CHAPTER 1

INTRODUCTION

1.1 HETEROCYCLIC COMPOUNDS

Heterocyclic compounds play a vital role among the pharmaceutically significant natural products and synthetic compounds. The heterocyclic nuclei have remarkable ability to serve both as biomimetics and reactive pharmacophores. This property has contributed them to have unique value as traditional key elements of numerous drugs [1]. New small organic molecules are very much needed in lead identification and lead optimization processes. Hence, the synthetic chemists are under pressure to synthesize compounds with enhanced biological activity and less side effects in short period of time.

1.1.1 Piperidin-4-ones

In recent years, there has been a growing interest with reference to the synthesis of bioactive compounds in the field of Organic Chemistry. Among the family of heterocyclic compounds, the nitrogen containing heterocycles, especially piperidin-4-ones is presumably gaining considerable importance owing to their varied biological properties as well as easy, economic and less time consuming preparation methods. Piperidine derivatives are formed by the reaction between acetone, ammonium acetate and substituted aromatic benzaldehydes. The parent molecule is flexible in nature and hence various derivatives can be easily prepared by altering its substituents. Piperidine compounds are reported to possess various biological activities, such as antiviral, antitumour, analgesic, local anesthetic, antimicrobial, bactericidal, fungicidal, herbicidal, insecticidal, CNS stimulant and depressant activities [2]. The skeletal ring of piperidine nucleus is often found in the molecular framework of many synthetic and natural medicaments [3]. Furthermore,
the significance of piperidin-4-ones as intermediates in the synthesis of a variety of physiologically active compounds has also been reviewed by Prostackov and Gaivoronskoya [4].

Piperidin-4-one compounds are also used as ligands in the preparation of complexes. Cockborn et al [5] have used N-benzylpiperidin-4-one as a ligand in the preparation of platinum and palladium complexes. Arunan et al [6] have studied the Cu(II) complexes of piperidin-4-ones and reported that piperidin-4-ones act as bidentate ligands co-ordinating through the ring nitrogen and carbonyl group.

Piperidin-4-ones have also been found to act as the potential inhibitors of human placental aromatase in vitro [7]. The 2,2,6,6-tetramethylpiperidin-4-one hydrochloride has been used as a spin trap in several EPR studies and its hydrazones are used as antioxidants and indocledin alkaloids. Many saturated and unsaturated piperidin-2-ones have been used as key chiral intermediates in the preparation of numerous natural and synthetic compounds with significant anticancer, anti-HIV and glycosidase inhibition activities. Piperidin-3-one derivatives are used as precursors for the synthesis of antimalarial agents viz., Febrifugine and Isofebrifugine. They are also reported to act as K⁺ channel blockers, hypoglycemic and hypolipidemic, anti-acetyl cholinesterase agents and opioid receptor antagonists. The 3,3-diethylpiperidin-2-one behaves as an effective anticonvulsant. The N-substituted-3-aminopiperidin-2-ones possess triptase inhibition activity. The 3-aminopiperidin-2-ones have been used as constrained surrogates of the SER – Leu dipeptides.

1.1.2 Diazepines

Diazepine is a heterocyclic compound [8] with two nitrogen atoms, five carbon atoms and possible number of cumulative double bonds. Benzodiazepines have been shown to be a very good scaffold for medicinal chemistry as a privileged structure i.e., a type of structure that is likely to bind to many types of targets. A reason for this is that the seven membered ring with the two nitrogen atoms mimics turns of endogeneius peptides. The first benzodiazepine, chlordiazepoxid (Librium)
was discovered accidently by Leo Standberch in 1955 and made available in 1960 and marketed as diazepam [Valium] since 1963. The general and simplest method for the synthesis of 1,5-benzodiazepines involves the acid catalyzed reaction of o-phenylenediamine with ketones, β-haloketons and α,β-unsaturated carbonyl compounds. Many catalysts have been reported in the literature for this reaction including BF$_3$-OEt$_2$, polyphosphoric acid-SiO$_2$, NaBH$_4$, MgO/POCl$_3$, Yb(OTf)$_3$, CH$_3$COOH using microwave, SO$_4^{2-}$/ZrO$_2$ and Al$_2$O$_3$-P$_2$O$_5$. Chemical process often employs large amounts of hazardous and toxic solvents. The choice of pursing a low-waste route and reusable reaction media to minimize the economic cost and environmental impact of a chemical process is becoming ever more urgent for the future. Therefore, there is pressure on organic chemists to investigate clean, economical and environmentally safer methodologies.

Benzodiazepines enhance the effect of neurotransmitter, gamma-amino butyric acid (GABA) which results in sedative, hypnotic (sleep inducing), anxiolytic (anti-anxiety), anticonvulsant, muscle relaxant, amnesic and antiinflammation action. Benzodiazepines have very recently been synthesized and utilized as both β-turn and γ-turn peptidomimetics. For a long time after the discovery of the effects of the 1,4-benzodiazepin-2-ones, there was a search for endogenous ligands to the benzodiazepine receptor subsite of the GABA$_A$ receptor. Better understanding of peptide interactions of the GABA receptor subtypes has lead to recent improved design on benzodiazepine ligands.

