammals’ skin [121]. The compounds of urea and thiourea have exercised as wrinkle proofing agents for cotton and cotton polyester fabrics [122, 123]. The utility of urea and thiourea derivatives were found in the preparation of triazines, isoxazoles and oxazoles. Triazines showed inhibitory activity against Bacillus subtilis and Candida albicans [124]. A series of diaryl substituted heterocyclic urea which were found to inhibit cholesterol O-acyl transferase (ACAT) as hypocholesterolemic agents’ in vitro and in vivo studies [125], but $N,N'$-disubstituted cyclic urea-3-benzamide was found to be HIV protease inhibitor; which may be useful in the treatment of AIDS [126]. Urea and thiourea compounds also could be used for elimination or detoxification of super antigens from body fluids [127-129], and for the treatment of haemoglobinopathies in the cases of sickle cell anemia and β-thalassemia [130]. A series of ureas and thioureas was
synthesized, and their inhibitory activities against NO (free radical) production in lipopolysaccharide-activated macrophages were evaluated [131]. Further, combinations of urea and thiourea derivatives with benzothiazole have produced DNA topoisomerase [132, 133] or HIV reverse transcriptase inhibitors [134, 135].

Interestingly urea bonds are also used as critical structural elements for enzyme inhibitory functions and as switching points in retro-inverso peptidomimetics [136, 137]. The expected increase in metabolic resistance as compared to native peptides as well as interesting hydrogen bonding properties of the urea backbone makes ureidopeptides attractive both in drug discovery and in the search of novel folded structures [138]. The structural similarity of oligopeptidylureas with polyamides makes them essential drug candidates for potent HIV protease inhibitors, CCK-B receptor antagonists and endothelin antagonists [139-141]. Urea derivatives have also been intensively investigated in the area of molecular recognition, stabilizing secondary structures like turns, helices or sheets and to recognize carboxylic acids, sulphonic acids, nitrates, etc. The advent of split synthesis [142] in combinatorial methodology [143] has opened new avenues for identification of many other urea based compounds with useful pharmaceutical properties. Ureas are also employed as catalysts in enantioselective and diastereoselective reactions [144]. Consequently, there is a need for efficient ways of preparation of urea precursors.

Kim et al., [145] synthesized a series of urea/thiourea derivatives and evaluated inhibitory activities of NO production in lipopolysaccaride-activated macrophages. Amongst, XLVIX have carboxymethyl group at N3 position of thiourea was the most
potent in the inhibition of NO production. They inhibited NO production through the suppression of iNOS protein and mRNA expression.

![Image of molecule](image.png)

In another study, Pete et al., [146] reported the synthesis of benzoylcarvacryl urea and thiourea derivatives. All the synthesized derivatives showed comparable insecticidal activity with the standard BPU lufenuron. Compound L showed potent activity against human pathogens. Moderate and selective activity was observed for other compounds. All the synthesized compounds were non-haemolytic. These compounds have potential application in agriculture and medicine.

![Image of molecule with R](image.png)

where, R = 2-F, 4-F and 2,6-F

Santos et al., [147] reported synthesis of a series of novel 1-phenyl-3-{4-[(2E)-3-phenylprop-2-enoyl]phenyl} urea/thiourea derivatives and their evaluation against writhing test in mice, following the aromatic substitution pattern proposed by Topliss. The results of the preliminary bioassays indicated that compound LI presents promising
anti-nociceptive activity in acetic acid-, formalin-, and glutamate-induced pain in mice, compared with some well-known non-steroidal anti-inflammatory and analgesic drugs.

A notable advance was achieved by Khan et al., [148] by synthesizing and screening a series of urea/thiourea derivatives of steroids for their antibacterial activity. The results revealed that steroidal thiourea derivatives inhibit growth as compared to steroidal urea derivatives. LII and LIII were found to be better antibacterial agents compared to standard chloramphenicol.

Azam et al., [149] reported design and synthesis of a series of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7-yl urea/thiourea derivatives. Majority of the compounds exhibited significant antiparkinsonian activity after intraperitoneal administration. Amongst, LIV possessed most potent inhibitory effect than standard drug levodopa.
In another study, Saeed and co-workers [150] efficiently synthesized a series of thiourea derivatives bearing benzisothiazole moiety for the evaluation of their biological profile. In preliminary MTT cytotoxicity studies, the thiourea derivatives LV were found to be the most potent.

Upadhayaya et al., [151] synthesized quinoline derivatives possessing triazole, ureido and thioureido substituents and evaluated their antimycobacterial properties. LVI and LVII inhibited *Mycobacterium tuberculosis* H37Rv up to 98% and 94% respectively, at a fixed concentration of 6.25 µg/mL.
Suresha et al., [152] reported the synthesis of a series of urea/thiourea/acetamide/sulphonamide derivatives of quinazolinones conjugated lysine and screened for their antibacterial studies. The activity profile revealed that the compounds containing urea and thiourea functionalities along with fluoro group exerted highly potent activity.

Forty five new derivatives of ureas and thioureas were synthesized by Suhas et al., [153] and were evaluated for their ability to inhibit the growth of a panel of microorganisms. All the synthesized compounds displayed an excellent antimicrobial
activity. It was found through this study that F and Cl containing urea/thiourea
derivatives of tricosamer conjugates LIX (1.25 µg/mL) and LX (1.75 µg/mL) showed
nearly 20-25 fold greater activity than the standard used.

Shantharam et al., [154] synthesized a new series of benzisoxazole conjugated
amino acids derivatives possessing ureido and thioureido substituents and screened them
for their antiglycation activity. It was found that compounds containing methoxy and
bromine substituents LXI have exerted highly potent activity. Thus, the compounds
represent novel class of potent antiglycating agents.
Suyoga Vardhan et al., [155] have synthesized various urea/thiourea derivatives of amino acids-piperazine conjugates and the study revealed that compounds containing electron withdrawing groups like halogens have exerted potent activity (LXII).
1.6. References

8. L. Sun, *Mod. Chem. Appl.*, 2013, 1, 1