Various diazepines have also been reported as fungicidal and herbicidal. Substituted 1,4-diazepine and their derivatives possess anti-HIV activity which is lesser than that of zidovudine (3’ – azidothymidin = AZT). They also show platelet activating factor (PAF) antagonistic and serotonergic $S_3$ antagonistic activities. Benzodiazepine receptor systems have been related to the development and evolution of various neurological and psychiatric disorders, including epilepsy, Huntington’s disease and Alzheimer’s disease. It has also been reported that benzdiazepam binds to benzodiazepine receptors in the brain and potentially useful radiopharmaceutical for PET (positron emission tomography) studies of cerebral
benzodiazepine receptor. 1,5-benzodiazepines have been tested against breast cancer and have shown moderate activity.

Other than their biological importance, benzodiazepine derivatives are also commercially used as dyes for acrylic fibres. Moreover, 1,5-benzodiazepine derivatives are valuable synthons that can be used in the preparation of other fused ring compounds, such as triazolo-, oxadiazolo-, oxazino-, or furano-benzodiazepines. Research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity.

1.1.3 Tetrazoles

Tetrazole has an aromatic azapyrrazole nucleus and it is a five membered ring compound containing one carbon, four nitrogen and two hydrogen atoms. It carries two double bonds and it exists in two tautomeric forms as shown below:

\[
\begin{array}{cc}
1H-\text{Tetrazole} & 2H-\text{Tetrazole} \\
1 & 2
\end{array}
\]

The first tetrazole derivative was synthesized by J. A. Bladin in 1885. From then, the tetrazole derivatives are investigated intensively as a subject of many possible applications. In the last decades, tetrazole has a renaissance especially as carboxylic acid mimicry and ligands for transition metal complexes.

It has been documented that 5-substituted-1H-tetrazoles (RCN₄H) can serve as a non-classical isostere for the carboxylic acid moiety (RCO₂H) in biologically active molecules [9]. According to the concept of non-classical isosterism, the functional groups that have similar physico-chemical properties are interchangeable, which will result in similar biological properties. Furthermore, a non-classical isostere may or may not have the same steric or electronic
characteristics, nor even the number of atoms has the substituent for which it is used as a replacement.

The advantage of tetrazolic acids over carboxylic acids is due to their resistance to many biological metabolic degradient pathways. It has been reported that tetrazole derived nicotinic acid analogues that were administered as drugs were excreted unchanged over a 24 h period, whereas nicotinic acid were rapidly metabolized.

Tetrazoles are ionized at physiological pH (7.4) similar to that of their carboxylic acid counterparts. Both carboxylic acid and tetrazole exhibit a planar structure. It has been reported by Hansch and Leo [10] that anionic tetrazoles are almost ten times more lipophilic than the corresponding carboxylates, the important factor which has to be born in mind during drug design. Moreover, another factor while considering tetrazole replacement is the effect of delocalization of the negative charge around the tetrazole ring. The charge distribution over a greater molecular surface area may be favourable for a receptor substrate interaction or it may complicate the contact, depending on the local charge density available at the interface. The larger size of the tetrazole ring compared with the carboxylic acid group may also have the tendency to reduce the binding affinity at the active site either by less favourable orientation of functional groups or by steric hindering of an active conformational change of the receptor complex.

Costantino and co-workers [11] have made an interesting comparison between the effective lengths of carboxylic acid versus tetrazole pharmacophore.
The authors have studied the structure activity relationship (SAR) of propellane analogues of L-glutamic acids as mGlul receptor agonists. They prepared the amino acids (3) containing distal carboxylic acid and amino acid (4) tetrazole units. From the models, it is observed that the distance between the two carboxylic acid functional groups amino acid (3) is 5.6 Å, whereas the distance has increased by 1 Å when a carboxylic group is replaced by in an amino tetrazole moiety (4). The *in vitro* analysis revealed that the compound (4) is 2.5 fold less potent than compound (3). This is due to the increased distance between the two acidic sites indicating an unfavourable fit between important synergistic positions.

It has been recently reported that the hydrogen bonding capability of tetrazolic anions with receptor recognition sites is responsible for the key interaction of enhanced binding affinity. It has been found that the tetrazole substrates form two hydrogen bonds with peptide residues in a biological target site which is responsible for the strong binding interaction. For instance, mutagenesis studies have revealed that the tetrazole moiety of several non peptide antagonists interact with a protonated lysine and histidine in the active site of angiotensin II receptor.

Researchers [12] have proved that the tetrazole groups in drug molecules could improve the interaction of drugs with the receptors in cell membrane. From this view point, tetrazole is expected to be an alternative group to carboxylic acid in cation exchanger and the resulting exchanger might be used to investigate the interaction between tetrazole moieties in drugs and receptor.

Losartan 5 is an angiotensin II antagonist and is commonly used for the treatment of hypertension.
Tetrazoles exhibit a strong networking ability usually as ligands. The four nitrogen electron donating atoms allow it to act as mono or bidentate or multidentate ligand. It has been reported [13] that the tetrazole ligands are able to participate in at least seven distinct types of coordination modes with metal ions in the construction of novel metal organic frameworks. Furthermore, numerous tetrazolyl ligands coordinated with metal centers such as, nickel, ruthenium, palladium or platinum have been tested in catalyzed reaction. A possible application for these materials as molecular host is in generating supramolecular arrays which embody additional functional groups capable of metal complexation. This would result in a metallo tetrazole framework with potential as new catalyst and therapeutic agents.

The tetrazole ring system has attracted much attention in medicinal chemistry [14]. Tetrazoles are reported to exhibit antihypertensive, antiallergic and antibiotic activity. For example, they are currently used as activators and anticonvulsants, as well as in cancer and AIDS treatment. Many patents are available concerning their varied biological activities, such as muscle relaxation, anti inflammatory, antiarthritic, analgesic, ulcer therapeutic and coccidiostatic properties. Tetrazoles are also used as plant growth regulators, herbicides and fungicides in agriculture, as stabilizers in photography and photoimaging. Another important application of tetrazoles is in the preparation of imidoylazides.

Due to high enthalpy of formation, tetrazole decomposition results in the liberation of two nitrogen molecules and significant amount of energy. Therefore, several tetrazole derivatives have been explored as explosives, propellant components for missiles and as gas generators for air bags in the automobile industry. Furthermore, the tetrazole ring has strong electron withdrawing properties and the tetrazole halides are employed in synthesis as derivatising agents for the chemical modification of alcohols [15].
1.1.4 Triazoles

1,2,3-Triazole is a basic aromatic heterocycle. It is one of a pair of isomeric chemical compounds with the molecular formula C₂H₃N₃. It is a five membered ring of two carbon atoms and three nitrogen atoms. It exists in two isomeric forms as represented below:

![1, 2, 3 – Triazole](image1) ![1, 2, 4 – Triazole](image2)

Triazoles are surprisingly stable compared to other organic compounds with three adjacent nitrogen atoms. However, flash vacuum pyrolysis at 500 °C leads to a loss of nitrogen to produce aziridine. Certain triazoles are relatively easy to be cleaved by ring chain tautomerism.

Triazoles are capable of hydrogen bonding. This property is responsible for binding biomolecular targets as well as the increased solubility of the compounds [16]. Triazoles can function as attractive linker units which could connect two pharmacophores to form an innovative bifunctional drug. Hence, these compounds have become increasingly useful and important in constructing bioactive and functional molecules. It is worthy to note that the bioisosteric replacement between the triazole moiety and its bioisostere triazole has received considerable attention in medicinal chemistry, which represented an efficient concept for the discovery and development of novel triazole drugs.

Triazole derivatives are known to exhibit various pharmacological properties such as, antimicrobial, antitubercular, anticancer, anticonvulsant, antiinflammatory, analgesic and antiviral. Triazoles have also been incorporated in a wide variety of therapeutically interesting drugs including H₁/H₂ histamine receptor blockers, CNS stimulants, antianxiety agents and sedatives. Many commercial
antifungal drugs contain triazole functional moiety. The best examples for such antifungal drugs are Fluconazole (8), Voriconazole (9), Ravuconazole (10), Itraconazole (11) and Posaconazole (12).

Triazoles are used in the control of variety of fungal diseases in fruits, vegetables, legumes and grain crops, both as pre and post harvest applications [17]. The biochemical mechanism of their antifungal effect is based on the inhibition of ergosterol biosynthesis thereby interfering with fungal cell-wall formation. They also inhibit sterol 14α-demethylase and hence considered steroid demethylation inhibitor.
The 3- Amino-1,2,4-triazole is an inhibitor of mitochondrial and chloroplast function. Commercial grade 3-amino-1,2,4-triazole is used as a herbicide and cotton defoliant. The triazole derivatives such as, S-3307, S-3308, triadimefon and paclobutrazol are recommended for use both as fungicides and plant growth regulators.

The corrosion inhibition properties of triazoles are also well documented [18]. Some triazole derivatives such as, 4-amino-5-mercapto-3-methyl-1,2,4-triazole(AMMT), 4-amino-5-mercapto-3-ethyl-1,2,4-triazole(AMET) and 4-amino-5-mercapto-3-propyl-1,2,4-triazole(AMPT) have been evaluated as new corrosion inhibitors for the corrosion of muntz alloy (Cu:Zn 60:40) in acidic and neutral solutions.

Many authors [19] have studied the properties of triazole compounds such as, crystal structure, quantum-chemical calculations on the tautomerism and protonation sites, spectroscopic properties and thermal decomposition products. Triazoles are reported to be a useful synthetic intermediate for many organic reactions.

1,2,4- Triazoles have also several other significant applications. They are used as dyes, biological reagents, photographic chemicals, precursors for the synthesis of peptidomimetics and synthesis of polymers. They are also used as agrochemicals, photostablizers in photographic materials, used as light stabilizers, fluorescent whiteners and optical seizures [20].

The complexes of triazoles are also applied as effective catalysts for allylic alkylation. The use of N-substituted triazoles as catalysts are dominant due to the well developed click chemistry in the synthesis of ligands. They act as good binding ligands even in a neutral form. Triazoles have also been suggested for water replacements in proton conductors used in fuel cells. Once the triazoles are dopped into membranes, they improve the conductivity of the membranes under anhydrous conditions due to their amphoteric nature. These compounds are used as proton...
acceptors and donors. The two main reasons which facilitate the triazoles to be considered for water replacement are their amphoteric nature and mobility.

### 1.2 PHARMACOLOGY

Throughout the history, humans have incidentally and intentionally discovered new drugs both in nature and through scientific experimentation. In addition to using plant, animal and mineral substances exclusively, science has progressed to using these substances as well as their modified forms and laboratory-derived chemical substances. New knowledge in the areas of chemistry, pharmacology and drug use has led to the phenomenal growth in the drug industry. New drugs that are both life enhancing and life prolonging are regularly discovered and developed. This is a surprising factor in history because so many beneficial drugs are available and are continuing to be developed.

There are many definitions existing for the term “drug”. A drug is defined as “an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans or animals.” Drugs affect human biological systems in both positive and negative ways. Humans have been gaining knowledge of the effects of drugs for thousands of years.

Pharmacology is one of the cornerstones of the discovery process. The word “Pharmacon” means “Drug” and “Logos” means “Science”. Oswald Schmiedeberg (1838 – 1921) is generally recognized as the founder of modern pharmacology. Pharmacology is defined as an experimental science which has for its purpose the study of changes brought about in living organisms in chemically active substances, whether used for therapeutic purposes or not.

Pharmacology is a study of the actions of chemicals on the body. It is defined as chemicals that can have a therapeutic action to treat disease. Pharmacology utilizes the basic principles of chemistry, biology, physiology, pathology and microbiology in its practice. Pharmacologic methodologies serve to describe how a drug acts in the body, and in many cases the scientific studies of
drug action are instrumental in developing an understanding of body function in health and disease. From the beginning of civilization, pharmacology has had a place in human history in all cultures.

History of pharmacology dates back to 16th century A.D and in those days, turpentine, poppy, beer and juniper berries were used to treat diseases. Around 46 B.C., the famous Greek Physician and Teacher of Medicine Hippocrates found that opium had narcotic properties and it could be used for treating internal diseases. Even today, opium is the source of key modern pain killers and exemplifies the value of pharmacology.

During the middle ages, much emphasis was placed on combining multiple ingredients in medicines so that they could be used for any ailment. During this time, the Arabs contributed to drug knowledge by recording new information about crude preparations. In 1948, the first official pharmacopeia was published in Florence and Italy. The goal was to provide a source of uniform pharmaceutical standards.

Drug discovery has gained momentum in the modern age. Scientists began developing biological in the late 1700s and throughout 1800s. Louis Pasteur, who is responsible for numerous scientific achievements discovered that weakened forms of microbes could be used as immunizations for more virulent forms of microbes. His work led to the development of vaccines for chickenpox, cholera, anthrax and swine erysipelas, as well as modern rabies vaccines for humans and dogs.

After 1920, human medicine grew significantly. The introduction of chemotherapy in 1936 and overall drug industry growth after World War II kept the momentum going. As these changes occurred, a greater emphasis was placed on pharmacology. In the 20th and 21st century, remarkable changes have occurred in the production and use of human and veterinary drugs [21].
A medicinal chemist may create a compound, but a pharmacologist is the one who tests it for physiologic activity. A promising compound is investigated by many other scientists, toxicologists, microbiologists and clinicians. In actual use, however, its meaning is limited to the study of action of drugs.

The main tasks of pharmacologists in the search and development for new medicines are

1. Screening for desired activity.
2. Determining the mode of action.
3. Quantifying the drug activity when chemical methods are not available.

Pharmacology, being considered as a branch of biology provides the required scientific data in both animals and humans. The science of pharmacology includes allied fields such as, pharmacognacy, pharmacy, pharmacodynamics, pharmacokinetics, therapeutics, toxicology and chemotherapy. Pharmacognacy is a science of identification of drugs, whereas pharmacy is the science of identification, selection, preservation, compounding and dispensing of drugs. In pharmacodynamics, the effect of the drug on the body is studied. This study gives the relation between the drug concentration and the biological effect (physiological and biochemical). For most drugs, it is necessary to know the site of action and mechanism of action at the level of the organ, functional system or tissue. Pharmacokinetics is the study of the effect of the body on the drug. To produce characteristic effects, a drug must be present in appropriate concentrations at its site of action. Hence, it is important to know the interrelationship of the absorption, distribution, binding, biotransformation and excretion of a drug and its concentration on its locus of action. The word therapeutics means “To Nurse” and this branch of medicine deals with the cure of diseases. The science of toxicology deals with the adverse effects of chemical agents. Toxicology concerns not only the drugs used in therapy, but also the other chemicals that may be responsible for environmental or
industrial intoxication. Chemotherapy is a science concerned with the effect of drugs on microorganisms and parasites.

Pharmacology depends largely on experiments conducted in laboratory animals and even human may be used as a test subject. The animals most frequently used in pharmacologic studies are mammals. Mice are preferred because of their small size, ease of breeding and short span of life generation time. Rats, guinea pigs, rabbits and dogs are also used. Each of them has special characteristics that make them optimal for certain types of tests. Experimental pharmacology uses animals in various phases. Intact animals are essential for the acute, subacute and chronic toxicity tests.

Organic chemists play a vital role in the pharmaceutical industry. They synthesize new compounds as well as isolate and characterize natural products. They have a specific reason for synthesizing a particular compound usually based on theoretical considerations, medicinal chemistry, biological mechanism or a combination of all the three. The pharmacological activities of a compound are involved function of the structure and even very small changes may profoundly modify the pharmacological effects. These structural modifications may involve replacing one group with another at a specific point in the molecule. Slight changes may sometimes completely reverse the action of the compound.

1.2.1 Antimicrobial Activity

1.2.1.1 Antimicrobial agents

An antimicrobial agent or an antibiotic is defined as any chemical substance produced by microbes or made synthetically and capable of selectively destroying or inhibiting the growth of other microbes, especially bacteria. Antibiotics may include antibacterial agents though antifungal and antiviral agents may also be called by the term.
1.2.1.2 Antimicrobial action through inhibition of cell metabolism

Para-amino benzoic acid (PABA) is an essential metabolite for a number of microorganisms [22]. PABA is a precursor of folic acid which is a prerequisite for the synthesis of nucleic acid. PABA is converted to folic acid through the action of dihydropteroate synthetase (folic acid synthetase). The antimicrobial agents compete with PABA for the active centre of the enzyme, as a result nonfunctional analogs of folic acid are formed, preventing further growth and multiplication of the bacterial cell.

There are two classifications of bacteria known as Gram positive and Gram negative bacteria. This classification is based on whether the organisms do not strain with the grams strain but has significance far beyond that of an embriical straining reaction. Gram positive and Gram negative organisms are different in several aspects. *Staphylococcus, streptococcus and pneumococcus* are some of the examples of Gram positive bacteria and *Escherichia coli, Salmonella, Bordetelle, Pseudomonas aeruginosa and Camphyliocovac* are some of the examples for Gram negative bacteria.

1.2.1.3 Mechanism of antibacterial action

There are four mechanisms by which the antibacterial agents act. The antibacterial agents inhibit the cell metabolism. They are called as antimetabolites. These compounds inhibit the metabolism of a microorganism. They inhibit an enzyme catalyzed reaction which is present in the bacterial cell. Sulfonamides are the best examples of antibacterial agents acting in this way of inhibiting the cell metabolism. Some antibacterial agents will act by inhibiting the cell wall synthesis leading to bacterial cell lysis (bursting) and death. Penicillins and Cephalosporins are the examples of these types of drugs. Some bacterial cells affect membrane permeability. This will result in the death of the cell. Polymyxins and Tyrothricin operate in this way.
1.2.1.4 Antifungal agents

Fungi cause a range of illnesses (mycoses) ranging from chronic to the serious. These mycoses can manifest themselves in a variety of ways. Infections can be superficial, that is situated at or close to the surface of the skin, or systemic which means they can affect the body as a whole rather than individual parts or organs.

1.2.2 Analgesic Activity

Pain is an unpleasant sensation which only the individual himself can appreciate. It cannot be objectively defined satisfactorily. The struggle to relieve pain began with the origin of humanity. Ancient writings, both serious and fanciful dealt with secret remedies, religious rituals and other methods of pain relief. Slowly the present modern era of synthetic analgesics evolved.

There is no doubt that pain act as a warning signal against disturbances both in the body and in the external environment of an individual. As a symptom, pain demands instant relief and in practice dramatic relief of pain by drugs highly impresses a layman.

Pain receptor organs are distributed throughout the body. Clinically pain can be classified as superficial or cutaneous pain, deep non-visceral pain from muscle joints, ligments and bones, visceral pain, referred pain or psychogenic pain.

An analgesic may be defined as a drug bringing about insensibility to pain without the loss of consciousness. Tainter has divided the history of analgesic drugs into four major eras, namely:

1) The period of discovery and use of naturally occurring plant drugs.

2) Isolation of pure plant principles (e.g. alkaloid).

4) Development of modern pharmacological techniques making it possible to undertake systematic testing of new analgesics.

It appears that peripheral sensation is transmitted by large diameter (L), fast conducting nerve fibres via small diameter (S) and slow conducting nerve fibres. Minimal stimulus such as, touch activates the L nerve fibres. Such an impulse, on reaching the spinal cord, activates the first transmission cell and also the collateral cells in the substantia gelatinosa (SG). Anatomically, these nerve fibres are carried in the dorsal nerve roots and end in the SG at the apex of the dorsal gray horn and the intermediate gray matter in the spinal cord. The SG cells inhibit the passage of signals and thus decrease the output reaching the higher centres. If, however, the peripheral stimulus is more intense, then both L and S nerve fibres are activated, resulting in higher output from transmission cells reaching the higher centres, leading to perception of pain. Thus, this gate control allows the sensory input to be decreased or augmented depending on the relative activity of L fibres and S fibres which in turn, depends upon the intensity of peripheral stimulus.

The secondary axons which arise from the dorsal horn cross over to the other side around the central canal of the spinal cord and from the spinothalamic tract. The intermediate gray matter gives origin to the spinoreticular fibres which intermingle with the spinothalamic tract. In the brainstem, the spinothalamic pathway retains a lateral position and finally terminates in the thalamus and the post central gyrus and is mainly responsible for localization of pain sensation. The other intermingled fibres which form an ascending multisynaptic pathway terminate in the thalamus and from there to frontal and limbic systems. This system is concerned with the emotional concomitants of pain.

It appears that higher centres, through their central inhibitory and facilitatory mechanisms, exert modulating influence on the gate mechanism. Thus, clinically the sensation of pain has several components including the emotional reaction. Painful stimuli may primarily be physical stimuli such as, pressure or heat, or they may be chemical stimuli from the products of inflammation.
A variety of naturally occurring compounds are capable of eliciting pain response in experimental animals, e.g., histamine, acetylcholine, bradykinin and 5-hydroxytryptamine. One or more of these substances are also present in various venoms. However, whether endogenous release of these substances is involved in the production of pathological pain is uncertain.

The laboratory evaluation of analgesics was formerly confined to studies on experimental pain in animals like rat, mice and guinea pigs. Pain can be evoked in these animals by intraperitoneal administration of chemicals, such as bradykinin, phenylbenzoquinone and acetic acid. Alternatively, methods such as the application of a clip of radiant heat to the rat tail, placing mice on a hot plate, electrical stimulation of the incisor tooth pulp or rectal mucosa of the guinea pigs or application of radiant heat to the guinea pig have also been extensively employed.

1.2.3 Antiinflammatory Activity

Inflammation is the body effort to inactivate or destroy invading organisms, remove irritants and set the stage for tissue repair. It is a normal protective response to tissue injury. Inflammation may be caused by microbiological agents, noxious chemicals or trauma. It can also be triggered by innocuous agents, such as pollen or by an autoimmune response, as in some asthmas or rheumatoid arthritis. In such cases, the defense reactions themselves may cause progressive tissue injury. Antiinflammatory or immune depressive drugs may be required to modulate the inflammation process. The inflammation subsides when the healing is complete.

Inflammation is triggered by the release of chemical mediates from the injured tissues and migrating cells. These specific chemical mediators may vary depending upon the type of inflammatory process. The chemical mediators include amines such as, histamine and 5-hydroxytryptamine. Lipids such as prostaglandins, small peptides such as bradykinin and larger peptides such as interleukin-1 are some of the chemical mediators. Discovery of such a variety of chemical mediators has clarified the apparent paradox that different drugs are effective in treating one form
of inflammation but not others. Thus, a drug may interfere with the action of particular mediator important in one type of inflammation, but be without effective in the inflammatory processes not involving the target mediator.

1.2.3.1 NonSteroidal antiinflammatory drugs (NSAIDs)

NSAIDs are the most widely used of all therapeutic agents. The examples for these type of drugs are aspirin, naprofen, ibuprofen, flurbiprofen, fenbufen, ketoprofen, indomethacin, sulindac, phenylbutazone, azapropazone, meclofenamic acid, mefenamic acid, talmatin, paracetamol etc. They are frequently prescribed for rheumatic musculo skeletal complaints. The primary action of the NSAIDs is the inhibition of arachidonate cyclooxygenase and thereby inhibiting the production of prostaglandins and thromboxane. Prostaglandins are unsaturated fatty acid derivatives containing 20 carbon atoms and a cyclic ring structure. Prostaglandins and related compounds are produced in minute quantities by virtually all tissues. They generally act on the tissues in which they are synthesized and are rapidly metabolized to inactivate products at their site of action. Therefore, prostaglandins do not circulate in the blood at significant concentrations.

1.2.3.2 Action of prostaglandins

Prostaglandins are one of the chemical mediators that are released in allergic and inflammatory process. Prostaglandins produced endogenously in the tissues act as local chemical signals that fine tune the response of a specific cell type. For example, the release of thromboxane from platelets is a signal to initiate recruitment of new platelets for aggregation (that is the first step in clot formation). However, in other tissues, elevated levels of thromboxane convey a different signal. For example, in certain smooth muscles, thromboxane induces contraction.

Many of the actions of prostaglandins are mediated by their binding to membrane receptors causing the subsequent activation or inhibition of adenyl cyclase. In some tissues, however prostaglandins appear to act by mechanisms that do not involve cAMP.
1.2.3.3 Inhibition of prostaglandin synthesis by NSAIDs

In the prostaglandin synthetic pathway, cyclooxygenase converts arachidonic acid to unstable intermediate PGG$_2$ and PGH$_2$. It is suggested that there are two types of cyclooxygenase namely, Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). COX-1 is constitutive enzyme found in blood vessels, stomach, kidney and in most tissues including blood platelets. It is involved in cell-cell signaling and in tissue homeostasis. COX-2 is induced in inflammatory cells when they are activated and is believed to be the enzyme that produces the prostanoid mediators of inflammation.

Most NSAIDs in current use are inhibitors of both isoenzymes, though they vary in the degree of inhibition of each. Clearly the antiinflammatory action of the NSAIDs is related to their inhibition of COX-2 and it is probable that when used as antiinflammatory agents, their unwanted effects are largely due to their inhibition of COX-1. New compounds with this selective action on COX-2 are in pipeline and could transform the approach to the treatment of inflammatory conditions.

Recently, it has been reported that nabumetone, a new NSAID, preferentially inhibits COX-2. This agent has antiinflammatory activity with little ulcerogenic effects. Similarly, new drugs like nimesulide and meloxicam are also reported to have greater affinity for COX-2 as compared to COX-1. Thus, the effort now is to develop NSAIDs which have greater affinity for COX-2 than COX-1.

1.2.4 Anticonvulsant Activity

Epilepsy is a common disorder affecting approximately 40 million people [23]. In 70 % of patients who have epilepsy, no specific cause can be determined. Though there is no recognizable cause for this disease, it may develop as a consequence of various kinds of brain damage, such as trauma, inhibition or tumor growth. It is a neurological condition characterized by recurrent, paroxysmal abnormal episodes of motor, sensory, autonomic or physic function. Epilepsy can affect anyone at any stage. However, the disease is more common in children than
adults, with prevalence rate between 4-8 per 1000 children below the age of seven years.

The characteristic event in epilepsy is seizure. Seizure is associated with the episodic high frequency discharge of impulses by a group of neurons in the brain. It initially starts as a local abnormal discharge and then spread to other areas of the brain. The site of the primary discharge and the extent of its spread determine the symptoms that are produced, which range from a brief lapse of attention to a full-blown convulsion losing for several minutes. The particular symptoms are produced depending upon the function of the region of the brain that is affected. Thus, the involvement of the motor cortex causes convulsions, involvement of the hypothalamus causes peripheral autonomic discharge and the involvement of the reticular formation in the upper brain stem leads to loss of consciousness.

In brain, the spread of convulsive activity is restricted probably by inhibiting mechanism. Certain physiological changes can themselves cause seizures. They may trigger the focus or may facilitate the spread of normal neurons. Changes in blood glucose concentration, blood gas tension, plasma pH, total osmotic pressure, fatigue and emotional stress are the physiological factors responsible for convolution.

The treatment of epilepsy includes the complete control of seizures, make the patient return to the normal life and make them free from side effects like impaired school performance. Charles Locock used KBr as the antiepileptic drug. It was the first antiepileptic drug used. But now it is obsolete. Fenobarbital was used in 1912 and Phenytoin (or diphenyl hydantoin) was used in 1938. The drug phenytoin was introduced in therapy by Merritt and Putnam in the year 1938. Phenytoin is the primary drug for all types of epilepsy except absence seizures. It exerts a selective antiepileptic action without causing drowsiness. The onset of action is low even on intravenous injection but the action persists for a considerable time after sedation or therapy.
Phenytoin inhibits the spread of seizure discharges in the brain and shortens the duration of after discharge. The drug is known to decrease the neuronal sodium concentration leading to a reduction in the post tetanic potentiation (PTP) and to increase the neuronal potassium concentration. PTP is an enhancement of synaptic transmission following repeated mechanic and high frequency simulation of the presynaptic fibers. The enhancement of synaptic transmission due to PTP may produce transmission tetanic impulses along the cerebral excitatory feedback circuits and the spread of such an activity across the entire brain would result in generalized grand seizures. Reduction in PTP by phenytoin blocks this process and stops the spread of the seizures discharge. The work of woodbury and associates indicates that the stabilizing effect of phenytoin is closely related to its ability to decrease the intraneuronal sodium concentration. Phenytoin also augments brain level of GABA (γ-amino butyric acid) as well as of 5HT and homovanillic acid. The restoration of a balance between the excitatory glutamate and inhibitory GABA pathways may be responsible for its antiepileptic action [24].

1.2.4.1 Mechanism of anticonvulsant drugs

The current anticonvulsant drugs are developed embrically on the basis of activity in animal models such as the electroshock seizure test. Their mechanism of action at the cellular level is not generally well understood. However, there are two main mechanisms. They are:

1) Enhancement of GABA action.

2) Inhibition of sodium channel function.

Enhancement of GABA Action

Many of the clinically effective anticonvulsants enhance the inhibitory effect of GABA, facilitating the GABA mediated opening of chloride channels. A recently introduced new drug namely, Vagabatrin acts by inhibiting the enzyme GABA-transaminase which is responsible for inactivating GABA, thereby
increasing the GABA content of the brain and heme enhancing its action as an inhibitory transmitter.

**Inhibition of Sodium Channel Function**

The antiepileptic drugs such as, phenytoin and carbamazepine effect membrane exitability by an action of voltage-dependent sodium channels which carry the inward membrane current necessary for the generation of an action potential. These antiepileptic drugs block the excitation of cells that are firing repeatedly. It is to be noted that higher the frequency of firing, the greater will be the blocks produced. This characteristic, which is relevant to the ability of drugs to block the high frequency discharge that occurs in an epileptic pit, without unduly interfering with low frequency firing of neurons in the normal state arises from the ability of blocking drugs to discriminate between sodium channels in their resting open and inactivated states. Depolarization of a neuron increases the proportion of the sodium channel in the inactivated state. Phenytoin and carbamazepine bind preferentially to channels in this state, preventing them from returning to the resting state and this reduces the number of functional channels available to generate action potentials.

1.3 **MOLECULAR DOCKING STUDIES**

Evolution of science has been benefiting mankind through a myriad ways. The notable advance in science is tracking and controlling diseases. We cannot imagine a world without drugs. The concept of drugs is as old as that of diseases. Drugs that are chemical in nature have side effects, though they are very much essential for life and diseases. Diseases are caused in the body due to the disharmony in the internal and external bodily parts.

To treat and control a disease, it is important to understand the biological processes involved in its evolution. All diseases, be it a simple headache or life-threatening cancer, all involve some biological processes such as, cell-to-cell communication, neural transmission, etc. It is very difficult to understand the
complex processes that are occurring in human body. It is time consuming, tedious and accurate results cannot be obtained.

The better way to understand the complex mechanism would be, to simulate the biological processes, and understand and design methodology for tackling the disease. *In vivo* and *in vitro* studies constitute the experiments for simulating them in the wet lab whereas *in silico* (computer aided) methods do not need animal models or enzymatic methods. *In silico* approaches have gained immense popularity recently and have become an integral part of the industrial and academic research that is directed towards drug design and discovery.

Molecular docking is a simulation process that predicts the conformation of a receptor-ligand complex, in which the receptor can be either a protein or a nucleic acid, and the ligand is a small molecule. This simulation can be visualized as analogous to the key and-lock problem. Here, the lock is the receptor and the key is the ligand. The aim of docking is to adjust the position of the key in the lock. It is possible to generate many possible positions for the key in the lock through a computer simulation. Therefore, there is a need for a criterion that will allow comparisons of all possible positions of the key and then select the best position.

High-throughput screening technique is the commonly used technique in drug discovery. It is used to evaluate the activity of a large number of compounds against a known target by trial and error means. This procedure is very costly and time-consuming. If the crystallographic structure of the protein target is available, then molecular docking simulations are very helpful computational approach in the process of drug-discovery

It has been well documented that the computer simulation process allows for faster and cheaper identification of promising drug candidates using structure-based virtual screening. After identifying the lead molecules by virtual screening process, *in vitro* tests can be performed to further evaluate the drug candidates.
The main aim in molecular docking is to find the fittest solution (pose) using fitness function (scoring function). The docking programs currently in use are DOCK AUTODOCK, GOLD, FLEXX, ZDOCK, M-ZDOCK, MS-DOCK, SURFLEX, MCDOCK, GLIDE, GEMDOCK and MOLDOCK. These programs were developed with the goal to get a rapid procedure capable of identifying a novel lead compound (in virtual screening) or replicating an experimental crystallographic structure (for validation with experimental data) that is as highly accurate as possible.

Docking programs can search for the best fit between two or more molecules by considering several parameters obtained from receptor and ligand atomic coordinates, such as geometrical complementarity, atomic VDW radius, charge, torsion angles, intermolecular hydrogen bonds and hydrophobic contacts. As a result, docking applications return the predicted orientations (poses) of a ligand in the target’s binding site. The posing process usually returns numerous possible conformations and several positions for a key. Scoring functions, which are able to evaluate intermolecular binding affinity or binding free energy are employed to optimize and rank the results to obtain the best orientation after the docking procedure and selecting the best key position.